Alterations in Ribonuclease Activities in the Plasma, Spleen, and Thymus of Tumor-bearing Mice

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SUMMARY

Six transplantable murine tumor models were evaluated for changes in RNase activity. This study was conducted with spleen and thymus homogenates, as well as with plasma collected from tumor-bearing mice. Nuclease activity directed against the synthetic substrates, polyadenylic acid, polyuridylic acid, and polycytidylic acid, was measured and the data obtained for tumor-bearing animals were compared to their normal counterparts. Elevated activity against polyuridylic acid was observed in the plasma of all tumor-bearing mice. Although not as all inclusive, RNase activity directed against the synthetic substrates demonstrated that, in most cases, two or more enzymes were being detected. The assay may have some eventual value in the monitoring of cancer.

INTRODUCTION

In at least 2 studies evaluating serum RNase activity in patients with various types of cancer, it has been shown that serum nuclease levels were elevated in certain classes of tumor (3, 4). Although no similar studies relating to murine tumor models appear to have been made, other alterations in RNase activity have been observed. For example, the increase in the activity for this enzyme in chick fibroblasts after transformation by the Rous sarcoma virus has been reported (5). Depression of spleen nuclease activity following injection of Friend disease virus in mice has been described also (2). Changes in spleen- and thymus-associated nuclease activities following immunization of mice have demonstrated that the change in the in vivo activity of this enzyme may result not only from neoplastic transformation but also from the interplay of normal biological systems such as immunogenesis (1).

Because of the possibility that this approach may represent a useful biological assay, 6 transplantable murine tumor models were evaluated for the changes in RNase activity wrought in the lymphoid tissue of tumor-bearing mice. Plasma nuclease levels were also assessed to determine whether or not any changes observed would be reflected in an easily obtainable blood fraction.

MATERIALS AND METHODS

Mice. Both C57BL/6 and BALB/c mice were obtained from the Chemotherapy Research Colony of the National Cancer Institute. C3H/HeJ mice were procured from The Jackson Laboratory, Bar Harbor, Maine.

Tumor Lines. Six transplantable tumors, 2 for each strain of mouse, were obtained from The Jackson Laboratory. BW10232, a spontaneous adenocarcinoma, and C1498, a spontaneous myeloid leukemia, were carried in C57BL/6 mice. Similarly, Harding-Passey, a spontaneous melanoma, and Sarcoma 180, a spontaneous pleomorphic cell sarcoma, were carried in BALB/c mice. Finally, C3HBA, a spontaneous adenocarcinoma, and 6C3HED, an induced lymphosarcoma, were carried in C3H/HeJ mice. Tumor lines were transplanted by s.c. injection of a cell preparation obtained by mincing tumor growths.

Polynucleotides. The synthetic polynucleotides, poly(A), poly(U), poly(G), and poly(C), were obtained from P-L Biochemicals, Milwaukee, Wis., and were the substrates for the nuclease assay at concentrations of 2.5 mg/ml.

Preparation of Cell-free Tissue Homogenates. Mice were sacrificed and organs from several mice were removed immediately and homogenized at 4° in homogenizers with Teflon pestles containing 6 ml Tris buffer (0.2 M, pH 7.5). Either 10 thymuses or 4 spleens were processed. Cell debris was removed by centrifugation for 15 min at 10,000 x g, and supernatant solutions were stored at —70°. Plasma was separated from whole blood that had been collected from the retroorbital plexus of anesthetized mice. The blood of 5 animals was pooled in the presence of EDTA (0.005%) and centrifuged at 1500 x g for 15 min.

Determination of Protein. The protein concentration of samples was measured by UV absorption (7), and the values obtained compared favorably to those determined by the fluoresceamine assay (11). Samples for which nuclease activity was to be compared were adjusted by the addition of buffer to equivalent absorbances at 280 nm. Cell-free samples were assayed generally at a concentration of 5 to 10 mg protein per ml. Plasma samples required no adjustment.

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2 The abbreviations used are: poly(A), polyadenylic acid; poly(U), polyuridylic acid; poly(G), polyguanylic acid; poly(C), polycytidylic acid.
**Assay of Nuclease Activity.** Nuclease activity was measured according to the method of Kalnitsky et al. (6). A typical incubation mixture contained 0.1 ml sample, 0.1 ml nucleic acid (2.5 mg/ml), 0.3 ml H2O, and 0.5 ml Tris-HCl (0.2 M), pH 7.5 (8). Controls consisted of a sample blank (containing no nucleic acid) and a substrate blank (containing no sample). These were handled identically to experimental mixtures. Nuclease activity was determined in a 15-min incubation at 37°, after which the reaction was terminated by 2.0 ml cold 12% perchloric acid containing 20 mm lanthanum nitrate (10). After cooling for 15 min at 4°, samples were centrifuged at 3000 x g, and the amount of degradation to acid-soluble nucleotides was determined at 260 nm, after the subtraction of both control values. Although RNase activity was determined following various incubation times, only the degradation of polynucleotides occurring in 15 min is presented in Table 1. This corresponded to the linear range of enzyme activity. A unit of RNase activity was defined as an increase of 0.1 unit absorbance at 260 nm under the conditions of the assay. Data were obtained with a Beckman model DB spectrophotometer using a cuvet with a 1-cm path length.

**RESULTS AND DISCUSSION**

The purpose of this study was to examine what changes in nuclease activity occur in lymphoid tissues of tumor-bearing mice and to determine whether such changes might be reflected in the plasma nuclease activity. Cell-free spleen and thymus homogenates were tested for nuclease activity against poly(A), poly(U), poly(G), and poly(C). No activity against poly(G) was observed in any of the preparations, and the degradation of poly(A) was negligible also. Synthetic substrates were used because activity observed against a natural RNA substrate could represent the summation of enzymes with varying specificities. In this fashion, it was hoped that differences would be detected optimally. Cell-free spleen homogenates from tumor-bearing mice exhibited generally elevated activity against either poly(U) or poly(C) (Table 1), except that the nuclease activity observed for C3H mice bearing the adenocarcinoma was depressed. The pattern of RNase activity observed in cell-free thymus homogenates was variable. The predominant type of activity in the thymus was found to be directed against poly(C). Growth of the 2 tumors in the C3H/HeJ strain resulted in a

### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Tumor Type</th>
<th>Substrate</th>
<th>Spleen* (units/mg)</th>
<th>Thymus* (units/mg)</th>
<th>Plasma* (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>Poly(U)</td>
<td>3.0 ± 0.2c</td>
<td>4.1 ± 0.7</td>
<td>708 ± 38</td>
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<tr>
<td></td>
<td>Poly(C)</td>
<td>7.2 ± 0.6</td>
<td>16.4 ± 1.1</td>
<td>1120 ± 105</td>
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<td>C57BL/6</td>
<td>Poly(U)</td>
<td>8.5 ± 0.9 (&lt;0.001)$!$</td>
<td>2.8 ± 0.8 (&lt;0.05)$!$</td>
<td>1900 ± 50 (&lt;0.001)$!$</td>
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<tr>
<td></td>
<td>Poly(C)</td>
<td>11.4 ± 1.1 (&lt;0.001)$!$</td>
<td>14.4 ± 1.1 (&lt;0.05)$!$</td>
<td>1500 ± 100 (&lt;0.02)$!$</td>
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<tr>
<td>C57BL/6</td>
<td>Poly(U)</td>
<td>5.1 ± 0.5 (&lt;0.001)$!$</td>
<td>3.6 ± 1.1 NS$!$</td>
<td>1283 ± 39 (&lt;0.001)$!$</td>
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<tr>
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<td>Poly(C)</td>
<td>12.4 ± 1.1 (&lt;0.001)$!$</td>
<td>25.8 ± 1.4 (&lt;0.001)$!$</td>
<td>1800 ± 200 (&lt;0.01)$!$</td>
<td></td>
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<tr>
<td>C3H/He</td>
<td>Poly(U)</td>
<td>6.2 ± 0.8</td>
<td>4.4 ± 1.1</td>
<td>688 ± 12</td>
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<td>Poly(C)</td>
<td>6.4 ± 0.3</td>
<td>14.4 ± 1.1</td>
<td>1033 ± 152</td>
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<td>C3H/He</td>
<td>Poly(U)</td>
<td>10.5 ± 0.5 (&lt;0.001)$!$</td>
<td>3.6 ± 1.1 NS$!$</td>
<td>1816 ± 104 (&lt;0.001)$!$</td>
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<tr>
<td></td>
<td>Poly(C)</td>
<td>18.9 ± 0.5 (&lt;0.001)$!$</td>
<td>3.6 ± 1.1 (&lt;0.001)$!$</td>
<td>1200 ± 200 NS$!$</td>
<td></td>
</tr>
<tr>
<td>C3H/He</td>
<td>Poly(U)</td>
<td>1.5 ± 0.3 (&lt;0.001)$!$</td>
<td>4.6 ± 1.5 NS$!$</td>
<td>1283 ± 104 (&lt;0.001)$!$</td>
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<tr>
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<td>Poly(C)</td>
<td>4.6 ± 0.8 (&lt;0.001)$!$</td>
<td>4.0 ± 0.7 (&lt;0.001)$!$</td>
<td>1200 ± 175 NS$!$</td>
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<tr>
<td>BALB/c</td>
<td>Poly(U)</td>
<td>6.4 ± 0.6</td>
<td>6.2 ± 1.4</td>
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<td></td>
<td>Poly(C)</td>
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<td>18.4 ± 1.2</td>
<td>1000 ± 50</td>
<td></td>
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<tr>
<td>BALB/c</td>
<td>Poly(U)</td>
<td>6.5 ± 0.4 NS$!$</td>
<td>7.4 ± 1.2 NS$!$</td>
<td>1716 ± 76 (&lt;0.001)$!$</td>
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<td>Poly(C)</td>
<td>16.2 ± 1.4 (&lt;0.001)$!$</td>
<td>16.4 ± 0.5 (&lt;0.01)$!$</td>
<td>1100 ± 100 NS$!$</td>
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<tr>
<td>BALB/c</td>
<td>Poly(U)</td>
<td>10.6 ± 0.8 (&lt;0.001)$!$</td>
<td>8.0 ± 1.0 NS$!$</td>
<td>1925 ± 66 (&lt;0.001)$!$</td>
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<tr>
<td></td>
<td>Poly(C)</td>
<td>17.2 ± 1.9 (&lt;0.001)$!$</td>
<td>18.4 ± 1.1 NS$!$</td>
<td>1067 ± 115 NS$!$</td>
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</table>

* Five experiments.
* Three experiments.
* Mean ± S.D.
* Numbers in parentheses, p value.
* NS, not significant.
depression in poly(C) activity. Growth of the BALB/c tumors resulted in no significant changes in thymus-associated activity.

Although it was hoped that some of the changes in nuclease activity observed for the lymphoid organs might be reflected in the plasma, no correlation was observed. Activity against poly(U) in the plasma was elevated in all cases. Such increases were generally comparable to the increases in activity noted in the spleen against poly(U), except that the depression of spleen-associated nuclease activity for C3HBA adenocarcinoma and the insignificant increase in spleen-associated activity for the Sarcoma 180 cell sarcoma broke the pattern. The lack of any apparent correlation among the nuclease activities observed for the spleen, thymus, and plasma of tumor-bearing mice compares favorably with the variety of changes observed in other tumor models (9). Such changes may merely represent changes secondary to metastases in the lymphoid tissues. Because the RNase activity directed against poly(U) was elevated in the plasma of all tumor-bearing mice tested, this type of approach may prove to be a helpful clinical assay in some circumstances or a useful device for the monitoring of the progression of tumor during therapy.

REFERENCES

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