Cytogenetic Studies of Lymphoma Cells From an American Patient With a Tumor Similar to Burkitt's Tumors In African Children

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SUMMARY—Cytogenetic studies were done on lymphoma cells from ascitic fluid of an American woman with a tumor similar to Burkitt's tumors in African children and on the established cell culture line (AL-2) derived from the ascites cells. More than 90% of the metaphases in direct preparations of the ascitic fluid contained an extra submedium chromosome, larger than pair #1, and lacked one chromosome from this #1 pair. The AL-2 line had a chromosome number distribution ranging from 42-86 with a modal number of 46, and the cells with 46 chromosomes had an apparently normal female karyotype. None of the AL-2 cells showed any marker chromosomes. The cultural conditions apparently favored the growth of those cells without the marker chromosome.—J Nat Cancer Inst 37: 885-891, 1966.

A NUMBER of cytogenetic studies of lymphoma cells from Africans with poorly differentiated lymphocytic lymphomas, called "Burkitt's tumor," have been reported (1-4). These studies were carried out both on direct smears of tumor cells and on several continuous cell lines derived from these tumors. In some cases, the lymphoma cells were predominantly diploid with no evident chromosome abnormalities, whereas others had pseudodiploid stem lines with one or more marker chromosomes, and some had hyperdiploid stem lines (1-4).

O'Conor and associates (5, 6) and Dorfman (7) recently called attention to the fact that lymphomas similar to those seen in African children also occur in the United States; however, they noted that the frequency is much lower than that in Africa and gross involvement of the jaw is much rarer.

In January 1965, an American Caucasian woman with a poorly differentiated lymphocytic lymphoma involving both ovaries was admitted to the Clinical Center of the National Institutes of Health. Her tumor had the morphological features of Burkitt's tumor as it is seen in Africans. She had ascites and the ascitic fluid contained large numbers of lymphoma cells. We studied the chromosomes of these cells in smears of the ascitic fluid and in a continuous line of lymphoma cells derived from the ascitic fluid which was designated AL-2. In a preliminary report (8), we described the presence of herpes-like particles, similar to those seen in the cell lines from African children, in the cells derived from this American patient. The present report describes cytogenetic studies of these cells.

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2 Medicine Branch, National Cancer Institute.
3 We are indebted to Dr. J. H. Tjio for his assistance and advice in the chromosomal analysis.
MATERIALS AND METHODS

Ascitic fluid from the patient was processed for chromosome analysis according to the technique of Tjio and Whang for direct marrow preparation (9).

The AL-2 line was established from the cells of the ascitic fluid. The cells were separated from the fluid by centrifugation and resuspended in medium #199 with 20% fetal bovine serum (FBS). Cultures were initiated in 60 ml prescription bottles and 960 ml bottles. After 2 days large numbers of round cells were floating in the medium and moderate numbers of elongated cells were growing on the dependent glass surface. During the subsequent 30 days the number of floating, round cells decreased and the number of elongated, fibroblast-like cells on the glass surface increased. Subcultures were prepared by trypsinization with methods similar to those previously reported (3), and the fibroblast-like cells on the glass predominated, with small numbers of floating, round cells in the medium. After approximately 60 days, the number of floating, round cells progressively increased and these cells formed clumps like those seen in our cultures of cells from an African child with lymphoma (AL-1) (3). Seventy days after initiation of the cultures, many clumps of round cells were growing in the fluid and these could be readily subcultured with the methods used previously in the growth of AL-1 cells (3).

Four months after initiation of the cultures, one of the bottles of AL-2 cells containing approximately $10^6.7$ cells was infected with $10^6$ TCID50 of simian virus 40 (SV40) in an attempt to accelerate the growth of the lymphoma cells. There were no changes in the growth characteristics or morphology of the lymphoma cells. After 2 months, SV40 virus could not be recovered from the cultures, and no SV40 “T” antigen could be demonstrated in the cells by immunofluorescence. 5

RESULTS

Tables 1, 2, and 3 present chromosome studies of cells from the ascitic fluid and from the established cell lines (AL-2) free of SV40 virus and infected with SV40 virus. Polyploid cells were rare in the direct smears of ascitic fluid, with only one tetraploid cell in 125 cells examined (1.8%). The AL-2 cells were examined 7-9 months after cultures had been initiated. In the AL-2 line free of SV40 virus, 3.7% of the cells were polyploid, whereas the SV40 virus-infected line contained 7.5% polyploid cells.

In the direct chromosome preparation of the ascitic fluid, only 2 of the 125 cells had a normal female karyotype (table 1). The remainder had a large extra chromosome with submedium centromere and one chromosome #1 missing (figs. 1 and 2). The cells with 47 chromosomes had both aberrations plus an extra chromosome in group 13-15 (fig. 2).

The marker chromosome was not seen many of the cells of the AL-2 line free of SV40 virus. The chromosomal number distribution in these cells, however, ranged from 42-86 with a modal number of 46 (tables 2 and 3). The karyotype of the cells with 46 chromosomes was that of a normal female (fig. 3).

DISCUSSION

A pseudodiploid karyotype with a marker chromosome in many of the lymphoma cells in the

5 We wish to thank Dr. R. A. Malmgren for his assistance in the immunofluorescence technique (40).

<p>| Table 1.—Chromosome counts of lymphoma cells in direct smears of ascitic fluid from an American patient with Burkitt-type lymphoma |
|--------------------|--------------------|--------------------|</p>
<table>
<thead>
<tr>
<th>With marker chromosome</th>
<th>Without marker chromosome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome count</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>Number of cells</td>
<td>1</td>
<td>73</td>
</tr>
</tbody>
</table>

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direct smears of our patient's ascitic fluid is in accord with previous observations of similar chromosome abnormalities in tumor cells and established cell lines from African patients with Burkitt's tumors (1, 2). The presence of predominantly diploid cells in the established line, AL-2, from our patient is in agreement with reports of predominantly diploid cells in several of the established lines derived from African patients (4). That most of the lymphoma cells in the ascitic fluid contained the marker chromosome, whereas none of the cells in the established line contained this marker suggests, however, that the culture conditions we have used favored growth of the lymphoma cells without the marker chromosome. It is of course possible, however, that the AL-2 cells actually were derived from non-neoplastic lymphoid cells present in the explants of tumor used to initiate the culture. It is of interest that, although we have apparently selected the relatively rare cells without the marker chromosome, some cells of the AL-2 line contained herpes-like particles identical to those seen in the lines established from African patients (8).

The addition of SV40 virus to the AL-2 cultures resulted in no significant change in karyotype. As noted, however, there is no virologic, electronmicroscopic, or immunologic evidence that infection of the cells by SV40 was ever established.

REFERENCES


Figure 1.—Metaphase plate and karyotype of a cell with 46 chromosomes including a marker of a cell from ascites of a patient with Burkitt-type lymphoma.
FIGURE 2.—Metaphase plate and karyotype of a cell from direct smear of ascitic fluid. There are 47 chromosomes with a marker as well as an extra chromosome in the 13-15 group.
Figure 3.—Metaphase and karyotype of a cell from AL-2 lines infected with simian virus 40. The cell has a normal female karyotype.