Heterogeneity of Acute Undifferentiated Leukemia at the Immunoglobulin and T-cell Receptor Genes Level

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Phenotypic markers of leukemic cells from 76 children with acute leukemia were examined. Of these cases, 7 were diagnosed as acute undifferentiated leukemia (AUL) whose leukemic cells were negative for myeloperoxidase and did not react with lineage-specific or lineage-associated monoclonal antibodies. Then, we analyzed the configuration of both immunoglobulin (Ig) and T-cell receptor β-chain (Tβ) gene in these 7 AUL cases. Three cases had no rearrangement of Ig or Tβ genes which was suggestive of non-lymphoid origin of these cases. In contrast, 4 cases showed rearrangements of Ig and/or Tβ genes. One of these 4 cases demonstrated Tβ gene rearrangement with retention of the germline configuration of Ig genes. Two cases with both Ig and Tβ gene rearrangements also showed kappa-chain gene rearrangements. These findings indicate the heterogeneity of AUL at the DNA level, and may cast new light on the early differentiation of hematopoietic progenitor cells.

Key words: Acute undifferentiated leukemia — Ig gene — T cell receptor β chain gene

Acute leukemias have traditionally been classified on the basis of the morphological and cytochemical characteristics of their leukemic cells. With the introduction of monoclonal antibodies, the cellular origin of leukemic cells can be more precisely determined. However, a small population of acute leukemias is difficult to classify due to the absence of reactivity with lineage-specific monoclonal antibodies. On the other hand, several reports have recently shown that analysis of immunoglobulin (Ig) and T cell receptor (TCR) β-chain (Tβ) genes by the Southern blotting technique can identify the cellular lineage and the stage of differentiation of individual leukemic cells. First demonstrated that the leukemic cells in common acute lymphoblastic leukemias (ALL) are committed to B-cell lineage by analysis of Ig gene rearrangement and showed the developmental hierarchy of the Ig gene in B-lineage cells. Minden et al. detected rearranged Tβ genes in all T-lineage ALLs. On the basis of these findings, it is likely that leukemic cells are frozen at some differentiation level in relation to their normal counterpart, and that rearrangements of Ig or TCR genes are essential for the differentiation to B-cell or T-cell lineage. Thus, characterization of the configuration of Ig and Tβ genes in leukemic cells from AUL is meaningful not only for the investigation of their ontogeny, but also for further refinement of the classifications of them. In the present work, we have studied 7 children with AUL, and found that 3 of them had the germline of both Ig and Tβ genes, one with rearrangements of the Tβ genes and three with rearrangements of both the Ig and Tβ genes. Based on the information gained from this study, the heterogeneity of AUL at the DNA level was obvious, and further classification seems to be possible.

MATERIALS AND METHODS

Patients Phenotypic markers of leukemic cells were examined in 76 children with acute leukemia by using our standard panel of monoclonal antibodies between July 1984 and September 1985. The results were as follows: acute myelogenous leukemia (13 cases), T-ALL (8 cases), B-ALL (2 cases), B-lineage ALL (46 cases) and

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HETEROGENEITY OF ACUTE UNDIFFERENTIATED LEUKEMIA (AUL) (7 cases). Leukemic cells from bone marrow or peripheral blood of the seven AUL patients were obtained before initiation of treatment. These leukemic cells were negative for myeloperoxidase and did not react with any lineage-specific or lineage-associated monoclonal antibodies. Cell morphology was examined by Wright-Giemsa and myeloperoxidase staining based on the FAB classification.\textsuperscript{1}

**Immunological Marker Studies** Mononuclear cells were isolated after Ficoll-Hypaque gradient centrifugation. Reactivity with a panel of monoclonal antibodies was assessed by indirect immunofluorescence using fluorescence microscopy. The panel of monoclonal antibodies consisted of OKT11, Leu1, B1, B4, slg, OKT10, Mo2, My7, Ia and J5.

**DNA Preparation** Mononuclear cells were obtained from 6 cases before initiation of treatment, and those from one case (case 4) were obtained during induction chemotherapy. High-molecular-weight DNA was extracted by routine methods from the mononuclear cells of the 7 children with AUL. The DNA samples were digested with either Eco RI, Bam HI, or Hind III restriction endonuclease. The digested DNA was subjected to electrophoresis in a 0.6% agarose gel and transferred to nitrocellulose filters by the Southern blotting technique.\textsuperscript{13} Filter-bound DNA fragments were then hybridized to nick-translated (\textsuperscript{32}P) probes and visualized on autoradiograms.\textsuperscript{14} The human Ig gene probes used in this study were the Ig heavy (H)-chain joining region JH probe (3 kb embryonic Eco RI-Hind III JH-containing fragment, provided by Dr. P. Leder)\textsuperscript{15} and the Ig kappa (\(\kappa\))-chain constant region C\(\kappa\) probe (2.5 kb embryonic Eco RI C\(\kappa\) containing fragment, provided by Dr. P. Leder).\textsuperscript{16} The human T\(\beta\) gene probe was the constant C\(\beta\)1 probe, which hybridizes to the C\(\beta\)1 and C\(\beta\)2 region (0.8 kb Bgl II-Eco RV C\(\beta\)1-containing fragment of the cDNA clone YT35, provided by Dr. T. W. Mak).\textsuperscript{17}

**RESULTS**

**Clinical Characteristics** The characteristics of the seven patients are presented in Table I. Four were males and three were females. The age range was from 2 months to 11 years. The initial leukocyte count ranged from 4,700 to 267,400/liter (mean 51,300/liter). Leukemic cells from 4 cases were classified as L1 morphology according to the FAB classification and the others were L2. Three of 6 examined cases were positive for periodic acid-Schiff (PAS) staining, and all cases were negative for myeloperoxidase. Six patients, including three with high risk factors (age <2, >10 yr, white cell count >50,000/liter),\textsuperscript{18} achieved complete remission, but one (case 7) did not. Among the 6 patients achieving complete remission, however, two cases (cases 3 and 5) relapsed 3 and 4 months later, respectively.

**Reactivity with Monoclonal Antibodies** The findings on the reactivity with monoclonal antibodies for the 7 patients are shown in Table II. Leukemic cells from all the cases did not react with lineage-specific or lineage-associated monoclonal antibodies such as B4, B1, slg, T11, Leu1, My7 and Mo2, and also did not react with J5. Four cases (cases 1 to 4) were Ia+ and two cases (cases 2 and 5) were T10+.

**Ig and T\(\beta\) Gene Analysis** In order to examine the Ig H-chain gene configuration, genomic DNAs from all 7 AUL patients were hybridized to the JH probe after Eco RI digestion (Fig. 1). Three cases (cases 3 to 5) showed rearrangements of the H-chain gene and 4 cases retained the germline configuration.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC at onset ((\times 10^9)/mm(^3))</th>
<th>FAB</th>
<th>PAS</th>
<th>POX</th>
<th>CR</th>
<th>Duration of CCR (mo)</th>
<th>Survival (mo)</th>
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<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>F</td>
<td>21.8</td>
<td>L(_1)</td>
<td>−</td>
<td>−</td>
<td>yes</td>
<td>10+</td>
<td>11+</td>
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<td>2</td>
<td>11</td>
<td>F</td>
<td>26.3</td>
<td>L(_2)</td>
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<td>−</td>
<td>39</td>
<td>42</td>
<td></td>
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<tr>
<td>3</td>
<td>2 mo</td>
<td>M</td>
<td>267.4</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>yes</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>M</td>
<td>9.4</td>
<td>L(_2)</td>
<td>+</td>
<td>−</td>
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<td>2+</td>
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<tr>
<td>5</td>
<td>9</td>
<td>F</td>
<td>4.7</td>
<td>L(_2)</td>
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<td>−</td>
<td>4</td>
<td>10+</td>
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<tr>
<td>6</td>
<td>2</td>
<td>M</td>
<td>16.5</td>
<td>L(_1)</td>
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<td>−</td>
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<td>6+</td>
<td>8+</td>
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<tr>
<td>7</td>
<td>1 yr 10 mo</td>
<td>F</td>
<td>13.3</td>
<td>L(_1)</td>
<td>−</td>
<td>−</td>
<td>no</td>
<td>4</td>
<td></td>
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ND, not done; FAB, French-American-British classification; CR, complete remission; PAS, periodic acid-Schiff staining; CCR, continuous complete remission; POX, peroxidase staining.
Table II. Results of Immunologic and Immunoglobulin and T-cell Receptor Gene Analysis

<table>
<thead>
<tr>
<th>No.</th>
<th>Ia</th>
<th>B4</th>
<th>J5</th>
<th>B1</th>
<th>Slg</th>
<th>T11</th>
<th>Leu1</th>
<th>T10</th>
<th>My7</th>
<th>Mo2</th>
<th>Jh</th>
<th>Cn</th>
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<tr>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>G</td>
<td>ND</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>(39)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>G</td>
<td>ND</td>
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<td>R</td>
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<td>+</td>
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<td>R</td>
<td>G</td>
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<tr>
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<td>-</td>
<td>-</td>
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<td>ND</td>
<td>-</td>
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<td>-</td>
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<td>G</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

(-) is <25%, (+) is >50%, specific values are 25–50% of the cells showing reactivity. ND, not done; R, rearranged configuration; G, germline configuration.

Fig. 1. The rearrangement patterns of immunoglobulin genes in patients with acute undifferentiated leukemia. The cases are numbered as in Table I. Lane C shows the germline control (an 18-kb fragment for the Jh probe and a 12.5-kb fragment for the Ck probe). Genomic DNAs from patients were hybridized to the Jh probe after Eco RI digestion (A) and to the Ck probe after Bam HI digestion (B). Arrows indicate rearranged bands. Because of the small proportion of leukemic cells in case 3, the density of the rearranged band in case 3 is diluted.

Fig. 2. The rearrangement patterns of T-cell receptor β-chain genes in the seven AUL patients. Lane C shows the germline control. Lanes 1 through 7 show the patterns after digestion with Eco RI (A) and Bam HI (B) for each patient identified in Table I. Triangles indicate the germline 11-kb and 4-kb Eco RI bands, and 24-kb Bam HI bands; arrows indicate rearranged bands.
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These findings were consistent with the results when Bam HI or Hind III was employed (data not shown). With consideration of the hierarchy of the Ig gene rearrangements (H-chain genes → κ-chain genes), further investigation was performed in three cases using the Cκ probe after Bam HI digestion. We found single allelic rearrangement of the κ-chain gene in 2 cases (cases 3 and 4). To determine whether the Tβ gene had rearranged, DNA samples were digested with Eco RI and Bam HI (Fig. 2). The Cβ probe used in this study hybridizes to two constant region genes (Cβ1, Cβ2). In the germline configuration, Bam HI generates a 24-kb fragment containing both Cβ1 and Cβ2, while Eco RI digestion yields 11- and 4-kb fragments containing Cβ1 and Cβ2, respectively. The 4-kb band does not move even in Cβ2 rearrangements, because an Eco RI restriction site lies just before Cβ2.19 Two cases (cases 1 and 5) showed deletion of both Cβ1 genes after digestion with Eco RI. In case 3, a single allelic rearrangement of Cβ1 gene was observed after Bam HI digestion. Compared to the faint rearranged bands, the intense germline bands in case 3 may have arisen from contaminating normal cells. In case 4, a single rearranged band was demonstrated with a faint germline band after Bam HI digestion. This may represent differential efficiency of transfer to the filter. However, the 11-kb band is also fainter than the 4-kb band in Eco RI digestion. Taken together, one allele of Tβ genes in germline configuration and a rearrangement of Cβ2 gene with deletion of Cβ1 gene in the remaining allele could be a most likely explanation for this case 4.

Finally, the 7 cases with AUL were divided into three subgroups at the DNA level: 3 cases (cases 2, 6 and 7) with the germline configuration of both the Ig and Tβ genes, one case (case 1) with rearrangement of only the Tβ genes, and 3 cases (cases 3 to 5) with rearrangement of both the Ig and Tβ genes (Table II).

DISCUSSION

Acute leukemia has been classified on the basis of the morphological features combined with the cytochemical staining characteristics according to the FAB classification.1) Recently, the introduction of immunologic techniques has made it possible to define the cellular lineage of leukemic cells in the majority of acute leukemias.2) However, a small number of acute leukemias do not have features matching any lineage, and such leukemias are termed AUL.3,4) Meanwhile, recent reports have shown that the analysis of the Ig and Tβ genes can be a powerful procedure for demonstrating the cellular lineage of individual leukemic cells.5-12) Analysis of the Ig and Tβ gene organization in AUL is of great value in distinguishing the cellular lineage, characterizing the hematopoietic stem cells and designing a treatment strategy for AUL.

We examined the phenotypic markers of leukemic cells from 76 children with acute leukemia, and 7 cases (9%) were classified as AUL. In the 7 cases with AUL, leukemic cells from three cases (cases 2, 6 and 7) showed the germline configuration of both the Ig and Tβ genes, and this suggests that they were not of lymphoid origin. It is conceivable that these leukemic cells might have been derived from either hematopoietic stem cells, or primitive myeloid cells.20,21) Since the detailed characteristics of hematopoietic stem cells are not known and there are no molecular genetic methods to distinguish myeloid cells, it is difficult to discern their cellular lineage precisely.

In one case (case 1), we found rearrangement of the Tβ gene, while the germline configuration was retained for the Ig gene. Since Minden et al.9) first reported that Tβ gene rearrangements were observed in all of 14 T-lineage ALL patients examined, it appears that TCR gene rearrangements are essential for the differentiation to the T-cell lineage and Tβ gene rearrangement has been used as the criterion to classify the cellular lineage of T-cell origin.22-24) Accordingly, leukemic cells from this case may be committed to the T-cell lineage. Contrary to these findings, however, we recently reported Ig gene rearrangements in a case of T-ALL25) and a case of acute myelogenous leukemia.26) Furthermore, Tawa et al.26) demonstrated that 10 out of 39 non-T, non-B ALL cases showed rearrangements of both the Ig and Tβ genes. These findings suggest that gene rearrangement of the Ig or Tβ gene does not always establish
B-cell or T-cell lineage, respectively. Similarly, we also found that 3 of our 7 cases (cases 3 to 5) had rearrangement of both the Ig and Tβ genes. In order to define the cellular lineage of individual leukemic cells from these cases, it is necessary to analyze the gene expression. Acute leukemia can be presumed to be a result of clonal expansion of cells which are frozen at different stages in relation to their normal counterpart. Therefore, cells with this combination may exist in the normal hematopoietic system, and analysis of these cases would provide information relating to the differentiation of lymphoid progenitor cells.

Based on the developmental hierarchy of Ig gene rearrangements, rearrangement of the light (L)-chain genes is observed in rather differentiated cells committed to B-cell lineage, and rearrangement of the L-chain genes has been considered to be a unique marker of the B-cell lineage. Two cases (cases 3 and 4) with AUL in this study showed rearrangements of the κ-chain genes in addition to H-chain and Tβ gene rearrangements. There are two possible explanations for this event; (1) that leukemic cells from these cases had lost their B-cell specific surface antigens, and (2) that these rearrangements of L-chain genes are aberrant. Recently, we found rearranged H- and L-chain genes with rearrangements of the Tβ genes in an apparent T-lineage lymphoma.29) Thus, detection of L-chain gene rearrangement does not always seem to indicate a B-lineage commitment.

The findings gained from this study confirmed that gene rearrangement is an early event which may be initiated in early hematopoietic cells. The findings also showed the heterogeneous nature of AUL at the DNA level.

All 7 patients were diagnosed as AUL on the basis of the morphological and cytochemical findings, and 6 were treated according to the standard protocol for ALL. Three patients relapsed early. Based on our results, AUL seems to be a more heterogeneous disease than has previously been believed, and a specific treatment strategy should be designed for each individual case with AUL taking into account the results of multimarker analysis.

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