Cytogenetic Study of a European Burkitt's Lymphoma Cell Line

Roland Berger, Alain Bernheim, Marc Fellous, and Jean-Claude Brouet

ABSTRACT—The chromosomes of an Epstein-Barr virus-negative European Burkitt's lymphoma cell line were studied. All the cells carried the t(8;14) translocation. One clone had 51 chromosomes and was (+1q, +7, +15, +16, +21), whereas another clone also had 51 chromosomes but was (+1q, +7, +16, +15, +21). A third clone had 46 chromosomes (4q±).

A consistent chromosome marker 14q± was noted in Burkitt's lymphoma cells by Manolov and Manolova in 1972 (1). Later, the 14q± marker was shown to have resulted from a t(8q−;14q+) translocation (2). In the present report, we analyze chromosomes of a newly derived cell line originating from a European Burkitt's lymphoma. Other characteristics of this cell line will be reported elsewhere.

MATERIALS AND METHODS

Case report.—A. CHEV. was a 14-year-old boy who had always lived in France. He had had no pertinent medical history. The boy was operated on for intestinal obstruction on October 2, 1976. A small intestinal tumor and adjacent lymph nodes were resected. Imprints of the tumor and cytologic examinations were fully consistent with a diagnosis of Burkitt's lymphoma. Prior to referral to the Department of Hematology, Hôpital Saint-Louis, the patient received abdominal X-rays (2,000 rads). Ascites and pleural effusion were present by the time the patient was referred to the Department of Hematology. The liver, spleen, and lymph nodes were not enlarged. Ascites cells were obtained for cultures prior to chemotherapy, which was begun on November 22, 1976. The child's clinical condition soon deteriorated, and he died on December 20, 1976.

Culture.—An ascites cell culture was established from 10⁶ cells. The cells were grown in 20 ml of RPMI-1640 medium supplemented with 20% fetal calf serum. The cells were first frozen on December 20, 1976. The culture has been continuously maintained. On occasions, lysis resulting in considerable cell mortality has necessitated the introduction of a feeder (either irradiated WI-38 cells or irradiated rat embryo fibroblasts) for the maintenance of the cell line.

The Giemsa standard technique and the following banding techniques were performed: Q-banding by quinacrine fluorescence, G-banding with the use of trypsin, C-banding following barium hydroxide treatment and with the use of Giemsa, and R-banding after heating (3, 4).

RESULTS

The chromosome counts are summarized in table 1. Two modal chromosome numbers were found: #51 and 46 (incomplete cells were due to a random loss of chromosomes). All karyotypes were abnormal.

Three karyotypes were present, all having the t(8;14)(q23;q32) translocation (fig. 1).

1) 51,XY,+1,+7,+15,+16,+21,t(8;14) (fig. 2): Two #1 chromosomes clearly had an unusual extra G-band at the end of the long arm (fig. 3).

2) 51,XY,(8;14)+1p+,+7,+15,+16,+21 (fig. 3): This line differed from the preceding one by the replacement of chromosome 1

3) 46,XY,4q± (fig. 4).

TABLE 1.—Chromosome studies of the cell line

<table>
<thead>
<tr>
<th>Date</th>
<th>Clone 1</th>
<th>Clone 2</th>
<th>Clone 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of chromosomes in a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>3/28/77</td>
<td>1</td>
<td>5</td>
<td>12 (5)</td>
</tr>
<tr>
<td>5/17/77</td>
<td>1</td>
<td>2</td>
<td>17 (3)</td>
</tr>
<tr>
<td>6/27/77</td>
<td>1</td>
<td>2</td>
<td>11 (3)</td>
</tr>
<tr>
<td>9/16/77</td>
<td>1</td>
<td>2</td>
<td>58 (8)</td>
</tr>
</tbody>
</table>

Table 1.—Chromosome studies of the cell line

a Numbers in parentheses refer to the No. of karyotypes.

ABBREVIATION USED: EBV = Epstein-Barr virus.

1 Received August 2, 1978; accepted October 30, 1978.
2 Laboratoire de Cytogénétique, Institut de Recherches sur les Maladies du Sang, Hôpital Saint-Louis, Paris, France.
3 Laboratoire d’Immuno-Hématologie, Hôpital Saint-Louis.
5 We gratefully acknowledge the technical assistance of Mrs. C. Chereau and Miss D. Vecchione.
of the #1 chromosome by a 1p+:Inv dup (1p) (qter-q23;p36-qter).

3) 46,XY,4q+t(8;14): The 4q+ chromosome was longer than the #1; it was apparently formed by a t(4;7)(q31;p21) translocation. The size of the centromere of the translocated chromosome #7 appeared to be reduced with the C-banding technique, which could be an example of a nonfunctional centromere (fig. 3).

Furthermore, in 14 cases the 11q+ was associated with dehydrogenase isoenzyme. In one case a second clone resulted from the reemergence of the primary malignant cell line.

DISCUSSION

The cytogenetic study of the Burkitt's cell line was undertaken not only to establish the karyotype of the line, but also to examine the #6 chromosomes inasmuch as the expression of HLA antigens was lacking. Both chromosomes #6 appeared to be normal in all cells. The same was true of chromosome #15, which was triplicated in the cells having 51 chromosomes, i.e., in accord with the expression of β2 microglobulin in the cell line.

Three clones with distinct chromosome complements were clearly present in the cultures. The two clones with 51 chromosomes were closely related but were not equally represented in each sample: The clone with 51,1p+ seemed to possess a selective advantage in vitro inasmuch as the proportion of these cells was greater in later samplings. The presence of the three clones in vivo was impossible to assess because of the lack of direct chromosome examination and could have been due to rearrangements occurring during culture. Because of this point, it is important to verify the karyotypes of all cell lines when any study is done, even if the karyotype has been studied before, because modifications may arise due to selection pressure during culture.

All the cells had in common the t(8;14) translocation. The fact that the surface markers were homogeneous contrasts with the variety of karyotypes and underlines once more the fact that the definition of a clone depends on the cell characteristics studied. Fialkow et al. (5) demonstrated the clonal origin of Burkitt's lymphoma cells by studies of glucose 6-phosphate dehydrogenase isoenzyme. In one case a second clone arising during the evolution of the disease did not result from the reemergence of the primary malignant cell line (6).

A chromosome #14 abnormality was detected in Burkitt's lymphomas and in some non-Burkitt's lymphomas and lymphoproliferative diseases. Chromosome 14q+ was found in 15 of 16 biopsy specimens of Burkitt's lymphoma patients (table 2) and in 20 of 23 cultivated cells or cell lines (table 2). Furthermore, in 14 cases the 14q+ was associated with a t(8q-;11q+) translocation. In the remaining cases, the translocation may have been present but was not demonstrated. The t(8;14) is not the only abnormality observed in Burkitt's lymphomas, but it seems to be the most constant one. The other abnormalities seem to vary from one case to another. A partial or total trisomy for chromosome #7 was present in 6 other cases in addition to the present one. No clear differences could be shown between EBV-positive and EBV-negative cell lines.

The problem of the specificity of the t(8;14) translocation has been questioned. Chromosome 14q+ has been irregularly found in some lymphosarcomas, reticulum cell sarcomas, Hodgkin's diseases, multiple myelomas, and in clones from patients with ataxia telangiectasia (table 3). In those cases in which the origin of the extra band on the #14 chromosome was ascertained, the origin was different from that of the chromosome #8. However in 2 patients with chronic lymphocytic leukemia, a t(8;14) translocation similar to that in Burkitt's lymphoma cells was observed (14).
The same translocation was also observed in a patient with histiocytic lymphoma (15) and in 2 patients with acute Burkitt's-type lymphoblastic leukemia (16).

One can conclude that the band 14q23 is often the site of a rearrangement associated with lymphoproliferative disorders, but that the t(8;14) translocation seems to be more specifically associated with Burkitt's lymphoma. An analogy can be drawn with chronic myelocytic leukemia in which the t(9;22) translocation, with histiocytic lymphoma. An analogy can be drawn with chronic myelocytic leukemia in which the t(9;22) translocation, 

### REFERENCES

32. HAYASHI K, SCHMIDT W: Tandem duplication q14 and dicentric formation by end to end chromosome fusions in ataxia-telangiectasia.

### Table 3. Chromosome 14q+ in various lymphoproliferative disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of cases</th>
<th>14q+</th>
<th>Origin of extra bands, chromosome No.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant lymphocytic leukemia</td>
<td>5</td>
<td>+</td>
<td>11</td>
<td>(14,17,18)</td>
</tr>
<tr>
<td>(poorly differentiated)</td>
<td>3</td>
<td>+</td>
<td>(14)</td>
<td></td>
</tr>
<tr>
<td>Histiocytic lymphoma</td>
<td>1</td>
<td>+</td>
<td>10</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+</td>
<td>? 10</td>
<td>(18)</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>1</td>
<td>+</td>
<td>8</td>
<td>(21)</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>5</td>
<td>+</td>
<td>? 10</td>
<td>(17,18,22,28)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>1</td>
<td>+</td>
<td>(24-26)</td>
<td>(28)</td>
</tr>
<tr>
<td>(plasma cell leukemia)</td>
<td>6</td>
<td>+</td>
<td>11</td>
<td>(26)</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>2</td>
<td>+</td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>Acute undifferentiated leukemia</td>
<td>1</td>
<td>+</td>
<td>11</td>
<td>(19)</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-type</td>
<td>1</td>
<td>+</td>
<td>11</td>
<td>(16)</td>
</tr>
<tr>
<td>L3 type</td>
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<td>+</td>
<td>11</td>
<td>(29)</td>
</tr>
<tr>
<td>B-type</td>
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<td></td>
<td>(20)</td>
</tr>
<tr>
<td>Ataxia-telangiectasia</td>
<td></td>
<td></td>
<td></td>
<td>(20-34)</td>
</tr>
</tbody>
</table>

**The same translocation was also observed in a patient with histiocytic lymphoma (15) and in 2 patients with acute Burkitt's-type lymphoblastic leukemia (16).**
Figure 1.—Translocation t(8;14) from different cells. G = G-bands; R = R-bands.
FIGURE 2.—Karyotype of clone 1 (R-banding): 51,XY,+1,+7,+15,+16,+21,t(8;14). Arrows indicate the rearranged chromosomes #8 and 14.