Marrow-Thymus Interactions during Radiation Leukemogenesis in C57BL/Ka Mice

Jacques Boniver, Alain Declève, Miriam Lieberman, Cyril Honsik, Marilyn Travis, and Henry S. Kaplan

ABSTRACT

Transplantation of thymus and bone marrow cells from irradiated C57BL/Ka mice demonstrated the presence of potentially neoplastic cells in the thymus at 30 to 60 days postirradiation. During the same interval, no such cells could be detected in the bone marrow; moreover, the capacity of bone marrow cells to repopulate the thymus was impaired severely. These observations suggest that the primary site of neoplastic transformation in irradiated C57BL/Ka mice is the thymus rather than the bone marrow and that impaired thymic regeneration is a critical step in radiation leukemogenesis in mice.

INTRODUCTION

Fractionated whole-body X-irradiation (4 × 175 R) induces a high percentage of thymic lymphomas in susceptible strains of mice (reviewed in Refs. 4 and 9). These tumors are derived from T-lymphocytes and bear murine T-lymphocyte surface antigens such as Thy 1 and TL (2, 14). In C57BL/Ka mice, radiogenic lymphomas appear to develop by a complex process in which, among other factors, the thymic microenvironment and the bone marrow have a crucial role (9). It has been shown that, under the influence of an irradiated host environment, radiogenic lymphomas can originate from cells present in nonirradiated thymus grafts (11, 13). It has been proposed that radiation injury to certain bone marrow cell populations alters the pattern of thymus repopulation and thus contributes to the initiation of the oncogenic process (9). The concept that radiation injury to the marrow can impair differentiation of thymocyte precursors and regeneration of the radiation-depleted thymus has been recently substantiated by the demonstration that the level of terminal deoxynucleotidyl transferase activity in murine bone marrow prothymocytes (12, 20) and thymocytes (12, 17) decreased dramatically during the preleukemic period in irradiated C57BL/6 mice (18). The interaction between marrow and thymus in C57BL/Ka mice exposed to leukemogenic doses of irradiation has been investigated further in experiments designed to answer the following questions. (a) Do the earliest preleukemic or leukemic cells appear in the thymus, the bone marrow, or in both sites in irradiated C57BL/Ka mice? (b) Is the bone marrow of irradiated animals capable of repopulating the thymus efficiently?

MATERIALS AND METHODS

Mice. The mice used in these experiments were of strain C57BL/Ka and (C57BL/Ka × BALB/c) F1 (hereafter called BCF1). In addition, mice of strain C57BL/Ka/Thy 1.1/Lb (known as BL/1.1 for convenience) were used also. This strain, recently established in this laboratory by M. Lieberman, is congenic with C57BL/Ka and bears the cell surface antigen Thy 1.1.

Sera. Anti-Thy 1.1 (C3H anti-AKR) serum was prepared according to the method of Reif and Allen (19) and used at a dilution of 1:20. Anti-Thy 1.2 (AKR anti-C3H) serum was used at a dilution of 1:10. Rat anti-mouse IgG: fluorescein isothiocyanate serum (Cappel Laboratories, Cochranville, Md.) was used at a dilution of 1:40.

Experiment 1. Female C57BL/Ka mice were irradiated with 4 weekly whole-body doses of 175 R starting at 33 ± 5 days of age and were sacrificed at intervals after the last exposure. Five × 106 thymus or bone marrow cells were inoculated i.t.4 into irradiated (400 R x 1), intact, 1-month-old BCF1 mice or i.v. into thymectomized hosts (thymectomy was performed 2 weeks before inoculation). Cells from each individual donor mouse were injected into a single host. Recipient mice were sacrificed when moribund or showing clinical evidence of disseminated lymphoma. The genetic origin of the tumors was tested by inoculating 2 × 106 lymphoma cells i.p. into both C57BL/Ka and BCF1 mice. Takes in both kinds of mice indicate tumors of the donor phenotype (C57BL/Ka), whereas takes in BCF1 mice only indicate that the tumor is of host origin.

Experiment 2. Female BL/1.1 mice were exposed to fractionated X-irradiation as in Experiment 1. At intervals after the last exposure, 3 mice were sacrificed, and 107 bone marrow cells from each donor were injected i.v. into one or 2 irradiated (400 R x 1) 2-month-old female C57BL/Ka mice. Three weeks later, thymus cell suspensions were prepared from each recipient and incubated with anti-Thy 1.1 serum and then with antimouse IgG: fluorescein isothiocyanate serum according to a published procedure (16). The percentage of Thy 1.1-positive donor cells was determined with a fluorescence-activated cell sorter (B) (FACS III; Becton-Dickinson, Mountain View, Calif.) and a Zeiss UV microscope.

Experiment 3. C57BL/Ka mice were given a course of fractionated X-irradiation as in Experiment 1. At various intervals thereafter, they were given i.v. injections of 107 bone marrow cells from normal 2-month-old female BL/1.1 mice. Three weeks later, 3 mice in each group were sacrificed, and the percentage of donor cells in the thymus was determined as described for Experiment 2 except that anti-Thy 1.2 serum was used.

1 These studies were supported by Research Grants CA-03352 and CA-10372 and by Contract NO1 CP-71052 from the National Cancer Institute, NIH, Department of Health, Education and Welfare.

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Received July 7, 1980; accepted October 16, 1980.
RESULTS

Experiment 1. The transplantation assay for the genotype analysis of Kaplan et al. (1) was used to determine the site of origin of neoplastic cells in irradiated C57BL/Ka mice. The results, shown in Table 1, indicate clearly that "preleukemic" cells in irradiated C57BL/Ka mice are detected first in the thymus. Inoculation of BCF, mice with C57BL/Ka thymus or bone marrow cells collected 7 to 60 days after the last of 4 fractionated X-ray exposures gave rise to lymphomas of donor origin when thymocytes were used and when the recipients were not thymectomized. The data demonstrate that potentially neoplastic cells are present in the thymus of C57BL/Ka mice at 30 to 60 days post-irradiation but that such cells are not yet autonomous, since they still require a period of residence in the thymic environment to evolve into frank lymphomas. The only lymphoma observed in mice inoculated with bone marrow cells harvested at 7 to 60 days proved to be of host origin.

Thymus-independent (autonomous) transformed lymphoid cells were detected in both the thymus and the marrow of irradiated C57BL/Ka donor mice 3 months after irradiation. By that time, 12 of 24 mice had developed frank thymic lymphomas, one half of them still localized to the thymus and the other half already disseminated. The thymus was slightly enlarged in 5 of the remaining mice and clearly atrophic in the other 7. However, thymus and marrow cells from these mice, regardless of thymic size, gave rise to lymphomas of donor origin in thymectomized hosts, indicating that neoplastic cells are present in these tissues 3 months after irradiation even in the absence of overt thymic lymphomas.

Splenec tumors of host origin were observed in 2 mice that had received thymocytes or bone marrow cells derived from normal unirradiated donors. Inoculation of spleens into C57BL/Ka mice 14 days after X-ray treatment. The genotype of the tumors as well as their long latency period suggest that they may have developed either spontaneously, as commonly observed for splenic reticulum cell sarcomas, or as the result of the transmission of leukemogenic virus by the thymus or bone marrow cells of the irradiated donors (7, 15).

Experiments 2 and 3. The possibility was entertained that the presence of "preleukemic cells" in the thymus and their absence from the marrow of preleukemic C57BL/Ka mice might be related to impaired regeneration of the radiation-damaged thymus, stemming from radiation-induced depletion of prothymocytes in the marrow. The capacity of irradiated

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**Table 1**

Detection of "preleukemic" and leukemic cells in thymus and bone marrow after fractionated irradiation

<table>
<thead>
<tr>
<th>C57BL/Ka donor mice</th>
<th>C57BL/Ka thymocytes</th>
<th>C57BL/Ka marrow cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacrifice (days post-irradiation)</td>
<td>Lymphomas</td>
<td>Origin</td>
</tr>
<tr>
<td>7</td>
<td>0/24</td>
<td>0/8</td>
</tr>
<tr>
<td>14</td>
<td>0/24</td>
<td>0/8</td>
</tr>
<tr>
<td>30</td>
<td>0/24</td>
<td>4/16 (128)</td>
</tr>
<tr>
<td>60</td>
<td>0/24</td>
<td>7/8 (94)</td>
</tr>
<tr>
<td>90</td>
<td>12/24</td>
<td>18/24 (50)</td>
</tr>
</tbody>
</table>

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**Table 2**

Repopulation capacity of bone marrow cells after fractionated irradiation

<table>
<thead>
<tr>
<th>Donor mice</th>
<th>Recipient mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval from last X-ray exposure to sacrifice (days)</td>
<td>Thymus wt (mg)</td>
</tr>
<tr>
<td>0</td>
<td>62.5 ± 1.7</td>
</tr>
<tr>
<td>7</td>
<td>35.8 ± 8.0</td>
</tr>
<tr>
<td>14</td>
<td>33.9 ± 3.6</td>
</tr>
<tr>
<td>28</td>
<td>48.7 ± 2.5</td>
</tr>
<tr>
<td>60</td>
<td>43.6 ± 6.6</td>
</tr>
</tbody>
</table>

**Table 3**

Repopulation of the thymus of irradiated mice grafted with normal bone marrow cells at various time intervals after the last X-ray exposure

<table>
<thead>
<tr>
<th>Time interval between last X-ray exposure and bone marrow graft (days)</th>
<th>% of donor cells in the thymus 3 wk after bone marrow graft</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>94.7 ± 4</td>
</tr>
<tr>
<td>7</td>
<td>91.0 ± 11</td>
</tr>
<tr>
<td>14</td>
<td>5.5 ± 4</td>
</tr>
</tbody>
</table>

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DISCUSSION

Our results are at variance with the findings of Haran-Ghera...
(5), who observed the presence of "preleukemic" cells in the bone marrow of C57BL/6 mice as early as 1 month after irradiation. Genetic differences between the C57BL/6 and C57BL/Ka substrains and their endogenous retroviruses (3, 5–7, 15) may account for this discrepancy. The present data confirm and extend previous studies on the pathogenesis of thymic lymphomas in irradiated C57BL/Ka mice. They suggest that one of the first effects of a leukemogenic course of irradiation is to deplete the marrow of prothymocytes capable of repopulating the thymus. The ensuing phase of impaired thymic regeneration is associated with a maturation arrest characterized by a sustained overabundance of large, immature lymphoid cells in the thymic cortex (1, 9). A subsequent event (virus infection, genetic mutation, or continuous immunogenic stimulation) is then apparently required to convert one or more cells of these preneoplastic thymocyte populations into fully transformed, autonomous clones. The production of specific growth or differentiation factors by the thymus and the role of this organ as a sanctuary, wherein the preleukemic cells can escape the immunological surveillance of peripheral T-cells, are additional mechanisms which, as previously suggested (9), may explain the requirement for an intact thymus for T-lymphomagenesis.

REFERENCES

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