Evidence Favoring Lineage Fidelity in Acute Nonlymphocytic Leukemia: Absence of Immunoglobulin Gene Rearrangements in FAB Types M4 and M5

By S.P. Ackland, C.A. Westbrook, M.O. Diaz, M.M. Le Beau, and J.D. Rowley

The concept of lineage fidelity in acute leukemia has recently been challenged by the finding of rearrangements of the immunoglobulin heavy chain genes in a leukemic cell line and in a small number of sporadic cases of acute nonlymphocytic leukemia with a monocytic phenotype. We therefore screened leukemic blood or bone marrow samples of 33 adult patients with acute nonlymphocytic leukemia of FAB types M4 (23 patients) and M5 (10 patients); 28 were obtained at diagnosis and 5 at relapse. All cases were well characterized pathologically and histochemically. Cytogenetic analysis performed in each case demonstrated karyotypes that were representative of those generally seen in these types of leukemia, with a clonal abnormality present in all except 9 of 32 patients who were successfully studied. DNA prepared from each sample was digested with the restriction enzyme BamHI and analyzed by Southern blot hybridization to probes for the Jκ region of the immunoglobulin heavy chain. All 33 cases had DNA retained in the germline configuration with no evidence of rearrangement. This finding supports the concept of lineage fidelity, and suggests that true interlineage infidelity, myeloid to lymphoid, is a rare occurrence in adult acute nonlymphocytic leukemia.

The concept of lineage fidelity in acute leukemia states that the leukemic clone is derived from a single phenotypically defined cell, that markers with specificity for normal differentiation pathways maintain that specificity in the leukemic clone, and that differentiation is arrested rather than being deranged.1-3 Furthermore, it implies that leukemic cells do not cross lineages. This concept remained largely unchallenged until recently when several investigators independently reported coexpression of lymphoid and myeloid markers in cells from some leukemic cell lines and from fresh leukemic tissue.4-6 This occurrence of "lineage infidelity" suggests that the leukemic cell may arise from a less well-differentiated stem cell precursor, which is capable of producing differentiated cells with features of both lineages.5 The investigation of leukemic lineage has led to many insights into the understanding of both normal and abnormal hematopoietic cell differentiation.

The hallmark of the B lymphocyte is the capacity to produce immunoglobulin. The loci for human immunoglobulin heavy, kappa, and lambda chain genes, have been mapped to chromosomes 14, 2, and 22, respectively.7-10 The genes coding for each immunoglobulin class are represented by a number of exons separated by noncoding regions along the chromosomes. For heavy chain genes at least four distinct elements are known, termed V (variable), D (diversity), J (joining), and C (constant) regions.11 The primary event leading to expression of the gene is a somatic recombination of the germline elements such that these regions are apposed.12 A developmental hierarchy exists such that heavy chain gene rearrangement represents the first stage of B cell commitment.13

Until recently, immunoglobulin gene rearrangements were considered to be restricted to normal B lymphocytes and B cell neoplasms. Demonstration of heavy chain gene rearrangements in cases of common "non-T/non-B" acute lymphoblastic leukemia (ALL),13 and cases of chronic myelogenous leukemia in lymphoid blast crisis14 and hairy cell leukemia15,16 indicate that these malignancies are of B cell origin. However, rearrangements of the heavy chain gene have also been detected in human and mouse T cell lines as well as in the leukemic cells of patients with T cell malignancies.12,17-19

Heavy chain immunoglobulin gene rearrangements have been reported in one human acute myelogenous leukemia cell line (ML), one cell line with both lymphoid and monocytoid features and a t(4;11) chromosomal rearrangement, as well as in fresh leukemic cells from three patients characterized phenotypically as acute nonlymphocytic leukemia (ANLL).10-12,21 Most of these cases had a monocytic phenotype. The finding of an irreversible stage of B cell commitment in some non-B cell neoplasms is surprising. Moreover, it casts doubt on the concept of lineage fidelity in leukemic blasts, and suggests instead that, in some cases of leukemia, transformation occurred in a progenitor cell capable of both B cell and myeloid differentiation, or that abnormal differentiation may include some switching between cell lineages.

To investigate this phenomenon, we studied leukemic cells from 33 adult patients with active acute myelomonocytic leukemia (AMoL) or acute monoblastic leukemia (AMOL; FAB types M4 and M5)22 for the presence of immunoglobulin heavy chain gene rearrangements. This patient population was well characterized phenotypically and cytogenetically. We report that the immunoglobulin genes retained germline configuration in all cases.

MATERIALS AND METHODS

For cytogenetic analysis, fresh leukemic cell specimens were obtained from peripheral blood buffy coat or bone marrow from patients at the University of Chicago (23 patients) and from other institutions (10 patients). These patients all had active ANLL that...
had been classified as FAB type M4 or M5 by morphology and by
cytochemical reactions according to established criteria.23

Cytogenetic analysis was performed on cells obtained at the time
of diagnosis or at clinical relapse as previously described.14 Meta-
phase chromosomes were analyzed using trypsin-Giemsa or quina-
crine fluorescence banding techniques. Chromosome abnormalities
were described according to the International System for Human
Cytogenetic Nomenclature (1978).26 An abnormal clone was
defined by the detection of at least two pseudo-diploid or hyper-
diploid cells, or at least 3 hypodiploid cells with identical chromo-
somal abnormalities.

High molecular weight DNA extracted from leukemic cell speci-
cmens that had been stored at -70 °C was digested with the
restriction enzyme Barn HI. The products were size-fractionated by
electrophoresis in 0.8% agarose gels, and the DNA fragments were
then transferred to activated nylon membranes (Gene Screen Plus,
New England Nuclear, Boston, Mass) by the technique of South-
em. Membranes were hybridized as described previously,18 with
nick-translated 32P-radiolabeled probes cloned in pBR 322 plasmids.
Probes used recognizing the joining region of the immunoglobulin
heavy chain gene (JH-region) have been previously described.16 After
extensive washing of filters, autoradiography was performed against
a sheet of x-ray film using a dual intensifying screen for 1 to 2 weeks
at -70 °C.

RESULTS

A total of 80 adult patients with a diagnosis of M4 or M5
ANLL were karyotyped at the University of Chicago
between 1981 and 1985. Thirty-three of these patients had
adequate numbers of leukemic cells frozen for DNA analysis
and these constitute the study population. The median age of
this group was 50 years (range 18 to 85 years). Twenty-three
patients were characterized as AMMoL (FAB type M4)
including one patient with ANLL following refractory ane-
mia. This patient (no. 214—see Table 1) had received
low-dose cytosine arabinoside prior to the diagnosis of
ANLL. Ten patients were characterized as AMoL (FAB
type M5) including one patient who developed ANLL fol-
lowing chemotherapy for lung carcinoma. Twenty-eight
patients had newly diagnosed ANLL and 5 patients were

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Source</th>
<th>Status</th>
<th>% ABN* Cells</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>PB</td>
<td>dx</td>
<td>58</td>
<td>46,XY(6%)/46,XY,-2,+der(2)t(11;1)45-13,del(9)(q21q34)(100%).</td>
</tr>
<tr>
<td>148</td>
<td>BM</td>
<td>dx</td>
<td>60</td>
<td>46,XX(83%)/46,XX,+8,+21.i(7)(17%).</td>
</tr>
<tr>
<td>150</td>
<td>BM</td>
<td>rel</td>
<td>80</td>
<td>46,XY(100%).</td>
</tr>
<tr>
<td>165</td>
<td>BM</td>
<td>dx</td>
<td>48</td>
<td>46,XY(100%).</td>
</tr>
<tr>
<td>166</td>
<td>BM</td>
<td>dx</td>
<td>83</td>
<td>46,XY(100%).</td>
</tr>
<tr>
<td>179</td>
<td>BM</td>
<td>dx</td>
<td>73</td>
<td>46,XY,+9,t(16;16)(11%)/48,XY,+9,+22.t(16;16)(86%)/SCA:48,XY,+8,+9,+22,t(16;16)(3%).</td>
</tr>
<tr>
<td>182</td>
<td>BM</td>
<td>dx</td>
<td>49</td>
<td>46,XX(39%)/46,XX,del(7)(q32q36)/inv(16)(p13q22)(16%).</td>
</tr>
<tr>
<td>183</td>
<td>BM</td>
<td>dx</td>
<td>76</td>
<td>46,XY(16%)/46,XY,del(7)(q12q31),inv(16)(p13q22)(84%).</td>
</tr>
<tr>
<td>194</td>
<td>PB</td>
<td>dx</td>
<td>58</td>
<td>46,XX(92%)/46,XX,del(6)(q17q23)(8%).</td>
</tr>
<tr>
<td>195</td>
<td>BM</td>
<td>dx</td>
<td>80</td>
<td>46,XY(14%)/46,XY,inv(3)(q12q26)(60%)/45,XY,−7,inv(3)(q12q26)(36%).</td>
</tr>
<tr>
<td>205</td>
<td>BM</td>
<td>dx</td>
<td>66</td>
<td>46,XX(13%)/46,XX,-16,-21,+der(21)t(16;21)(p11;q22),+mar(G size)(87%).</td>
</tr>
<tr>
<td>212</td>
<td>PB</td>
<td>dx</td>
<td>79</td>
<td>46,XY(100%).</td>
</tr>
<tr>
<td>214</td>
<td>BM</td>
<td>dx†</td>
<td>67</td>
<td>46,XX(93%)/46,XX,inv(9)(p23q23)(7%).</td>
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<tr>
<td>215</td>
<td>BM</td>
<td>dx</td>
<td>50</td>
<td>46,XY(91%)/46,XY,del(16)(q22)(9%).</td>
</tr>
<tr>
<td>231</td>
<td>BM</td>
<td>dx</td>
<td>85</td>
<td>46,XX(100%).</td>
</tr>
<tr>
<td>238</td>
<td>BM</td>
<td>dx</td>
<td>70</td>
<td>46,XX(43%)/46,XX,inv(16)(p13q22)(57%).</td>
</tr>
<tr>
<td>8003</td>
<td>BM</td>
<td>dx</td>
<td>70</td>
<td>46,XX(13%)/46,XX,-16,-21,+der(21)t(16;21)(p11;q22),+mar(G size)(87%).</td>
</tr>
<tr>
<td>R-15</td>
<td>PB</td>
<td>rel</td>
<td>73</td>
<td>46,XY,inv(16)(p13q22),r(17)(p13q25)(59%)/47,XY,+8,inv(16)(26%)/46,XY,-17,+8,inv(16)(15%).</td>
</tr>
<tr>
<td>R-28</td>
<td>PB</td>
<td>dx</td>
<td>75</td>
<td>47,XX,+22.t(16;16)(p13q22)(95%)/SCA:46,XX,t(16;16)(p13q22)(5%).</td>
</tr>
<tr>
<td>R-29</td>
<td>BM</td>
<td>dx</td>
<td>95</td>
<td>47,XY,+22.t(16;16)(p13q22)(77%)/46,XY,t(16;16)(p13q22)(73%).</td>
</tr>
<tr>
<td>R-31</td>
<td>BM</td>
<td>dx</td>
<td>90</td>
<td>46,XY(15%)/46,XY,+11q(58%)/46,XY,-16,-del(11)(p13q11),r(17;20)(p12q11)(32%)/SCA:47,XY,same,+10(5%).</td>
</tr>
<tr>
<td>R-32</td>
<td>BM</td>
<td>dx</td>
<td>90</td>
<td>47,XY,+Y(100%).</td>
</tr>
<tr>
<td>R-33</td>
<td>BM</td>
<td>dx</td>
<td>83</td>
<td>46,XX(100%).</td>
</tr>
</tbody>
</table>

*Percentage of leukemic cells identifiable by morphology.
†Leukemia following refractory anemia.

 Patients 128 to 183 and R-15 were previously reported.24,25 R refers to patient specimens referred to the University of Chicago. PB, peripheral blood, BM, bone marrow. Status: dx, diagnosis of leukemia, rel, relapse.

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studied at the time of relapse. Leukemic cells constituted 48% to 99% of nucleated cells in the specimens obtained, as assessed by morphology (peripheral blood in 11, bone marrow in 22 patients).

The karyotypic abnormalities identified in these patients were consistent with those generally seen in these types of leukemia (see Tables 1 and 2). Specifically, the inv(16) or t(16;16) that has been associated with AMML was observed in 9 patients, and the t(9;11), a rearrangement commonly seen in AMoL, was noted in 2 patients. Chromosomal anomalies seen in other types of ANLL, such as the t(8;21) and t(15;17) in M2 and M3, respectively, were not seen in this group. One patient (no. 188) who had a hyperdiploid clone with 57 chromosomes had an extra chromosome 14; otherwise no abnormalities involving chromosome 14 were found. This observation is not surprising since nonrandom abnormalities of chromosome 14 have not been reported in ANLL. A gain of chromosome 22 was observed in 3 patients. In each case this abnormality was associated with an inv(i6) or a t(i6;i6), a phenomenon that has been reported previously. A single patient (no. 148) had a rearrangement involving chromosome 2; however, this rearrangement involved a breakpoint in band p25, a site that is distal to the immunoglobulin light chain locus at p11–p13. Only 9 patients failed to show a clonal abnormality.

All 33 cases were analyzed for immunoglobulin gene rearrangements. In each case DNA was retained in the germline configuration (single 18 kilobase fragment) with no evidence of rearrangements (see Fig 1).

### DISCUSSION

In our study, all of the adult patients with the M4 or M5 subtype of ANLL clearly retained the immunoglobulin heavy chain gene in its germline configuration. We decided to investigate the monocytic and myelomonocytic subgroups, since immunoglobulin gene rearrangements in ANLL seem to be more common in these subgroups. All our patients were well characterized as M4 and M5 and no patients had ambiguous markers or lymphoid phenotypes. These findings
are in contrast to two recent series; Rovigatti et al demonstrated a Cμ segment rearrangement in 2 of 14 samples examined from children with ANLL, and Ha et al found a rearrangement in one adolescent patient classified as FAB type M1, but only germline configuration of immunoglobulin genes in 8 other children and 10 adults with ANLL.

Of six myeloid cell lines studied to date, only one (ML) has shown a Cμ rearrangement; HL-60, K562, U937, THP1, and KG-1 show no such rearrangement. The cell line established from a 32-year-old leukemic patient with a t(4;11) translocation also had a Cμ rearrangement. This information is difficult to interpret because this cell line simultaneously displays lymphoid and monocytic features. Patients with this karyotype may be diagnosed as acute lymphoblastic, acute myelomonocytic, or biphenotypic leukemia.

In a variety of hematopoietic malignancies that have nonspecific surface markers, the presence of rearrangement of immunoglobulin genes from the germline configuration is considered a marker of clonal B cell differentiation. However the observations of such rearrangements in a few cases of myeloid leukemia have led to the suggestion that the initial event in immunoglobulin gene organization occurs during an early stage of hematopoietic cell development, at a time when the cells are not restricted to B lineage development, but may differentiate along more than one pathway.

This concept is supported by the demonstration of heavy chain rearrangements in an occasional T cell neoplasm.

All of these exceptions raise questions regarding the concept of lineage fidelity in acute leukemia. In contrast, our findings support the concept of lineage fidelity, and suggest instead that true interlineage infidelity, myeloid to lymphoid, is either an extremely uncommon occurrence in adult ANLL, or that its appearance is confined to childhood ANLL.

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