Table 1. Proviral DNA and Antibody Titers Among ATL Patients by Clinical Subtype

<table>
<thead>
<tr>
<th>Subtype</th>
<th>N</th>
<th>Median White Blood Count (× 10⁹)</th>
<th>Median Proviral DNA Levels (copies/10⁶ lymphocytes)</th>
<th>Median Antibody Titers (reciprocal titer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>11</td>
<td>50.0 (23.9, 178.7)</td>
<td>30,409 (17,100, 72,946)</td>
<td>9,162 (4,406, 249,459)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>13</td>
<td>11.9 (7.2, 12.2)</td>
<td>4,266 (3,177, 13,489)</td>
<td>23,281 (5,916, 68,234)</td>
</tr>
<tr>
<td>Chronic</td>
<td>6</td>
<td>17.1 (14.8, 38.9)</td>
<td>81,283 (34,914, 144,877)</td>
<td>97,949 (59,020, 163,305)</td>
</tr>
</tbody>
</table>

*As defined in Shimoyama.†
†25th and 75th percentile values.

REFERENCES

Tyrosine Phosphorylation of Shc Proteins in Normal CD34⁺ Progenitor Cells and Leukemic Cells

To the Editor:

After the Shc gene was identified, biological function studies have focused on the oncogenic potentials of Shc proteins, especially their role in the Ras-dependent mitogen-activated protein kinase activation.1,2 Jucker et al1 reported in Blood that Shc proteins are constitutively tyrosine-phosphorylated in primary acute myelogenous leukemia (AML) cells but not in primary cell cultures or normal tissues.3,4 The presence of constitutively phosphorylated Shc proteins found in the AML peripheral blood (PB) cells, but not in normal PB cells, led to the suggestion that the Ras pathway may be constitutively activated in AML.2 However, it should be noted that in these studies Shc phosphorylation was compared in PB cells of AML and PB cells of healthy donors. One potential problem with their conclusion is that AML cells are immature myeloid cells while normal PB cells are end-stage mature cells. Hence, the Shc phosphorylation difference between these two groups of cells might reflect difference in the level of cell maturation rather than a difference between leukemic and normal myeloid cells. To distinguish between Shc phosphorylation in these two groups of cells, one must compare Shc phosphorylation in AML cells and in normal bone marrow (BM) cells that are at a comparable level of maturation.

We evaluated Shc protein expression and phosphorylation in normal hematopoietic progenitor cells and leukemia hematopoietic cells. Five normal individuals and 10 AML patients participated in this study. Three leukemic cell lines (HL-60, K562, and KG-1) were also studied. BM and PB specimens were collected before any treatment from the 10 patients with newly diagnosed AML. BM aspirates were obtained from the five normal donors. Standard Ficoll density centrifugation was performed to collect mononuclear cells (MNCs). MNCs from BM specimens of two patients with AML and five normal donors were subjected to CD34 separation by MACS separation columns (Miltenyi Biotec Inc, Auburn, CA). Cell pellets of PB and BM were lysed in cell
tyrosine-phosphorylated Shc proteins were expressed constitutively in µg/mL). (4G10) (0.5 µg/mL) (Upstate Biotechnology Inc). Antiphosphotyrosine blots were stripped and reprobed with anti-Shc anti-serum (3 µg/mL).

Our data show that among the 10 AML patients reported here, tyrosine-phosphorylated Shc proteins were expressed constitutively in PB cells of all 10 AML patients. Phosphorylated p66Shc, p52Shc, and p46Shc was found in 8, 10, and 7 of 10 AML PB samples, respectively. Shc tyrosine phosphorylation was examined in CD34+ and CD34− cells separated from BM aspirates of two AML patients. The expression of Shc proteins was found in both subsets. However, tyrosine-phosphorylated Shc proteins were expressed only in CD34+ cells and not in the CD34− leukemia cells. Among five normal BM specimens, tyrosine-phosphorylated Shc proteins were expressed exclusively in the CD34+ cell compartment, but not in CD34− cells or in the unseparated cells. Phosphorylated p66Shc, p52Shc, and p46Shc was found in 2, 5, and 5 of 5 normal CD34+ samples, respectively. With respect to tyrosine-phosphorylated Shc proteins, no significant difference was observed between CD34+ leukemia cells and CD34− normal cells (Fig 1).

Phosphorylation of p66Shc, p52Shc, and p46Shc on tyrosine was detected in K562 and KG-1 cells, but not in HL-60 cells.

Our study shows that the presence of constitutively tyrosine-phosphorylated Shc proteins does not distinguish between normal progenitor cells and AML cells, suggesting that phosphorylation of Shc proteins might be associated more with cell maturity than with malignant transformation.

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REFERENCES


To the Editor:

In a recent issue of Blood, Loh et al1 reported a low frequency of TEL-AML1 fusion (translocation t(12;21)[p13;q22]) in relapsed childhood acute lymphoblastic leukemia (ALL) treated initially on four different DFCI ALL consortium protocols from 1981 until 1995. The incidence of 1 TEL-AML1−positive ALL among 28 B-cell precursor (BCP-) ALL relapses (3.6%) is exceptionally low, although in subsequent reports the frequency was approximately 10% (5 of 49; ALL subtype not specified)2 to 13% (4 of 30; BCP-ALL).3 These results are in contrast to previously communicated Japanese and German retrospective studies on the frequency of TEL-AML1 fusion in relapsed childhood ALL (19% to 28%) questioning the favorable risk feature of TEL-AML1 rearrangement in initial ALL.4,5 These data underline the necessity of prospective analysis to clearly determine the relapse rate of TEL-AML1−positive BCP-ALL following specific frontline treatment.

In our recently published study,6 (the only analysis with a substantial number of patients to date) on incidence and prognostic significance of TEL-AML1 positivity in relapsed childhood ALL, 22.5% of relapsed BCP-ALL were TEL-AML1−positive (32 of 142 patients). TEL-AML1−positive patients had a significantly longer duration of complete remission (CR), and the majority relapsed off-therapy and were younger at initial diagnosis. Furthermore, probability of event-free survival (EFS) for TEL-AML1−positive children enrolled in multicentric Berlin-Frankfurt-Münster (BFM) ALL relapse trials ALL-REZ BFM 90/96 was significantly better.

The ongoing study used nested reverse transcriptase-polymerase chain reaction (RT-PCR) to determine the frequency and the prognostic impact of TEL-AML1 fusion in relapsed childhood ALL.6 All samples were subjected to duplex-PCR including the simultaneous amplification of an ABL transcript to confirm the presence of intact cDNA. Positive results were confirmed by analyzing at least one additional sample independently, and in 16 cases by fluorescence in situ hybridization (FISH). Updating our data, we have analyzed bone marrow samples from a total of 340 children with recurrent ALL retrospectively (117 patients) and prospectively (223 patients). Sixty-two of 310 (20%) relapsed BCP-ALL were TEL-AML1−positive. None of the 30 relapsed T-cell lineage ALL showed a TEL-AML1 fusion. In prospective analysis performed since September 1996, 40 of 202 relapsed BCP-ALL samples were TEL-AML1−positive (19.8%), and all 21 T-cell ALL were TEL-AML1−negative. The frequency of TEL-AML1 positivity at first relapse of BCP-ALL was 16.7% (28 of 168), and even in samples available at second relapse, 12 of 34 patients were TEL-AML1−positive. A similar frequency was obtained for patients with first relapse of BCP-ALL screened prospectively and initially treated uniformly on a single BFM frontline protocol ALL BFM 90 (26 of 123; 21.1%). All TEL-AML1−positive patients had been assigned to standard or intermediate-risk arms of trial ALL BFM 90, whereas 15 of the TEL-AML1−negative patients belonged to the high-risk group. None of the TEL-AML1−positive patients have thus received epipodophyllotoxins reported to be involved in therapy-related secondary leukemia. The
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