On the Mechanism of Urethan Leukemogenesis in Newborn C57BL Mice

I. Influence of Injections of Syngeneic Bone Marrow Cell Suspensions

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Summary

Injections (i.v.) of syngeneic bone marrow cell suspension failed to inhibit urethan leukemogenesis in newborn C57BL/6 mice, in contrast to the known inhibition of radiation leukemogenesis by bone marrow in adult mice of the same strain. Other differences in the mode of action between whole-body X-irradiation and urethan are discussed in the light of the present results.

Introduction

The role of urethan in experimental leukemogenesis (thymic lymphosarcoma induction) differs from that of whole-body X-irradiation in at least 2 respects: (a) urethan alone displays a fairly pronounced leukemogenic action when treatment is started soon after birth (8, 10, 24, 29), with little or no effect in adults (4, 22, 31), whereas whole-body X-irradiation is potently leukemogenic in adult (12, 19, 23) as well as in newborn mice (30); and (b) if the 2 are administered together to adult C57BL mice, synergism is observed when acting concurrently (22) or when the urethan treatment is given after the irradiation (4), but not when the sequence is reversed (4).

To throw more light on these differences in action, experiments were undertaken to test whether certain factors, known to modify the leukemogenic effect of X-irradiation in adult mice, also operated in the case of urethan leukemogenesis in newborn mice. One such factor is the capacity of injected syngeneic bone marrow cells to inhibit the leukemogenic action of radiation (20); another factor is the interference of radiation leukemogenesis by thymectomy and the reversal of the effect by subsequent reimplantation of normal (nonirradiated) thymus (16, 21). The possible effect of bone marrow on urethan leukemogenesis in newborn mice is described in the present communication; that of thymectomy and reimplantation of normal thymus on urethan leukemogenesis in newborn mice is dealt with in the following communication (2).

Materials and Methods

Newborn C57BL/6 mice, of a line originally derived from the Jackson Laboratories, Bar Harbor, Maine, and subsequently bred in this laboratory by brother-sister mating, were used for this investigation. The newborn mice remained with their mothers, except for the brief periods of treatment, until they were 9 weeks old. This delayed weaning time was necessitated by the fact that the young were late in reaching maturity as a consequence of the urethan treatment. After weaning, they were divided according to sex and placed in separate cages, 8–10/cage. The mothers, and subsequently the young, were fed Purina laboratory chow, occasionally supplemented with barley and sunflower seeds, and provided with tap water ad libitum. The animals were kept in stainless steel cages, bedded with sawdust, and housed in an air-conditioned room at 21°–25°C.

Urethan treatment was begun within 24 hr after birth and consisted of 10 weekly i.p. injections of a solution of the compound (British Drug Houses Ltd.) in distilled water, at a dose level of 1 mg/gm of body weight, with a 5% solution used for the 1st injection and a 10% solution used for the 9 subsequent injections. The total dose of urethan ranged from 120 to 150 mg, depending on the different rates of weight increase of the animals. There was a considerable mortality during the initial 3–4 days of life, due in part to the toxicity of the urethan, and accentuated by cannibalism by the mothers, probably as a consequence of the young being handled at the time of the 1st injection.

About half the survivors received, in addition to the urethan, i.v. injections of a bone marrow cell suspension, and the remainder were left as controls. The bone marrow preparation was derived from syngeneic, 5- to 8-week-old donor mice, by aspiration (30) ; and (b) if the 2 are administered together to adult C57BL mice, synergism is observed when acting concurrently (22) or when the urethan treatment is given after the irradiation (4), but not when the sequence is reversed (4).

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Urethan treatment was begun within 24 hr after birth and consisted of 10 weekly i.p. injections of a solution of the compound (British Drug Houses Ltd.) in distilled water, at a dose level of 1 mg/gm of body weight, with a 5% solution used for the 1st injection and a 10% solution used for the 9 subsequent injections. The total dose of urethan ranged from 120 to 150 mg, depending on the different rates of weight increase of the animals. There was a considerable mortality during the initial 3–4 days of life, due in part to the toxicity of the urethan, and accentuated by cannibalism by the mothers, probably as a consequence of the young being handled at the time of the 1st injection.

About half the survivors received, in addition to the urethan, i.v. injections of a bone marrow cell suspension, and the remainder were left as controls. The bone marrow preparation was derived from syngeneic, 5- to 8-week-old donor mice, by aspiration of the long bones (2 femurs and 2 tibiae from each animal), the material being suspended in Tyrode's solution to provide 107 cells/injection. The recipients received 4 such injections, the 1st, after the 1st urethan injection, administered into the sigmoid portion of 1 of the transverse lateral sinuses of the brain, and the 2nd, similarly administered, after the 2nd urethan injection; the 3rd, after the 5th urethan injection, and the 4th, after the 10th (last) urethan injection, were given by the tail vein.

Because newborn mice catabolized urethan more slowly than adult mice (28) and to avoid any possible damage to the injected bone marrow cells by residual urethan in the blood stream, the 1st and 2nd bone marrow injections were given 4 days after the corresponding urethan injections, and the 3rd and 4th were given 24 hr after the respective (5th and 10th) urethan injections.

The animals were examined periodically for signs of leukemia and were killed and autopsied when such signs were found;
tissues from the thymus, liver, kidney, lymph nodes, and other organs that showed pathologic features were kept for histologic diagnosis. All survivors at the end of 60 weeks were killed and similarly autopsied, and the appropriate organs were examined histologically.

**Results**

Three days after the 1st urethan injection given to the group receiving urethan plus bone marrow, there were 50 survivors, of which 46 (22 males and 24 females) were alive at the time of the 1st appearance of leukemia in the group. Of this effective

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**Chart 1.** Cumulative incidence of leukemia with urethan alone and with urethan plus bone marrow (B.M.) injections.

**Chart 2.** Survivors during the course of the experiment, with urethan alone and with urethan plus bone marrow (B.M.) injections.
Evidence of differences in mechanism of action in the two types of experiment: (a) four injections of bone marrow were given—1 after the 1st urethan injection given to the control group (urethan alone), there were 60 survivors, of which 49 (23 males and 26 females) were alive at the time of the 1st appearance of leukemia in that group. Of these, 19 developed leukemia (9/23 in males and 10/26 in females), representing an incidence of 39%. The cumulative incidence rates in the 2 groups are plotted against time in Chart 1, from which it will be noted not only that the total incidence is closely similar for the 2 groups but also that the latent periods are almost identical. Chart 2 shows the actual numbers of survivors during the experiment in the 2 groups.

Of the 17 leukemias in the experimental group (urethan plus bone marrow), 15 developed in the thymus and infiltrated other organs, and 2 were confined to the thymus. Of the leukemias in the controls, 16 developed in the thymus and infiltrated other organs, and 2 affected the thymus only. One mouse in this group had a generalized lymphatic leukemia without involvement of the thymus.

Discussion

The object of this investigation, and the one following it (2), was to try to account for certain differences between urethan and radiation leukemogenesis in mice. The fact that injections of syngeneic bone marrow cell suspension, known to inhibit radiation leukemogenesis in adult mice (20), did not interfere with urethan leukemogenesis in newborn mice, provides further evidence of differences in mechanism of action in the 2 types of leukemogenesis.

The actual technic adopted in the present experiment calls for some comments. Whereas the inhibitory effect of bone marrow on radiation leukemogenesis is based on experiments performed in adult C57BL mice (20), its influence on urethan leukemogenesis could only be carried out in newborn mice, since urethan is only weakly leukemogenic for adult C57BL mice. Furthermore, urethan is very slowly catabolized in newborn mice (28); the time required for 50% elimination is 38 hr in 1-day-old mice, 15.5 hr in 10-day-old mice, and 4.5 hr in 30-day-old mice (6). To avoid the possibility of the injected bone marrow cells being damaged by the urethan persisting in the blood stream, and yet to assure that these cells are administered as soon as possible after the urethan treatment, and in adequate amounts, the following principles were used in the design of the experiment: (a) 4 injections of bone marrow were given—1 after the 1st urethan injection, 1 after the 2nd, 1 after the 5th, and 1 after the 10th (last) injection—even though, for inhibition of radiation leukemogenesis in adult mice, a single injection of bone marrow after the last irradiation is sufficient (20); and (b) the timing of the bone marrow injections was 4 days after the 1st and 2nd urethan injections and 24 hr after the 5th and 10th urethan injections. In spite of these exacting conditions, leukemia was not inhibited.

The persistence of urethan in newborn mice may be partly responsible for the greater sensitivity of newborn mice to urethan carcinogenesis (24) and particularly to urethan leukemogenesis (8, 10, 24, 29). On the other hand, such differences in the rate of catabolism cannot account for strain differences in response to urethan carcinogenesis (32) or to the synergistic action of urethan in relation to radiation leukemogenesis, when the urethan is administered after the irradiation (4). This is because the rate of urethan catabolism does not vary significantly among the different strains of newborn mice tested, nor is it different in irradiated and nonirradiated adult mice (6). The rate of urethan catabolism is, therefore, not a critical factor in accounting for the dissimilarity in leukemogenic response to X-irradiation and urethan action.

The participation of a virus in mouse leukemogenesis also comes into consideration for at least 2 reasons: (a) because in the case of the transmission of mouse leukemias by virus (14) the use of newborn recipients appears to be critical, and (b) because a defective immune response could play an essential role in virus-induced tumors (9). The analogy between neonatal sensitivity to viral action and to urethan leukemogenesis may, however, be fortuitous. The high responsiveness to the leukemia virus disappears within a matter of days after birth (14), as would be expected if a defective immune response were involved, whereas the responsiveness to urethan persists for several weeks (1, 25). The fact that a leukemogenic virus can be recovered from X-irradiated mice but not from the corresponding normal controls (13, 26) could conceivably be linked with the notion that depression of the immune response in adult mice, as the result of the irradiation, permitted a preexisting virus to manifest itself (17). However, no such evidence of the manifestation of a preexisting leukemia virus has so far been found following urethan action alone in newborn (11) or adult mice (7). To explain the synergistic action of urethan and X-irradiation, an incomplete (possibly a precursor) virus has been postulated, liberated by the radiation and requiring urethan for its effective leukemogenic action (5). This would not, however, readily explain the responsiveness to urethan of newborn mice, in which the immune response is still defective.

An alternative approach is to consider the problem from the viewpoint of the target organ(s). Since the leukemia under consideration has its origin in the thymus gland, as a localized lymphosarcoma in that organ, and since thymectomy interferes with this form of leukemogenesis (16), the thymus can be considered as the ultimate target organ. On the other hand, since shielding of the hematopoietic tissue during the radiation (18) or subsequent injection of syngeneic bone marrow (20) inhibits radiation leukemogenesis, the bone marrow is clearly also involved. Indeed, according to Kaplan (17), injury and subsequent recovery of both the bone marrow and the thymus are essential prerequisites for mouse leukemogenesis. In a recent report (15) attention is drawn to the fact that urethan administered to newborn or young mice up to 1 month causes injury to both the thymus and the bone marrow, and under these conditions, it acts as a leukemogenic agent; however, in adult mice, the injurious action of urethan is restricted to the thymus, and under these conditions, it is no longer effectively leukemogenic.

According to this hypothesis, compensation for the injury to the bone marrow, by replacing the injured cells with normal cells, should, in the case of urethan treatment to the newborn, antagonize the leukemogenic action. This is not borne out by the present results, showing no inhibition of leukemogenesis when syngeneic bone marrow cells were injected in the course of the urethan treatment. This supports the earlier findings (3) that in
the case of leukemogenesis in adult mice by means of irradiation followed by urethan treatment, bone marrow injections inhibited the former but not the latter component. Other forms of leukemogenesis are, in fact, not inhibited by bone marrow injections (27). The role of bone marrow seems, therefore, to be restricted to an antiradiation effect rather than to a specific antileukemic influence.

The present results thus add to the inherent complexity of the biologic chain reaction in leukemogenesis and further emphasize the difference in mode of action between whole-body X-irradiation and urethan with respect to leukemia induction in mice.

Another aspect of the problem, from the viewpoint of the participation of the thymus, is discussed in the following communication (2).

References
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