Cytogenetic Evidence for Involvement of B Lymphocytes in Acquired Idiopathic Sideroblastic Anemias

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We studied the cellular distribution of an unusual chromosomal abnormality, an interstitial deletion of the long arm of chromosome 13, in the peripheral blood lymphocytes of two patients with acquired idiopathic sideroblastic anemia (AISA). We found no metaphases containing the 13q— abnormality in preparations of phytohemagglutinin (PHA)-stimulated lymphocytes from either patient. In both cases, however, some metaphases from Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines contained the clonal karyotypic abnormality. These observations indicate that B lymphocytes but not T cells are expressed as members of the clonal cohort of cells. Our results strongly suggest that the initial pathogenetic events that led to expansion of the 13q— clone occurred in a progenitor cell capable of giving rise to both hematopoietic and B lymphoid cells.

A M P L E E V I D E N C E , using both chromosomal studies and analysis of glucose-6-phosphate dehydrogenase (G6PD) isoenzymes, shows that B lymphocytes are progeny of the neoplastic stem cells in several hematopoietic disorders, including chronic myelocytic leukemia, polycythemia rubra vera, and essential thrombocytopenia.

Acquired idiopathic sideroblastic anemia (AISA) is a clonal hemopathy classified as one of the myelodysplastic syndromes and characterized by anemia, ringed sideroblasts in the marrow, and minimal disturbance of other cell lines. The level of progenitor cell involvement in this disease is unclear. Using the mosaicism of G6PD isoenzymes in a heterozygous female with AISA, Prchal found that the peripheral blood lymphocytes were clonal and displayed the same isoenzyme as the RBCs, granulocytes, and platelets, suggesting that lymphocytes were part of the neoplastic clone. Raskind et al reported another female, heterozygous for G6PD, with AISA whose Epstein-Barr virus (EBV)-transformed lymphoblastoid lines were clearly clonal whereas her cultured T cells were not.

We have observed two patients with AISA and an unusual interstitial deletion of chromosome 13. We investigated the involvement of T and B lymphocytes in these two individuals by seeking the 13q— abnormality in phytohemagglutinin (PHA)-stimulated lymphocytes and in EBV-transformed B lymphoblastoid cells. In both cases, we found cytogenetic evidence for partial involvement of B lymphocytes but not T cells.

MATERIALS AND METHODS

Subjects

Patient 1 (R.T.) was a 72-year-old man who came to our hospital for evaluation of a macrocytic anemia. His laboratory evaluation revealed a hematocrit of 32%, mean corpuscular volume (MCV) of 101 μL with normal WBC and platelet counts. Bone marrow aspiration revealed a cellular marrow with erythroid hyperplasia and mildly megaloblastic maturation. Prussian blue stain of the marrow slides revealed numerous ring sideroblasts. A diagnosis of AISA was made. Trials of pyridoxine and androgens were ineffective.

Karyotypic Analysis

Heparinized (1,000 U/mL) bone marrow (0.1 mL) was incubated overnight at 37°C in 5 mL RPMI 1640 medium with 10% fetal calf serum (FCS) and 1% gentamicin (50 μg/mL). Cells were harvested, and slides were made. Chromosome preparations were G-banded for chromosome analysis. Approximately 20 metaphases were analyzed.

PHA-stimulated peripheral blood. Heparinized whole blood (0.5 mL) was inoculated into culture tubes containing 5 mL RPMI 1640 medium with 10% FCS gentamicin, 2% glucose, and 1% PHA (Wellcome, Research Triangle Park, North Carolina). Cells were harvested according to established procedures. Slides were prepared and stained using the G-banding technique. Approximately 20 to 40 metaphases were examined.

EBV-transformed peripheral blood lymphocytes. Peripheral blood mononuclear cells (PBMCs) were obtained from heparinized venous blood by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density-gradient centrifugation. Preferential transformation of B lymphocytes in the PBMCs was accomplished by adding EBV (100 μL/mL of EBV suspension containing 1 x 10^6 transforming units/mL) to suspensions of PBMCs (1 x 10^4 cells/mL) in RPMI 1640 with 20% FCS and 2% glucose. Cultures were harvested at 96 hours, slides were made, and preparations were stained with quinacrine. Between 100 and 150 cells were analyzed and photographed using a Zeiss microscope III. In cells demonstrating the deleted 13q, fluorescent variant regions were observed to determine whether the deletion was consistently in the same homolog.

RESULTS

Cytogenetic studies of unstimulated bone marrow cells were performed in both cases. Metaphases from both cases...
cent chromosome 13 heteromorphisms was consistent with examples of AISA for which there is cytogenetic evidence of the deletion involving the same homolog in each cell examined. The normal 13 has bright short arms, medium-length stalk region, and bright medium-size satellites. The deleted 13 has bright short arms, long stalks, and small, pale satellites. 

displayed an interstitial deletion of chromosome 13 with or without other karyotypic abnormality. In case 1, six of 18 cells displayed a 46,XY,del(13)(q11;q21) karyotype. In case 2, 21 of 21 cells had a del(13)(q12;q14) (Fig 1). In addition, three of 21 metaphases were missing a number 21 chromosome.

Cytogenetic evaluation of PHA-stimulated lymphocytes was carried out on blood samples from both patients to assess whether T cells were involved in the neoplastic clone. In each case, some metaphases containing the 13q deletion were identified. Comparison of fluorescent variant regions of the chromosomes 13 of each pair are normal. Right chromosomes 13 have an interstitial deletion, bands q12 → q14. Fluorescent variant regions of the chromosomes were consistent with the deletion occurring in the same homolog in each cell examined. The normal 13 has bright short arms, medium-length stalk region, and bright medium-size satellites. The deleted 13 has bright short arms, long stalks, and small, pale satellites.

**DISCUSSION**

These cases are, to the best of our knowledge, the first examples of AISA for which there is cytogenetic evidence of involvement of lymphocytes. Raskind et al were able to demonstrate B cell involvement in a case of AISA using G6PD isoenzymes but not by karyotypic analysis. They hypothesized a two-step mutational process, the first involving B lymphocytes as well as other hematopoietic cell lines and manifested by a single G6PD isoenzyme in all involved cells, and the second with more restricted involvement of nonlymphoid hematopoietic cells and manifested by a clonal chromosomal abnormality.

Our results, unlike those of Prchal, have not documented that T lymphocytes are part of the clonal disorder. Our findings are consistent with most observations in other clonal hematologic diseases such as polycythemia rubra vera and chronic myelogenous leukemia, although T cell involvement has been suggested by some studies. In our cases, the extent of T lymphocyte involvement may have been small and might have been detected if more metaphases had been available for analysis.

Other evidence for involvement of lymphocytes in AISA and in myelodysplastic syndromes is less clear-cut. A case of acute lymphocytic leukemia (ALL) has been reported in a patient with AISA, but the diagnosis of ALL was made primarily by morphological criteria and not by immunophenotyping. Functional abnormalities of lymphocytes in myelodysplasia have been reported by other researchers. One curious observation was a lack of receptors for EBV with resultant resistance to in vitro infection with the virus. EBV-transformed lymphoblastoid cell lines were easily established from peripheral blood of both our patients.

In summary, our studies have provided clear cytogenetic evidence of partial involvement of B lymphocytes, but not T cells, in the neoplastic clone in AISA. Whether this pattern of lymphocytic involvement is specific for patients with the 13q- abnormality awaits further study of cases of AISA with different karyotypic abnormalities.

**REFERENCES**

8. Hungerford DA: Leukocytes cultured from small inocula of

**Table 1. Frequency of Abnormal Metaphases in Marrow Cells and Peripheral Blood Lymphocytes**

<table>
<thead>
<tr>
<th>Patient</th>
<th>BM*</th>
<th>PHA†</th>
<th>EBV‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/18§</td>
<td>0/40</td>
<td>3/100</td>
</tr>
<tr>
<td>2</td>
<td>21/21</td>
<td>0/20</td>
<td>20/151</td>
</tr>
</tbody>
</table>

*BM = unstimulated bone marrow cells.
†PHA = stimulated peripheral lymphocytes.
‡EBV = transformed peripheral lymphocytes.
§Number of metaphases containing 13 q- deletion/total number of metaphases examined.
whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl. Stain Technol 40:333, 1965
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