Effect of Starvation of Intact and Adrenalectomized Mice Bearing Lymphosarcoma P1798 on Tumor Regression and Ribonuclease Activity

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SUMMARY

Starvation of BALB/c mice bearing Lymphosarcoma P1798 resulted in regression of both the corticoid-resistant and corticoid-sensitive tumor strain. Increased acid-specific ribonuclease activity of whole tumor homogenates accompanied regression of the corticoid-sensitive strain from intact animals but not the corticoid-resistant strain. Adrenalectomy prevented the increase in tumor RNase activity induced by starvation in the corticoid-sensitive strain. Enhancement of specific RNase activity in the corticoid-sensitive strain of P1798 after starvation may be due to increased endogenous glucocorticoid secretion secondary to stress, but increased tumor RNase activity is not necessary for tumor regression to occur. Both the corticoid-sensitive and -resistant strains of P1798 regressed after starvation, and adrenalectomy of the host did not prevent regression of corticoid-sensitive P1798. Therefore, starvation-induced regression of the corticoid-sensitive tumor may not be mediated by endogenous glucocorticoid secretion, and the mechanism of starvation-induced regression remains unclear.

INTRODUCTION

Glucocorticoid-mediated regression of the corticoid-sensitive strain of mouse Lymphosarcoma P1798 and rat thymus is accompanied by an increase in specific acid RNase activity of tumor or thymus (9, 14). In addition, thymic regression after corticoid administration is accompanied by increased specific alkaline RNase activity. The administration of vinblastine sulfate to mice bearing either the corticoid-sensitive or corticoid-resistant strain of P1798 (7) resulted in tumor regression without an increase in RNase activity (15). Similarly, thymus regression in rats treated with vinblastine sulfate was not associated with an increase in RNase activity (15). It would appear that a unique effect of glucocorticoid administration on corticoid-sensitive lymphoid tissue is enhancement of specific RNase activity in that tissue. This study was designed to investigate the effect of prolonged starvation on regression and RNase activity of Lymphosarcoma P1798 and to evaluate whether this method of producing tumor regression resembles glucocorticoid-induced regression (increased tumor RNase activity accompanying regression) or vinblastine-induced regression (no increased tumor RNase activity).

MATERIALS AND METHODS

Male BALB/c mice obtained from Arthur D. Little, Corp., Cambridge, Mass., and from Microbiological Associates, Bethesda, Md., were inoculated with the appropriate strain of P1798 as previously described (9). After 2 weeks the tumors were approximately 2 cm in diameter and were used for experimentation. Groups of mice bearing the corticoid-sensitive or -resistant strain of P1798 were starved for 1, 2, or 3 days or fed ad libitum and used as controls. Similar groups of adrenalectomized mice were prepared. Adrenalectomized animals were treated by subcutaneous daily injection with 0.5 mg deoxycorticosterone acetate. Two days after adrenalectomy starvation was begun. Animals were weighed and then sacrificed by decapitation. Whole tumor weights were recorded.

RNase activity in the 10,000 X g supernatant of 10% tumor homogenates was assayed at pH 5.7 as previously described (9, 14). Yeast RNA purified by the method of Crestfield et al. (2), and purchased from the Sigma Chemical Company, St. Louis, Mo., was used as substrate. Tumor homogenate supernatant, 0.1 ml, was incubated with 1.0 mg yeast RNA dissolved in 0.8 ml 0.05 M acetate buffer, pH 5.7, to which 0.001 M EDTA was added, and the reaction was stopped after 12 min by the addition of 0.2 ml of a cold 25% perchloric acid solution containing 0.75% uranyl acetate. The mixture was centrifuged at 1000 X g and the A260 of the supernatant fraction was determined. Specific RNase activity was recorded as ΔA260/12 min/mg protein. Protein was measured by the method of Lowry et al. (8).

RESULTS

Effect of Starvation on Host and Tumor Weight. Table 1 shows that body weight decreased in all 3 animal groups progressively. Therefore, starvation was in fact achieved. Concurrently, tumor weight in all 3 animal groups progres-
Whole body and tumor weight and tumor ribonuclease activity after starvation of mice bearing Lymphosarcoma P1798

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. animals</th>
<th>Body weight (g)(^a)</th>
<th>Maximal change (%)</th>
<th>Tumor (g)(^a)</th>
<th>Maximal change (%)</th>
<th>Specific RNase activity(^b)</th>
<th>Maximal change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact, corticoid-sensitive strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>26</td>
<td>34.0 ± 2.0</td>
<td>4.27 ± 0.11</td>
<td>370 ± 27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day starved</td>
<td>24</td>
<td>30.0 ± 1.8</td>
<td>3.49 ± 0.12</td>
<td>500 ± 23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 day starved</td>
<td>25</td>
<td>24.2 ± 1.9</td>
<td>2.53 ± 0.20</td>
<td>699 ± 45</td>
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<tr>
<td>3 day starved</td>
<td>26</td>
<td>21.0 ± 2.0</td>
<td>-40%</td>
<td>1.90 ± 0.09</td>
<td>-56</td>
<td>761 ± 11</td>
<td>+105</td>
</tr>
<tr>
<td>Intact, corticoid-resistant strain</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>25</td>
<td>37.1 ± 0.5</td>
<td>5.01 ± 0.07</td>
<td>472 ± 25</td>
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<tr>
<td>1 day starved</td>
<td>24</td>
<td>31.8 ± 1.7</td>
<td>4.69 ± 0.11</td>
<td>465 ± 16</td>
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<tr>
<td>2 day starved</td>
<td>25</td>
<td>27.0 ± 1.8</td>
<td>4.10 ± 0.17</td>
<td>327 ± 14</td>
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<td></td>
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<td>3 day starved</td>
<td>25</td>
<td>23.2 ± 1.6</td>
<td>-37</td>
<td>3.76 ± 0.12</td>
<td>-25</td>
<td>262 ± 21</td>
<td>-56</td>
</tr>
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<td>Adrenalectomized, corticoid-sensitive strain</td>
<td>22</td>
<td>30.2 ± 1.3</td>
<td>4.80 ± 0.09</td>
<td>328 ± 36</td>
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</tr>
<tr>
<td>1 day starved</td>
<td>23</td>
<td>27.6 ± 0.4</td>
<td>3.96 ± 0.11</td>
<td>315 ± 25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 day starved</td>
<td>22</td>
<td>23.6 ± 1.1</td>
<td>3.51 ± 0.09</td>
<td>342 ± 23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 day starved</td>
<td>24</td>
<td>20.0 ± 1.6</td>
<td>-34</td>
<td>2.21 ± 0.12</td>
<td>-54</td>
<td>381 ± 19</td>
<td>+10</td>
</tr>
</tbody>
</table>

\(^a\)Values are mean ± S.E.
\(^b\)A\(_{260}\)/12 min/mg protein.

Effect of Starvation on Tumor Acid RNase Activity. Table 1 shows that as starvation progresses and tumor regression occurs tumor-specific acid RNase activity increases in the intact corticoid-sensitive, but not in the corticoid-resistant, strain group. Tumor-specific RNase activity in the adrenalectomized corticoid-sensitive strain group remains relatively unchanged.

DISCUSSION

MacLeod et al. (9) have shown that administration of a potent synthetic glucocorticoid to mice bearing the corticoid-sensitive strain of Lymphosarcoma P1798 resulted in a prompt regression of the tumor. This regression was accompanied by a selective decrease in tumor RNA and was preceded by an increase in tumor acid RNase activity. Regression of tumor and activation of tumor RNase were not observed in the corticoid-resistant strain of P1798 after the same steroid was administered to tumor-bearing mice. Ambellan and Hollander (1) have conclusively shown by means of glass-bead chromatography that the glucocorticoid-induced increase in RNase activity in homogenates of corticoid-sensitive P1798 is due to increased enzyme activity in tumor lymphocytes and not to the addition of RNase activity to the tumor by macrophages, which invade the regressing tumor in large numbers.

Increased lymphoid cell RNase activity in corticoid-sensitive lymphoid tissue may be a specific effect of glucocorticoid. Rat thymus has been demonstrated to exhibit increased acid and alkaline RNase activity during corticoid-induced regression (14), while neither thymus nor corticoid-sensitive or -resistant P1798 showed such increased RNase activity during regression induced by vinblastic sulfate (15). The concept of enhancement of RNase activity as a unique primary effect of glucocorticoid in sensitive lymphoid tissue has been challenged by Ambellan and Hollander (1). These authors reported increased acid RNase activity in the corticoid-sensitive strain of P1798 after treatment not only with 9a-fluoroprednisolone but with other chemotherapeutic agents as well, including vinblastine. In each case in which RNase activation resulted from treatment, tumor regression followed. Chemotherapeutic agents that caused no tumor regression had no effect on RNase activity. These authors suggested that enhanced RNase activity accompanied lymphoid tissue regression irrespective of the method by which regression was accomplished. In this study, tumor RNase activity is compared in 3 groups of mice after starvation: intact mice bearing corticoid-sensitive P1798, intact mice bearing corticoid-resistant P1798, and adrenalectomized mice bearing corticoid-sensitive P1798. In all 3 groups tumor regression occurred over a 3-day period of starvation. A significant increase in RNase activity of tumor homogenates was seen only in corticoid-sensitive tumors from intact animals. Adrenalectomy of the host prevented the increase in RNase activity that accompanied starvation-induced regression of corticoid-sensitive P1798. Therefore, tumor regression in both strains of P1798 can occur without an increase in RNase activity. When endogenous glucocorticoid secretion is increased (by starvation), tumor-specific RNase activity is enhanced although the rate of tumor...
regression is not accelerated. As others have noted (1), the case for ribonuclease activation representing a specific manifestation of glucocorticoid action on sensitive lymphoid tissue is strengthened indirectly by the observation that corticosteroids cause the breakdown of polysomes in thymus lymphocytes (6), reduce incorporation of amino acids into proteins (5, 12), and alter nucleic acid metabolism (4).

Whether or not enhanced RNase activity accompanies the regression of lymphoid tissue brought about by agents other than corticosteroids is unclear. Total-body irradiation causes regression of mouse thymus, and the regression is accompanied by increased thymus RNase activity (10, 13). The enhancement of RNase activity can be virtually nullified by shielding the head of the animal (10) and may be due to release of adrenocorticotropic hormones from the irradiated pituitary. Mashburn and Wriston (11) have reported that the regression of asparaginase-sensitive Lymphosarcoma 6C3HED induced by L-asparaginase administration to host animals is preceded by an increase in tumor alkaline RNase activity. No tumor regression or increased RNase activity was produced by L-asparaginase in the asparaginase-resistant strain of 6C3HED. Since no adrenalectomized animals were used as hosts, it is impossible to assess whether or not increased endogenous production of glucocorticoid secondary to L-asparaginase administration or stress in general played a role in increasing tumor RNase activity. Alkaline RNase activity was not determined in our experiments.

Erbe et al. (3) have shown that in vitro treatment of Ehrlich ascites tumor cells with a variety of cytostatic agents results in increased tumor cell RNase activity. They conclude that increased RNase activity is a nonspecific reaction of proliferating cells to cytostasis. The present study suggests that enhancement of specific RNase activity in the corticoid-sensitive strain of Lymphosarcoma P1798 may be a direct effect of glucocorticoid but that increased RNase activity is not necessary for tumor regression to occur. Regression of the corticoid-resistant strain in intact animals, as well as the corticoid-sensitive strain of P1798 in adrenalectomized animals, was induced by starvation of the host. Therefore, starvation-induced regression of P1798 cannot be ascribed to endogenous glucocorticoid effect, and the mechanism by which starvation induces regression of this tumor remains unclear.

REFERENCES

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