Development of Neoplasia and Karyotype Analysis in Mice with Graft-versus-Host Reaction

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SUMMARY

When F1 hybrid mice from a cross between strains CBA/H-T6T6 and SJL/J were given parental (SJL/J) spleen cells or cell-free preparations of parental spleen cells, reticulum cell sarcoma developed with increased incidence in both groups, compared with noninjected controls. The presence of graft-versus-host reactions in the cell-inoculated animals did not influence tumor development. When CBA/H-T6T6 X C3H F1 hybrids were given C3H spleen cells, visible GVH reactions did not develop. The incidence of mammary tumors in the cell-inoculated females of this hybrid cross was significantly greater than that in controls not receiving cell inoculations.

Nonthymic lymphomas occurred in CBA/H-T6T6 X SJL/J hybrids and hepatomas developed in male CBA/H-T6T6 X C3H mice with equal incidence in all experimental groups.

Karyotype analysis of the reticulum cell neoplasms showed small numbers of donor cells and aneuploid (malignant) cells of host origin.

No evidence was found in the mouse strains tested in this study to support the hypothesis that the immunological disorder associated with a graft-versus-host reaction could eventuate in lymphoma.

INTRODUCTION

In the preceding paper (5), studies evaluating the role of the immunological stress of a GVH2 reaction in lymphoma development showed that, when F1 hybrid mice were inoculated with viral-infected parental spleen cells, lymphoma occurrence was related to the presence of virus in them. The GVH reactions that developed in this situation did not influence the incidence of lymphoma. In their studies reporting lymphoma development associated with GVH reactions in F1 hybrid mice, Armstrong et al. (1) describe RCS's occurring in animals during the 2nd year of life, morphologically similar in their development and final expression to the type of lymphoma found in SJL mice. Since SJL mice have a high natural incidence of RCS, the use of this strain in a hybrid combination could test the effect of the production of GVH reactions on this type of lymphoma development in a situation in which genetic factors favor its occurrence. In our previous studies (5), a SJL X AKR hybrid was used. However, thymic lymphomas developed in high incidence during the 1st year of life in animals given viral-infected parental spleen cells. Therefore, the observation of animals into the age range at which RCS might be expected to develop could not be accomplished.

Studies of cellular origin of lymphomas developing after the occurrence of GVH reactions have yielded conflicting results, when analyzed by cell transfer to parental and hybrid animals. Schwartz and Beldotti (8) found the developing neoplasms to be of host origin. Lymphomas occurring after GVH reactions in a different hybrid cross were found by Cole and Nowell (2) to be derived from donor cells.

This study was designed to look at the effect of GVH reactions on lymphoma development in a different SJL/J hybrid (CBA/H-T6T6 X SJL/J). SJL cells injected into these hybrids would be expected to produce marked GVH reactivity, because the 2 strains differ at H-2. The CBA/H-T6T6 parent would provide a marker chromosome so that the cellular origin of lymphomas could be evaluated. A hybrid cross without a genetic predisposition to lymphoma and which would not be expected to exhibit fatal GVH reactions after receiving parental spleen cells was also studied.

MATERIALS AND METHODS

Mice. The mouse strains used were SJL/J, high-incidence RCS; CBA/H-T6T6, a strain with a double marker chromosome; and C3H/HeJ, high incidence of mammary carcinoma in females and hepatomas prevalent in males. All neoplasms of these strains characteristically occur in the 2nd year of life.

Cell Suspensions and Cell-free Centrifugates. These preparations were made as described in the preceding paper (5).

Observations. Those animals that either died from acute GVH reactions within 3 weeks of the last cell inoculation, developed tumors, or were sacrificed at 19 to 20 months of age were given complete autopsies. Microscopic sections were prepared of the liver, kidney, and lymphoid tissues.

Chromosome preparations and tumor transfers were done as described in the preceding paper (5). The F1 hybrids of CBA/H-T6T6 mice showed a single marker chromosome in all of their metaphases.

1 This investigation was supported by the University of California and by Contract AT(04-1) GEN-12 between the Atomic Energy Commission and the University of California.

2 The abbreviations used are: GVH, graft-versus-host; RCS, reticulum cell sarcoma.

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RESULTS

Inoculation of CBA/H-T6T6 X SJL/J F, (TSF,) Hybrids with SJL/J Spleen Cells. Littermates were inoculated with 8 to 30 X 10⁶ adult spleen cells or with cell-free extract made from an equivalent amount of spleen at 8 to 59 days of age. The age at inoculation was increased and the size of the inoculum was decreased to reduce the mortality from acute GVH reactions, which was 55% in the cell-inoculated mice. Animals given 3 inoculations of 10, 5, and 3 million SJL spleen cells at 3-week intervals starting at 44 days of age had a 57% mortality from acute GVH reactions. Table 1 records tumors found in the cell-inoculated animals surviving GVH reactions and their controls. RCS was found in all groups of animals, all but 1 animal developing it during the 2nd year of life. The 3 nonthymic lymphomas occurring in animals receiving multiple cell inoculations developed at 8, 9, and 11 months of age; the other nonthymic lymphomas and tumors (mammary, lung, and adrenal) developed between 12 and 19 months of age.

The incidence of RCS in the group given a single inoculation of cells and the cell-free-preparation-inoculated group was compared with that of the noninjected animals, with the use of the χ² test. The p value was 0.05. However, there was no significant difference in RCS incidence between the cell-inoculated and cell-free-preparation-inoculated groups. When the RCS incidence in the multiple injection group was compared with that in the animals not given injections, it was significantly increased (p < 0.01). Nonthymic lymphoma occurred with similar incidence in all the groups of mice in the study.

The RCS's were morphologically similar in all groups. They were morphologically identical to the spontaneously occurring RCS of the SJL/J strain (11). The nonthymic lymphoma showed replacement of spleen and lymph nodes with lymphoblasts without primary involvement of the thymus.

Inoculation of CBA/H-T6T6 X C3H/HeJ Hybrids with C3H Spleen Cells. In this experiment, the F₁ hybrids were inoculated at 3 to 7 days of age with 10 to 20 X 10⁶ C3H spleen cells. Noninoculated control animals were littermates or were from alternate litters of the inoculated mice. These parent strains do not differ at H-2, and no GVH reactions were seen. No lymphomas developed but, during the 2nd year of life, hepatomas and mammary adenocarcinomas appeared in both groups of animals. When these tumors were analyzed by sex, there was a significantly increased incidence of mammary tumors (p < 0.01) in the females receiving the cell inoculum, while hepatomas, which were found predominantly in males, were of equal distribution in the cell-inoculated and noninoculated groups (Table 2).

Chromosome Studies. These studies were performed on the TSF₁ mice. In such animals, the spleen and mesenteric lymph node were subjected to karyotype analysis and, whenever possible, 50 metaphases from each organ were evaluated. The results of these studies in individual mice are presented in Table 3.

Four mice with acute GVH reactions were studied. The

<table>
<thead>
<tr>
<th>Material injected</th>
<th>No. of mice</th>
<th>No. of inoculations</th>
<th>RCS</th>
<th>Nonthymic lymphoma</th>
<th>Other tumors</th>
<th>No. of mice</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen cells</td>
<td>36</td>
<td>1ᵃ</td>
<td>10</td>
<td>29</td>
<td>2</td>
<td>5.5</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>3</td>
<td>9</td>
<td>47</td>
<td>3</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Cell-free preparations of spleen cells</td>
<td>27</td>
<td>1ᵃ</td>
<td>8</td>
<td>30</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>None</td>
<td>45</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>7</td>
<td>6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>13</td>
<td>33</td>
</tr>
</tbody>
</table>

ᵃ Littermates.

<table>
<thead>
<tr>
<th>Cell inoculation</th>
<th>Sex</th>
<th>No. of mice</th>
<th>No. with hepatoma</th>
<th>%</th>
<th>No. with mammary tumor</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Male</td>
<td>21</td>
<td>6</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>Male</td>
<td>26</td>
<td>7</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Yes</td>
<td>Female</td>
<td>21</td>
<td>0</td>
<td>14ᵃ</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Female</td>
<td>17</td>
<td>0</td>
<td>4</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ Two mice had mammary tumor and hepatoma.
Table 3
Chromosome studies in TSF₁ mice
Metaphases: host/donor

<table>
<thead>
<tr>
<th>Acute GVH reaction</th>
<th>RCS</th>
<th>No inoculation</th>
<th>19-month-old normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cells&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CFC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No inoculation</td>
</tr>
<tr>
<td>Spleen Node</td>
<td>Spleen Node</td>
<td>Spleen Node</td>
<td>Spleen Node</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1/1</td>
<td>0/5</td>
<td>49/1</td>
<td>50/1</td>
</tr>
<tr>
<td>46/1</td>
<td>0/31</td>
<td>45/0</td>
<td>49/1</td>
</tr>
<tr>
<td>0/50</td>
<td>0/7</td>
<td>50/0</td>
<td>50/0</td>
</tr>
<tr>
<td>47/62</td>
<td>0/93</td>
<td>446/49</td>
<td>441/25</td>
</tr>
</tbody>
</table>

<sup>a</sup> SJL/J spleen cells.
<sup>b</sup> Cell-free centrifugate of SJL/J spleen cells.
<sup>c</sup> Host animal given 3 inoculations of SJL/J spleen cells.
<sup>d</sup> Animal with lymphoma in pancreatic node only; normal spleen and mesenteric node.

Table 4
Representative karyotypes from 5 TSF₁ mice with RCS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age (mo.)</th>
<th>&lt;39 M&lt;sup&gt;a&lt;/sup&gt;</th>
<th>39 U</th>
<th>40 M</th>
<th>41 U</th>
<th>42 M</th>
<th>43 U</th>
<th>44 M</th>
<th>&gt;44 U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>19</td>
<td>3</td>
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<td>8</td>
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<tr>
<td>15</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>0</td>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>No injection</td>
<td>15</td>
<td>7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>28&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> M, presence of a single T6 marker (host cells); U, unmarked or donor cells.
<sup>b</sup> Host animals given 3 inoculations of SJL/J spleen cells.
<sup>c</sup> Host animal given 1 inoculation of SJL/J spleen cells.
<sup>d</sup> Some metaphases with 2 or 3 T6 markers.

Spleens showed 47 host (marked) and 62 donor (unmarked) metaphases. No host metaphases and 93 donor metaphases were found in the mesenteric lymph nodes of these animals. The results in the tumors were somewhat different. Donor cells were found in low frequency, and host cells predominated in RCS's occurring in mice given both 1 and 3 cell inoculations. A somewhat greater number of donor cells were found in the nonthymic lymphomas. Aneuploidy involving at least 1 or 2 metaphases, and frequently more, was found in all of the tumor-bearing animals, regardless of experimental group. It was not present in any of the animals with acute GVH reactions or in the 19-month-old animals sacrificed without tumor. Aneuploidy of 10 or more metaphases was found in 8 of 15 RCS's and in 2 of 7 nonthymic lymphomas and was not characteristic of any particular experimental group. The aneuploid cells were nearly all of host origin and were frequently associated with duplication or triplication of the T6 marker. The donor cells were for the most part diploid; a few showed either 39 or 41 chromosomes. Representative karyotypes from 5 mice with RCS are presented in Table 4.

In 1 mouse given a single inoculation of SJL spleen cells, there was a large metacentric chromosome in nearly all of the cells of both the spleen and lymph node (mouse chromosomes are all telocentric normally). Several cells in this animal had 2 of these metacentric chromosomes, and aneuploidy was present. The single T6 marker of the host was also present, and 2 unmarked cells were found which also had the metacentric chromosome.

**DISCUSSION**

This study shows that, in the TSF₁ mice, conditions which produced high mortality from GVH reactions did result in...
an increased incidence of lymphoma. The fact that RCS incidence was significantly increased in animals receiving cell-free extracts of SJL spleen cells, as well as in those given intact cells, suggests the presence of a lymphomagenic virus in the inoculated material. Although oncogenic properties of cell-free filtrates of SJL lymphomas were not tested in this study, there is evidence in the literature for a viral etiology of SJL lymphoma, in that cell-free filtrates of neoplasm were found to transmit the disease to syngeneic hosts (3). Other investigators have demonstrated the group-specific (gs) antigen of murine leukemia virus in SJL/J mice (4). The results of the studies with C3H and CBA-T6 hybrids also support the concept of virus transmission by the parental cells, in that the incidence of mammary tumors, which are of known viral etiology, was increased in the animals receiving the C3H cells, while that of hepatomas, a tumor known to be common in C3H mice (6) but not of proven viral etiology, was not augmented by the inoculation of parental spleen cells.

The F1 hybrid-parental spleen cell system provides a model in which histoincompatible lymphocytes can exist within a host. It has been proposed by several authors (7, 9, 10) that such a situation could result in lymphoma because of continued proliferation of the donor immunocompetent cells. The findings in the present study did not, however, confirm this attractive hypothesis. The chromosome studies showed that the tumors had a small but definite donor cell population but that the aneuploid cells were always of host origin. The presence of aneuploidy in all animals with lymphomas, regardless of whether or not they were given cells, suggests that the aneuploid is the malignant cell. Further evidence to correlate aneuploidy with the tumor cells is that it was not found in the metaphases of normal lymphoid tissues. The donor metaphases were found to be most numerous in the animals with acute GVH reactions occurring several weeks after the inoculation of cells, which is what might be expected if immunocompetent donor cells were reacting against a foreign host. The small number of donor cells in animals surviving the acute GVH reaction suggests that they are not an expanding cell population and that their proliferation during the early weeks may be etiologically related to the GVH reaction.

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REFERENCES

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