Effects of Infant Thymectomy and Antilymphocyte Serum on Xenotransplantation of a Human Leukemia in the Hamster

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SUMMARY

The effects of neonatal thymectomy and antilymphocyte serum (ALS) treatment on the transplantability of a human leukemia were studied in hamsters of varying ages. This tumor, H-HM-1, is normally transplantable only in ALS-treated neonatal hamsters. Multiple doses of ALS prolonged the period of susceptibility to tumor implantation, as compared to a single dose at the time of tumor inoculation. Neonatal thymectomy alone did not substitute for ALS administration, but a combined program of neonatal thymectomy and multiple doses of ALS greatly depressed the host resistance to this tumor xenograft, allowing successful transplantation as late as 20 days after birth. These studies suggest that a thymic-dependent immune mechanism is involved in the rejection of a human tumor xenograft by hamsters.

INTRODUCTION

Xenograft rejection is traditionally considered to be mediated by humoral antibody, and its immunological nature has been reaffirmed recently by the demonstrations that heterotransplantation of human melanoma (13) or choriocarcinoma (3) in the hamster cheek pouch and a rat carcinosarcoma in the mouse (7) are facilitated by ALS administration. A more recent study involving suppression of rat-to-mouse skin xenograft rejection by ALS has been interpreted by Lance (8) to imply a certain degree of mediation of such rejection by cells. As further evidence, thymic-dependent, cytotoxic cellular immunity to a mouse mastocytoma has been directly demonstrated in rats by Jose and Good (6).

In our laboratories, experience with more than 30 neoplasms of human lymphoid origin in serial transplantation in hamsters indicates that such tumors are transplantable only in hamsters younger than 3 to 4 days and that ALS treatment is required for the progressive growth of most of these tumors. Hamsters that reject such tumors contain serum antibodies specific for human cells that are detectable by immuno-fluorescence techniques (4), and antiserum raised in adult hamsters against the human leukemic cells can confer on newborn hamsters the ability to reject a human leukemia xenograft (unpublished data). Further, heterologous rabbit antiserum or spleen cells from specifically sensitized or normal hamster donors also protect newborn hamsters in the same manner (1).

The purpose of the present study was to determine the extent to which thymic-dependent mechanisms are involved in the acceptance or rejection of such human tumor xenografts. Neonatal hamsters were thymectomized, subjected to various regimens of ALS treatment, and challenged with an ALS-dependent tumor at various times up to 30 days of age, well beyond the age at which normal hamsters have developed immunocompetence. Thymectomy was found to potentiate the facilitative effects of ALS on growth of the test human tumor.

MATERIALS AND METHODS

Thymectomy. Pregnant hamsters (strain LGV:LAK, a noninbred but closed colony) were obtained from Lakeview Hamster Colony, Newfield, N.J. Infant hamsters were thymectomized at 1 to 3 days of age by standard aspiration technique under light Nembutal anesthesia (approximately 0.1 ml Diabutal (Diamond Laboratories, Des Moines, Iowa), diluted 1:60 in sterile water). Operative mortality was negligible. Sham-thymectomized hamsters and normal controls were included in each litter.

Antilymphocyte Serum. Antihamster thymus serum was prepared in these laboratories by giving New Zealand white rabbits injections of hamster thymic cells as described previously (2). All hamsters received the same dose of ALS (0.05 ml i.p.), regardless of age or weight.

Treatment with ALS Alone. ALS was administered to 3 groups of animals. One group (111 hamsters) received ALS only at time of tumor inoculation (Regimen A); a 2nd group (55 hamsters) received ALS only at birth (Regimen B); and a 3rd group (120 hamsters) received 2 doses of ALS, once at birth and again at time of tumor inoculation (Regimen C) (see Table 1).

Treatment with Thymectomy and ALS. Three groups of thymectomized and control animals were used. One group (79 hamsters) was thymectomized only; no ALS was administered. Group 2 (93 hamsters) received ALS once, at time of tumor inoculation; and the 3rd group (161 hamsters) received 2 doses of ALS, 1 on day of thymectomy and 1 on day of tumor inoculation (see Table 2).

Tumor. H-HM-1, the ALS-dependent tumor selected for study, was originally established by direct i.p. implantation into ALS-treated neonatal hamsters of bone marrow cells from

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2 The abbreviation used is ALS, antilymphocyte serum.

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a male pediatric patient with acute lymphoblastic leukemia, as described in further detail elsewhere (Ref. 9; R. A. Adams, L. Pothier, E. E. Hellerstein, and G. Bórleau, to be published). The tumor has been maintained by serial i.p. transplantation in neonatal hamsters treated with one 0.05-ml administration of ALS and is currently in its 55th passage. It progresses to acute leukemia in about 30% of implanted hamster neonates, with total white blood cell counts ranging from 20,000 to 400,000/cu mm or more. In the remainder, the solid tumor that grows in the peritoneal cavity invades most of the major organs, including liver, spleen, kidneys, and gonads, and metastasizes to deep and superficial lymph nodes, thymus, lung, and brain. Death generally occurs between 10 and 20 days postgrafting. Throughout serial transplantation, the tumor cells have retained human species-specific antigens at the cell surface, as demonstrated by immunofluorescence methods, and have retained a diploid male human karyotype.

For maintenance of the carrier line of tumor or for challenge of the experimental hamsters, tumor was dissected from the abdomen, minced in 2% penicillin : streptomycin in 0.9% NaCl solution, and injected i.p. in a concentration of approximately 20% (about $1.0 \times 10^8$ trypan blue unstained cells) in 0.2 ml inoculum. All hamsters received the same inoculum of tumor, regardless of age or weight. Successful implantation of tumor was defined as progressive growth of tumor to death of the host; in a few instances, tumor grew temporarily and then regressed, and these instances were classed as “no growth.” The effectiveness of immunosuppression was evaluated in terms of the incidence of tumor and the prolongation of the time interval after birth at which tumor could be successfully transplanted.

RESULTS

Effects of ALS Treatment Alone on Tumor Implantation. We examined 286 unthymectomized hamsters for the effects of varying the time of ALS and tumor administration. Three regimens were examined: ALS at time of tumor inoculation, ALS at birth, or ALS at birth and again at time of tumor inoculation (Table 1). When ALS was administered once at the time of tumor inoculation (Regimen A), tumor (which is normally transplantable in 100% of 0- to 24-hr-old neonates) was not transplantable in hamsters beyond 3 days of age. Of the hamsters given implants at 2 to 3 days, 32.6% died from their tumors. When ALS was administered immediately at birth (Regimen B), successful tumor implantation could be delayed to 4 to 5 days of age, while administration of ALS at birth and again at the time of tumor implantation resulted in progressive tumor growth, in some cases as late as 6 to 7 days of age. Hence, ALS has a definable effect in facilitating implantation of this tumor in the neonatal hamster.

Effects of Thymectomy and ALS Combined on Tumor Implantation. Table 2 illustrates tumor growth in thymectomized hamsters as compared with sham-thymectomized and normal control hamsters. These control data have been combined because no differences were found. The dependence of this tumor on ALS is clearly seen in the failure of the tumor to grow in either the thymectomized or control hamsters not given ALS (Table 2, Column 1). Clearly, thymectomy alone was totally ineffective in the 0- to 10-day age range, and thus

Table 1
Facilitative effects of rabbit anti-hamster thymocyte serum on xenotransplantability of human acute lymphoblastic leukemia H-HM-I in neonatal hamsters

<table>
<thead>
<tr>
<th>Age at tumor implantation (days)</th>
<th>Rabbit anti-hamster thymocyte serum at time of tumor inoculation (Regimen A)</th>
<th>Rabbit anti-hamster thymocyte serum at Day 0 (Regimen B)</th>
<th>Rabbit anti-hamster thymocyte serum at Day 0 and day of tumor inoculation (Regimen C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>28/28 (100%)</td>
<td>9/9 (100%)</td>
<td>22/28 (78.6%)</td>
</tr>
<tr>
<td>2–3</td>
<td>15/46 (32.6%)</td>
<td>8/31 (25.8%)</td>
<td>18/42 (42.9%)</td>
</tr>
<tr>
<td>4–5</td>
<td>0/27</td>
<td>0/15</td>
<td>7/36 (19.4%)</td>
</tr>
<tr>
<td>6–7</td>
<td>0/8</td>
<td>0/8</td>
<td>0/14</td>
</tr>
<tr>
<td>&gt; 7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Number of hamsters dying with tumor/total inoculated.

Table 2
Facilitative effects of neonatal thymectomy at 1 to 3 days and rabbit anti-hamster thymocyte serum combined on heterotransplantability of human acute lymphoblastic leukemia H-HM-I in hamsters

<table>
<thead>
<tr>
<th>Age at tumor implantation (days)</th>
<th>No rabbit anti-hamster thymocyte serum</th>
<th>Rabbit anti-hamster thymocyte serum once at tumor transplantation</th>
<th>Rabbit anti-hamster thymocyte serum at thymectomy and tumor transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controlb</td>
<td>Thymec tomized</td>
<td>Thymec tomized</td>
</tr>
<tr>
<td>0–5</td>
<td>0/34</td>
<td>0/24</td>
<td>9/11 (81.8%)</td>
</tr>
<tr>
<td>6–10</td>
<td>0/8</td>
<td>0/13</td>
<td>16/19 (84.4%)</td>
</tr>
<tr>
<td>11–15</td>
<td>0/8</td>
<td>4/25 (16.0%)</td>
<td>4/23 (17.4%)</td>
</tr>
<tr>
<td>16–20</td>
<td>0/5</td>
<td>0/12</td>
<td>0/34</td>
</tr>
<tr>
<td>21–30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Number of hamsters dying with tumor/total inoculated.

b Sham-thymectomized + nonthymectomized.
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did not substitute for the administration of ALS (see Table 1). By contrast, when thymectomy was coupled with any of the ALS regimens used in the study, significant prolongations in the period of susceptibility to tumor implantation could be demonstrated (Table 2, Columns 2 and 3). Infant thymectomy coupled with the most effective ALS regimen, i.e., one administration at birth and another at tumor implantation, resulted in the most significant prolongation, tumor being transplantable in a low but significant percentage of thymectomized hamsters as late as 16 to 20 days of age.

White Blood Cell Counts and Histology. White blood cell counts and histological examinations were performed on selected animals. As with unthymectomized hamsters, metastatic foci occurred (Fig. 1), total white blood cell counts were significantly elevated in more than 50% of those examined, and conversion to leukemia was frequent (Fig. 2). In all tumors examined (usually 1 per litter), the tumor cells retained human species-specific antigens at the cell surface, as determined by immunofluorescence. Thus, these biological properties were not altered by the growth of this human tumor in older, thymectomized and/or ALS-treated hamsters.

DISCUSSION

The results of the present study indicate that multiple doses of ALS prolonged the period of perinatal susceptibility to implantation of a human tumor and that neonatal thymectomy significantly potentiated this suppressive effect of ALS on the host resistance to tumor implantation, but that thymectomy without ALS treatment was inadequate immunosuppression. Thus, analogous to the case of the humoral immune response to sheep erythrocytes in mice, for example (11), thymic-dependent mechanisms may figure significantly in the acceptance or rejection of these leukemic xenografts in the hamster.

It is not easy to understand how a thymic-influenced mechanism could be so difficult to demonstrate by thymectomy without adjunctive ALS treatment. One among the several possibilities is that the newborn hamster may contain sufficient numbers of thymic-dependent immunocompetent cells that, while thymectomy removes the source of the thymic-dependent reaction, ALS may be required to block or remove those thymic-dependent peripheral lymphoid cells still present after thymectomy. Other published evidence supports this argument; thymectomy alone in adult mice is ineffective in reducing immunological competence; however, coupled with ALS treatment, it produces a long-standing lymphopenia both in peripheral blood and in the lymphoid organs, as well as a significant impairment of the skin allograft rejection reaction (12). Such a mechanism might also underlie results recently reported with another allogeneic system (5), in which thymectomy was found to potentiate the effects of ALS in increasing the number of metastases associated with implantation of Sarcoma 180 in mice.

However effective thymectomy in combination with ALS treatment may be in prolonging the period of susceptibility to human leukemic grafts in the postnatal hamster, tumor failed to grow in animals older than 20 days. It is possible that weight-adjusted doses of ALS coupled with thymectomy might result in successful grafting in older hamsters. Another tumor, H-EB-3, derived from Burkitt lymphoma (2) can grow in comparably ALS-treated thymectomized hamsters when implanted as late as 30 days after birth (unpublished data). Such evidence might suggest that variable antigenicity of human tumor cells may also be an important factor. Experiments are in progress to elucidate the nature of thymic dependence in this xenogeneic system, further to define whether the thymus provides antigen-recognizing "helper" cells or effector "killer" cells (10) or whether both humoral and cellular mechanisms may function simultaneously. This technique is being utilized as well in attempts to facilitate the primary isolation of human tumors in hamsters.

REFERENCES


Fig. 1. Brain of neonatally thymectomized hamster 40 days after i.p. implantation of H-HM-1 tumor cells at 9 days of age. Note infiltration and perivascular cuffing (arrows) by tumor cells. H & E, x 500.

Fig. 2. Peripheral blood of neonatally thymectomized hamster 15 days after i.p. implantation of H-HM-1 tumor cells at 9 days of age. Total white blood cell count of this animal was 257,000/cu mm. Wright and Giemsa, x 800.
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