Acid Hydrolase Activity in the Leukocytes of Tumor-bearing Rats

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SUMMARY

Acid hydrolase activities were measured in separated lymphocytes, polymorphonuclear leukocytes, and the total leukocyte population of normal and tumor-bearing rats. Only aryl sulfatase activity of the lymphocytes from tumor-bearing rats was significantly increased. Those showing significant decreases in tumor bearers were cathepsin in polymorphonuclear leukocytes and β-galactosidase and acid phosphatase in both cell types. The activity per unit volume of blood was increased in the tumor-bearing rats for all enzymes except acid phosphatase. The increase in activity per unit volume of blood in the tumor-bearing animals was attributed to an increase in total leukocytes and a higher percentage of polymorphonuclear leukocytes.

INTRODUCTION

The cytoplasmic granules found in leukocytes are membrane-bound structures and contain various digestive enzymes. These granules have most of the same acid hydrolases found in the granules of liver cells and seem to qualify fully as lysosomes (9, 11, 25). A disappearance of the granules has been observed after phagocytosis of bacteria or other particles by leukocytes (8, 24).

Lysosomes occur not only in granulocytes but also in lymphoid tissue (3) and lymphocytes (5). There is evidence that several distinctly different populations of lysosomes are present in leukocytes (2). The lysosomes of the leukocytes show altered acid hydrolase activity or abnormal staining reactions during bacterial (17) or viral infections (15) or with other types of tissue injury (6, 10, 12, 19, 26). We, therefore, wanted to see if an alteration of acid hydrolase activities occurred in the leukocytes during tumor growth and to determine if the lymphocytes or PMN1 leukocytes were the cells responsible.

MATERIALS AND METHODS

Animals and Tumors. Female Holtzman rats weighing 180 to 250 g were maintained at 72°F with 12 hr of light and 12 hr of darkness and fed Purina rat chow and water ad libitum. The tumors used were Jensen sarcoma and Walker carcinoma 256, and they were transplanted by injecting 0.2 ml of a 50% suspension of tumor cells into the rectus femoris. The suspension was prepared by forcing viable tumor tissue through a 30 mesh stainless steel wire cloth in the barrel of a syringe. The tumors used were Jensen sarcoma and Walker carcinoma 256, and they were transplanted by injecting 0.2 ml of a 50% suspension of tumor cells into the rectus femoris. The suspension was prepared by forcing viable tumor tissue through a 30 mesh stainless steel wire cloth in the barrel of a syringe. The tumors used were Jensen sarcoma and Walker carcinoma 256, and they were transplanted by injecting 0.2 ml of a 50% suspension of tumor cells into the rectus femoris. The suspension was prepared by forcing viable tumor tissue through a 30 mesh stainless steel wire cloth in the barrel of a syringe.

Leukocyte Isolation and Separation. The total blood leukocytes were isolated by a slight modification of the method used on mouse blood by Hammerstrom and Stoner (7). A PVP solution was made by adding 5% PVP and 5 units/ml of sodium heparin to a 0.9% sodium chloride solution and adjusting the pH to 7 with 1.0 N disodium phosphate. Blood from the heart of anesthetized rats was collected into a heparin syringe. The whole blood was added to a tube containing an equal volume of the 5% PVP solution and mixed by inverting the tube. After 20 to 50 min, a demarcation between the erythrocytes and leukocytes was clearly visible. The top layer containing the leukocytes was removed and centrifuged for 10 min at 2000 x g. The leukocytes were resuspended in 0.9% NaCl solution, and 3 volumes of distilled water were added to rupture the remaining erythrocytes. Within 30 sec after the addition of the distilled water, 1 volume of 3.5% sodium chloride was added to restore isotonicity. The leukocyte preparation was then centrifuged and diluted to the desired concentration.

For separation of lymphocytes and PMN, modifications of the procedures of Johnson and Garvin (13) and Rabinowitz (20) were used. A column (1.8 x 31 cm) fitted with a water jacket maintained at 37° was used. Three g of glass wool were packed tightly in the bottom of the column, which was then filled with 3-mm glass beads. Heparinized blood from 5 to 10 rats was added to the column. The erythrocytes and lymphocytes flowed through the column and those that remained were washed through with 200 to 300 ml of heparinized 0.9% NaCl solution. The lymphocytes were isolated from this solution by the PVP procedure described above. The PMN cells were eluted from the column with a buffered EDTA solution (20) which was allowed to remain on the column for 3 hr, followed by 2 or 3 washings with the same solution. Leukocyte counts and slides for differential counting were made before and after separation. The recovery of lymphocytes from the column for normal and tumor-bearing animals averaged 60 ± 6% of those present in whole blood. The recovery of PMN cells was 22 ± 5% for normal rats, 48 ± 7% for Walker tumor-bearing rats, and 33 ± 5% for rats bearing the Jensen tumor.

Enzymatic Activity. The leukocytes were stored overnight at 4° in 0.9% NaCl solution. They were frozen and thawed rapidly 5 times; Triton X-100 was added to 0.2% concentration, followed by 20 strokes with a Potter-Elvehjem tissue homogenizer. The activities of cathepsin (1), aryl sulfatase (21), β-galactosidase (22), and acid phosphatase (16) were assayed by previously described methods (14). The activities are expressed as μmoles of product/1 X 10^9 leukocytes/min.

1 The abbreviations used are: PMN, polymorphonuclear; PVP, polyvinylpyrrolidone.

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Table 1

Leukocyte numbers and differential counts in tumor-bearing rats

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. of groups</th>
<th>Body weight (g)</th>
<th>Tumor age (days)</th>
<th>Tumor weight (g) (cu mm X 10³)</th>
<th>Leukocyte differential (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L</td>
</tr>
<tr>
<td>None</td>
<td>20</td>
<td>227 ± 5d</td>
<td>0</td>
<td>0</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>Walker</td>
<td>8</td>
<td>232 ± 4</td>
<td>6</td>
<td>24 ± 1</td>
<td>16.3 ± 0.8</td>
</tr>
<tr>
<td>Walker</td>
<td>6</td>
<td>259 ± 7</td>
<td>13</td>
<td>76 ± 4</td>
<td>34.0 ± 1.1</td>
</tr>
<tr>
<td>Jensen</td>
<td>8</td>
<td>213 ± 4</td>
<td>7</td>
<td>24 ± 3</td>
<td>15.5 ± 0.6</td>
</tr>
<tr>
<td>Jensen</td>
<td>6</td>
<td>244 ± 5</td>
<td>14</td>
<td>64 ± 4</td>
<td>20.6 ± 0.7</td>
</tr>
</tbody>
</table>

*Each group contained 5 to 10 rats.

bMean ± S.E.

dTo be multiplied by 10³.

Table 2

The acid hydrolase activity in the total, lymphocyte, and PMN leukocyte populations of normal and tumor-bearing rats

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Leukocyte preparation used</th>
<th>Activity (μmoles product/1 X 10¹⁰ cells/min)</th>
<th>No. of groups</th>
<th>Aryl sulfatase</th>
<th>β-Galactosidase</th>
<th>Acid phosphatase</th>
<th>Cathepsin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>20</td>
<td>4.7 ± 0.3</td>
<td>3.4 ± 0.3</td>
<td>301 ± 19</td>
<td>31.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte</td>
<td></td>
<td>18</td>
<td>4.8 ± 0.5</td>
<td>3.6 ± 0.3</td>
<td>483 ± 38</td>
<td>30.5 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>PMN</td>
<td></td>
<td>18</td>
<td>21.5 ± 1.6</td>
<td>5.9 ± 1.3</td>
<td>79 ± 12</td>
<td>43.1 ± 3.4</td>
</tr>
<tr>
<td>Walker</td>
<td>Total</td>
<td></td>
<td>14</td>
<td>12.5 ± 1.9</td>
<td>2.2 ± 0.3</td>
<td>141 ± 22</td>
<td>27.3 ± 3.5</td>
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<tr>
<td></td>
<td>Lymphocyte</td>
<td></td>
<td>14</td>
<td>9.2 ± 1.2</td>
<td>2.8 ± 0.3</td>
<td>340 ± 43</td>
<td>29.3 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>PMN</td>
<td></td>
<td>14</td>
<td>22.8 ± 1.4</td>
<td>1.9 ± 0.3</td>
<td>44 ± 6</td>
<td>17.4 ± 1.1</td>
</tr>
<tr>
<td>Jensen</td>
<td>Total</td>
<td></td>
<td>13</td>
<td>11.4 ± 0.7</td>
<td>2.3 ± 0.3</td>
<td>93 ± 9</td>
<td>33.6 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte</td>
<td></td>
<td>13</td>
<td>6.0 ± 0.6</td>
<td>2.4 ± 0.3</td>
<td>334 ± 44</td>
<td>34.8 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>PMN</td>
<td></td>
<td>13</td>
<td>21.7 ± 1.5</td>
<td>2.3 ± 0.3</td>
<td>66 ± 14</td>
<td>21.8 ± 1.3</td>
</tr>
</tbody>
</table>

RESULTS

A description of the normal and tumor-bearing rats from which the leukocytes were obtained for enzymatic assay is given in Table 1. The number of leukocytes per unit of blood increased approximately 2-fold in the 6- or 7-day-old Walker or Jensen tumor-bearing rats. This was due in a large degree to the multiplication of neutrophils in the blood stream of the tumor-bearing animals. With older and larger tumors, the host rats had an increase in total leukocytes which appeared to be due to increases in both lymphocytes and PMN leukocytes.

After separation of the lymphocytes and PMN leukocytes, the cells were smeared on glass slides, and Wright's stain was used in making differential counts. In normal rats, the lymphocyte preparation averaged 95% lymphocytes, with a range from 88 to 100%, whereas the PMN preparation had an average contamination of 6% lymphocytes. In the tumor-bearing animals, with older and larger tumors, the host rats had an increase in total leukocytes which appeared to be due to increases in both lymphocytes and PMN leukocytes.

As tumor growth occurs, the activity of acid hydrolases from the leukocytes of normal rats and those bearing the Walker or Jensen tumors is shown in Table 2. The aryl sulfatase activity of 1 × 10¹⁰ total leukocytes from normal rats was similar to the activity found in the same number of lymphocytes. The activity found in the PMN leukocytes was 4-fold higher, but due to the small percentage of these cells they did not have a corresponding effect on the total activity. Both the Walker and Jensen tumor bearers had a 2-fold increase over the normal animals in the aryl sulfatase activity in the total leukocyte population. This was due in part to an increase in the activity of the lymphocytes and in part to an increase in the percentage of PMN's in the total cell population. The aryl sulfatase activity per 1 × 10¹⁰ PMN leukocytes was not significantly altered during tumor growth.

In normal rats, the β-galactosidase activity was found to be higher in PMN leukocytes than in the lymphocytes. During tumor growth the activity decreased significantly for both lymphocytes and PMN leukocytes, the latter having the most pronounced change.

Acid phosphatase activity was higher in lymphocytes than in PMN leukocytes in both normal and tumor-bearing rats. The activities were lower than normal for both cell types in the tumor bearers, and this contributed, along with the shift to higher PMN percentages, to the decline in activity of the total cell population in these animals.

In normal rats, the PMN leukocytes had a slightly higher cathepsin activity than the lymphocytes. In the tumor-bearing animals, there was a significant decrease of the cathepsin...
activity in the PMN leukocytes. This decrease did not cause a significant change in the cathepsin activity of the total leukocyte population in the tumor-bearing rats.

**DISCUSSION**

There were significant alterations in the acid hydrolase activities of the leukocytes during tumor growth in the rat. Aryl sulfatase increased in lymphocytes, cathepsin decreased in PMN leukocytes, and β-galactosidase and acid phosphatase decreased in both cell types. No change in activity occurred for cathepsin in lymphocytes and for aryl sulfatase in PMN's. A large part of the change in activity in the total leukocyte population of the tumor-bearing rats could be attributed to the increased percentage of PMN leukocytes. The activity per unit volume of blood was increased for all enzymes, except acid phosphatase, due to the increase in the total number of leukocytes.

It is generally accepted that some patients with cancer are more susceptible to bacterial infection. One defense mechanism which has been shown to be altered is the phagocytosis of bacteria by leukocytes from these patients (4, 18). Another factor which could contribute to increased susceptibilities to bacterial infection would be an alteration in the intracellular killing. The acid hydrolases from the leukocyte lysosomes represent one possible mechanism of bacterial destruction. The decrease in 3 of the 4 acid hydrolases measured in these experiments may therefore be an indication of a reduced capacity for intracellular digestion of foreign materials.

Selvaraj et al. (23) studied respiratory activity, glucose oxidation, and lactate production and found that overall metabolism of leukocytes from patients with lymphoproliferative disorders was lower than normal on a per cell basis. However, many of the metabolic activities of leukocytes from these patients were normal when presented on the basis of unit blood volume. The acid hydrolase activities reported herein are also normal or above normal activity when expressed on the basis of leukocytes per unit volume of blood.

**REFERENCES**

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