Influence of Thymectomy and Subsequent Thymus Implantation on Leukemia Induction in Adult C57BL Mice by Radiation and Urethan

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Summary

A single exposure of adult C57BL/6 mice to 400 r total-body X-irradiation yielded 5% leukemia; thymectomy reduced the incidence to zero, while subsequent implantation of normal thymus reversed the process. Subsequent urethan treatment raised the incidence in nonthymectomized, irradiated mice to 34% and in thymectomized, irradiated, and thymus-implanted mice to 17%. When the thymus implant was given after instead of before the urethan treatment, the incidence remained low (4%). These results confirm in adults, what had previously been found in newborn mice, that thymus implantation cannot restore urethan leukemogenesis in thymectomized mice, as it does in the case of radiation leukemogenesis.

Introduction

The fact that leukemogenesis (thymic lymphosarcoma induction) in C57BL mice by fractional total-body X-irradiation is prevented by prior thymectomy (8), and rendered effective again by subsequent implantation of nonirradiated syngeneic newborn thymus (10), is generally accepted as evidence that the leukemogenic action of X-irradiation on the thymus is able to operate in an indirect way (5).

Urethan leukemogenesis differs from radiation leukemogenesis in many respects. When, for instance, the technic of thymectomy followed by reimplantation of normal thymus was applied to urethan leukemogenesis in newborn C57BL mice (1), the reversal effect by the reimplantation was not observed. [The use of newborn mice was necessitated by the fact that urethan, acting alone, is very weakly leukemogenic to adult C57BL mice (1) but fairly effective when the treatment is begun soon after birth (1, 6, 7, 12).] A plausible explanation of the failure of thymus reimplantation to reverse the inhibition of thymectomy in the case of urethan was that, unlike radiation leukemogenesis, urethan leukemogenesis operates directly on the thymus (1).

In the present investigation, advantage was taken of the fact that urethan can potentiate radiation leukemogenesis in adult C57BL mice—an effect observed not only when the 2 treatments are given concurrently (11) but also when the urethan treatment is begun 2 weeks after completion of the radiation treatment, though not when the sequence is reversed (2). Using this system for testing the influence of thymectomy and reimplantation of normal thymus, it was possible not only to test in adults the validity of the observation made in newborn mice, but also to check, in the same experiment, the effect of thymus reimplantation separately on the radiation component and on the urethan component of the 2-stage leukemogenic action (i.e., on the assumption that, under such conditions, a single dose of radiation acts mainly as initiator and the subsequent urethan treatment as promoter).

Materials and Methods

The animals used were C57BL/6 mice, originally derived from a pair obtained from the Jackson Laboratories, Bar Harbor, Maine, and since bred here by brother-sister mating. They were kept in stainless steel cages, bedded with sawdust, housed in an air-conditioned room at 21-25°C, fed Purina Laboratory Chow, occasionally supplemented with barley and sunflower seeds, and provided with water ad libitum.

Mice of both sexes were thymectomized when 5-6 weeks old, and 2 weeks later, submitted to a single exposure of 400 r total-body X-irradiation. (Physical conditions for radiation: 250 kv, 15 ma, with 1 mm Al and 0.5 mm Cu filters; at a dose rate of 67 r/min). After an interval of 1-2 hr, each animal was grafted with a thymus from a newborn mouse of the same strain, either s.c. (Group V) or under the kidney capsule (Group VI). Two weeks later, urethan treatment was begun, consisting of 10 weekly, i.p. injections of a 10% aqueous solution, the dose per injection being 1 mg/gm body weight. In Group IX, the order was changed: the thymectomized and irradiated mice were 1st given the course of s.c. urethan injections and then grafted with a newborn thymus.

The controls included: (a) thymectomized, irradiated mice receiving urethan injections (Group I); (b) nonthymectomized mice receiving irradiation only (Group II); (c) thymectomized and irradiated mice without urethan treatment or thymus reimplantation (Group III); (d) thymectomized and irradiated mice receiving s.c. thymus graft, without urethan treatment (Group IV); (e) similar to Group IV, but with the grafting performed 2 months later (Group VIII), to serve as control for experimental Group IX; and (f) thymectomized and irradiated mice receiving urethan treatment, but without reimplantation of thymus (Group VII).

The mice were examined every 2 weeks for signs of leukemia or, in the case of the grafted animals, for enlargement of the thymic implants. Those showing changes suggestive of leukemia were killed and autopsied and the affected tissue kept for histologic examination. Mice that died before the 21st week (the time of the 1st appearance of leukemia) or those with thymic remnants at

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Table 1

| GROUP | 1st | 2nd | 3rd | 4th | NO. OF MICE USED | EFFECTIVE TOTAL (SURVIVORS AT 21ST WK.) | THYMIC LYMPHOSARCOMAS | OTHER LEUKEMIAS
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85</td>
<td>76</td>
<td>6/20 26/76 = 34%</td>
<td>27 R.C.S.</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>90</td>
<td>0/5 5/90 = 5%</td>
<td>37 R.C.S.</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td>400 r</td>
<td></td>
<td>65</td>
<td>60</td>
<td>0/0 0/60 = 0%</td>
<td>100 R.C.S.</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>58</td>
<td>57</td>
<td>0/2 2/57 = 3%</td>
<td>100 R.C.S.</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>52</td>
<td>48</td>
<td>1/7 8/48 = 17%</td>
<td>34 Myel. L</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45</td>
<td>39</td>
<td>0/7 7/39 = 18%</td>
<td>36 R.C.S.</td>
</tr>
<tr>
<td>VII</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>57</td>
<td>0/0 0/57 = 0%</td>
<td>100 R.C.S.</td>
</tr>
<tr>
<td>VIII</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>59</td>
<td>53</td>
<td>0/0 0/53 = 0%</td>
<td>100 R.C.S.</td>
</tr>
<tr>
<td>IX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>55</td>
<td>0/2 2/55 = 4%</td>
<td>62 Myel. L</td>
</tr>
</tbody>
</table>

Other leukemias: R.C.S., reticulum cell sarcoma; Myel. L, myelogenous leukemia.

Two of the generalized leukemia, though lymphatic in type, were nonthymic.

Discussion

The low incidence of leukemia following a single irradiation of 400 r (5%) is in keeping with the earlier finding (4, 9) that radiation leukemogenesis in mice is far less effective when the irradiation is given as a single dose than in divided doses; while the augmentation by subsequent urethan treatment (to 34%) confirms our earlier results that the role of urethan is not merely additive—urethan alone eliciting 2-6% leukemia under similar conditions (1, 3)—but more in keeping with a 2-stage process of initiation and promotion (2).

The crucial results of this experiment are concerned with the groups of mice receiving all 4 treatments—thymectomy, X-irradiation, reimplantation of thymus, and urethan treatment—with the thymus reimplantation performed before or after the urethan treatment (i.e., Groups V and IX). The fact that the former yielded 17% leukemia while the latter only 4%, shows that the reimplantation was only effective on the radiation component of the radiation-urethan 2-stage process of leukemogenesis. The possible influence of reimplantation of thymus, in the case of Group IX, could only be considered in relation to the radiation component, since the long interval after the radiation rendered it ineffective in relation to the radiation component (cf. control Group VIII). The observed 4% leukemia incidence in Group IX would seem to be a simple urethan effect and not a reversal to the radiation-urethan synergism.

These results are in keeping with those reported in newborn mice treated with urethan alone (1), and provide more satisfactory evidence that while reimplantation of normal thymus reverses, to a large extent, the inhibiting effect of thymectomy in the case of radiation leukemogenesis, it does not do so in the case of urethan leukemogenesis. This supports the view that whereas radiation leukemogenesis can operate indirectly on the thymus, the urethan action is probably directly on that organ (1).
References


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