Stimulation of Resistance to Tumor Growth of Athymic Nude Mice Pretreated by Combined Local Hyperthermia and X-Irradiation

A. Yerushalmi and Y. Weinstein

ABSTRACT

Host resistance to tumor growth was studied in athymic nude mice of C57BL background. Animals were pretreated in the left hind leg by local hyperthermia, local X-irradiation, or combined local hyperthermia and X-irradiation. Twenty-four hr posttreatment, the animals were inoculated with the metastatic Lewis lung carcinoma tumor. Half of the animals in each group were inoculated in the pretreated leg and animals of the other half of the group were inoculated in the contralateral untreated leg. An increase of 30 to 45% in life span was achieved in normal and nude mice pretreated by combined local hyperthermia and irradiation. The increase in life span was similar in animals inoculated in the pretreated leg or in the untreated contralateral leg. These results indicate that T-lymphocytes are not involved in the protection against tumor growth.

INTRODUCTION

Experimental and clinical evidence proves that hyperthermia and combined local hyperthermia and ionizing radiation are inactivators of malignant cells (1, 6, 9, 17). There exists contradictory evidence about the relationships between heated tumor cells and the immune status of the host. There are suggestions that heat lowers the host immune response (5), has no effect on the immune system sufficient to influence tumor growth (12), or increases the host's immune response (4). However, there exists clinical evidence that hyperthermia stimulates patient antitumor immunity. In some patients with more than one tumor, hyperthermic treatment of one tumor resulted in regression of both the treated and untreated tumors (2, 13). Hyperthermic treatment of tumor-bearing patients may be associated with modified immunogenicity of treated tumor cells or with nonimmunological processes of the host. The relationship(s) between the tumor cell population and the above-mentioned mechanisms of the host and the manner in which these relationships are affected in the host could be of clinical importance. Previous experiments carried out in this laboratory have shown an increased resistance to tumor development in animals pretreated by combined simultaneous local hyperthermia and X-irradiation prior to tumor inoculation (18). This stimulated resistance to tumor development was found in animals inoculated in the pretreated leg as well as in animals inoculated in the contralateral untreated leg. The aims of this study were to test the contribution of T-lymphocytes to the stimulation of resistance against local tumor growth of hosts pretreated by combined local hyperthermia and X-irradiation. Therefore, congenitally athymic nude mice were selected as hosts.

MATERIALS AND METHODS

Congenitally athymic nude mice (nu/nu) of C57BL background together with phenotypically normal animals of the same litters (nu/+ ) were used as hosts. The mice were bred in the Weizmann Institute of Science. The tumor was the 3LL. This malignant metastasizing tumor originated spontaneously in a C57BL/6 mouse (14). Tumor maintenance and details of tumor cell suspension preparation have been described elsewhere (16). Viable tumor cells (0.5 x 10^6) in 0.5 ml were injected i.m. into the left or right hind leg. Heating System. The heating system has been described elsewhere (17). Briefly, it consisted of a hot-air blower, a heat-insulated cylinder, and a temperature control unit. For local heating, the left hind limbs of 4 mice were inserted into 4 holes (forming a Maltese cross) drilled in the upper part of the cylinder. The local heating was confined to the hind left leg. Intralimb temperatures were 42.7 ± 0.1° or 44.5 ± 0.1°. Simultaneous temperature measurements were made in the cylinder, in the limb, and in the ear (to record skin temperature). Temperature measurements were taken by Copper-Constantan thermocouples (IT-1; Bailey Instrument Co., Saddle Brook, N. J.). The leads from the installed thermocouples were connected to an electronic control unit, which included a continuous digital temperature readout (BAT-8, Bailey) and an X-Y recorder. Irradiation. The X-ray machine used was a Picker Vanguard. Irradiation specifications were 250 kV; 15 ma; half-value layer, 1.35 mm copper; focus skin distance, 34 cm; and exposure rate, 100 R/min. Except for the irradiated limbs, the animals were shielded by half-cylinders of lead 6.5 mm thick. Experimental Schedule. Healthy normal C57BL (nu/+ ) and athymic (nu/nu) animals were pretreated in the left

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1 Supported in part by the Gulton Foundation, Englewood, N. J.
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Received October 25, 1978; accepted December 21, 1978.
In untreated animals, tumor growth was the same in normal and athymic mice inoculated with the same dose of tumor cells. Table 1 summarizes the AST of animals subjected to the different treatments. In Table 2, we have calculated the EASTR. Each value in Table 2 is the AST of animals inoculated with 3LL pretreated by combined local hyperthermia and X-irradiation. Twenty-four hr after treatment, the animals were inoculated i.m. with $0.5 \times 10^8$ viable tumor cells. Half of the animals in each group were inoculated in the pretreated left hind leg, and the animals of the other half of the group were inoculated in the contralateral untreated hind leg. Untreated animals were inoculated at the same time as the pretreated animals and served as controls. All animals, including untreated controls, were anesthetized during treatment with an i.p. injection of pentobarbital sodium. This enabled positioning of the limb into the hot-air cavity without mechanical fixing. By this technique, blood supply to the limb was not impaired during treatment.

**RESULTS**

In untreated animals, tumor growth was the same in normal and athymic mice inoculated with the same dose of tumor cells. Table 1 summarizes the AST of animals subjected to the different treatments. In Table 2, we have calculated the EASTR. Each value in Table 2 is the AST of the control untreated animals. Tables 1 and 2 show that local preheating of the left hind limb and inoculation of the left hind limb or right limb did not cause delay in tumor growth. The same applies to left hind limb preirradiated and left hind limb or right limb-inoculated animals. Combining the 2 modalities increased the life span of the pretreated animal by 30 to 45% ($p < 0.001$). This applies both to normal and nude mice inoculated in the same pretreated leg ($p < 0.001$) and to animals inoculated in the untreated contralateral leg ($p < 0.001$). The results presented here are in agreement with previous results obtained by the same strategy and technique with normal animals (18).

**DISCUSSION**

The nude mutation in the mouse is associated with dysgenesis of the thymus (7). In the adult nude homozygote, the thymus is represented only by a few cysts composed of glandular and ciliated cells (3). Cellular immunity is absent hind limb by (a) local hyperthermia alone, (b) local exposure to X-rays alone, or (c) simultaneous local heat and X-irradiation. Twenty-four hr after treatment, the animals were inoculated i.m. with $0.5 \times 10^8$ viable tumor cells. Half of the animals in each group were inoculated in the pretreated left hind leg, and the animals of the other half of the group were inoculated in the contralateral untreated hind leg. Untreated animals were inoculated at the same time as the pretreated animals and served as controls. All animals, including untreated controls, were anesthetized during treatment with an i.p. injection of pentobarbital sodium. This enabled positioning of the limb into the hot-air cavity without mechanical fixing. By this technique, blood supply to the limb was not impaired during treatment.

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### Table 1

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Pretreated leg</th>
<th>Inoculated leg</th>
<th>AST (days)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
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<tr>
<td>Untreated</td>
<td>Left</td>
<td>Left or right</td>
<td>17.25 ± 1.16 (20)</td>
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<tr>
<td>Heat, 42.7°C, 45 min, or X-irradiation, 800 R</td>
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<td>Left</td>
<td>18.60 ± 1.00 (10)</td>
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<tr>
<td>Heating + X-irradiation</td>
<td>Left</td>
<td>Right</td>
<td>17.50 ± 0.90 (10)</td>
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<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Pretreated leg</th>
<th>Inoculated leg</th>
<th>AST (days)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
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<tr>
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<td>Left</td>
<td>23.60 ± 1.40 (9)</td>
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<td>42.7°C, 45 min, + 800 R</td>
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<td>24.30 ± 1.70 (10)</td>
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<tr>
<td>44.5°C, 60 min, + 800 R</td>
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<td>Right</td>
<td>22.50 ± 1.20 (4)</td>
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### Table 2

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<th>Inoculated leg</th>
<th>EASTR'</th>
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<tbody>
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<td></td>
<td></td>
<td>Normal</td>
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<td>1.39</td>
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<td>Left</td>
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<tr>
<td>800 R</td>
<td>Left</td>
<td>Right</td>
<td>1.43</td>
</tr>
</tbody>
</table>

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**a** Animals were inoculated with $0.5 \times 10^8$ viable tumor cells 24 hr after treatment.

**b** Mean ± S.D.

**c** Numbers in parentheses, number of mice per group.

**d** Significantly different from untreated controls ($p < 0.001$).
REFERENCES


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