Induced Resistance to an Indigenous Transplantable Mouse Tumor*

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Although the literature contains many references to successful attempts to immunize animals against transplantable tumors, few deal with transplantable spontaneous tumors in inbred strains, and, in general, the results of these latter experiments have been negative.

Aptekman and his co-workers (1-3), in a series of studies with an alcohol-soluble extract of methylcholanthrene-induced transplantable rat sarcoma, produced oncolysis of tumors that arose in rats of their own inbred strain. A greater percentage of the animals so treated were resistant to subsequent implants of these tumors. However, these investigators failed to induce resistance in a recognized pure strain of mice using a transplantable spontaneous tumor.

In 1943 Gross (7), using intradermal injections of living tumor cells, reported the induction of resistance in the inbred C3H strain of mice against a transplantable sarcoma induced by a carcinogen in that strain. However, with the same technique, he failed to demonstrate resistance in that strain to a transplantable spontaneous carcinoma known to have arisen in the C3H mice.

Foley (6), by total ligation of established tumors, produced their regression and induced resistance in C3H mice to a tumor initiated in that strain with methylcholanthrene. Foley pointed out that results in experiments on resistance to tumors produced by carcinogens are subject to the criticism that the tumor cells may have undergone genetic changes. Foley (5), like Gross, failed to demonstrate resistance in an inbred strain of mice to an indigenous mammary carcinoma.

In our laboratory, Fardon and Prince (4), using the method of tumor ligation described by Lewis and Aptekman (10, 11), attempted unsuccessfully to induce resistance in DBA/1 mice to the indigenous dbrB adenocarcinoma.

The present report deals with successful attempts to induce resistance in an inbred strain of animals to a transplantable indigenous tumor. These experiments include studies of the effect of size of the dose of immunizing agent and the quantity of challenge tumor material used.

The importance of the size of the challenge dose has already been emphasized by Gross (8). In one series of our experiments the immunizing material was administered subcutaneously in the tail, and in another series the immunizing dose was given intravenously. In all experiments the site selected for administration of the second or challenge dose of tumor tissue was under the skin on the dorsum.

MATERIALS AND METHODS

**Animals and tumors.**—Female DBA/1 mice under 1 year of age were used in these experiments and were procured as young adults 4-12 weeks old from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The dbrB adenocarcinoma was secured from the same laboratory and was maintained in the DBA/1 strain by routine transplants made subcutaneously into the groin in 0.1- and 0.2-cc. quantities of 5-25 per cent suspensions of tumor tissue. Each mouse was housed in an individual cage with food and water supplied ad libitum.

**Tumor tissue suspensions, types A and B.**—Two standard types of suspensions were prepared differing in the size and number of cell aggregates comprising them. All procedures were routinely conducted under aseptic conditions, and only selected portions of non ulcerated tumors with a minimum of necrosis were used.*

The larger aggregates of the Type A suspension were formed by pressing the tumor tissue through a monel metal screen with openings approximately 0.2 sq. mm., with 1 ml. of Tyrode's solution used as the dispersion medium. Three dilutions were made from these aggregates, and the one giving the best results was used in experiments. *The tissue-dispersing screen may be obtained from C. O. Jelliff Corp., Southport, Conn.

2The technical assistance of Dr. A. G. Duarte is herewith acknowledged.

3A metal spatula with a rounded bend 1.6 cm. from the tip was used to exert a rolling action on the tissue against

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1 Personal communication, M. R. Lewis.

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tions of this suspension were prepared containing 1 part of the dispersed tissue in 2, 50, and 500 parts, respectively, of Tyrode’s solution.

The smaller cell aggregates for the Type B suspension were obtained by dilution of the concentrated Type A suspension to 5 per cent with Tyrode’s solution in a 10-cc. syringe. The suspension was gently discharged from this syringe onto a nylon tricot jersey cloth and filtered by gravity to screen out the larger cell aggregates. The concentration of this Type B suspension was determined by centrifuging a small sample (later discarded) in a protein sedimentation tube, calibrated in 0.01 ml., for 15 minutes at 2,400 r.p.m. The figure thus obtained for the packed material per unit volume served as the basis for calculating the dilution of the noncentrifuged portion of the suspension for experimental use. The dilutions of the Type B suspensions used in these experiments were 1 part in 1,500 and 1 part in 3,000 of Tyrode’s solution. The cell aggregates in

Dosages in control animals.—Control animals of the same age as the experimentals were given only the challenge dose of tumor tissue (Tables 1 and 2). To insure uniformity, in each experiment the inoculations were alternated between the control and experimental animals in such a way that the first and last recipients of the challenge implants were in the control group.

Tumor volume determinations.—The volume of the subcutaneous tumors on the tail and on the dorsum was determined by measuring the tumor mass in its three dimensions. The lungs of animals developing pulmonary tumors after intravenous treatment were removed and immersed in 10 per

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>SUBCUTANEOUS IMMUNIZATION</strong></td>
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<table>
<thead>
<tr>
<th>SERIES</th>
<th>GROUP</th>
<th>INITIAL NO. MICE</th>
<th>PRIMARY SUBCUTANEOUS TREATMENT (0.05 cc.)</th>
<th>NO. MICE WITH PRIMARY TUMORS ON 28TH DAY</th>
<th>CHALLENGE SUBCUTANEOUS DRESS AT 28 DAYS (0.05 cc.)</th>
<th>SURVIVORS 28 DAYS POST-CHALLENGE TUMORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>10</td>
<td>1 in 2</td>
<td>5</td>
<td>1 in 500</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>10</td>
<td>1 in 2</td>
<td>9</td>
<td>1 in 500</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>10</td>
<td>1 in 50</td>
<td>9</td>
<td>1 in 500</td>
<td>6</td>
</tr>
<tr>
<td>(Control)</td>
<td>6</td>
<td>5</td>
<td>1 in 50</td>
<td>9</td>
<td>1 in 500</td>
<td>6</td>
</tr>
</tbody>
</table>

* The interval between the primary and challenge dose was 28 days.

† Three additional animals developed tail tumors by the 40th day after the primary treatment (12th day after administration of the challenge dose).

these Type B suspensions were sufficiently small to permit safe intravenous administration. To insure successful implantations with these high dilutions, forced passage of the suspension through small openings had to be kept at a minimum, and vigorous agitation of the suspensions at any time was carefully avoided.

Primary or immunizing dosages.—The total volume of the injected material, regardless of the dilution, was 0.05 cc. Dilutions of 1 in 2 and 1 in 50 parts of Tyrode’s solution (Type A suspension) were given subcutaneously in the tail with subsequent development of tumors at the site of the injection (Table 1). Dilutions of 1 in 1,500 and 1 in 3,000 parts of Tyrode’s solution (Type B suspension) were administered intravenously into the tail, with the subsequent development of tumors in the lungs of a large percentage of the animals (Table 2).

Secondary or challenge dosages.—The total volume of the challenge implant was 0.05 cc., regardless of the dilution, and was administered subcutaneously in the dorsum in both series of experiments (Tables 1 and 2). Twenty-eight days elapsed between the primary and challenge dosages in the subcutaneously immunized series of animals (dilutions Type A, 1 in 50 and 1 in 500, Table 1), and 4, 15, 35, and 29 days in the intravenously immunized series of animals (dilutions Type B, 1 in 1,500 and 1 in 3,000, Table 2). The metal screen to minimize the shearing effect of the slap and drag action usually associated with the use of a spatula.

cent neutral formalin for 24 hours. The tumor masses from each pair of lungs were shelled out and immersed in formalin in a calibrated glass tube. The volume of displaced fluid was noted and recorded as the tumor volume.

RESULTS

Resistance has been demonstrated in an inbred strain of mice (DBA/1) following primary administration of a suspension of the dbB adenocarcinoma, whether the primary treatment was given subcutaneously or intravenously (Tables 1 and 2, Charts 1 and 2).

Primary subcutaneous treatment.—Examination of the data in Table 1 reveals that the average volume for the challenge tumors was smaller than that of their control partners when the more concentrated suspension was used for the primary treatment; the size of the challenge dose was the same for both groups (Groups A-1, B-3, and C-5). In confirmation of the work of Gross, the data show that, with a constant level of the immunizing dose, resistance was masked by the use of the
larger challenge dose (Table 1, A-1, C-5, A-2, and C-6; Chart 1).

No consistent relationship was apparent between the average volumes of the primary and of the challenge tumors (Table 3). For example, in the presence of the smallest primary tumor (Group B-4, Table 3, 0.095 cc., av.), the average volume for the challenge tumors was the largest in the series (0.276 cc.). In contrast, in the presence of the largest primary tumors (Table 3, Group A-2, 3.075 cc., av.), the challenge tumor value was by no means the smallest (0.910 cc., av.). Further, although one animal in Group B-4 (Table 3) failed to develop a challenge tumor, this did not correspond to either of the two animals in this same experimental group that failed to develop tumors after the initial inoculation.

Intravenous inoculation.—Complete resistance to the dbrB adenocarcinoma developed in DBA/1 mice 18–29 days after intravenous inoculation of dilute suspensions (one in 1,500 and one in 3,000 parts of Tyrode's solution) of the tumor (Table 2, Chart 2). Slight resistance was apparent as soon as 4 days after the primary inoculation. Although deaths occurred in six of the experimental animals prior to the 29th day, necropsy indicated that these deaths were due to the overwhelming effect of the primary pulmonary tumors, since there was no evidence of challenge tumors developing in any of these animals.

Despite the fact that the challenge dosages were quite small in these intravenous experiments, it can be seen in Table 2 that they were sufficient to produce tumors of considerable size in each control mouse. Disregarding the time factor, it is also evident from the same table that there is no apparent relationship between the size of the primary tumors and the average volumes of the challenge tumors. For example, in the presence of the smallest primary tumor (Group B-4, Table 3, 0.095 cc., av.), the average volume for the challenge tumors was the largest in the series (0.276 cc.). In contrast, in the presence of the largest primary tumors (Table 3, Group A-2, 3.075 cc., av.), the challenge tumor value was by no means the smallest (0.910 cc., av.). Further, although one animal in Group B-4 (Table 3) failed to develop a challenge tumor, this did not correspond to either of the two animals in this same experimental group that failed to develop tumors after the initial inoculation.

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mary tumor and resistance to the challenge implant. It is of interest to note a progressive decrease in the number of primary tumors, with the increase in the interval between administration of the primary and challenge tumors (4–23 days).

In comparing the results of the subcutaneous and intravenous experiments (Charts 1 and 2), it is apparent that resistance was greater following use of very small amounts of tumor tissue intravenously than when larger amounts of tissue were used subcutaneously.

**DISCUSSION**

It might be argued that in the experiments described in this paper no true resistance has been induced, but that the effects noted are due to the fact that the challenge tumor has been restricted in its growth by a limited energy supply due to the requirements of the initial tumor. However, as stated previously, a comparison of the volumes of primary and challenge tumors shows that no such relation existed.

There is the possibility that (a) the tumor has changed in our laboratory, (b) the tumor in its many transplants at the Roscoe B. Jackson Laboratory has changed, and (c) the DBA/1 strain is not genetically pure.

That the tumor in our laboratory does not differ in this respect from the one now available at the Roscoe B. Jackson Laboratory has been verified by repeating the experiments, with the same results, with a fresh tumor and a new supply of animals.

Even though the other possibilities still remain, these results seem interesting in view of the negative results reported previously (5) with similar animals and tumor.

**SUMMARY**

Partial resistance has been induced in an inbred strain of mice (DBA/1) by the subcutaneous injection, and complete resistance by the intravenous injection, of suspensions of viable dbBr adenocarcinoma, a transplantable tumor indigenous to this strain.

The resistance can be masked by the use of too large a challenge dose. The degree of resistance is dependent on the amount of material injected initially as the immunizing dose in the subcutaneous series of experiments. Intravenous administration is more effective, requiring only a fraction of the material needed for immunization by the subcutaneous route. The resistance to a challenge im-

**TABLE 3**

**SUBCUTANEOUS EXPERIMENTS**

Lack of relationship between volume of primary and challenge tumors

<table>
<thead>
<tr>
<th>SERIES</th>
<th>GROUPTREATMENT</th>
<th>VOLUME (0.05 cc.)</th>
<th>DILUTION</th>
<th>VOLUME 8TH DAY Av. (range)</th>
<th>DILUTION</th>
<th>VOLUME 14TH DAY Av. (range)</th>
<th>TUMOR VOLUME PRIMARY IN TAIL Av. (range)</th>
<th>TUMOR VOLUME CHALLENGE IN DORMIN Av. (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>1 in 2</td>
<td>0.123(0.019–0.784)</td>
<td>1 in 500</td>
<td>1.41(0.46–4.550)</td>
<td>0.03(0.000–0.105)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-1</td>
<td>1 in 50</td>
<td>0.008(0.000–0.046)</td>
<td>1 in 500</td>
<td>0.38(0.001–1.377)</td>
<td>0.076(0.000–0.482)</td>
<td></td>
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</tr>
<tr>
<td>A-2</td>
<td>1 in 2</td>
<td>0.056(0.38–0.750)</td>
<td>1 in 50</td>
<td>3.075(0.32–5.760)</td>
<td>0.210(0.024–0.526)</td>
<td></td>
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</tr>
<tr>
<td>B-4</td>
<td>1 in 50</td>
<td>0.007(0.000–0.024)</td>
<td>1 in 50</td>
<td>0.085(0.000–0.359)</td>
<td>0.276(0.000–0.816)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interval between initial treatment and challenge dose was 28 days.

* One animal in Group B-4 failed to develop a challenge tumor. This did not correspond to either of the two animals that failed to develop primary tumors in this group (see Table 1).
plant of tumor tissue is independent of the size attained by the primary tumor. Resistance to a challenge tumor appears to be, at least in part, the result of a reaction of the host to a primary implant, requiring between 4 and 18 days to become effective.

REFERENCES
8. ———. The Importance of Dosage in the Intradermal Immunisation against Transplantable Neoplasms. Ibid., pp. 770–73.
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