On the Mechanism of Urethan Leukemogenesis in Newborn C57BL Mice

II. Influence of Thymectomy and of Subsequent Thymus Reimplantation

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Summary

Urethan leukemogenesis was found to be fairly potent in C57BL mice when the treatment was begun soon after birth (35% incidence) but very weak when the treatment was begun at about 45 days of age (6% incidence). The inhibitory effect of thymectomy on urethan leukemogenesis was not reversed by subsequent implantation of syngeneic newborn thymus, in contrast to such a reversal in the case of radiation leukemogenesis. Possible reasons for the difference are discussed.

Introduction

In the foregoing communication (1), an attempt was made to elucidate the differences in leukemogenic action between whole-body X-irradiation and urethan, by testing whether the inhibitory effect of injections of syngeneic bone marrow cell suspension on radiation leukemogenesis in adult mice (12) also operated with respect to urethan leukemogenesis in newborn mice. [The comparison could not have been made in adult mice, since urethan is effectively leukemogenic only in newborn mice (4, 5, 19); see also urethan controls in the present series.]

In the present communication, another modifying factor is used as a criterion for comparison, namely, whether interference of urethan leukemogenesis by thymectomy is reversed by subsequent reimplantation of normal thymus, as is the case with radiation leukemogenesis (11, 13).

Materials and Methods

The source of the C57BL/6 mice and their diet and maintenance were the same as described in the foregoing communication (1).

In the experiment proper (Groups I and II), the urethan treatment was begun within 24 hr after birth and consisted of 10 i.p. injections of a solution of the compound (British Drug Houses Ltd.) in distilled water, at a dose level of 1 mg/gm body weight, with a 5% solution used for the 1st injection and a 10% solution used for the 9 subsequent injections. In the adult series (Groups III and IV), used for comparison, 10 weekly injections of 10% urethan were given, starting at 45 ± 3 days of age. The total dose ranged from 120 to 150 mg in the newborn and from 200 to 250 mg in the adult series. Thymectomy, performed 1 day after the last urethan injection, was carried out in about half the survivors (Groups I and III), with the technic described by Kaplan (9). About 5 hr later, after recovery from the operation, a thymus of a newborn animal of the same strain was implanted s.c., through an 18-gauge trocar, into the right axillary region of the thymectomized animals, care being taken that donor and recipient were of the same sex. The nonthymectomized urethan-treated animals (Groups II and IV) were left as controls.

The mice were examined periodically for signs of enlargement of the graft or for other signs of leukemia. These were killed and autopsied, and the implanted thymus and other tissues showing pathologic changes were kept for histologic diagnosis. All survivors at the end of 60 weeks were killed and similarly examined. Those with thymic remnants at the original site, indicating incomplete thymectomy, were not included in the evaluation of results.

Results

The results, summarized in Table 1, confirm that urethan alone is fairly potent leukemogenic when treatment is begun soon after birth (i.e., 35% incidence) but very weakly leukemogenic when the treatment is begun in young adult life (i.e., 6% incidence), and they show an inability of the reimplanted normal thymus to reverse the inhibitory effect of thymectomy on urethan leukemogenesis. (The incidence of spontaneous leukemia in our C57BL/6 strain is less than 1%.)

In the newborn control Group II—i.e., urethan treatment alone without thymectomy and reimplantation of normal thymus—15 of the 19 leukemias were of thymic origin, with dissemination to other organs, and 4 were confined to the thymus. The average latent period was 27.5 weeks. In the adult control Group IV, the 3 leukemias were all of the generalized type, involving the thymus, with an average latent period of 40 weeks. The 2 reticulum-cell sarcomas in this group were of Type B, observed at the 60th week.

In the newborn experimental Group I—i.e., urethan treatment followed by thymectomy and reimplantation of normal thymus—the single lymphatic leukemia was of the generalized kind.
without involvement of the thymus graft, and appeared at the 23rd week. The myelogenous sarcoma in this group also appeared at the 23rd week, and the reticulum-cell sarcoma appeared at the 33rd week.

In the adult experimental Group III—i.e., urethan treatment followed by thymectomy and reimplantation of normal thymus—the single lymphatic leukemia was also generalized, without involvement of the thymic graft, and appeared at the 60th week. The 2 reticulum-cell sarcomas, Type B, in this series also appeared at the 60th week.

Discussion

The object of this investigation, as of the preceding one (1), was to try to account for the differences between urethan and radiation leukemogenesis in mice, e.g. (a) that urethan, acting by itself, displays a pronounced leukemogenic action only in newborn mice (4, 5, 19), whereas whole-body X-irradiation is potently leukemogenic both in newborn (20) and in adult mice (10), and (b) that when radiation and urethan are administered together to adult C57BL mice, synergism is observed when the 2 are acting concurrently (14) or when the urethan treatment is given after the irradiation, but not when the sequence is reversed (2).

In the preceding communication (1), evidence is presented of the inability of syngeneic bone marrow to inhibit urethan leukemogenesis in newborn C57BL mice. In the present communication, experiments are described on the influence of thymectomy, and of subsequent reimplantation of normal thymus, on urethan leukemogenesis in newborn C57BL mice. It was found that the interference of urethan leukemogenesis in the newborn by thymectomy was not reversed by subsequent reimplantation of normal newborn thymus, contrary to the situation with respect to radiation leukemogenesis in adults (11, 13).

The methods employed in the 2 cases were, however, not identical. In the case of radiation (11, 13), the animals were 1st thymectomized, then given whole-body radiation in fractional doses at weekly intervals, and then provided with a new implant of thymus tissue from a normal (nonirradiated) newborn mouse of the same strain. This procedure could not be followed in the present experiment (a) because newborn mice would not have tolerated neonatal thymectomy followed by prolonged urethan treatment and (b) because after initial thymectomy and a short period of recovery, the urethan treatment would no longer have

TABLE 1

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>AGE AT START OF URETAN TREATMENT</th>
<th>NO. OF MICE USED</th>
<th>LYMPHATIC LEUKEMIA INCIDENCE (%)</th>
<th>OTHER TYPES OF LEUKEMIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Urethan × 10</td>
<td>Thymectomy and thymus grafting</td>
<td>10-24 hr</td>
<td>65</td>
<td>1/44* = 2</td>
</tr>
<tr>
<td>II</td>
<td>Urethan × 10</td>
<td>None</td>
<td>10-24 hr</td>
<td>70</td>
<td>19/54 = 35</td>
</tr>
<tr>
<td>III</td>
<td>Urethan × 10</td>
<td>Thymectomy and thymus grafting</td>
<td>45 ± 3 days</td>
<td>65</td>
<td>1/63* = 1</td>
</tr>
<tr>
<td>IV</td>
<td>Urethan × 10</td>
<td>None</td>
<td>45 ± 3 days</td>
<td>53</td>
<td>3/47 = 6</td>
</tr>
</tbody>
</table>

* Leukemia without thymic graft involvement.
* R.C.S., reticulum-cell sarcoma.
* M.L., myelogenous leukemia.
reversal effect of reimplemented thymus tissue, are the facts that (a) a causative virus is known to be involved in the spontaneous disease in AK mice (8) and (b) in low-leukemia strains, in which such a virus cannot normally be detected, it does become demonstrable in the leukemic tissue induced by radiation (7, 17). Although no such virus has as yet been demonstrated in urethan-induced leukemia (3, 6), it would be hazardous to speculate, without more convincing evidence, that a virus is involved only in 1 kind of leukemogenesis and not in another.

Assuming that the difference is one of demonstrability, rather than of the actual presence or absence of a causative virus, the possibility of a breakdown of an immune response to a preexisting virus, in the case of adult mice, and of a naturally defective immune response in the case of newborn mice (18) might come under consideration. But then the question would remain: why was urethan needed at all for leukemogenesis in the newborn C57BL mice.

As for the alternative idea—that the reversal effect of reimplantation of normal thymus, in the case of radiation leukemogenesis, is dependent on the “repopulation” of the implanted thymus by leukemic cells (15, 16)—here, too, the question arises: why should not the same also operate in relation to urethan leukemogenesis in newborn mice.

Aside from such fundamental questions as virus action versus leukemia cells invading the thymus, there is a relatively simple explanation to account for the fact that implantation of normal thymus is effective in reversing the inhibitory effect of thymectomy in the case of radiation leukemogenesis, but not in the case of urethan leukemogenesis, and that is on the assumption that urethan acts directly on the thymus, whereas radiation acts indirectly. Although further work would be needed to substantiate or disprove this postulated explanation, the present results do provide additional evidence of significant differences in the mode of action between whole-body X-irradiation and urethan leukemogenesis in mice.

References
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