Chromosome Studies of Cell Lines and Tumors Derived from a Single Specimen of Human Leukemic Blood by Cell Culture and Heterotransplantation

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SUMMARY

Lymphoblasts isolated from the peripheral blood buffy coat of a pediatric patient and maintained in continuous suspension culture (CCRF-SB) have a diploid karyotype of 46 chromosomes. Tumors initiated and transplanted in hamsters from this cell line with the help of antilymphocytic serum showed a high degree of pseudodiploidy and aneuploidy without any consistent recognizable marker chromosomes. A serially transplantable tumor isolated directly in hamsters (H-SB-2) from the same sample of the buffy coat had a distinctive extra F-group chromosome. The H-SB-2 tumor cell population was near-diploid in the earlier serial passages, but by the 46th serial passage it had evolved into a predominantly near-tetraploid cell population which retained the extra F-group chromosomes. This extra chromosome was also seen in hamster tumor cells grown in suspension cultures and in i.p. transplants of hamster tumors in rats.

INTRODUCTION

The serial heterotransplantation of lymphoblasts from human leukemic blood in newborn Syrian hamsters had been reported by Adams et al. (1, 2). A later report (7) described the evolution of a near-tetraploid karyotype in 3 sublines of these lymphoblasts (CCRF-CEM), which were grown initially in suspension cultures and subsequently transplanted serially in newborn Syrian hamsters. In contrast, the original cell line in suspension culture has maintained a near-diploid karyotype through more than 5 years of continuous growth, thus showing that in vitro growth was more conducive to the maintenance of diploidy than the in vivo condition.

The present report describes the chromosomal constitution of a 2nd series of human cell lines and tumors which were grown in vitro in continuous suspension cultures (5) and by i.p. implantation in newborn Syrian hamsters with cells taken directly from the patient (1). Although both cell line CCRF-SB and the transplantable tumor originated from the same sample of the peripheral blood, observations in the present study show karyotypic differences between the two and the gradual evolution of a near-tetraploid cell population in the serially transplanted in vivo tumor.

MATERIALS AND METHODS

CCRF-SB (Suspension Cultures). This cell line, isolated in May 1966 from the peripheral blood buffy coat of an 11.5-year-old boy with acute lymphoblastic leukemia, has been maintained in continuous suspension cultures (5). Aliquots of cells from the present study were removed in December 1966.

H-SB9 (ALS-dependence CCRF-SB Hamster Transplants). This serially propagated line was initiated from CCRF-SB suspension culture cells which were inoculated into newborn Syrian hamsters treated with ALS (R. A. Adams et al., to be published). Tumor cells from these hamster transplants were examined in the 6th serial passage (January 1969).

H-SB2 (Syrian Hamster Serial Transplants). This tumor was established by the i.p. implantation of cells from the above-mentioned specimen of peripheral blood buffy coat into newborn Syrian hamsters treated with ALS (R. A. Adams et al., to be published). Tumor cells from these hamster transplants were examined in the 30th passage (May 1967), the 40th passage (August 1967), and the 46th passage (October 1967).

R-SB9 (i.p. Transplants in Rats). Cells from the 24th serial transplant of H-SB2 tumor were grown s.c. in newborn Syrian hamsters (H-SB6). Tumor cells from the 14th passage of this s.c. transplantable tumor in hamsters were transplanted in rats (August 1967). Material for the present study was taken from a 3rd serial passage in rats (September 1967).

H-SB2 (Transplanted Tumor Growing in Suspension Cultures). Tumor cells from the 24th serial passage of the H-SB2 cells in Syrian hamsters were grown in suspension cultures. Chromosome preparations were made from these cells after approximately 3 months of cultivation in continuous suspension cultures.

Cells from suspension cultures were incubated with 0.01 μg/ml of colchicine for 3 to 4 hr. Animals with i.p. transplants...
were given injections of vinblastine sulfate (Velban, Eli Lilly and Co., Indianapolis, Ind.), 1 μg/g of body weight, 4 to 5 hr before sacrifice. The tumors were removed, and a cell suspension was prepared in 0.75% sodium citrate. Cell suspensions, whether from the cultures or from the i.p. tumors, were incubated in hypotonic sodium citrate (0.75%) for 30 min at 37°C. After centrifugation, cell buttons were fixed in 1:3 acetic acid:alcohol, and squash preparations were stained in carbol-fuchsin.

RESULTS

CCRF-SB Suspension Cultures. A majority of metaphase plates (18 of 35 counted) from this sample had a normal human male diploid complement of 46 chromosomes (Table 1). Plates with 45 chromosomes did not show the consistent absence of any particular chromosome, thus ruling out the possibility that a stem line with 45 chromosomes was present.

H-SB9 ALS-dependent CCRF-SB Hamster Transplants. Cells from the 6th serial transplant were predominantly pseudo-diploid and 13 of a total of 21 plates had 46 chromosomes. Seven karyotypes made from this passage (5 with 46, 1 with 45, and 1 with 48 chromosomes) revealed a highly aneuploid/pseudodiploid chromosome condition with extra and missing chromosomes from nearly all the chromosome groups.

H-SB2 Hamster Transplants. Cells from the 30th serial passage of this tumor had a predominantly near-diploid karyotype, as evident from the earlier immunofluorescent labeling studies (1) that the serially transplantable tumors resulting from the implantation of buffy coat cells from human leukemic blood in newborn Syrian hamsters are of human origin and were not induced in the host animals by an oncogenic agent (or agents) inoculated with the human lymphoblasts.

A later report (7) described the karyotype evolution in transplantable tumors initiated in newborn hamsters by the i.p. inoculation of cells from suspension cultures (CCRF-CEM) of human leukemic lymphoblasts. In contrast to this, the present in vivo series of transplantable tumors was initiated by the direct implantation of cells from the peripheral blood buffy coat of a leukemic patient into the newborn hamsters. The in vitro suspension cultures were also started from the same sample of the buffy coat. In spite of their common origin, prominent karyotypic and morphological differences are seen between cells from the tissue culture cell line and the transplantable tumors. CCRF-SB, the cell line isolated in vitro, has maintained a typical human diploid karyotype, as have

<table>
<thead>
<tr>
<th>Material</th>
<th>Chromosome No.</th>
<th>Total no. of cells</th>
</tr>
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<tbody>
<tr>
<td>CCRF-SB continuous suspension culture</td>
<td>2 4 10 18 1</td>
<td>35</td>
</tr>
<tr>
<td>H-SB9 ALS-dependent transplant of CCRF-SB</td>
<td>1 1 2 13 1 3</td>
<td>21</td>
</tr>
<tr>
<td>H-SB2 serial hamster transplants</td>
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<td></td>
</tr>
<tr>
<td>30th passage</td>
<td>1 2 2 2 5 4 1</td>
<td>72</td>
</tr>
<tr>
<td>40th passage</td>
<td>1 2 2 4 10 3 6 1</td>
<td>50</td>
</tr>
<tr>
<td>46th passage</td>
<td>1 19 2 1 3 1 6 13 44 6 1 1 1 1 112</td>
<td></td>
</tr>
<tr>
<td>R-SB rat transplants of H-SB6</td>
<td>1 5 14 1</td>
<td>21</td>
</tr>
<tr>
<td>H-SB2 hamster transplants in culture</td>
<td>2 35 1</td>
<td>38</td>
</tr>
</tbody>
</table>

DISCUSSION

The karyotype analysis considered herein confirms the evidence from the earlier immunofluorescent labeling studies (1) that the serially transplantable tumors resulting from the implantation of buffy coat cells from human leukemic blood in newborn Syrian hamsters are of human origin and were not induced in the host animals by an oncogenic agent (or agents) inoculated with the human lymphoblasts.
several other established human cell lines (6, 7, 9). No marker chromosomes have been recognized either in CCRF-SB culture cells or in cells from the i.p. tumors resulting from the implantation of these cells in ALS-treated newborn Syrian hamsters.

H-SB2 tumor derived from the same buffy coat specimen by direct implantation in newborn Syrian hamsters, on the other hand, has consistently shown the presence of an extra F-group chromosome in the diploid and tetraploid hamster transplants, in rat transplants derived from tumor serially transplanted in hamsters, and in suspension cultures initiated from the hamster transplants.

CCRF-SB cells in culture and H-SB2 tumor cells have also shown differences in their fine structure (10) and in their biochemical and immunological character (4, 8). CCRF-SB cells from cultures and from ALS-dependent transplantable tumors (H-SB9) often have a prominent complex of granules and tubules in association with the endoplasmic reticulum (10). Similar structures associated with the endoplasmic reticulum have been reported in a number of normal and leukemic human cell lines (3). These observations offer additional support to the suggestion that the 2 different modes of isolation (as hamster transplants or in suspension cultures) may have selected 2 different cell types from an apparently homogenous population of cells from peripheral blood buffy coat (8).

The other significant difference has been in the retention of diploidy by the CCRF-SB cells through more than 3 years of continuous growth in suspension cultures, in contrast to the gradual evolution of near-tetraploidy in the H-SB2 cells consequent to serial transplantation in the Syrian hamsters. The similar evolution of a tetraploid cell population consequent to serial transplantation of CCRF-CEM human leukemic lymphoblasts in newborn Syrian hamsters has been reported (7). In either case, CCRF-CEM and CCRF-SB cells had been serially transplanted in untreated newborn Syrian hamsters long before the development of tetraploidy; hence, tetraploidy was not a requisite for successful heterotransplantation of these human leukemic lymphoblasts in untreated neonatal Syrian hamsters, but rather developed as a result of selective pressures consequent to serial in vivo transplantation.

ACKNOWLEDGMENTS

We extend our sincere thanks to Dr. George E. Foley, Dr. Richard A. Adams, Dr. Betty G. Uzman, and Dr. Herbert Lazarus for their critical suggestions and help; to Mrs. Patricia Hutchins for technical assistance; and to Miss Eleanor Monkouski for secretarial help.

REFERENCES


Figs. 1 and 2. Karyotype and its metaphase plates from the 30th serial passage of the hamster tumor H-SB2 showing a near-diploid (47) chromosome complement with an extra chromosome in the F group.

Figs. 3 and 4. Karyotype and its metaphase plate from the 46th serial passage of the tumor H-SB2 showing a near-tetraploid (94) chromosome complement with 2 extra chromosomes in the F group.
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