Direct Implantation and Serial Transplantation of Human Acute Lymphoblastic Leukemia in Hamsters, SB-2

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SUMMARY

Human acute lymphoblastic leukemia cells have been established as a serially transplantable lymphosarcoma in newborn Syrian hamsters by the direct inoculation of cells from the peripheral blood of a boy in the terminal stages of lymphosarcoma. The experimental neoplasm, currently in its 70th serial passage, is metastatic and occasionally progresses to frank leukemia. In later passages, it has become transplantable in the newborn hamster every 10-12 days. Immunofluorescence evidence indicates that it is still composed of human cells after continued passage in the experimental host. Cytogenetic examination shows the cells to be of male human karyotype. Electron-microscopic examination reveals them to be free of morphologically recognizable virus.

INTRODUCTION

The implantation of cultured human leukemia lymphoblasts (10) in first passage and the subsequent serial transplantation of the resultant lymphosarcoma in experimental animals has been reported previously from these laboratories (4, 6, 9).

The present communication details experiments reported in brief elsewhere (2), which, in contrast to all previous studies, have eventuated in the successful establishment of a serially transplantable human lymphosarcoma, designated SB-2 (or lately, H-SB2), directly from the patient.

This neoplasm, which occasionally progresses to leukemia, is currently in its 70th passage in newborn hamsters. It was established in April 1966 by the intraperitoneal implantation into Syrian hamsters of peripheral blood buffy coat cells obtained from a boy in the terminal stages of lymphosarcoma that had progressed to acute lymphoblastic leukemia. As in previous work (5, 6), it has been considered important to provide immunologic evidence that the tumor cells have remained human after continued passage in the hamster.

MATERIALS AND METHODS

Hamsters. Newborn hamsters were bred by mating proven stock males with young females obtained from a professional breeder. Details of handling and husbandry have been presented elsewhere (6).

Leukemic Cells. The leukemic cells were separated by slow centrifugation of a heparinized specimen of the child's peripheral blood. The total white count was 345,000 cells per cu mm, 90% of which were lymphoblasts (Fig. 1).

Transplantation. The leukemic cells were injected i.p. into each of 8 intact newborn hamsters at inocula of $2.0 \times 10^8$ cells per 0.1-0.2 ml in autologous plasma. Subsequent serial transplantation of the resultant lymphosarcomas was done by i.p. administration of 20-50% suspensions of tumor in 2% streptomycin-penicillin saline, using 1-ml Tuberculin syringes and 21-gauge needles.

Guinea Pig Antibody. Antiserum was raised in different groups of 10 guinea pigs to the cells of a normal human peripheral blood buffy coat or to cells from serial passages 8, 13, and 20 of SB-2, essentially according to the methods of Stulberg (12). The cells were washed twice and suspended in serum-free Eagle's minimal essential medium (8) for injection. Each guinea pig was sensitized with a total of $1.0 \times 10^7$ cells, half given i.p. and the remainder given s.c. as a 1:1 emulsion in complete Freund's adjuvant. The guinea pigs were exsanguinated by cardiac puncture 4 weeks later, and serum was stored at $-72^\circ$C.

Prior to use, aliquots of the serum were thawed and freshly absorbed with human type O Rh-negative red blood cells (12), bovine liver powder and hamster spleen cells and/or red blood cells. For control purposes, antiserum to hamster spleen cells was similarly prepared but not absorbed with hamster tissues, except in experiments designed to confirm specificity of the antihamster antibody.

Indirect Fluorescent Antibody Methods. The cells to be examined were washed three times in phosphate-buffered

1 These studies were supported in part by research Grants C-6516 from the National Cancer Institute, and FR-05526 from the Division of Research Facilities and Resources, NIH; the legacy of Loula D. Lasker; the Albert and Mary Lasker Foundation, New York; and the Alvan T. and Viola D. Fuller Cancer Research Unit Grant, American Cancer Society (Massachusetts Division) Inc.

2 The prefix H is used to distinguish cells established and grown in hamsters from those (prefixed CCRF) isolated in cell culture in the Laboratories of Microbiology of this Foundation.

Received December 20, 1967; accepted February 25, 1968.
saline (PBS), reacted with the unconjugated guinea pig antiserum for 30 min at room temperature, and again washed three times in PBS. In a second step, the cells were reacted for 30 min at room temperature with commercially obtained fluorescein-conjugated globulin fraction of rabbit antibody to guinea pig globulin, mixed 5:1 with Rhodamine-bovine albumin. (Both were obtained from Hyland Laboratories, Los Angeles, California.) The cells were washed again three times in PBS and were mounted directly on glass slides under coverslips sealed with clear lacquer. The slides were examined immediately with a Reichert Zetopan microscope. The criterion for a specific reaction was yellow-green peripheral fluorescence (5, 6, 12).

### RESULTS

#### Growth in First Passage

At 30 and 41 days postimplantation, intraabdominal tumor was observed in 2 of the 8 inoculated young. The total white counts just prior to sacrifice were 10,400 per cu mm and 18,400 per cu mm respectively, slightly elevated above the 6,000 per cu mm normal at this age. These elevations, however, were not due to the presence of lymphoblasts. The abdominal tumor in both hamsters was large, soft, pale, and substantially free of necrosis.

Besides the intraabdominal tumor in the 30-day specimen, there were 2.5 ml of milky abdominal ascites having a cell density of about $2.0 \times 10^8$ cells per ml. Most of these cells were lymphoblasts of the type seen in the tumor. In Wright- and Giemsa-stained smears, the ascites tumor cells were morphologically indistinguishable from the inoculated cells.

A variable quantity of ascites tumor has been an unpredictable concomitant of tumor growth from passage to passage. Although such ascites can be used to pass the tumor successfully, an ascites subline of the tumor has not yet been established.

#### Growth in Serial Passage

Certain of the growth characteristics of SB-2 in the first 10 serial passages are summarized in Table 1, which shows that the median age at death with tumor or at sacrifice for transplantation decreased to 13 days by the 10th passage. The tumor in subsequent passages has become transplantable at 10-12 days.

One risk of serial transplantation in neonatal hamsters is that of cannibalization of traumatized young in the first 1-3 days postimplantation or of young sick or dying with tumor at later times: in this study, roughly a third of the experimental group were cannibalized. This and the accelerated growth rate combine to make estimates of tumor incidence inexact. Nevertheless, by the most conservative estimate, approximately half of the 538 survivors beyond 10 days (roughly a third of the total tumor-inoculated group) had histologically verified or grossly evident tumor postmortem. Of the remaining third of the group negative for tumor at autopsy, 38 presumably had regressed their tumor, since abdominal masses had been palpated initially.

#### Progression to Leukemia

The progression to frank leukemia has been a rare event, as judged by preliminary examination of smears of blood exhibiting counts in excess of 10,000 per cu mm. In the first 10 passages, in only 7 of 11 hamsters with counts elevated above 10,000 per cu mm (i.e., 2% of the population surviving beyond 10 days) was there unequivocal evidence of progression to leukemia. Among these the percentage of lymphoblasts varied from 16% in a hamster with a count of 62,800 per cu mm, to 99% in a hamster with a count of 372,000 per cu mm. Assessment of progression to leukemia on the basis of the hematopathologic examination of the bone marrow is in progress and could conceivably alter these estimates.

#### Histologic Appearance

The histologic appearance (Fig. 2) is that of well-vascularized lymphosarcoma composed of mitotically active lymphoblasts having scant cytoplasm and deeply basophilic nuclei, growing in stroma presumably of hamster origin. The histologic appearance is in general little different from that of a serially transplantable lymphosarcoma initiated with the CCRF-CEM cell cultures (6).

Invasion of the kidney via the renal pelvis or by a subcapsular infiltrate of tumor cells, infiltration of the diaphragm, and invasion of the capsular infiltrate of tumor cells, infiltration of the diaphragm, and invasion of the abdominal cavity by tumor mass have been observed.

#### Table 1

<table>
<thead>
<tr>
<th>Passage</th>
<th>No. of litters</th>
<th>No. of hamsters cann.*/ no. of hamsters inoc.</th>
<th>No. of hamsters with tumor*/ no. uncanibalized</th>
<th>Age at sacrifice or death (days)</th>
<th>Median</th>
<th>Range</th>
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<tbody>
<tr>
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<td>1</td>
<td>2/8</td>
<td>2(0)/6</td>
<td>30-41</td>
<td>30</td>
<td>41</td>
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<tr>
<td>2</td>
<td>7</td>
<td>17/55</td>
<td>7(1)/38</td>
<td>22-25</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>8/51</td>
<td>12(1)/43</td>
<td>17-43</td>
<td>21</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>38/117</td>
<td>34(4)/74</td>
<td>14-39</td>
<td>20</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>22/99</td>
<td>46(3)/77</td>
<td>16</td>
<td>13-28</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>36/106</td>
<td>32(2)/70</td>
<td>15</td>
<td>11-22</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>30/104</td>
<td>51(11)/74</td>
<td>14</td>
<td>11-22</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
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<td>17/125</td>
<td>52(4)/80</td>
<td>13</td>
<td>11-17</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>9</td>
<td>22/50</td>
<td>24(3)/28</td>
<td>11</td>
<td>8-14</td>
<td>11</td>
<td>14</td>
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<td>25/73</td>
<td>27(9)/48</td>
<td>13</td>
<td>10-20</td>
<td>13</td>
<td>20</td>
</tr>
</tbody>
</table>

* Serial transplantation of human leukemia in the newborn Syrian hamster: transplantation characteristics of SB-2 in the first 10 passages.

* Number cannibalized in the first 1-10 days postimplantation.

* By palpation and gross and histologic evidence; numbers in parentheses refer to those judged to have regressed.
Human metastatic, and can on occasion progress to frank leukemia. The resultant lymphosarcoma is serially transplantable, metastasizing to the brain, periportal infiltration of the liver and disruption of the architecture of the spleen and of the sternal bone marrow in instances of conversion to leukemia have all been observed. These features are suggestive of initiation of tumor growth by retroperitoneal implantation of the inoculum just as with cultured CCRF-CEM tumor cells, as indicated by studies to be reported elsewhere of the early pathology of inoculated, tritium-labeled CCRF-CEM cells.

Species Identification of the Tumor Cells. Results of attempts to demonstrate human species-specific antigen in the serially transplanted tumor cells are summarized in Table 2. The cells examined included 25th-passage SB-2 tumor cells, normal human peripheral blood buffy coat cells, hamster spleen cells, and cells of an ascites neoplasm of thymic origin originally discovered in a hamster and maintained in hamsters by serial passage. The antisera to three different passages of SB-2 cells and the antiserum to human buffy coat cells gave a strong positive yellow-green fluorescence with test human buffy coat cells and with 100% of 25th-passage SB-2 cells; such sera did not react with the Syrian hamster normal or malignant cells, respectively of splenic and thymic origin. Conversely, the antiserum to hamster spleen cells reacted strongly with hamster cells but failed to react with either human buffy coat cells or cells obtained from the serially transplanted tumor.

The unconjugated specific antiserum may be diluted 320-fold with retention of specific stainability, suggesting the retention in the passed tumor cells of substantial quantities of human species-specific antigen. (A more detailed consideration will be presented elsewhere of direct and indirect fluorescent antibody methods in the species identification of transplantable CEM and SB-2 tumors, incorporating the appropriate specific inhibition and absorption confirmatory procedures.)

DISCUSSION

These experiments indicate that human leukemia cells have been directly implanted from the patient into neonatal hamsters. The resultant lymphosarcoma is serially transplantable, metastatic, and can on occasion progress to frank leukemia.

The similarities and differences between SB-2 and a histologically similar neoplasm resulting from the implantation of cultured CCRF-CEM human malignant lymphoblasts have been emphasized elsewhere (3). The striking recapitulation of the human disease in both instances suggests the potential utility of both neoplasms as models for the study of the progression of lymphosarcoma to leukemia.

These experiments are the most recent in a series designed to elucidate some of the factors which bear on the hetero-transplantation of leukemia. They were initiated because of the repeated perplexing observation similar to that of Zlotnick et al. (13), that cells of human acute leukemia could not be perpetuated beyond one passage in the cheek pouch of the normal or cortisone-conditioned adult hamster. Such observations were at variance with reports of the successful establishment in the hamster cheek pouch (11) of serially transplantable lines of murine leukemia or of solid tumors of man.

In order to better understand the factors governing hetero-transplantation of leukemia, murine leukemia cells were examined in less usual transplantation systems: intravenous infusion of the leukemic cells into whole-body X-irradiated adult hamsters (1) or intraperitoneal implantation of such cells in newborn hamsters (7). Subsequently, these methods were successfully used to grow the cultured human leukemia lymphoblasts (CCRF-CEM cells) in first passage in whole-body X-irradiated adult (5) or normal neonatal hamsters (4) and, later, in serial intraperitoneal passage in newborn Syrian hamsters under 24 hr of age (6, 9).

The present demonstration of the implantation and serial transplantation of human malignant lymphoblasts directly from the peripheral blood augments the earlier work with cultured CCRF-CEM human leukemia lymphoblasts, implying that the prior establishment of the cells in culture was probably neither the sole nor the major factor determining their transplantability. These experiments further suggest that transplantation approaches of this kind, when better studied, might be used profitably for analysis of events in the course of the human disease. The utility of this and other transplantation systems for the "monitoring" of cell cultures of malignant human lymphoblasts has been stressed elsewhere (3, 5, 6, 9).

The results of the species-identification studies indicate that
the passaged cells are with little doubt progeny of the inoculated human lymphoblasts. Conversely, they also indicate that the tumor cells were probably not induced in hamster tissues by putative leukemogenic virus present in or on the cells of the original inoculum. These findings are borne out by concomitant ultrastructural studies. Electron microscopic examination of the original buffy coat and of thirty-odd separate specimens of eleven passages of serially transplanted SB-2 tumors have shown no morphologic evidence for the presence of virus. Hence, whatever the etiology of this lymphosarcoma and leukemia in the patient, the malignant behavior of the cell is not necessarily dependent upon the presence of morphologically recognizable virus.

Cytogenetic studies in progress at the moment reveal the presence in the transplanted tumor cells of a human near-diploid male karyotype, providing confirmatory evidence that these cells are of human origin. These studies, to be reported in detail later, also indicate that SB-2 cells may be distinguished from cell cultures of SB cells—CCRF-SB cells derived from the same patient buffy coat (4)—by the presence of one additional chromosome. The results suggest that the present transplantation system and spinner cell-culture methods may have selected different stem lines from a heterogeneous population of cells in the patient’s blood; and they are further suggestive, therefore, of additional areas for future study.

ACKNOWLEDGMENTS

We thank Mrs. Charles Aster for technical assistance in the immunofluorescence studies; Dr. Betty Uzman for the electron microscopic examinations; Dr. Awtar Krishan for the cytogenetic examinations; and Mr. John Carabites for the photomicrographs.

REFERENCES

Fig. 1. Peripheral blood smear of SB on April 11, 1966, when the WBC was 143,000, 57% of which were lymphoblasts. Note mitotic figure (arrow). Twelve days later, when the WBC was 345,000 with 90% lymphoblasts, circa $2.0 \times 10^8$ buffy coat cells were implanted i.p. to establish the first passage in newborn hamsters. Wright's and Giemsa's stains, $\times 780$.

Fig. 2. Abdominal tumor in the ninth serial i.p. passage in newborn hamsters, Sept. 28, 1966. Twelve days after transplantation. Hematoxylin and eosin, $\times 910$. 
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