Leukemia: Serial Transplantation of Human Leukemic Lymphoblasts in the Newborn Syrian Hamster

RICHARD A. ADAMS, GEORGE E. FOLEY, BETTY G. UZMAN, SIDNEY FARBER, HERBERT LAZARUS, AND LAWRENCE KLEINMAN

The Children’s Cancer Research Foundation, and the Department of Pathology, Harvard Medical School, at The Children’s Hospital, Boston, Massachusetts 02115

SUMMARY

Human leukemia cells are being regularly grown intraperitoneally in the intact newborn Syrian hamster as 3 serially transplantable sublines of lymphosarcoma. These sublines, established from suspension cultures of human leukemic lymphoblasts (CCRF-CEM cells) by the implantation of large numbers of cells, exhibit similar transplantation and growth characteristics, and are presently in their 11th, 8th, and 6th serial passages, respectively. The intraabdominal lymphosarcomas frequently metastasize to the central nervous system, and in some instances progress to leukemia—a striking recapitulation of the human disease from which the CCRF-CEM cells were originally isolated.

Immunofluorescence evidence clearly indicates the presence of human species-specific antigens in the cells of the serially transplanted lymphosarcomas. The morphology of these cells, as determined by electron micrography, does not differ significantly from that of the CCRF-CEM cells in suspension culture. There is as yet no morphologic evidence for the presence of virus associated with growth of these cells, either in suspension cultures or in newborn hamsters.

INTRODUCTION

Although several reports describe the heterotransplantation of human lymphoma and leukemia cells in first-passage (18, 33), the serial transplantation of such cells, in sharp contrast to that of other kinds of neoplastic cells, has not been reported heretofore.

The present communication describes the intraperitoneal implantation and growth of cultured human leukemic lymphoblasts (CCRF-CEM cells), and the serial transplantation of the resulting lymphosarcomas in the newborn Syrian hamster. These studies are based on evidence that CCRF-CEM cells were equivalent to the buffy coat cells from which they were derived with respect to their capacity to implant in the bone marrow of the lethally X-irradiated adult Syrian hamster (2, 4), and that they grew predictably in first-passage in the intact newborn Syrian hamster (3).

Subsequent reports will describe the transplantation of these human leukemia cells in newborn mice (Adams et al., unpublished work) and the direct implantation and serial transplantation of peripheral blood buffy coat cells from another patient with acute lymphatic leukemia in newborn Syrian hamsters (Adams et al., unpublished work).

MATERIALS AND METHODS

Animals

Syrian hamsters obtained from a professional supplier (Pure-bred Animals for Research, Billerica, Mass.) were bred on the premises. Regular litter production, which is essential for the serial transplantation of tumor by the methods here described, was maintained according to the recommendations of Cusick and Cole (8). All breeding stocks were housed in individual suspended wire mesh cages. Water and a diet of Purina laboratory chow supplemented with wheat germ were provided ad libitum. Four days prior to delivery, pregnant females were removed to whelping cages. Foster-nursing has been employed successfully when indicated.

Cells

The human lymphoblastic cells used in these experiments were derived from the peripheral blood buffy coat of a child (CEM) with acute lymphoblastic leukemia who had initially presented with lymphosarcoma (16). The isolation (16), the cytogenetic (23), and the cytochemical characteristics (22) of the CCRF-CEM cells in suspension culture have been described elsewhere, and the morphologic characteristics of these cells have been documented extensively by electron micrography (16, 32).

These cells have been maintained for more than 1.5 years in continuous log-phase suspension cultures in Eagle’s minimal essential medium modified for suspension culture (11) and supplemented with 10% whole fetal calf serum (MEM). Their generation time is approximately 24 hr, and population densities of 3.0–4.0 × 10^6 cells/ml can be maintained in cultures containing 10 or more liters of medium. At various times, cells from aliquots of the stock cultures have been preserved in media containing 15% dimethylsulfoxide (16) by freezing and storage in liquid nitrogen as described by Stulberg et al. (30).
Electron Microscopy

Monium sulfate and dialyzed according to standard procedures were pooled. The globulins were precipitated with am

Immunofluorescence

CCRF-CEM cells from culture were washed, suspended in serum-free MEM, and injected into guinea pigs according to the methods described by Stulberg et al. (31). Four weeks later the guinea pigs were bled by cardiac puncture and the sera thus obtained were pooled. The globulins were precipitated with ammonium sulfate and dialyzed according to standard procedures (7), redissolved in minimal quantities of bicarbonate-buffered saline at pH 9.0, and then by modifications of the method de-

RESULTS

Three serially transplantable sublines are now being regularly maintained in newborn hamsters: 1 subline established from the parent suspension culture which has been maintained in log-phase growth since its isolation, and 2 sublines established from reconstituted aliquots which had been preserved in liquid nitro-
Adams et al.

Gross Appearance and Distribution of Tumor

The intraabdominal tumors are characteristically soft, white masses enveloping the abdominal viscera (Figs. 1, 2). The liver is grossly uninvolved and the spleen slightly to moderately shrunken. The diaphragm is often swollen and pale. There may be slight to moderate enlargement of the thymus and the mediastinal lymph nodes, and occasionally of the axillary and inguinal lymph nodes. There may be a nodule of tumor in the abdominal musculature at the site of inoculation, and abdominal or pleural effusion may be present.

Tumor growth also has been achieved occasionally in the subcutaneous space of normal hamsters implanted subcutaneously at birth with CCRF-CEM cells (Fig. 3), and in the cheek pouches of cortisone-conditioned adult hamsters (Fig. 4) implanted with inocula derived either from suspension cultures or from the tumor grown by serial transplantation in newborn hamsters. Growth in either of these sites, however, has not been as predictable as routine passage in the newborn hamster, although cheek pouch tumors have been maintained for more than 6 serial passages.

Histologic Appearance

The intraabdominal tumors are composed of mitotically active small and medium-size cells, cytologically indistinguishable from the CCRF-CEM cells in culture, growing in a delicate, well-vascularized stroma of supporting connective tissue (Figs. 5, 6). Tumor cells are seeded along the capsules of the liver and kidney, frequently with direct extension into the renal pelvis, and occasionally into the renal cortex. Commonly, there is infiltration or total replacement of the pancreas (Fig. 7), but direct infiltration of the liver is unusual. The spleen in all instances has been enveloped by tumor, but parenchymal replacement is uncommon; in many instances, the histologic picture has been that of a reactive spleen with well-developed, mitotically active white pulp of normal architecture. There is massive infiltration of the diaphragm, the intercostal musculature, and the mesenchymal tissues of the peristernal region (Figs. 8, 9). Nodules of tumor cells have been seen occasionally on the pleura of the lungs, in association with diaphragmatic involvement. The thymus is invariably surrounded by large masses of tumor cells which occasionally penetrate the thymic capsule. The thymus itself is for the most part intact, despite the surrounding tumor masses, and may exhibit slightly increased mitotic activity in the cortex.

Metastasis to the brain occurs frequently as slight to marked meningeal infiltration; in more advanced lesions there is diffuse infiltration with perivascular cuffing and destruction of brain tissue. The sequestration of masses of lymphoblasts in the brain has been observed in hamsters in which the implant otherwise failed to “take”; and has been the presumed cause of death in hamsters in which regression of a well-established intraabdominal tumor occurred (Figs. 10–13). The heterotransplantability of the CCRF-CEM cells directly into the newborn hamster brain has been reported elsewhere (3).

In those newborn hamsters in which progression to leukemia has occurred, the bone marrow is partially to completely filled

TABLE 2

<table>
<thead>
<tr>
<th>Passage</th>
<th>No. of litters</th>
<th>No. of animals</th>
<th>Incidencea</th>
<th>Age of hamster (days) at sacrifice for passage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>5</td>
<td>2/2</td>
<td>16; 23</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>9</td>
<td>7/9</td>
<td>13 × 3; 15; 26; 27</td>
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<td>3</td>
<td>7</td>
<td>60</td>
<td>4/23</td>
<td>13; 16 × 2; 17</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>49</td>
<td>7/26</td>
<td>14 × 2; 16 × 2; 18</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>76</td>
<td>6/26</td>
<td>15 × 5; 16</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>52</td>
<td>9/18</td>
<td>7; 11 × 2; 13 × 2; 14; 16 × 2</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>78</td>
<td>22/59</td>
<td>10; 11; 12 × 2; 13 × 2; 14 × 2; 15 × 2; 16 × 2</td>
</tr>
</tbody>
</table>

a Number of histologically verifiable tumors over the total number of surviving (uncannibalized) young.
Electron Microscopy

Sections of tumor masses from the 2nd, 4th, 5th, and 6th passages of Line 39 (Table 1) have been examined. The tumor cells grow either as compact masses (Fig. 23), or as very loosely associated cells (Fig. 25) in a connective tissue stroma. No virus particles have been observed. Phagocytosis of tumor cells by macrophages (Fig. 24), phagocytosis of cellular debris by tumor cells (Fig. 25), the presence of large numbers of lysosomes (Fig. 23), or sporadic necrosis of tumor cells has been characteristic of one or another of these passages. The morphology of the cells in these tumors does not differ significantly from that of CCRF-CEM cells in culture as described in detail elsewhere (16, 32).

Immunofluorescence

The interpretation of staining reactions was based upon the criterion for specific staining proposed by Stulberg et al. (31), namely, the presence of peripheral staining. Cells which exhibited mottled or solid fluorescent areas in the absence of peripheral staining were interpreted as evidence of a negative or nonspecific reaction (31).

CCRF-CEM cells reisolated in culture after 6 consecutive passages in the newborn hamster, and CCRF-CEM cells obtained by titration of 7th-passage intraabdominal tumor stained peripherally, as did CCRF-CEM cells in the parent cultures (Table 3). Cross-reactivity with KB, a human cell line of malignant origin (10), and with WI-38, a normal human diploid fibroblast (19), and the absence of cross-reactivity with cultured Syrian or Chinese hamster normal cells or with a Syrian hamster thymoma maintained in vivo, indicate retention of human species-specific antigen by the CCRF-CEM cells during serial propagation in culture and following serial passage in the newborn hamster.

DISCUSSION

The serial transplantation of human leukemia cells in experimental animals, heretofore not accomplished, has been achieved by the implantation of large numbers of cells into immature Syrian hamsters. Although the "immunologic immaturity" (receptivity to alien tissue grafts) of the newborn rodent has long been known, the suitability of such animals for the serial transplantation and growth of human leukemia cells was not tested until preliminary experiments with murine leukemia (1, 5, 6) and human leukemia cells (2-4) indicated that given the proper conditions of age and route of inoculation, successful implantation was critically dependent upon the use of large inocula. Previously reported failures to establish human leukemia cells in first-passage (9) or to transplant serially beyond first-passage in newborn hamsters (18) may thus be attributable to the use of inadequate inocula. The successful propagation of the CCRF-CEM cells in baby mice (Adams et al., unpublished work) provides further evidence for the validity of this approach and further suggests that human leukemia cells may be transplantable to any species sufficiently immature at birth, or perhaps by inoculation in utero, provided large enough numbers of cells are implanted.

The implantation and serial transplantation of human leukemia cells directly from a patient into newborn hamsters in which the resulting lymphosarcomas were similar in many respects to those here described will be reported elsewhere (Adams et al., unpublished work). In view of this observation, it is unlikely that the transplantation of CCRF-CEM cells in hamsters is dependent in any way on their propagation in culture. Moreover, the CCRF-CEM cells were neither more nor less transplantable than the original buffy coat cells in first-passage in X-irradiated adult hamsters (2, 4), and there have been no differences in the transplantability of the parent stock culture and the cultures reconstituted from aliquots preserved in liquid nitrogen shortly after initiation of the parent stock cultures (Table 1).

Although there may be some changes in histocompatibility requirements consequent to the serial propagation of murine leukemia in monolayer culture (15), little is known of this parameter following isolation and propagation of leukemic cells in sus-
pension culture. In the case of monolayer cultures of murine leukemia cells, serial transplantation and the ability to metastasize and produce generalized leukemia in the adult hamster is independent of loss or retention of histocompatibility requirements (15, 17, 18). Similarly, in view of the results of heterotransplantation studies with other kinds of cells (12-14), there seems to be little reason thus far to relate heterotransplantability of CCRF-CEM cells to changes in chromosome number. Detailed karyotypic analyses of the serially transplanted tumors (Krishan et al., unpublished work) and of the cells reisolated in suspension cultures from such tumors (McCarthy et al., unpublished work) will be described elsewhere.

As has been discussed previously (2), it is important in experiments involving the transfer of human leukemic tissues to experimental animals (25, 26) to ascertain whether the development of leukemia in the recipients represents the growth of transplanted human cells or is the result of leukemogenesis occurring in host tissue. The time-intervals involved, transplantability, histopathology, etc., may be helpful in deciding between these alternatives, but immunofluorescence evidence is more reliable: immunologic methods are, as pointed out by Stulberg et al. (31), by far the most specific among the methods presently available for the species-identification of mammalian cells. In the present experiments, immunofluorescence evidence clearly decides in favor of transplantation by establishing the human origins of the cultured cells and those which are serially transplantable as lymphosarcomas in the newborn hamster, and further imply that the transplantability of the CCRF-CEM cells cannot be attributed to a complete loss of species characteristics consequent to serial propagation in vitro or in vivo. Aside from this evidence of their human origin, the malignant origin of the CCRF-CEM cells is evidenced by their capacity to induce serially transplantable lymphosarcoma with metastasis and conversion to leukemia—a striking recapitulation of the human disease from which the cells were originally isolated.

The lack of morphologic evidence for the presence of virus in the CCRF-CEM cells in culture (16, 32), and in the tumor cells serially transplanted in newborn hamsters, suggests the possibility that the malignant behavior of these cells may be independent of the presence of morphologically recognizable virus, irrespective of whether or not such virus was the initial cause of malignancy in the patient. It might be argued that the lack of such evidence does not definitively exclude the possibility that the lymphosarcomas in newborn hamsters originated from hamster cells “transformed” by oncogenic virus in, or on the implanted CCRF-CEM cells. Accordingly, the immunofluorescence reactions demonstrated with these tumor cells should then be attributable only to the presence of virus-associated or “T”-antigen present in hamster cells so transformed, rather than to the presence of human species-specific antigen in the progeny of the implanted cells. Until such time as there is acceptable evidence for oncogenic activity in cell-free extracts of filtrates of the CCRF-CEM cells, such an hypothesis appears to be untenable. It seems more likely that the present experiments should be interpreted as the actual implantation, serial transplantation, and growth of human leukemia cells in an heterologous host.

ACKNOWLEDGMENTS

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REFERENCES


FIG. 1. Intraabdominal tumor, first-passage, Line 39, 16 days following intraperitoneal implantation of \(1.0 \times 10^6\) cultured human leukemic lymphoblasts at birth.

FIG. 2. Intraabdominal tumor, first-passage, Line 39, 23 days following implantation of \(1.0 \times 10^6\) cultured human leukemic lymphoblasts at birth.

FIG. 3. Subcutaneous tumor (arrows), first-passage, Line 33 (cf. text and Table 1), 14 days following subcutaneous implantation of \(1.0 \times 10^6\) cultured human leukemic lymphoblasts at birth. Note enlargement of contralateral axillary lymph node.

FIG. 4. Tumor in cheek pouch of cortisone-conditioned adult hamster, first-passage, 10 days following intrabuccal implantation of \(1.0 \times 10^6\) cultured human leukemic lymphoblasts (cf. text).

FIG. 5. Typical histologic appearance of intraabdominal tumor, Line 15 (cf. Table 1), first-passage, 13 days following implantation as described in Fig. 1. H & E, \(\times 600\).

FIG. 6. Different field, same specimen as in Fig. 5. \(\times 1200\).

FIG. 7. Infiltration of pancreas, third-passage, Line 39, 16 days following transplantation of second-passage tumor. H & E, \(\times 250\).

FIG. 8. Massively infiltrated tumor cells in skeletal muscles around sternum. Line 39, fifth-passage, 15 days following transplantation of fourth-passage tumor. H & E, \(\times 150\). Bone marrow was apparently uninvolved. Note megakaryocytes (arrows). Compare with Fig. 16 showing massive replacement of marrow by tumor cells.

FIG. 9. Different field, same specimen as in Fig. 8. \(\times 680\).

FIG. 10. Massive infiltration of brain by tumor cells in a hamster in which the intraabdominal tumor had regressed markedly in size. Line 39, fourth-passage, 36 days following transplantation of third-passage tumor. H & E, \(\times 185\).

FIG. 11. Different field of same specimen as in Fig. 10. \(\times 300\).

FIG. 12. Perivascular infiltration by tumor cells. Arrows in Figs. 10 and 12 point to same mitotic figure. H & E, \(\times 875\).

FIG. 13. Diffuse infiltration of brain by tumor cells in hamster with large intraabdominal tumor. Line 39, second-passage, 48 days following transplantation of first-passage tumor. Arrow indicates ependymal lining of floor of fourth ventricle. H & E, \(\times 250\).

FIG. 14. Peripheral blood of hamster with intraabdominal tumor and leukemia. WBC at time of sacrifice was 179,000/cu mm. Line 48, sixth-passage, 12 days following transplantation of fifth-passage tumor. Wright's and Giemsa's, \(\times 1200\).

FIG. 15. Different field, same specimen as in Fig. 14. \(\times 1000\).

FIG. 16. Sternal bone marrow and peristernal tissues of hamster with intraabdominal tumor and leukemia. WBC at time of sacrifice was 106,400/cu mm. Arrow indicates area in which tumor cells have replaced cortical bone and periosteal tissue. Line 48, second-passage, 14 days following transplantation of first-passage tumor. H & E, \(\times 225\).

FIG. 17. Spleen of same hamster as in Fig. 16. Perisplenic tumor to left of arrows which indicate capsule. Both capsule and splenic pulp (to right of arrows) are infiltrated by tumor cells. H & E, \(\times 600\).

FIG. 18. Same as Fig. 17. \(\times 1200\).

FIG. 19. Periportal infiltration in the liver. A few tumor cells may be seen in the sinusoids. Examination of bone marrow at time of sacrifice indicated that 90% of nucleated cells were lymphoblasts. Line 39, fifth-passage, 15 days following transplantation of fourth-passage tumor. H & E, \(\times 150\).

FIG. 20. Infiltration of liver. Peripheral WBC at time of sacrifice was 7,200/cu mm, but differential indicated 86% lymphoblasts. Line 48, second-passage, 14 days following transplantation of first-passage tumor. H & E, \(\times 700\).

FIG. 21. Infiltration of kidney, same hamster as in Figs. 16-18. H & E, \(\times 180\).

FIG. 22. Same as Fig. 21. \(\times 710\).

Figs. 23-25. Electron micrographs of intraabdominal tumors from early passages (second-fourth) of Line 39. Compact masses of tumor cells seen in Fig. 23 contrast with the loosely associated tumor cells seen in Fig. 25. Tumor cells (outlined by arrows) in Fig. 24 have been phagocytized by a macrophage. Tumor cells (indicated by arrows) in Figs. 23 and 25 contain large masses of phagocytized cellular debris, and large numbers of lysosomes.
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