Antilymphocyte Serum and Allogeneic Inhibition

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SUMMARY

Antimouse lymphocyte serum completely abrogated the phenomenon of allogeneic inhibition. Tumors grew as well in F₁ hybrid mice receiving antimouse lymphocyte serum as in those from a similarly treated parental strain. In both tumor incidence and growth were markedly enhanced. The mechanism(s) whereby antimouse lymphocyte serum produced these results is considered.

INTRODUCTION

Since 1958, when Snell (12) first reported that a transplanted C57BL lymphoma grew better in syngeneic C57BL mice than in F₁ hybrids (C57BL X allogeneic strain), this phenomenon has been demonstrated with a variety of tumor lines. Such deficient tumor growth in hybrids was subsequently designated "allogenic inhibition" by the Hellströms (5—7). As a result of their extensive in vivo and in vitro investigations and those of others (9, 10), it has been postulated that the findings are due to a nonimmunological interaction between tumor cells and cells of the F₁ hybrid which contain H-2 isoantigens (or cellular material closely associated with these antigens) foreign to the tumor cells. Such a hypothesis has been given preference to other explanations. It is considered unlikely that the results are related to general host factors which differentiate homozygous from F₁ hybrid recipients or to the possibility that the tumor contains antigens present in the homozygous, but absent from the F₁ hybrid host, against which the F₁ hybrid can react immunologically. It has been proposed that this phenomenon may serve as a cell-to-cell surveillance mechanism against neoplasia.

Recently, we have observed (2) that administration of antilymphocyte serum enhanced the incidence, growth, and metastases of transplanted mouse mammary tumors and of 3-methylcholanthrene-induced tumors in their syngeneic hosts (1). While the precise mechanism responsible for such augmentation of tumor growth and metastases, as well as enhanced survival of allogeneic grafts and transplants after ALS² treatment remains to be elucidated, it is generally considered to be the result of the immunosuppressant action of this agent.

Consequently, it seemed pertinent to evaluate its effect on allogeneic inhibition with the hope that the findings might provide information relative to ALS action and/or the mechanism of this tumor-inhibitory phenomenon.

MATERIALS AND METHODS

A spontaneous C3H mammary carcinoma in the ninth transplant generation was used in these studies. Cell suspensions prepared from tumors cytosieved and diluted with 0.9% NaCl solution to contain either 5 × 10⁵ or 5 × 10⁶ cells/0.1 ml were inoculated s.c. into the left hind legs of C3HeB/FeJ or C3D2F₁/J(C3H/HeJ × DBA/2J) females. Isogenicity was confirmed by the acceptance of skin grafts between members of the same line.

ALS was prepared in New Zealand female rabbits by the method of Gray et al. (3) with lymph node and thymus from C3HeB mice. The serum was heated to inactivate complement, sterilized by filtration, and stored at —20° until used. Sera obtained from 6 rabbits were combined to provide a sufficient amount for the experiment.

The leukoagglutination titer of the pooled serum was 1:256. Normal rabbit serum similarly prepared had a titer of zero. The potency of all batches of ALS was determined by their ability to prolong skin allograft survival and to reduce the number of circulating lymphocytes.

Serum (ALS or NRS) was administered i.p. 0.25 ml daily (Monday through Friday) beginning the day of tumor cell injection, and was continued until termination of experiments.

All animals, C3HeB and C3D2F₁, were inoculated with the same tumor cell suspension. Each strain was arbitrarily divided into 2 groups. One was treated with ALS; the other was treated with NRS. All were examined daily for appearance and growth rate of tumors.

In Experiment I animals were inoculated with 5 × 10⁵ tumor cells and were sacrificed either when tumors measured 2 cm or 28 days after injection if the tumors failed to attain that size. In Experiment II animals treated with 5 × 10⁶ cells were sacrificed when tumors reached 2 cm or 42 days from the time of tumor cell inoculation if tumors did not attain such a size.

RESULTS

In Experiment I groups of C3HeB and C3D2F₁ mice were inoculated with 5 × 10⁵ tumor cells. One-half of each group...
was the recipient of ALS, and the other one-half was given NRS. No significant difference was observed (Table 1) between the C3HeB and the F₁ hybrid animals insofar as numbers of animals growing tumor, time of tumor appearance, and subsequent growth of tumors were concerned. Evidence of “allogeneic inhibition” in the F₁ hybrid was nonexistent. The effect of ALS in enhancement of tumor growth was equal in the 2 animal strains. Tumors appeared and grew with greater rapidity in both.

Because of failure to demonstrate a difference in tumor growth between normal C3HeB mice and their F₁ hybrids, a second experiment was carried out similar to the first, except that all the animals were inoculated with $5 \times 10^5$ cells. A striking difference in the number of control animals (NRS-treated) growing tumors in the 2 strains was evident (Chart 1). Whereas 90% of the C3HeB mice demonstrated tumors by 42 days, only 50% of the F₁ hybrids had tumors within that time. Such tumors appeared on an average of 22.0 days after cell inoculation in the former and 31.0 days in the latter. Subsequent tumor growth was more rapid in C3HeB animals (Table 1). Such a difference in tumor appearance and subsequent growth between the 2 strains was no longer evident when animals received ALS (Chart 1, Table 1). The appearance and growth of tumor in both strains were markedly accelerated. All of the C3HeB mice receiving ALS demonstrated tumor with an average appearance time of 9.1 days, and 100% of the F₁ hybrids similarly treated had tumors by 10.2 days.

**DISCUSSION**

Findings indicate that the ALS treatment of C3D2F₁ mice abrogates the phenomenon of allogeneic inhibition. Tumors grew as well in such F₁ hybrids as in syngeneic hosts. If the effect of ALS is due to its immunosuppressive activity, as is generally considered to be its mode of action in prolonging allogeneic transplants, then it is likely that allogeneic inhibition is the result of immunological mechanisms rather than of the nonimmunological phenomena postulated by the Hellströms (5–7) and others (9, 10). However, the possibility that ALS may exert its effect by interfering with the

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**Table 1**

Antilymphocyte serum and allogeneic inhibition

<table>
<thead>
<tr>
<th>Mice</th>
<th>Tumor cells</th>
<th>Type of serum</th>
<th>No. mice</th>
<th>No. with tumor*</th>
<th>Tumor appearance (av. day)</th>
<th>No. tumors 1 cm*</th>
<th>Av. day became 1 cm</th>
<th>No. tumors 2 cm*</th>
<th>Av. day became 2 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td>NRS</td>
<td>$5 \times 10^5$</td>
<td>10</td>
<td>9</td>
<td>16.0 (8–25)</td>
<td>5</td>
<td>14.0 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3HeB</td>
<td>ALS</td>
<td>$5 \times 10^5$</td>
<td>9</td>
<td>9</td>
<td>7.5 (7–9)</td>
<td>9</td>
<td>10.0 (9–14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NRS</td>
<td>$5 \times 10^5$</td>
<td>10</td>
<td>10</td>
<td>13.2 (8–18)</td>
<td>10</td>
<td>17.3 (14–21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3D2F₁</td>
<td>ALS</td>
<td>$5 \times 10^5$</td>
<td>9</td>
<td>9</td>
<td>7.3 (7–10)</td>
<td>9</td>
<td>11.2 (9–15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment II</td>
<td>NRS</td>
<td>$5 \times 10^5$</td>
<td>10</td>
<td>9</td>
<td>22.0 (18–30)</td>
<td>9</td>
<td>25.9 (21–32)</td>
<td>9</td>
<td>34.1 (29–42)</td>
</tr>
<tr>
<td>C3HeB</td>
<td>ALS</td>
<td>$5 \times 10^5$</td>
<td>10</td>
<td>10</td>
<td>9.1 (8–11)</td>
<td>10</td>
<td>12.6 (11–14)</td>
<td>10</td>
<td>19.9 (17–23)</td>
</tr>
<tr>
<td></td>
<td>NRS</td>
<td>$5 \times 10^5$</td>
<td>5</td>
<td>4</td>
<td>31.0 (28–37)</td>
<td>4</td>
<td>33.5 (32–35)</td>
<td>1</td>
<td>42.0 (32–35)</td>
</tr>
<tr>
<td>C3D2F₁</td>
<td>ALS</td>
<td>$5 \times 10^5$</td>
<td>12</td>
<td>12</td>
<td>10.2 (9–12)</td>
<td>12</td>
<td>15.2 (11–21)</td>
<td>11</td>
<td>22.4 (20–26)</td>
</tr>
</tbody>
</table>

*aAt termination, Experiment I, 28 days, Experiment II, 42 days.
interaction of tumor cells and host cells containing foreign H-2 antigen (allogeneic inhibition) cannot be dismissed. Cortisone, but not X-irradiation, has similarly been shown (7) to abrogate inhibition in vivo. Its ability however to interfere with this phenomenon in vitro as well has suggested (5) that the results are not caused by the depressive effect of cortisone on immunological reactivity, but are more likely due to either interference by cortisone with the interaction of allogeneic antigens and target cells or to the making of target cells more resistant to the cytotoxic effects of such an interaction. In this regard, Rosenau and Moon (11) have observed that the addition of cortisone to a culture of sensitized lymphocytes and target cells failed to interfere with aggregation of the former around the latter, but the cytotoxic effect of the lymphocytes was eliminated. If ALS should be found, as was cortisone, to abrogate allogeneic inhibition in vitro, not only would the existence of this phenomenon be further substantiated, but such a finding would suggest that this mechanism of action of ALS is not the result of immunity depression in the usual sense, but is more like that postulated for cortisone. Possibly, ALS prevents the interaction of target cells and incompatible cells containing foreign H-2 antigens (lymphocytes) from interacting by a process similar to the “blindfolding” described by Levey and Medawar (8), or perhaps the ALS coats the target cells, as suggested by Guttman et al. (4), making them resistant to the action of allogeneic cells. Just as allogeneic inhibition has not been observed when the number of target cells has been large, so may the phenomenon be absent when the number of incompatible host cells (e.g., lymphocytes) is too small to effect destruction. Consequently, the lymphocytopenia resulting from ALS administration might be a significant factor responsible for the ALS effect.

Should it be demonstrated that ALS abrogates allogeneic inhibition by interference with a nonimmune mechanism, then it may be equally possible that its action in enhancing allogeneic transplant survival may, at least in part, be similarly mediated.

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REFERENCES

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