Lineage Infidelity of a Human Myelogenous Leukemia Cell Line

By Antonio Palumbo, Jun Minowada, Jan Erikson, Carlo M. Croce, and Giovanni Rovera

We have analyzed the organization and expression of the immunoglobulin heavy and light chain gene in the human myeloblastic leukemic sublines, ML1, ML2, and ML3, and in the human myeloid leukemic cell lines, HL-60, U937, THP1, and K562. ML1, ML2, and ML3 cells, despite a predominant granulocytic phenotype, express a rearrangement of the immunoglobulin heavy chain gene that typically occurs during the early stages of the B cell differentiation pathway. No rearrangement was found in any of the other cell lines tested. These findings strongly support the notion that, at least in some cases, acute myeloid leukemia (AML) cells represent highly atypical cells with profoundly altered gene expression, rather than cells arrested at a well-defined stage of the myeloid lineage.

TWO MODELS have been advanced to explain the derivation of acute leukemia cells. One model proposes that the cells result from either an impairment in differentiation along early stages of the lymphoid or myeloid pathway or from an uncoupling between the ability to proliferate and the ability to differentiate. An alternative model proposes that, in at least some cases of acute myeloid leukemia (AML), the cells are highly atypical and coexpress genes specific for the granulocytic-monocytic lineage with genes specific for the lymphoid or erythroid lineage. The latter model has been suggested by McCulloch and co-workers, who have shown that both the K562 myelogenous leukemia cell line and blast cells from patients with AML coexpress a wide variety of phenotypic markers characteristic of the granulocytic, erythroid, and B lymphocytic lineages. The presence within the same leukemic cells of markers specific for different hemopoietic lineages has been termed "lineage infidelity" and has been observed thus far in only a fraction of the leukemic cell populations examined. A possible objection to the lineage infidelity model is that the simultaneous but transient expression of markers selectively retained in the granulocytic, erythroid, and B lymphocytic lineages may occur in normal hemopoietic progenitor cells. In contrast, gene rearrangements involving the heavy and light immunoglobulin chain loci are believed to be irreversible, since they involve DNA deletions. These rearrangements are considered a marker event, reflecting the commitment of pluripotent stem cells to differentiation along the B lymphocytic lineage.

In this article, we examine the genotypic and phenotypic characteristics of a human myelogenous leukemia line (ML) established recently in the laboratory of one of us. ML cells, despite a rearrangement of the immunoglobulin heavy chain genes, clearly exhibit a predominant granulocytic phenotype and can be induced to differentiate in vitro into cells expressing markers of the granulocytic and monocytic lineages.

MATERIALS AND METHODS

Cells
Human myeloblastic leukemia ML sublines, ML1, ML2, and ML3, human promyelocytic leukemia HL-60, monocytic leukemia U937 and THP1, and erythroleukemia K562 cell lines were grown to densities of $5 \times 10^6$ to $1 \times 10^7$ in RPMI medium supplemented with 10% fetal bovine serum.

DNA Extraction
Cells ($5 \times 10^6$) were washed twice in 100 mmol/L Tris-Cl, 10 mmol/L EDTA, and resuspended in 2 mL of a solution containing 100 mmol/L EDTA, 10 mg Na-lauroyl-sarcosine (Sigma, St Louis, Mo), and 0.5 mg Proteinase K (Boehringer Mannheim, West Germany) and incubated at 50 °C for three hours. Suspensions of lysed cells were then extracted three times with saturated phenol and dialyzed extensively in 50 mmol/L Tris-Cl, 10 mmol/L EDTA, and 10 mmol/L NaCl. Samples were incubated with RNase-free RNase (100 μg/mL) at 37 °C for three hours, extracted twice with a mixture containing 50% (vol/vol) phenol/chloroform, and dialyzed against 1 L of 10 mmol/L Tris-Cl. The extracted DNA was precipitated with ethanol and quantitated by UV spectrophotometry.

DNA Gel Electrophoresis and Southern Transfer
Agarose gel (0.8%) electrophoresis was carried out in 40 mmol/L Tris-Cl, 5 mmol/L NaOAc, 2 mmol/L EDTA, pH 8.0. HindIII-digested λ-phae DNA molecular weight markers (Bethesda Research Labs, Md) were included on every gel. Cellular DNA samples were digested with BamHI, EcoRI, or HindIII and subjected to electrophoresis in a horizontal agarose (Bethesda Research Labs) slab gel. Gels were stained for ten minutes with ethidium bromide (1 μg/mL) and photographed under UV light. Transfer of DNA from gels to nitrocellulose sheets (Millipore, Bedford, Mass) was performed essentially as described by Southern.

Preparation of Labeled Probe DNAs
The probes for the μ, J, x, and λ regions used in these studies are schematized in Fig 1. The Jw probe consisted of a 3.3-kb germline EcoRI-HindIII fragment. The human J region was obtained by subcloning a 3.3-kb fragment of the human genomic DNA clone (H18CP10). This fragment contains 2.2 kb of the human joining (J) region DNA and 1.1 kb of the 3' end flanking sequences. The Cu is a 1.2-kb EcoRI fragment subcloned in pBR322, encompassing the...
sequences encoding the Cmu, Cmu, and Cmu fragments. Cx is a 300-bp Mbol–Hindl fragment subcloned in M13 mp7, equivalent to residues 115 to 415 (T. Rabbits, MRC Laboratory of Molecular Biology, University Medical School, Cambridge, England; manuscript in preparation). Cx is an 8-kb EcoRI fragment subcloned in pBR322 containing the Ckeoz Ckeoz genes.

The Jmu, Cmu, Cx, and Ca probes were labeled with 11P to a specific activity of 0.5 to 2 x 106 cpm/μg of DNA before use and hybridized with DNA.

Hybridization

DNA on nitrocellulose filters was hybridized to 32P-labeled probe DNA in a hybridization solution containing 50% (vol/vol) formamide, 0.9 mol/L NaCl, 50 mmol/L HEPES, 5 mmol/L EDTA, 0.2 mg/mL sonicated salmon sperm DNA, 1X Denhardt’s solution at 42°C. After hybridization, the filters were washed, air-dried, and exposed to Kodak XRP-5 film for various periods.

Phenotypic Analysis

Cytocchemical stainings (peroxidase and ASD chloroacetate esterase) were performed as described by Kaplow15 and Yam et al.18

Cell surface phenotype was analyzed by indirect immunofluorescence (Ortho Cytofluorograf 5OHH, Ortho Instruments, Westwood, Mass) using monoclonal antibody R1B19, which is specific for the granulocytic lineage; monoclonal antibodies LB3.45 and 54.7, specific for cells of the myelomonocytic lineage; monoclonal antibodies (CALLA). Antibody BA, specific for a B cell subset antigen; SK3.7, specific for HLA-DR; and J5, specific for common acute lymphocytic leukemia antigen (CALLA). Membrane IgM, cytoplasmic IgM, and the ability to form rosettes with sheep erythrocytes (SRBC) were assayed as described.21,22

RESULTS

DNA samples extracted from ML1, ML2, and ML3 sublines and from human HL-60 promyelocytic leukemia, U937 and THP1 monocytic leukemia, and K562 erythroleukemia cells13 were digested with BamHI restriction endonuclease, separated by agarose gel electrophoresis and transferred to nitrocellulose filters. The resulting blots were hybridized to a 32P-labeled DNA probe specific for the constant region of the immunoglobulin heavy chain gene (Cmu).13 One germline band of approximately 17 kb was detected in all the myeloid cell lines tested (Fig 2). ML1, ML2, and ML3 cells showed an additional band of approximately 13.5 kb. Similarly, the joining (Jmu) segment of the K gene, detected after hybridization of HindIII-digested DNA from the cell lines with a 32P-labeled Jmu probe, resolved as a single 9-kb band in germline configuration in HL-60, U937, THP1, and K562 cells (Fig 3A). However, the Jmu segment was rearranged in ML1, ML2, and ML3 cells, as evidenced by a 9-kb germline band and a second band of 8 kb. Rearrangement of the Jmu segment was also detected when the DNA samples were digested with EcoRI restriction endonuclease (Fig 3B). In this case, we observed a 16-kb germline band and a second band of 12 kb, representing the rearranged Jmu segment. The rearrangement of both the Cmu gene and the Jmu segment suggests that, in ML cells, the target for malignant transformation was a cell that had completed the process of rearrangement of a heavy chain immunoglobulin gene. However, no K-heavy chain immunoglobulin mRNA could be detected by mRNA dot blot (data not shown) under conditions that allow detection of approximately ten molecules per cell of specific mRNA.24

The Cx and the Ca genes were detected in a germline configuration in all the myelogenous leukemia cell

![Fig 1. Schematic representation of the human immunoglobulin gene probes used to detect germline and rearranged genes. (A) The 3.3-kb human Jmu region and the 1.2-kb Cmu, encoding the Cmu, Cmu, and Cmu fragments; (B) the 300-bp Cx region; (C) the 8-kb Ca fragment containing the Ckeoz Ckeoz genes.](image)

![Fig 2. Cx-related sequences in DNA of human myeloid leukemic cell lines. BamHI-digested genomic DNA, prepared from HL-60, ML1, ML2, ML3, U937, THP1, and K562 cells, was electrophoresed, transferred to nitrocellulose paper, and hybridized to a 32P-labeled Cmu probe. All the cell DNA samples tested show a 17-kb germline band, whereas ML1, ML2, and ML3 DNA show an additional band of approximately 13.5 kb.](image)

![Fig 3. Configuration of Jmu segment in human myeloid leukemic cell lines. DNA samples were digested with HindIII (A) and EcoRI (B) restriction endonucleases, electrophoresed and hybridized to the 32P-labeled Jmu probe. (A) HL-60, U937, THP1, and K562 DNA show only a single germline band of 9 kb. ML1, ML2, and ML3 DNA show a rearranged configuration with a 9-kb germline and a rearranged 8-kb band; (B) similar results were obtained after EcoRI digestion of ML1, ML2, and ML3 DNA: 16-kb germline band and a 12-kb rearranged band.](image)
Table 1. Characteristics of the Human Myeloid Leukemic Cell Line ML3

<table>
<thead>
<tr>
<th>Cytochemical and Immunologic Markers</th>
<th>Lineage Specificity</th>
<th>Percent Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase</td>
<td>Granulocytic</td>
<td>80</td>
</tr>
<tr>
<td>ASD chloroacetate esterase</td>
<td>Granulocytic</td>
<td>60</td>
</tr>
<tr>
<td>R1B19</td>
<td>Granulocytic</td>
<td>95</td>
</tr>
<tr>
<td>S4.7</td>
<td>Myelomonocytic</td>
<td>95</td>
</tr>
<tr>
<td>LB3.45</td>
<td>Myelomonocytic</td>
<td>95</td>
</tr>
<tr>
<td>SK37.7</td>
<td>HLA-DR (a)</td>
<td>75</td>
</tr>
<tr>
<td>BA1</td>
<td>B subset</td>
<td>0</td>
</tr>
<tr>
<td>J5</td>
<td>CALLA</td>
<td>80</td>
</tr>
<tr>
<td>Membrane IgM*</td>
<td>B cell</td>
<td>0</td>
</tr>
<tr>
<td>Cytoplasmic IgM*</td>
<td>pre-B cell</td>
<td>0</td>
</tr>
<tr>
<td>SRBC*</td>
<td>T cell</td>
<td>0</td>
</tr>
</tbody>
</table>

*At least 200 cells were examined with the optical or fluorescent microscope. Values are the means of at least two separate experiments.

Fig 4. Southern blot of restriction endonuclease digests of human myeloid cell lines to human Cx (A) and CX (B) probes. (A) BamH1-digested DNA samples were hybridized with 32P-labeled Cx probe, detecting a single germline band of 12 kb. (B) Hybridization of same DNA samples digested with EcoRl with 32P-labeled CX probe reveals a germline configuration in all the samples tested, whereas K562 DNA shows an amplified germline configuration.

Lineage infidelity in leukemia

The ML1, ML2, and ML3 sublines were established from the peripheral blood of a patient with acute myelomonocytic leukemia. This leukemia arose two months after the patient underwent chemotherapy for a malignant lymphoma of pre-T cell type. A direct relationship between the T lymphoma cells of the patient and the myelogenous leukemia cells could not be established, since the karyotype of the lymphoma cells was diploid, while that of the myeloblastic leukemic cells was polyploid, with a unique marker chromosome. The leukemic sublines established in culture contained the same chromosomal abnormalities that were present in the leukemic cells of the patient. Therefore, the three sublines were considered to be of clonal origin and were not further recloned for these studies. Cells from these sublines exhibit myeloblastic and promyelocytic morphology and granulocytic lineage-specific immunologic and histochemical markers. They can be induced to differentiate in vitro into mature cells expressing several granulocyte- and monocyte-specific markers by treatment with the phorbol diester 12-0-tetradecanoyl-13-acetate, cytosine arabinoside, or dimethyl sulfoxide.

However, the ML cell line contains a rearrangement of the JH region of the immunoglobulin chain locus, but not of the light chain loci, which is a pattern observed in cells at an early stage of maturation along the B cell pathway, as in the REH common ALL cell line. Since several restriction enzymes were used in this study, the data support a recombination of the JH antigens, respectively. However, because CALLA is also expressed at low levels in granulocytic cells, its presence cannot be considered to be indicative of a pre-B marker. No cytoplasmic or membrane immunoglobulin or T cell markers were observed in ML3 cells.

**DISCUSSION**

The results of surface marker analysis, using immuno-fluorescence and histochemical markers of the ML3 subline, are summarized in Table 1. Cells of the ML3 subline were 80% reactive with peroxidase and 60% reactive with ASD chloroacetate esterase, two granulocytic lineage-specific markers. More than 95% of the cells were positive for monoclonal antibody R1B19, which is specific for the granulocytic lineage, and for monoclonal antibodies LB3.45 and S4.7, specific for cells of the myelomonocytic lineage. The high (approximately 100%) percentage of cells positive for myelomonocytic markers, together with the presence of two bands with similar intensity in the Southern blots analyzing the μ-gene and J segment, rules out the possibility that the ML3 line consists of two separate populations, one myeloid and one lymphoid.

The B subset antigen detected by antibody BA1 was not detected in ML3 cells, whereas 80% of the cells were weakly positive for antibodies SK37.7 and J5, which are specific for the HLA-DR and CALLA antigens, respectively. However, because CALLA is also expressed at low levels in granulocytic cells, its presence cannot be considered to be indicative of a pre-B marker. No cytoplasmic or membrane immunoglobulin or T cell markers were observed in ML3 cells.
region. A deletion with the intervening sequence can also be ruled out, because the different rearranged restriction fragments do not differ from their normal counterparts by the same amount. Since we were unable to detect an abnormal chromosome 14, we could rule out a chromosome translocation involving the heavy chain immunoglobulin locus.

Immunoglobulin gene rearrangement, common in cases of human B lymphomas, is a rare event in the case of T lymphoma. In fact, Korsmeyer et al. examined 12 cases of T cell lymphomas and found only one case (in the HSB2 cell line) in which Cγ and Jγ were rearranged.

The lack of available information about the conformation of the IgG genes in the T lymphoma cells of the patient from which the ML cells were derived precludes any definitive conclusion on whether or not the leukemia cells were derived from the malignant lymphoma T cells. This possibility cannot be ruled out in light of the case reported by Murphy et al., in which the direct transition from T leukemic cells to myeloid leukemia cells was observed following therapy. In any case, the observations reported here indicate that a recombination of the Jγ region does not restrict the commitment of a pluripotent cell to the lymphoid lineage, but still allows the cells to differentiate into cells with a myeloid phenotype. Since recombination of the Jγ region is usually lineage-specific, it is possible that lineage infidelity is observed only in those types of leukemias in which the target for the malignant transformation process is a very immature stem cell. The findings of lineage infidelity in the K562 line10 could be explained by the fact that K562 cells derive from a patient with chronic myelogenous leukemia, a type of leukemia that has been shown to originate from pluripotent stem cells. Recent reports indicate that myeloblastic leukemia cells freshly obtained from patients with chronic myelogenous leukemia in blastic crisis had immunoglobulin gene rearrangement in the case of lymphoid but not myeloid blastic crisis. It will be of interest, however, to determine whether or not other AML cells, freshly obtained from patients, contain immunoglobulin gene rearrangements.

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