Enhanced Tumor Transplantability Following Second Transplantation in Mouse of Original Strain

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The XYZ factors have been prepared from tumor cells by keeping the tumor tissue frozen for a period of 10 days or longer, either by anaerobic storage (4, 8, 22) or by prolonged desiccation (lyophilization) (4, 24, 25). Ordinarily, tumor cells are killed by this treatment, although the presence of viable tumor cells does not neutralize or diminish the XYZ effect (4, 9, 16, 17). Furthermore, tumor cells have a different thermal death point from the XYZ factors (44° C. for the Z8352 tumor cells and 56° C. for the Z8352 [23] and the Brown-Pearce XYZ factors [13]); and at least two XYZ factors may be obtained from their respective fresh untreated tumors in full potency, by using the supernate from repeated centrifugation for 1 hour at 1200 r.p.m. (14, 22). Despite the presence of the XYZ factors in fresh tumor, a second inoculation of tumor cells 2 weeks after a first does not result in enhanced tumor growth (10). It remains to be proved that the XYZ factors actually influence ordinary tumor growth and spread in the original animal host or even in the inbred strain in which the tumor arose, although it has been proved that the injection of the XYZ factor into the strain of origin 2 weeks before transplant resulted in enhanced tumor growth and earlier mortality—E 0771 mammary carcinoma in C57BL/6Jax mice (11, 12) and Z8352 tumor in C3H mice (23). To ascertain whether the XYZ factors actually influence tumor growth in the inbred strain of origin E 0771/Jax mammary carcinoma was reinoculated into BALB/c mice ordinarily resistant to this neoplasm. As controls BALB/c mice were inoculated with tumor taken from C57BL/6Jax controls singly inoculated with E 0771/Jax.

MATERIALS AND METHODS

Trocar implants of E 0771/Jax into C57BL/6Jax mice resulted in death from tumor growth at 14 days in 50 per cent of the mice. Therefore, emulsions were used when it had been determined that 0.1 cc. of 25 per cent emulsion of E 0771/Jax tumor cells in saline would kill 50 per cent of C57BL/6Jax mice by the 23rd day. The mice, C57BL/6Jax and BALB/c, 3–8 months of age when used, and the tumors (E 0771/Jax, 11–14 days old after trocar implant) were obtained in each experiment from the inbred nucleus at the Jackson Memorial Laboratory at Bar Harbor, Maine. Additional mice, C57BR/cdJax X BALB/cJax, hybrids bred in the Baptist Hospital laboratory, were inoculated in parallel experiments not recorded in detail here. The mice were 3–6 months of age and were divided and caged upon receipt into control and experimental groups. There were three experiments (Table 1), and 0.1 cc. of a saline emulsion of tumor cells was injected subcutaneously into the groin in all instances. Tumors were measured twice weekly in three dimensions with calipers.

Experiment I.—E 0771 tumor (from C57BL/6Jax mouse 5405) implanted by trocar in the Jackson Laboratory 12 days before was suspended in normal saline after aseptic removal; 0.1 cc. of a 0.75 per cent saline cell suspension was injected subcutaneously into the left groin of 20 C57BL/6Jax mice. Twelve days later ten of the mice (those with even numbers) were given subcutaneous injections into the right groin of 0.1 cc. of a 25 per cent cell suspension of E 0771/Jax tumor cells. The tumor (not ulcerated and from C57BL/6Jax mouse 5406) had been injected by trocar at the Jackson Laboratory 12 days before (and from same tumor as 5405). Seventeen days after the second implant of E 0771, a mouse (55064) with good tumors, neither of which was ulcerated, was killed for transfer. The tumor in the left groin (inoculated 29 days before with 17 per cent emulsion) measured 2.55 X 2.45 X 1.85 cm. and that in the right groin 2.05 X 1.35 X 1.25 cm. The latter tumor (second inoculation, 17 days old) was minced, ground in a mortar, and suspended in normal saline, and 0.1 cc. of a 25 per cent cell suspension was injected subcutaneously into the left groin of four BALB/cJax, three C57BR/cdJax.


(resistant to E 0771), and eight C57BR/cd × BALB/c hybrids. Controls for the BALB/c mice were 47 BALB/c mice injected with E 0771 taken from a third C57BL/6Jax mouse implanted by trocar in the Jackson Laboratory.

Experiment II—C57BL/6Jax mouse (5585) given inoculations by trocar at the Jackson Laboratory with E 0771/Jax was killed 11 days after transplant and 0.1 cc. of a 30 per cent suspension of tumor cells injected subcutaneously into the right groin of seven C57BL/6Jax mice, nine BALB/c mice, and ten cd/c (C57BR/cd × BALB/c hybrids). Fourteen days later one of the mice (C57BL/6Jax mouse 5510) with an unulcerated tumor was killed, and 0.1 cc. of 25 per cent tumor cell suspension in normal saline, by grinding in a mortar with saline, was injected subcutaneously into the right groin of five C57BL/6Jax mice (even numbers) inoculated with E 0771/Jax was killed, and the 13-day-old tumor in the left groin, measuring 2.55 × 2.0 × 1.55 cm.; and in right, 1.75 × 1.25 × 1.3 cm.; the tumor from the right groin (second inoculation, 14 days old) was emulsified in normal saline, and 0.1 cc. of a 20 per cent suspension of tumor cells injected subcutaneously in the left groin of eight BALB/c mice and six cd/c mice not previously inoculated.

RESULTS

The results are tabulated (Table 1 and Chart 1). Mice of the C57BL/6Jax strain (in which E 0771 originally arose) had no difference in the cumulative mortality curve in days after inoculation or in

<table>
<thead>
<tr>
<th>TRANSPLANTS INTO C57BL/6JAX MICE</th>
<th>TRANSPLANTS INTO BALB/c MICE</th>
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<tr>
<td>(Original inbred strain)</td>
<td>(Foreign inbred strain)</td>
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<tr>
<td>E 0771 +</td>
<td>E 0771</td>
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<td>Died/Hosts</td>
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<td>Experiment No.</td>
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<td>E 0771</td>
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<td>1/27 3/4</td>
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<td>2</td>
<td>1/9 3/8</td>
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<tr>
<td>3</td>
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<tr>
<td>Subtotal 17/17 18/18 P = 0.001 (sig.)</td>
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<tr>
<td>Other exp. 52/52 Total 69/69 18/18 P = 0.001 (sig.)</td>
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1.05 × 0.9 × 0.9 cm. (the tumor in right groin measured 2.55 × 2.0 × 1.55 cm.), was emulsified in normal saline, and 0.1 cc. of a 20 per cent tumor cell suspension injected subcutaneously into the right groin of five C57BL/6Jax mice (5590–5), eight BALB/c mice (5820–5, 5880–1), and into three cd/c mice (5874–6).

Experiment III.—C57BL/6Jax mouse (5540) inoculated 15 days before by trocar at the Roscoe B. Jackson Memorial Laboratory with E 0771/Jax was killed; the tumor was suspended in normal saline by grinding in a mortar with saline, and 0.1 cc. of a 25 per cent tumor cell suspension was injected subcutaneously in the left groin of ten C57BL/6Jax mice (5575–85) into four BALB/c mice. Afterwards, a C57BL/6Jax mouse (5576) inoculated 15 days prior with E 0771/Jax was killed, and the tumor was emulsified in normal saline and grinding in mortar; 0.1 cc. of a 30 per cent suspension of tumor cells was injected subcutaneously into the right groin of five C57BL/6Jax mice previously inoculated with the E 0771/Jax (Nos. 5577, 5579, 5581, 5583, 5585, odd numbers); and into six C57BL/6Jax mice (5582–81) into five BALB/c and into seven cd/c (C57BR/cd × BALB/c hybrids) not previously inoculated. Approximately 27 days after the first and 14 days after the second inoculation, a C57BL/6Jax mouse (#5580) doubly inoculated with E 0771/Jax tumor was killed (tumor in left groin, 2.55 × 2.0 × 1.55 cm.; and in right, 1.75 × 1.25 × 1.3 cm.); the tumor from the right groin (second inoculation, 14 days old) was emulsified in normal saline, and 0.1 cc. of a 20 per cent suspension of tumor cells injected subcutaneously in the left groin of eight BALB/c and six cd/c mice not previously inoculated.

the percental accelerant index (22), when inoculated only once with E 0771 or when given a second transplant 12–14 days later.

In each of the three experiments E 0771 carcinoma taken from a doubly inoculated C57BL/6Jax mouse grew in and killed 50 per cent or more of the resistant BALB/cJax mice without any treatment whatever. On the other hand, E 0771 carcinoma taken from a second transplant into a C57BL/6Jax mouse; (4) 45 BALB/c and controls transplanted with E 0771 carcinoma taken from a first transplant in C57BL/6Jax mice.
from the doubly inoculated C57BL/6Jax mice. The tumor mortality among these hybrids both in the primary and in the serial transfers was about as great as in the pure line BALB/cJax mice. There were fifteen deaths among 57 hybrids thus inoculated in primary and serial transfer, as compared to one death among 22 hybrid controls (transplanted from a singly inoculated C57BL/6Jax mouse; \( \chi^2 = 4.6, n = 1, P = 0.03, \) probably sig.). In other experiments this hybrid strain has had the same death rate from E 0771/Jax as the pure BALB/cJax (i.e., 3 per cent).

**DISCUSSION**

When the growth of tumors is induced in a foreign resistant mouse strain by the use of trypan blue (1), flannel (15), x-ray (18, 26), cortisone (26), or by repeated hemorrhage (2) the tumors do not become adapted to the new hosts and fail to grow in serial transplants. Only the XYZ factors are known to induce permanent adaptations of a tumor to a foreign strain unless the factor described in CSH hybrids is not an XYZ factor (2, 3). This premise is now being tested by serial transfer of the E 0771 tumor in BALB/cJax and cd/c mice. Thus far, seventeen of 54 BALB/c mice have died in serial passages from E 0771 tumor adapted to BALB/c and cd/c mice in the three experiments herein reported (\( P = 0.01 \), sig.). It would seem, therefore, that an XYZ effect was induced in mice of the original strain by the simple procedure of reinoculating with the same neoplasm in a different site 13-14 days later, even though the doubly inoculated animals did not show any direct evidence of it.

A search of the literature reveals other instances of an XYZ effect when animals bearing spontaneous tumors were given auto-transplants of the same tumor into a different site. Thus, the well known Rous chicken tumor VII, an osteochondrosarcoma (20, 21, 27), and the Brown-Pearce carcinoma of the rabbit (19) were originally transplanted to foreign strains only after doubly transplanting in the original host carrying the spontaneous tumor. It has been reported (10) that the Rous chicken tumor No. 1 was adapted to foreign chickens only after preliminary XYZ treatments. Since inoculations of fowls or rabbits with tumor from the primary site were unsuccessful, the very existence and usefulness of these well known neoplasms were made possible by using for transfer tumor taken from an autograft or by direct XYZ treatment.


**REFERENCES**

6. ———. Failure of a Mouse Carcinoma Material To Enhance a Mouse Sarcoma. Ibid., pp. 1085–86.


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