Induced Susceptibility and Resistance to an Indigenous Transplantable Mouse Tumor with Autolyzed and Alcohol-killed Tumor Tissue*

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In a previous paper (3) describing experiments in which DBA/1 female mice and the transplantable dbrB adenocarcinoma indigenous to that strain were used, it was demonstrated that the inoculation of living tumor cells resulted in a substantial increase in resistance to subsequent tumor implants. Under certain conditions the technic employed rendered the animals completely refractory to secondary implants.

Earlier unpublished experiments in our laboratory demonstrated that the subcutaneous inoculation into mice of tumor tissue stored for 24 hours at 37.5° C. resulted in an increased growth of subsequent tumor implants. These suspensions which do not produce tumors upon inoculation have been referred to as "autolyzed." Using the ZBC mouse and a Z(C3H) tumor, Miroff et al. (2) found a growth-accelerating effect from the tumor tissue heated at 70° C. for 1 hour.

Since suspensions of living tumor cells had elicited resistance and cell suspensions of tumors stored for 24 hours had increased the susceptibility of the host, the effect of storage for periods varying from 1 to 24 hours has been investigated. Thus, at one extreme (tissue inoculated 1 hour after removal from the host) mice received viable tissue known to produce tumors and induce resistance, and at the other extreme (24 hours' storage) mice received autolyzed tissue known to produce no tumors and to increase the susceptibility of the host.

Because injured cells produce comparatively large amounts of growth-stimulating substances, in contrast to cells that have not been injured and cells that have been killed quickly (Fardon et al. [1]), it was thought that enhanced growth resulting from the use of "autolyzed" tumor suspensions might be due to the production of growth-stimulating substances by the slowly dying tumor cells. To test this hypothesis, suspensions of tumor cells killed quickly by immersion in ethyl alcohol were used in one series of experiments.

In the present experiments no attempt has been made to influence the progress of the growing tumors. These investigations have been confined to a study of the resistance and susceptibility of the treated host to subsequent tumor implants.

MATERIALS AND METHODS

DBA/1 female mice under 1 year of age and the dbrB mammary carcinoma obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were used throughout these experiments. Details of animal and tumor maintenance have been given elsewhere (3).

Autolyzed tumor tissue.—Suspensions of the fresh tumor were prepared by careful pressing of selected necrosis-free portions of tumor through a monel metal screen having 0.2-sq. mm. openings; 0.5 cc. of the dispersed tissue suspended in 9.5 cc. of Tyrode's solution was placed in a 15-ml. centrifuge tube, stoppered tightly, then placed in an incubator at 37.5° C., undisturbed, for the desired period of time. The incubation time for a given suspension was terminated by immersing the tube in ice water for 5 minutes. If any delay was encountered between this step and the next, the suspension was held at 4° C.

For safe intravenous injection and uniform particle size at high dilution, the incubated suspensions were gently screened through "nylon tricot jersey" cloth. This technic has been previously described in detail (3).

In the first series of experiments, four groups of animals were treated with tumor tissue which had been incubated for 1, 5, 10, and 24 hours, respectively. In a second series of experiments, mice

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were treated with suspensions incubated for 5, 6, 7, 8, and 10 hours.

**Alcohol-killed tumor tissue.**—Small blocks of the fresh tumor, 1–2 cu. mm. in size, were immersed in 70 per cent ethyl alcohol for 30 minutes at room temperature. Upon removal, the tissues were washed thoroughly in Tyrode’s solution to remove the alcohol before passage through the monel metal screen and nylon cloth in the manner described above for the autolyzed tissue. Tissue treated in this manner was considered dead, since it failed to produce tumors when injected into mice even in large doses. Further evidence which tends to confirm the fact that the alcohol-treated cells are not living was deduced from methylene blue staining and tissue culture tests.

**Dosages.**—All inoculated suspensions from fresh or modified tissue were diluted 1 part tissue in 1500 parts Tyrode’s solution, according to a previously described technic (8). The experimental mice were treated intravenously through a vein in the tail with 0.05 cc. of the modified tissue. The challenge dose of fresh tumor suspension was likewise 0.05 cc., being given subcutaneously in the dorsum. Control mice received only the challenge implant of tumor.

**Interval between treatment and challenge dosages.**—The test animals in the first experiment were divided into two groups—the first receiving the challenge dose of viable tissue subcutaneously 4 days after the intravenous administration of the incubated tissue, the second group receiving the challenge dose 18 days after the initial intravenous treatment (Table 1).

In the experiment with alcohol-killed tumor tissue the interval between treatment and challenge ranged from 17 to 19 days (Table 2).

**Observations.**—Growth of subcutaneous challenge tumors was determined by measurement over three dimensions with vernier calipers beginning on the 15th day and at intervals through the 30th day post-transplant and at the death of the mouse. In the second series of experiments with the incubated tumor tissue (Table 3) the mice were sacrificed on the 19th day after the challenge implant in order that the lungs might be examined for the presence of tumors. In all cases the lungs were examined at necropsy for lung tumors.

**RESULTS**

The results indicate that the time of incubation of the immunizing dose as well as the period between the immunizing and challenge doses are important in determining the inhibition or stimulation of the growth of challenge tumors.

For example, it can be seen from the size of the challenge tumors in the group inoculated 18 days after the immunizing dose was given (Table 1)

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**TABLE 1**

**The Effect of Incubation Time of Immunizing Material and Length of Immunizing Period on Challenge Tumor Growth**

<table>
<thead>
<tr>
<th>Incubation Time (Hours)</th>
<th>No. Mice with Tumors</th>
<th>Volume (cc.)</th>
<th>No. Mice with Tumors</th>
<th>Volume (cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.10</td>
<td>3</td>
<td>0.32</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1.56</td>
<td>5</td>
<td>3.52</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>2.55</td>
<td>5</td>
<td>5.51</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>0.45</td>
<td>5</td>
<td>1.55</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* One of the five original mice died with tumor on the 18th day.
† Challenge tumor negative.
that, in general, the longer the incubation period of the immunizing dose, the less the resulting inhibition. It will be further noted that the 24-hour incubated tissue resulted in stimulation rather than inhibition. By comparing the 4- and 18-day immunizing periods (Table 1) it will be seen that the longer period (18 days) gave a substantially higher degree of resistance.

The experiments in which the incubation time of the tumor suspension was varied from 5 to 10 hours (Table 3) gave results similar to those of the first series of experiments with the 18-day immunizing period (Table 1).

It is of interest to note that, in the preceding experiments, the resistance effect was more pronounced in the mice with pulmonary tumors than in those without such tumors. From this it might be concluded that resistance can be induced only by the inoculation of living cells. That substances other than living tissue can induce resistance is illustrated in the series of experiments in which alcohol-killed tumor tissue was used as the immunizing agent. It will be noted (Table 2) that a moderate degree of resistance resulted from the injection of a suspension prepared from tumor tissue immersed in 70 per cent alcohol for 30 minutes, a period sufficient to kill all cells.

**SUMMARY**

1. The injection of dbdB tumor tissue could result in either induced resistance or increased susceptibility of the host, depending upon the time the

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**TABLE 2**

**IMMUNIZING EFFECT OF ALCOHOL-KILLED TUMOR TISSUE**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. Mice</th>
<th>15th Volume</th>
<th>20th Volume</th>
<th>21st Volume</th>
<th>22nd Volume</th>
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<tr>
<td>I</td>
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<td>7</td>
<td>0.01</td>
<td>8</td>
<td>0.27</td>
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<td>10</td>
<td>7</td>
<td>0.00</td>
<td>9</td>
<td>0.95</td>
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<tr>
<td>II</td>
<td>10</td>
<td>10</td>
<td>0.34</td>
<td>10</td>
<td>1.41</td>
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<tr>
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<td>10</td>
<td>10</td>
<td>0.44</td>
<td>10</td>
<td>1.65</td>
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<tr>
<td>III</td>
<td>10</td>
<td>9</td>
<td>0.21</td>
<td>9*</td>
<td>0.51</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>9</td>
<td>0.19</td>
<td>9*</td>
<td>1.01</td>
</tr>
</tbody>
</table>

* All of the ten original mice died with tumors.

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**TABLE 3**

**THE EFFECT OF INCUBATION TIME OF IMMUNIZING MATERIAL ON PULMONARY TUMOR INCIDENCE AND CHALLENGE TUMOR GROWTH**

<table>
<thead>
<tr>
<th>Incubation Time (Hours)</th>
<th>No. Mice</th>
<th>Pulmonary Tumors</th>
<th>Challenge Tumors</th>
<th>Av. Volume, cc.</th>
<th>Group Av.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>No. Mice</td>
<td>With</td>
<td>Without</td>
<td></td>
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<tr>
<td>5</td>
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<td>2</td>
<td>2</td>
<td>0.31</td>
<td>0.12</td>
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<tr>
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<td>4</td>
<td>1</td>
<td>3</td>
<td>0.53</td>
<td>0.40</td>
</tr>
<tr>
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<td>7</td>
<td>2</td>
<td>3</td>
<td>0.21</td>
<td>0.33</td>
</tr>
<tr>
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<td>8</td>
<td>3</td>
<td>2</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
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<td>4</td>
<td>4</td>
<td>2</td>
<td>1.07</td>
<td>1.07</td>
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<tr>
<td>Control</td>
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<td>4</td>
<td>4</td>
<td>0.92</td>
<td>0.92</td>
</tr>
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</table>

* One mouse displayed neither pulmonary nor challenge tumors.
tissue was permitted to stand in the incubator prior to injection. Specifically, mice challenged 4 days after treatment, with tumor tissue permitted to incubate at 37.5°C for 1–5 hours, showed resistance, while those inoculated with tumor tissue incubated for longer periods of time (5–24 hours) showed increased susceptibility to a challenge tumor.

When mice were challenged 18 days after the initial treatment, this difference between resistance and susceptibility was not evidenced until the tissue had been incubated for 24 hours. The reason for this is not understood.

2. The inoculation of dbBR tumor tissue which has been killed by immersion in alcohol for 30 minutes results in a demonstrable increase in the resistance of DBA/1 mice to this tumor, if the challenge dose is sufficiently small.

REFERENCES


Induced Susceptibility and Resistance to an Indigenous Transplantable Mouse Tumor with Autolyzed and Alcohol-killed Tumor Tissue

John E. Prince, Carl M. Morgan, John C. Fardon, et al.


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