Testosterone Prevention of Post-Irradiation Lymphomas in C57 Black Mice*

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It was recently reported that testosterone strikingly inhibits radiation-induced lymphoid tumor development in intact and castrate adult male C57 black mice (5). This result is consistent with the earlier observations of Murphy (9) on spontaneous leukemia and of Gardner (1) in irradiated female BC mice.

The present report is concerned with two additional experiments on C57 black mice. In one, the inhibitory effect of testosterone was observed only when it was given concurrently with irradiation. In the other, the degree of inhibition has been shown to be essentially the same in female mice, as previously reported for males of this strain, though somewhat greater in spayed than in intact animals.

METHODS

Experiment 1.—Intact male C57 black mice were distributed among seven experimental groups and given whole-body irradiation to a total dose of 675 r in four equally fractionated treatments, at intervals of 4 days, starting at 60 days of age.1 Six groups received intramuscular injections of 0.5 mg. testosterone in 0.05 cc. of stabilized aqueous suspension twice weekly for 5 weeks. The testosterone-injected groups differed only in the time at which testosterone injections were started in relation to the time of irradiation, as indicated in Table 1. The seventh group received similarly timed placebo injections of the suspension medium alone, starting concurrently with the first irradiation and continued for 5 weeks.

Experiment 2.—Litter-mate female C57 black mice were distributed among four groups at the time of weaning. Groups II and IV were spayed at 2 months of age. The intact and spayed groups then received the same dose of fractionated whole-body irradiation as in Experiment 1, beginning 2-4 days postoperatively. Groups III and IV received testosterone injections intramuscularly twice weekly for 10 weeks, the dose of testosterone and volume of injection being the same as in Experiment 1. Groups I and II received placebo injections of the suspension medium alone for 10 weeks. All injections were started immediately after the first x-ray treatment.

In both experiments, all animals were identically caged and maintained on Purina Laboratory Chow and water ad libitum. Mice that died of any cause before the time of appearance of the first lymphoid tumor have been omitted. All animals were carefully examined at autopsy. In most instances the diagnosis of a mediastinal or disseminated lymphoma could be made on gross examination, but diagnoses were established histologically in all questionable instances.

RESULTS

Experiment 1.—The data are summarized in Table 1, and cumulative incidence curves are seen in Chart 1. There was a striking inhibition of lymphoma development in Group II, in which testosterone injections were started concurrently with irradiation. There was no protection when testosterone injections were started at 1 month of age and completed by the time irradiation began. There was a slight increase in latent period when testosterone was not started until 3 weeks after the first x-ray treatment, but the ultimate incidence in this group did not differ significantly from placebo-injected irradiated controls. No inhibition was observed when testosterone was started 6-12 weeks after irradiation.

Experiment 2.—The data are similarly presented in Table 2. Testosterone yielded a pronounced inhibition of lymphoid tumor development in both intact and spayed female mice. The degree of inhibition was somewhat greater in the spayed than in the intact group, although the differences are of borderline significance at the 0.05 level. The degree of inhibition in the spayed group was about the same as that previously noted in male mice treated with testosterone.

DISCUSSION

Cortisone has been shown to inhibit lymphoma development significantly when started as late as 6 weeks after the completion of x-radiation (6). In contrast, the data of Experiment 1 indicate that testosterone is effective only when given concurrently with irradiation. This suggests that testosterone and cortisone exert their inhibitory effects upon the leukemogenic process independently of one another. Further studies on the acute effects of both agents upon irradiated lymphoid tissues are needed.

It is of interest that pretreatment with testosterone at 1 month of age did not decrease...
**TABLE 1**

**TIME OF TESTOSTERONE INJECTION AND LYMPHOMA DEVELOPMENT IN IRRADIATED C57 BLACK MALE MICE**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MATERIAL INJECTED</th>
<th>TIME OF TESTOSTERONE INJECTION</th>
<th>NO. OF MICE</th>
<th>PERIOD</th>
<th>MEAN LATENT PERIOD D (DAYS)</th>
<th>NO. DEAD</th>
<th>NO. ALIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Placebo</td>
<td>simultaneously with x-ray</td>
<td>28</td>
<td>20</td>
<td>71</td>
<td>176</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>Testosterone</td>
<td>simultaneously with x-ray</td>
<td>30</td>
<td>6</td>
<td>80</td>
<td>218</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>Testosterone</td>
<td>4 weeks before x-ray</td>
<td>29</td>
<td>21</td>
<td>72</td>
<td>101</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>Testosterone</td>
<td>5 weeks after first x-ray</td>
<td>30</td>
<td>19</td>
<td>63</td>
<td>193</td>
<td>6</td>
</tr>
<tr>
<td>V</td>
<td>Testosterone</td>
<td>6 weeks after first x-ray</td>
<td>30</td>
<td>25</td>
<td>87</td>
<td>189</td>
<td>1</td>
</tr>
<tr>
<td>VI</td>
<td>Testosterone</td>
<td>9 weeks after first x-ray</td>
<td>31</td>
<td>94</td>
<td>77</td>
<td>199</td>
<td>6</td>
</tr>
<tr>
<td>VII</td>
<td>Testosterone</td>
<td>12 weeks after first x-ray</td>
<td>32</td>
<td>97</td>
<td>54</td>
<td>170</td>
<td>2</td>
</tr>
</tbody>
</table>

* 360 days after first x-ray.

**TABLE 2**

**EFFECT OF TESTOSTERONE INJECTIONS ON RADIATION-INDUCED TUMORS IN C57 BLACK FEMALE MICE**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MATERIAL INJECTED</th>
<th>NO. OF MICE</th>
<th>PERIOD</th>
<th>MEAN LATENT PERIOD D (DAYS)</th>
<th>NO. DEAD</th>
<th>NO. ALIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact Placebo</td>
<td>30</td>
<td>27</td>
<td>98</td>
<td>155</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>Spayed Placebo</td>
<td>25</td>
<td>23</td>
<td>92</td>
<td>170</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>Intact Testosterone</td>
<td>28</td>
<td>7</td>
<td>17</td>
<td>870</td>
<td>16</td>
</tr>
<tr>
<td>IV</td>
<td>Spayed Testosterone</td>
<td>27</td>
<td>2</td>
<td>4</td>
<td>311</td>
<td>15</td>
</tr>
</tbody>
</table>

* 360 days after first x-ray.

**CHART 1.**—Time of testosterone injection and lymphoma development in irradiated C57 black male mice.
lymphoma incidence, since testosterone provokes a striking and selective thymic involution which might, on a priori grounds, have been considered analogous to thymectomy. It has previously been shown that thymectomy prior to irradiation virtually abolishes lymphoma development in C57 black mice (4). From this result, it would appear that the size of the thymus at the time of irradiation is unrelated to leukemogenic susceptibility.

Testosterone is also an effective inhibitor of lymphoma development in female mice. The apparently greater degree of suppression exerted in spayed females is of considerable interest, if confirmed by subsequent experiments, because it is the first suggestion that endogenous estrogen secretion may contribute to leukemogenic susceptibility in mice of this strain. Estrogens are well known to be leukemogenic for some strains (3), and Kirschbaum, Shapiro, and Mixer (8) have previously shown that exogenous estrogen may act synergistically with irradiation in eliciting thymic lymphomas in a susceptible strain. However, strain C57 black mice tolerate exogenous estrogens poorly, and estrogens do not appear to be leukemogenic for this strain (2, 7). Moreover, the incidence of lymphomas in irradiated females, whether immature (4) or adult, is not altered by gonadectomy. The present experiment suggests that spayed females are more susceptible to the lymphoma-inhibiting action of testosterone; that this may be due in part to the "neutralization" of endogenous estrogen is a plausible explanation deserving further experimental study.

SUMMARY

In two experiments concerned with radiation-induced lymphoid tumors of C57 black mice, it has been shown that: (a) testosterone inhibits lymphoma development only when given concurrently with irradiation and (b) the inhibitory effect of testosterone previously noted in male mice of this strain is also observed in females, the degree of inhibition being apparently somewhat greater in spayed than in intact animals.

REFERENCES

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