Studies on Radiation-induced Leukemia in Mice

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During the past several years we have been carrying out a series of experiments dealing with an attempt to induce leukemia in mice of different strains by total-body x-radiation.

The main purpose of this study was to familiarize ourselves with the incidence of leukemia that could be induced in mice of otherwise low-leukemic strains by ionizing radiation, to observe the differences in the incidence of leukemias induced according to the strain of animals used, and also to study the nature of leukemia thus induced.

Mice of the high-leukemic Ak strain were also irradiated to determine the possible influence of total-body x-radiation on the incidence of spontaneous leukemia in these mice.

Furthermore, a group of C3H mice was given inoculations, when newborn, of the mouse leukemia agent (6), and then irradiated, to determine whether the development of leukemia in such mice could thus be accelerated (or delayed) by total-body irradiation.

MATERIALS AND METHODS

The Incidence of Spontaneous Leukemia in Mouse Strains Used in This Study

C3H mice. — The C3H and foster-nursed C3H(f) mice used in this study have been raised in this laboratory by brother-to-sister mating from a litter obtained from Dr. J. J. Bittner in November, 1944 (3). The incidence of spontaneous leukemia in our untreated C3H and C3H(f) mice has been less than 0.5 per cent, occurring, if at all, in mice over 15 months old. We have observed only a single spontaneous parotid tumor among more than 10,000 C3H mice observed.

C3H(An) mice. — Another strain of the C3H mice (free from the milk agent by foster nursing) was obtained in 1945 from Dr. H. B. Andervont, National Cancer Institute, and then raised in our laboratory by brother-to-sister mating (8). This strain was designated by the symbol "C3H(An)." Although we have seen no spontaneous leukemia developing in untreated mice of this C3H strain in our laboratory, it is quite probable that the incidence of spontaneous leukemia in these mice is essentially similar to that in C3H mice of the Bittner strain.

C57BR/cd mice. — A small colony of C57BR/cd mice has been maintained in our laboratory by brother-to-sister mating since 1952 (4). We have not seen more than about 2 per cent spontaneous leukemias in our untreated C57BR mice, occurring, as a rule, in older mice. At the Jackson Memorial Laboratory, where our initial nucleus of these mice was obtained, the incidence of spontaneous leukemias (usually lymphosarcomas) does not usually exceed 4 per cent.

A mice. — Our colony of A mice (9) has been raised by brother-to-sister mating from a litter obtained from Dr. G. W. Woolley of the Sloan-Kettering Institute. In our limited experience, with relatively small numbers of these mice, we have never seen any spontaneous leukemia or lymphosarcoma in untreated males or females of this strain. It is probably safe to assume that the incidence of spontaneous leukemia in mice of this strain is below 1 per cent. Two groups of A mice were used: One was the original A strain carrying the mammary carcinoma agent; the other group was free from the mammary tumor agent by foster nursing and was designated by the symbol "A(f)."

I mice. — We have had very limited experience with these mice. Since it was our understanding that I mice have a low susceptibility to spontaneous tumors and leukemias, a litter was obtained from Dr. H. B. Andervont, National Cancer Institute, and a small colony of I mice has since been raised in our laboratory by brother-to-sister mating. We have never observed any spontaneous leukemias among the rather limited number of I mice (less than 80) raised in our laboratory. Again, it appears that the incidence of spontaneous leukemia in mice of this strain is very low, certainly not higher than in C3H or A mice.

Ak mice. — Our colony of Ak mice has been raised by brother-to-sister mating from a litter obtained in November, 1945, from Dr. Jacob Furth, then at Cornell University (3). Spontaneous leukemia develops in Ak mice of both sexes. Our experience referring to spontaneous Ak leukemia has been, with only few exceptions, limited to female mice. The incidence of spontaneous leukemia in a sample of 500 Ak females was, in our laboratory, 94 per cent at the average age of 8.6 months. Spontaneous leukemia, usually of the lymphatic type, begins to develop in Ak females approximately at 4 months of age; this incidence rises rapidly, reaching its peak at 7–9 months, then declines gradually.

Irradiation of Mice

The mice were irradiated in the Radiotherapy Department of this hospital under the direct supervision of one of us (B. R.). All mice received total-body irradiation. From eight to twelve mice were irradiated together at each exposure in a specially constructed plastic cage. Most irradiations were fractional and repeated at weekly intervals, although a few single irradiations were also carried out in some experiments. The weekly

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dose most frequently employed was 150 r. In most experiments four to six fractionated irradiations were applied. The details are indicated in the respective tables. The technical factors were as follows: 200 kv., 0.5 mm. Cu and 1 mm. Al filter, 1.5 mm. Cu HVL, 100 cm. distance (target to midplane of mouse), 18 ma., 15 r/min (rate of output), and a portal size of 15 × 15 cm. A light localizer was employed to clearly delineate the treatment portal. The x-ray therapy machine was calibrated regularly by the staff physicist.

RESULTS

Irradiation of mice of low-leukemic strains.—It is evident from Table 1 that leukemia could be induced without difficulty in C3H mice by fractionated total-body x-radiation. The incidence varied from 13 to 77 per cent. Females were more susceptible than males, mice irradiated at 1-3 months of age more susceptible than those in the older group. Although our figures are small, C3H and C3H(f) mice of the Bittner substrain appeared more susceptible than those obtained from Dr. Andervont's laboratory. There was no spontaneous leukemia among the 153 nonirradiated control mice.

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>Age at 1st irradi. (mo.)</th>
<th>Fract. each dose</th>
<th>Total dose</th>
<th>Leuk. inc. (per cent)</th>
<th>Avg. age</th>
<th>No. parot. tu-</th>
<th>No. dev. tumor</th>
<th>Av. age</th>
<th>Nonirradiated litter-mate controls</th>
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</thead>
<tbody>
<tr>
<td>C3H</td>
<td>1-3</td>
<td>150- 200 F</td>
<td>600- 800 M</td>
<td>34 13 38 9 0 0</td>
<td>124 0</td>
<td>79 0</td>
<td>45 0</td>
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</tr>
<tr>
<td>C3H</td>
<td>3-5</td>
<td>150 F</td>
<td>600 M</td>
<td>8 1 13 11 0 0</td>
<td>164 0</td>
<td>124 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H(f)</td>
<td>1-3</td>
<td>150 750 F</td>
<td>600- 750 M</td>
<td>30 23 77 8 1 0</td>
<td>9 0</td>
<td>20 0</td>
<td></td>
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<tr>
<td>C3H(An)</td>
<td>1-3</td>
<td>150 750 F</td>
<td>600- 750 M</td>
<td>33 12 36 10 0 0</td>
<td>9 0</td>
<td>28 0</td>
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</tbody>
</table>

* Of 280 mice in irradiated groups, 141 died without signs of tumors or leukemia at 14 mo. av. age.
† In the control group 153 mice died without signs of tumors or leukemia at 14 mo. av. age.
‡ Among 153 mice which developed radiation-induced leukemia, 96 (78 per cent) developed generalized leukemia, and 37 (28 per cent) thymic lymphosarcomas. In the irradiated group several mice developed ovarian tumors. Among C3H females in both irradiated and control groups, several developed mammary carcinomas and/or pulmonary adenomas.
§ Three mice in addition developed subcutaneous fibrosarcomas at 15 mo. av. age.
(average of eighteen smudge cells per 100 white cells). Thirty-three per cent of them showed the presence of nucleated red cells in peripheral blood. The dominant white cell was the lymphocyte, many of them showing atypical forms (17 per cent). Most of the leukemic mice showed marked to moderate anemia ranging from 3.5 to 14.0 (average, 11.1 gm Hb/100 ml as compared with 15.3 in normal C3H mice). The radiation-induced leukemia was readily transplantable, by cell graft, into adult mice of the inbred line. Within 2–3 weeks after intraperitoneal inoculations of cell suspensions prepared from radiation-induced leukemic donors, multiple lymphoid tumors developed in the peritoneal cavity of the inoculated hosts, followed within a few days by a generalized leukemia. All radiation-induced leukemias proved to be transplantable in their respective strains.

### TABLE 2

**RESULTS OF TOTAL-BODY FRACTIONATED X-RADIATION OF C57BR, A, AND I MICE**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age</th>
<th>Fract.</th>
<th>1st dose</th>
<th>Total dose</th>
<th>No. mice</th>
<th>Leuk. (per cent)</th>
<th>No. leuk. (per cent)</th>
<th>Av. age</th>
<th>Leuk.</th>
<th>Av. age</th>
<th>Leuk.</th>
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<tbody>
<tr>
<td>C57BR</td>
<td>1–3</td>
<td>150</td>
<td>600–750</td>
<td>F</td>
<td>34</td>
<td>9</td>
<td>9</td>
<td>15</td>
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<td>15</td>
<td>1</td>
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<td>A</td>
<td>1–2</td>
<td>150</td>
<td>750</td>
<td>F</td>
<td>32</td>
<td>9</td>
<td>9</td>
<td>15</td>
<td>1</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>A(t)</td>
<td>1–2</td>
<td>150</td>
<td>750</td>
<td>F</td>
<td>38</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>1</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>1–2</td>
<td>150</td>
<td>750</td>
<td>F</td>
<td>36</td>
<td>11</td>
<td>9</td>
<td>15</td>
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<td>15</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>M</td>
<td>24</td>
<td>11</td>
<td>9</td>
<td>15</td>
<td>1</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

* Among the 96 mice which developed radiation-induced leukemia, 67 per cent in the C57BR group, 82 per cent of the A and A(t), and 43 per cent of the I mice developed generalized leukemia; the remaining mice developed thymic lymphosarcomas. One C57BR mouse developed both generalized leukemia and also typical parotid gland tumors at 6 mo. age, and one A(t) mouse developed parotid gland tumors at 8 mo. of age. 29 C57BR, 53 A and A(t), and 38 mice of the I line died without signs of tumors or leukemia at 13 mo. av. age; several of the A and A(t) mice, however, developed multiple pulmonary adenomas.

† Of the 61 control C57BR, 60 died without signs of tumors or leukemia at an average age of 14 mo. There were no littermate controls in the A and A(t) group; however, we have not seen any spontaneous leukemia developing among A or A(t) mice of our small breeding colony of this strain during the past several years.

### TABLE 3

**RESULTS OF TOTAL-BODY FRACTIONATED X-RADIATION OF HEALTHY, YOUNG ADULT AK FEMALE MICE**

<table>
<thead>
<tr>
<th>Age</th>
<th>Fract.</th>
<th>1st dose</th>
<th>Total dose</th>
<th>No. mice</th>
<th>Leuk. (per cent)</th>
<th>No. leuk. (per cent)</th>
<th>Av. age</th>
<th>Leuk.</th>
<th>Av. age</th>
<th>Leuk.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk.</td>
<td>150</td>
<td>600–750</td>
<td>F</td>
<td>30</td>
<td>20</td>
<td>87</td>
<td>5.7</td>
<td>29</td>
<td>29</td>
<td>79</td>
</tr>
<tr>
<td>1–3</td>
<td>10–200</td>
<td>100–1000</td>
<td>F</td>
<td>288</td>
<td>242</td>
<td>94</td>
<td>8.4</td>
<td>198</td>
<td>181</td>
<td>91</td>
</tr>
</tbody>
</table>

* Among the 268 irradiated mice which developed leukemia, 245 (91 per cent) developed generalized leukemia; 29 (9 per cent) thymic lymphosarcomas; 39 mice in this group died without signs of tumors or leukemia at 9 mo. av. age.

† Among the 204 litter-mate control mice which developed leukemia, 199 (98 per cent) developed generalized leukemia, and five (2 per cent) thymic lymphosarcomas; 23 of the control mice died without signs of tumors or leukemia at 13 mo. av. age.
Irradiation of mice of the high-leukemic Ak strain.—Table 3 summarizes results of total-body x-radiation of Ak mice. The incidence of spontaneous leukemia in irradiated groups was 93 per cent, with leukemia developing at an average age of 7.8 months, as compared with an incidence of 90 per cent developing in untreated litter-mate controls at an average age of 8.5 months. The incidence of leukemia was thus slightly higher and the latency period slightly shorter in the irradiated group. These differences are slight, however, and it is difficult to accept them as significant without reservations.

Several usually low-leukemic strains would develop leukemia following total-body x-radiation (1, 2, 9, 11-15, 17, 20). Experiments reported in this paper confirm and extend these findings. The previously observed curious sex difference in susceptibility to radiation-induced leukemia (10, 18) has also been recorded in our series.

The nature of radiation-induced leukemia has not yet been completely elucidated. Recent studies suggest however that radiation-induced leukemia could be transmitted by filtrates prepared from organs of leukemic donors (7, 8, 16).

It was interesting to note that three C3H mice

| TABLE 4 |

RESULTS OF INOCULATION OF CELL-FREE LEUKEMIC EXTRACTS* INTO NEWBORN C3H OR C3H(f) MICE, FOLLOWED BY TOTAL-BODY X-RADIATION

<table>
<thead>
<tr>
<th>Age at 1st</th>
<th>Fract.</th>
<th>Total</th>
<th>No.</th>
<th>No.</th>
<th>Av.</th>
<th>Av.</th>
</tr>
</thead>
</table>
| Irrad. (mo.) | Isom. | dose | Total dose | mice | Leuk. inc. (per cent) | Leuk. dev. | age parot. tumor dev. (mo.) | Leuk. filtrate, or supernate, then x-ray
| 1-2 ½ | 150 | 300-750 | 49 | 16 | 33 | 9 | 1 | 7 |
| 1-5 | 150 | 600-750 | 49 | 16 | 33 | 9 | 1 | 7 |
| Total, 1-5 | 108 | 46 | 43 | 7 | 1 | 7 |
| 1-2 ½ Single dose | 200 | 26 | 17 | 65 | 4 | 0 | 0 |
| Total: | | 131 | 43 | 33 | 6 | 7 | 7 |

* These extracts (filtrate or 7,000 g supernate) were prepared from Ak spontaneous leukemias or from CSH donors in which leukemia was induced with Ak extracts.
† 61 mice died without signs of tumors or leukemia at av. age of 10 mo.
‡ 79 mice died without signs of tumors or leukemia at av. age of 18 mo.; two mice developed subcutaneous fibrosarcomas at av. age of 9 mo.
§ One mouse developed parotid tumor and also adrenal medullary tumors.

Irradiation of C3H mice that had been inoculated with the leukemic (Ak) agent.—In another series C3H and C3H(f) mice were inoculated within 16 hours after birth with cell-free (7,000 g supernate or Selas 02 or 03 filtrate) extracts prepared in the usual manner either from spontaneous Ak leukemia, or from C3H leukemic donors in which leukemia was induced by inoculation of cell-free Ak leukemic extracts. After the inoculated mice reached adult age, they were irradiated as specified in Table 4. The incidence of leukemia developing in the irradiated group was higher (48 per cent) than in the litter-mate control group which received only the inoculation of filtrates (83 per cent).

DISCUSSION

It has long been observed that x-radiation is carcinogenic and leukemogenic and that mice of
newborn with Ak or r of the high-leukemic Ak strain had practically no radiated mice after a latency varying from 5 to 6 months. This observation was similar to those previously reported and dealing also with irradiation of other leukemic strains (19). These results were particularly impressive when compared with experiments dealing with the irradiation of mice of the low-leukemic strains such as the C3H, in which fractional irradiation induced leukemia in a high number of animals, with practically none developing leukemia in the nonirradiated litter-mate controls.

Mice of both the Ak and C3H strains may carry a masked oncogenic agent having a leukemogenic potential. In the high-leukemic strains, certain not yet fully understood inducing factors may become operative in middle-aged hosts, prompting then activation of the hitherto latent agent and causing the development of “spontaneous” leukemia in over 85 per cent of these mice. In certain low-leukemic strains, on the other hand, such as the C3H or C57BR, a potentially leukemogenic agent may be also present, at least in some hosts, but would usually remain inactive, because no inducing mechanism would be available under natural conditions of life. Fractional total-body x-radiation, however, would create conditions favoring activation of the hitherto masked agent and would prompt the development of leukemia in mice of these usually low-leukemic strains. On the other hand, similar irradiation of mice of a high-leukemic strain such as Ak would only increase by a small margin the development of “spontaneous” leukemia, because in such mice the inducing mechanism would become operative “spontaneously,” without the aid of irradiation.

In some instances, however, irradiation of mice of a high-leukemic line may under certain not yet precisely defined conditions lower the incidence of the spontaneous leukemia (19). Recently, Reverdy, Rudali, Duplan, and Latarjet (21) observed that total-body x-radiation, with a dose varying from 50 to 500 r of 1107 newborn (less than 16 hours old) Ak mice, lowered the development of spontaneous leukemia slightly among females and more markedly among males.

SUMMARY

1. Fractionated total-body x-radiation of young adult mice of C3H, C57BR/cd, A, and I strains induced leukemia in up to 77 per cent of the irradiated mice after a latency varying from 5 to 6 months.

2. Fractionated total-body irradiation of mice of the high-leukemic Ak strain had practically no effect on the incidence of spontaneous leukemia.

3. A group of C3H mice were inoculated when newborn with Ak or C3H leukemic filtrates and then irradiated. The incidence of leukemia in this group was 45 per cent, as compared with 33 per cent in the litter-mate control group which received only the inoculation of the agent.

REFERENCES

Studies on Radiation-induced Leukemia in Mice

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