RAPID COMMUNICATION

Monoclonal Nature of Transient Abnormal Myelopoiesis in Down's Syndrome

By Hiroki Kurahashi, Junichi Hara, Keiko Yumura-Yagi, Norihide Murayama, Masami Inoue, Shigehiko Ishihara, Akio Tawa, Shintaro Okada, and Keisai Kawa-Ha

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 myeloproliferative 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 accompa-

AN EXCESS OF BLASTS in peripheral blood is occasionally observed in neonates with Down's syndrome. Although a majority of cases also show marked hepatosplenomegaly and are sometimes complicated with severe coagulopathy, this disorder usually spontaneously resolves within several months. Based on these clinical characteristics, this disorder has been considered to be distinct from neoplasia and is called transient abnormal myelopoiesis (TAM). The expanding blasts frequently express platelet associated surface antigens and electron microscopic platelet peroxidase, suggesting that these blasts belong to the megakaryocyte lineage.

In females, one of the X chromosomes in each cell is randomly inactivated at an early stage of embryogenesis. This activation pattern of the X chromosomes remains unchanged throughout the individual's life and in progeny cells. Many genes are activated or inactivated by changes in methylation of cytosine residues in the genes. Because X chromosome inactivation occurs randomly on either the paternal or maternal allele, in polyclonal cells half of the inactivated alleles are paternal and the other half are maternal. On the other hand, in a monoclonal cell population, the same alleles are exclusively inactivated. When restriction fragment length polymorphism (RFLP), which distinguishes paternal and maternal alleles, is observed in given cell samples, it is possible to examine the clonality of these cells by analysis of the methylation patterns of the X chromosomes. To know whether TAM is caused by monoclonal proliferation or simply polyclonal reactive conditions, we used the hypoxanthine phosphoribosyltransferase (HPRT) and phosphoglycerate kinase (PGK) genes located on the X chromosome to analyze the methylation patterns.

MATERIALS AND METHODS

Patients. Eight peripheral blood samples from neonates with Down's syndrome associated with TAM were referred to Osaka University Hospital for phenotypic analysis between January 1987 and December 1989. Four were females, and three of them had polymorphic restriction sites on the HPRT and/or PGK genes. In this study, these three cases were analyzed. Each of the three cases showed leukoerythroblastosis, and the percentage of blasts ranged from 20% to 56% of the white blood cells. The blasts from these cases were morphologically myeloblasts or megakaryoblasts, and negative for peroxidase. Results of phenotypic analysis of mononuclear cells are shown in Table 1. Expression of GpIIb/IIIa on the cell surface or positivity for electronmicroscopic platelet peroxidase confirmed that the blasts belonged to the megakaryocyte lineage. Karyotypic analysis of both somatic cells and blasts in these cases showed trisomy 21, but no other abnormalities were identified. Case 1 died at 9 days of life because of complicated congenital heart disease. Case 2 required total exchange transfusion for the treatment of severe coagulopathy before the blasts spontaneously disappeared from the peripheral blood in 1 month. In case 3, the blasts also disappeared in 1 month. However, acute megakaryoblastic leukemia (AMKL) developed 1 year later, and complete remission was achieved by intensive chemotherapy.

Southern blotting. DNA was extracted from mononuclear cells isolated by Ficoll density gradient centrifugation. Digestion with BamHI and Pvu II was performed for screening for BamHI RFLP of the HPRT gene, while Bgl I, Bgl II and Eco RI were used for screening for Bgl I RFLP of the PGK gene. Results of RFLP analysis of the HPRT and PGK genes are shown in Table 1. DNA samples with heterozygosity of the HPRT or PGK gene were digested with the respective enzymes mentioned above, and then equally divided into two aliquots. On one aliquot, second digestion was done with methylation sensitive enzyme Hpa II. Agarose gel electrophoresis was performed in pairs of samples with or without Hpa II digestion. Nylon filters immobilized with DNA were hybridized with a radiolabeled HPRT or PGK probe. The DNA probes used in this study were as follows: HPRT probe, a 600-bp Hpa II fragment from the first intron of HPRT gene; and PGK probe, an 800-bp BamHI-EcoRI fragment from the 5' end of the PGK gene. These DNA clones were kindly provided by Dr B. Vogelstein.

© 1991 by The American Society of Hematology.
Table 1. Summary of Three Females With TAM

<table>
<thead>
<tr>
<th>Case</th>
<th>% Blasts*t</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>6</td>
<td>2</td>
<td>6</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>9</td>
<td>46</td>
<td>15</td>
<td>4</td>
<td>22</td>
<td>6</td>
<td>ND</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 6.5kb 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 I 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-

Fig 1. Southern blots of the cases with Down's syndrome associated with TAM. Case numbers are noted below each blot. Sizes of bands are indicated in kilobases. Lanes A through F show Southern blots hybridized with the HPRT probe after digestion with BamHI and Pvu II (lanes A, C, and E) and digestion with BamHI, Pvu II, and Hpa II (lanes B, D, and F). Lanes G through N show Southern blots hybridized with the PGK probe after digestion with Bgl I, Bgl II, and EcoRI (lanes G, I, K, and M) and digestion with Bgl I, Bgl II, EcoRI, and Hpa II (lanes H, J, L, and N).
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.9,10 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.

ACKNOWLEDGMENT

We are indebted to Drs K. Noma, K. Nishiike, and T. Hashizume for providing the cell samples, and Dr B. Vogelstein for the HPRT and PGK probes.

REFERENCES

7. Keith DH, Singer-Sam J, Riggs AD: Active X chromosome DNA is unmethylated at eight CCGG sites clustered in a guanine-plus-cytosine-rich island at the 5' end of the gene for phosphoglycerate kinase. Mol Cell Biol 6:4122, 1986
Monoclonal nature of transient abnormal myelopoiesis in Down's syndrome

H Kurahashi, J Hara, K Yumura-Yagi, N Murayama, M Inoue, S Ishihara, A Tawa, S Okada and K Kawa-Ha