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.

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

PHA-stimulated peripheral blood. Heparinized whole blood (0.5 mL) was inoculated into culture tubes containing 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.

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 deleted 13 has 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 both cases, only normal metaphases were seen (Table 1). To assess B cell involvement, karyotypic analysis of EBV-transformed lymphocytes was performed on blood samples from both patients. In each case, some metaphases containing the 13q interstitial deletion were identified. Comparison of fluorescent chromosome 13 heteromorphisms was consistent with the deletion involving the same homolog in each cell examined (Fig 1).

**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.\(^6\) 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,\(^3,5\) although T cell involvement has been suggested by some studies.\(^13\) 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.\(^16\) Functional abnormalities of lymphocytes in myelodysplasia have been reported by other researchers.\(^17,18\) 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>
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<tbody>
<tr>
<td>1</td>
<td>6/18§</td>
<td>0/40</td>
<td>3/100</td>
</tr>
<tr>
<td>2</td>
<td>2/21</td>
<td>20/151</td>
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</tr>
</tbody>
</table>

*BM — unstimulated bone marrow cells.*
†PHA — stimulated peripheral lymphocytes.
‡EBV — transformed peripheral lymphocytes.
§Number of metaphases containing 13q~ 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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