Monoclonal Nature of Transient Abnormal Myelopoiesis in Down's Syndrome

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Neonates with Down’s syndrome occasionally show an excess of blasts in their peripheral blood. This disorder spontaneously resolves within several months and is called transient abnormal myelopoiesis (TAM) or transient myelo-proliferative disorder. It has been uncertain whether the excess of blasts in TAM is a result of a clonal proliferation or a polyclonal reactive condition. The clonality of cells in females can be examined by analysis of the methylation patterns of the X chromosomes of proliferating cells using restriction fragment length polymorphism (RFLP). Using this strategy, we studied three females with Down’s syndrome accompanied by TAM who showed heterozygosity in RFLP of either the hypoxanthine phosphoribosyltransferase or phosphoglycerate kinase gene. Analysis of the methylation patterns of these genes demonstrated a clonal nature for blasts in three patients. Thus, TAM is a clonal proliferative disorder. In addition, lymphocytes with a normal appearance contained in analyzed samples from these patients also showed a monoclonal pattern, suggesting that TAM may be a disorder of multipotent stem cells.

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### Table 1. Summary of Three Females With TAM

<table>
<thead>
<tr>
<th>Case</th>
<th>% Blast*</th>
<th>HLADR</th>
<th>CD13</th>
<th>CD33</th>
<th>CD34</th>
<th>CD7</th>
<th>CD41</th>
<th>CD14</th>
<th>CD3</th>
<th>CD20</th>
<th>PPO</th>
<th>HPRT</th>
<th>PGK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20%</td>
<td>2</td>
<td>13</td>
<td>2</td>
<td>67</td>
<td>72</td>
<td>16</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>52%</td>
<td>1</td>
<td>42</td>
<td>4</td>
<td>67</td>
<td>35</td>
<td>66</td>
<td>66</td>
<td>16</td>
<td>34</td>
<td>16</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>56%</td>
<td>89</td>
<td>ND</td>
<td>2</td>
<td>ND</td>
<td>56</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>ND</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3 (1 y)</td>
<td>78%</td>
<td>40</td>
<td>76</td>
<td>7</td>
<td>46</td>
<td>15</td>
<td>4</td>
<td>22</td>
<td>6</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*Percentage of blasts in blood samples.
†Percentage of positive cells in mononuclear cells.
‡A plus sign denotes heterozygote at the BamHI site of the HPRT gene or at the Bgl I site of the PGK gene.

### RESULTS

Two samples (cases 1 and 2) with heterozygosity at the BamHI site of the HPRT gene yielded 12-kb and 18-kb restriction fragments following BamHI and Pvu II digestion (Fig 1, lanes A and C). After additional digestion with Hpa II, the 12-kb and 18-kb bands completely disappeared, and 0.6-kb and 2.1-kb bands were derived from the active and inactive alleles, respectively (lane B). These findings indicated that mononuclear cells in case 1 were clonally derived.

In case 2, DNA samples were obtained at the time of onset of TAM and 7 months later. She was treated with total exchange transfusion on day 3, and the blasts completely disappeared from the peripheral blood within 2 weeks. Following Hpa II digestion of the DNA sample at the time of onset, 18-kb and 12-kb bands completely disappeared. Instead, a 0.6-kb band from active alleles and a 6.5-kb band from inactive alleles were observed (lanes C and D). Because the cell sample from which this DNA sample was derived contained not only megakaryoblasts but also significant number of T cells (Table 1), this observation indicated that at least T cells with a normal appearance as well as blasts were clonally derived. In the cell sample at 7 months of age, 18-kb and 12-kb bands were reduced in intensity but did not completely disappear. Several bands, including a faint 6.5-kb band, appeared, indicating this cell sample consisted of polyclonal cells (lanes E and F).

Heterozygosity of the Bgl I restriction site of the PGK gene was observed in two cases (cases 1 and 3), and digestion with Bgl I, Bgl II, and EcoRI yielded 1.7-kb and 1.3-kb bands. Lanes G through N (Fig 1) show Southern blots of DNA samples from these two cases hybridized with the PGK probe. Following Hha II digestion of the DNA sample from case 1, the 1.3-kb band completely disappeared, indicating clonal origin of mononuclear cells in case 1 (lanes G and H).

Case 3 showed spontaneous regression within 4 weeks and developed AMKL 1 year later. Each sample obtained at the time of onset of TAM or AMKL showed a monoclonal pattern with deletion of the 1.3-kb band (Lanes I through L). With intensive chemotherapy, complete remission was achieved, and the 1.3-kb band reappeared (lanes M and N). In this case as well as in case 2, the mononuclear cell samples obtained at the time of onset of TAM and AMKL included 10% and 22% of T cells, respectively (Table 1). Therefore, T cells in these conditions also appeared to be clonal and derived from the identical clone as blasts.

### DISCUSSION

TAM in neonates with Down's syndrome is a unique disorder, and analysis of this disorder may provide invaluable information for understanding tumorigenesis. Al-
though proliferation of blasts in TAM is self limited and spontaneously resolves, the blasts are morphologically indistinguishable from true neoplastic cells. It has remained controversial as to whether this disorder may consist of proliferation of monoclonal cells or polyclonal reactive cells. In this study, using analysis of the methylation patterns of the HPRT and PGK genes, TAM was demonstrated for the first time to be a monoclonal disorder.

Of particular interest was that, despite the contamination of the cell samples with significant number of T cells, monoclonal patterns with HPRT and PGK probes were observed. These findings suggest that both cells with a morphologically normal appearance and blasts in this disorder may belong to an identical clone. Although analysis of myeloid lineage cells was not performed, it is possible that TAM may be a disorder of multipotent stem cells.8,10 Infants with Down's syndrome occasionally show hematologic abnormalities other than TAM, such as thrombocytopenia and neutropenia.11 These conditions, without an excess of blasts or morphologic abnormalities, also spontaneously resolve. Such disorders may be clonal disorders like TAM, and analysis is in progress.

Case 3 experienced TAM and then developed AMKL 1 year later. The blasts at the time of onset of TAM and AMKL blasts showed disappearance of the 1.3-kb band and were found to have identical monoclonal patterns by PGK gene analysis. The incidence of leukemia in children with Down's syndrome is higher than that in the normal population. Hayashi et al showed that whereas the cytogenetic abnormality of blasts in TAM was 21 trisomy alone, additional chromosomal abnormalities were observed in leukemic cells.12 It is interesting to know whether the AMKL clone with additional advantage for proliferation, such as chromosomal abnormalities other than 21 trisomy, might originate from the surviving TAM clone.

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