

# Host-induced Alterations in Strain Specificity of Sarcoma I in Mice: Reversibility of the Change\*

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The alteration in strain specificity of Sarcoma I evidenced by its successful homotransplantation to normal adult mice of three inbred mouse strains in which it ordinarily will not grow progressively has been reported (19).

Many investigators have used various methods to induce the survival or rejection of transplanted homografts in both normal and altered hosts (1-18, 20-22). Barrett and Deringer (1) reported an alteration in strain specificity of a C3H tumor via transplantation in (C3H × C)F<sub>1</sub> hybrids and termed this change an induced adaptation in the tumor. They further reported that growing the adapted tumor in the strain of origin for only five generations did not result in a return of the tumor to its original state (2). In a study of the specificity of the adaptation, Barrett, Deringer, and Hansen (3) found that the specificity involved appeared to extend to relatively small genetic differences. Our findings indicate that an adaptation of the tumor is achieved by treatment of the recipient host (C57BL/6 mice) with A/Jax tissues and that it results in a change in which the tumor grows on direct transplantation to adult C3H mice.

The altered Sarcoma I derived in our earlier experiments (19) was continued in serial passage in normal, adult mice of the C3H, BALB/c and C57BL/6 strains,<sup>1</sup> with cross-transplantation between these strains and the strain of origin. In our present study, a process of continuing adaptive change in different host-strain environments, which is reversible to the specificity of the strain

of origin, has been elicited. These data are the subject of the present communication.

## METHODS AND RESULTS

The initial findings were previously reported (19) and are included here in brief outline for continuity of presentation. The altered tumor was obtained from C57BL/6 mice treated, prior to receiving live graft, with lyophilized Sarcoma I tissue. Three doses of lyophilized Sarcoma I tissue, 15 mg. each (dry weight), were injected intraperitoneally at 5-day intervals. Ten days after the last injection a viable Sarcoma I graft was implanted subcutaneously by trocar as described below. The subsequent progressively growing Sarcoma I graft was then transplanted directly to normal adult C3H mice, both male and female, and carried in serial passage. After the sixth serial passage in C3H mice, the Sarcoma I graft (designated Sarcoma I/Mo to differentiate it from the A-strain specific Sarcoma I) was successfully further homotransplanted to normal adult BALB/c and C57BL/6 mice, in which two strains it was also carried in serial passage.

All mice, approximately equal numbers of males and females, were between 8 and 24 weeks old. The serial passages were performed male to male, female to female, male to female, and female to male, frequently enough to control factors of sex differences.

Transplantation was made by a 13-gauge × 4½-inch trocar with a tightly fitted obturator by subcutaneous insertion near the tail, with the trocar tip directed subcutaneously to the nuchal area, depositing the sarcoma fragment. Eight to twelve recipient mice were used for each serial passage. For transplant two to four donors were sacrificed, the tumor was removed aseptically, and a portion was fixed for histologic study;<sup>2</sup> the remainder, finely minced with sterile scissors, was used for

<sup>2</sup> A separate report of the detailed morphologic study of the tumor graft from its original source through serial homotransplant passages is in preparation by our colleague, Dr. Lawrence W. Smith.

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<sup>1</sup> All inbred strains were obtained from the Roscoe B. Jackson Memorial Laboratory except for approximately 25 per cent of the animals, which were bred in our own colony from Jackson Laboratory breeders.

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implantation. The remaining mice in each serial passage were observed for progress of the tumor implant and ultimate fate of host. All mice were observed, and tumor outlines were recorded by bi-weekly palpation until death due to very large tumors. In those animals in which the live tumor implant failed to grow or regressed, observations were made for 60 days. All mice were necropsied to assure that death was due primarily to the implanted tumor growth.

At frequent intervals, parallel with the homotransplantation of Sarcoma I/Mo, control passages of Sarcoma I (carried routinely in serial passage in the A strain mice) were implanted to normal adult mice of each of the three strains used for the experimental groups. The control tumor did not grow progressively, and the implant regressed in all mice of the C3H, BALB/c, and C57BL/6 strains, respectively.

Each inbred strain subline in which the altered sarcoma was serially passaged is reported below. The total passages, number of mice per passage, and ultimate fate of the tumor implant and host are given. In addition, after approximately ten serial passages in each respective subline, the sarcoma was also implanted into mice of each of the other strains, as well as A strain mice.

At the present time, all the homotransplant sublines have been discontinued, and the tumor is being maintained in our colony in C3H or (C3H × BALB/c)F<sub>1</sub> mice.

#### C3H HOMOTRANSPLANT SUBLINE

This subline originated directly from the treated C57BL/6 mice and was carried for 55 serial passages during a 24-month period. In total, 533 C3H mice were grafted with live tumor, and 492 (92.3 per cent) mice died with large, progressively growing grafts. Each passage was made into eight to twelve mice, with two mice bearing tumors approximately 1 × 2 cm. in size from the previous passage used as donors. Owing to a shortage of C3H mice in ten of the passages (3, 4, 7 through 13, 44), only six mice were used. In all passages subsequent to the third passage, 100 per cent of the mice died with very large subcutaneous tumors extending from the point of grafting along the entire animal body. There appeared to be no sex differences in the growth. Tumors grew equally well in male to male, female to female, or male to female serial passage. In a few instances in passages 41, 43, 45, 48, 49, 51, and 52, the tumor graft was caseous in 10–25 per cent of the mice. Cultures revealed bacterial contamination in the tumor graft. Cultures from mice used in passages 41–50 were positive for *Salmonella*, and the animals exhibited diarrhea

and severe weight loss. We are certain that this accounts for the small number of tumors regressing after 40 continuous serial passages with 100 per cent deaths and no regressions.

This finding of intercurrent and latent infections in some mice is pertinent to the question of frequently reported erratic behavior of tumors and tumor grafts. Sarcoma I has been carried routinely in A strain mice since 1951, and erratic behavior, in that the tumors will at times regress, has been observed. In these instances, the bacteriologic controls have always revealed either direct infection of the tumor or an acute systemic infection which has arisen from an existing latent infection. In general, our procedures are similar to those of other groups in which normalcy of animals is assumed by gross observation of appearance, growth, and behavior. Latent infections such as *Bartonella* are virtually impossible to uncover, and *Salmonella* and several virus diseases of rodents frequently cannot be uncovered owing to an immune host-parasite status similar to a carrier state. The additional physiologic-pathologic stress of a tumor graft, x-ray exposure, or other debilitating influence often permits a latent infection to flare up and become acute, and at that time it can be bacteriologically or histopathologically demonstrated. We are certain that factors such as these account for instances of unexplained erratic behavior of tumor grafts.

The last passages in the C3H mice and fifteen subsequent routine passages in (C3H × BALB/c)F<sub>1</sub> have resulted, to date, in 100 per cent death in all mice. After the fifth, fifteenth, and 25th serial passages in C3H mice the Sarcoma I/Mo was returned to A strain mice and, after one passage, was reimplanted into the subline strain, e.g., from C3H to A to C3H mice in series. In each of these instances the tumor grew progressively, and all the implanted mice (groups of ten to twelve mice each) died with very large tumors, including the C3H mice receiving the Sarcoma I/Mo from a single passage through the A mice.

Outstrain tests by passage from the C3H subline series were made into C57BL/6, BALB/c, and A strain mice at periodic intervals, and the results found are given in Table 1.

Failure was noted in all attempts at direct homotransplantation to C57BL/6 mice. The tumor did, however, retain its ability to grow progressively in the A strain and, to a lesser degree, was homotransplantable to BALB/c mice. The acquisition of ability for successful homotransplantation to BALB/c mice (absent initially) after the sixth and later passages in the C3H subline indicates that the specificity of the tumor is

further altered during continuous residence in the C3H strain.

#### BALB/c HOMOTRANSPLANT SUBLINE

The BALB/c subline originated from the sixth passage in C3H mice by direct implantation from three of the C3H mice to a total of twelve normal, adult BALB/c mice. Each C3H donor tumor was implanted into two females and two males, respectively (19). From this initial, direct homotransplantation through 50 serial passages during 20 continuous months, this tumor subline has grown progressively, and, of a total of 457 BALB/c mice implanted, 423 (92.6 per cent) have died with large tumors. The tumors in twelve mice regressed in passages 38-40 and 45. In these latter passages both bacterial contamination and mite infestation were found after the graft broke through the skin and formed an open lesion, thus accounting for

TABLE 1

#### OUTSTRAIN TESTS FROM C3H SUBLINE

Passage no. in C3H	A mice (per cent)	BALB/c mice (per cent)	C57BL/6 mice (per cent)
29	100 (10/10)*	0 (0/10)	0 (0/10)
34	100 (8/8)	50 (4/8)	0 (0/8)
40	100 (8/8)	25 (2/8)	0 (0/8)
48	100 (16/16)	50 (8/16)	0 (0/16)
54	100 (8/8)	50 (4/8)	0 (0/8)

\* Per cent died with tumor (number of deaths/total implanted).

this small percentage (7.4 per cent) of implants which regressed.

Outstrain tests by direct passage to the other mouse strains were made after the 9th, 22d, 28th, 33d, 44th, and 50th serial passages in BALB/c mice. The results are given in Table 2.

The BALB/c subline tumor retains its ability for direct transplantation back to the A strain mice in which Sarcoma I originated, and to a lesser degree remains homotransplantable to the C3H mice during the 50 passages tested. Another factor which appears and may indicate a further adaptation of the sarcoma graft when carried in BALB/c mice is the acquisition of homotransplantability to C57BL/6 mice. This was absent and not achieved through 55 passages in the C3H subline. Indeed, the 28th passage of the BALB/c subline served as the origin of the C57BL/6 subline reported below.

#### C57BL/6 HOMOTRANSPLANT SUBLINE

The Sarcoma I tumor, originally implanted into C57BL/6 mice treated with lyophilized Sarcoma I tissue and thereafter directly implanted into other C57BL/6 mice, failed to grow (19).

A third attempt at direct homotransplantation

to C57BL/6 mice was made from the 28th serial passage in normal BALB/c mice. In this series, the Sarcoma I/Mo was carried for 25 passages continuously during 12 months. The majority of these passages took in 30-75 per cent of the implanted mice, and in six passages (not consecutive) 100 per cent of the mice died with large, progressing tumors. For the 25 passages, 238 C57BL/6 mice were implanted with a live tumor graft, and in 92 (38.8 per cent) mice regressions occurred, an over-all "take" rate in 146 mice (61.2 per cent). The serial passages were discontinued at this point because of the unavailability of suitable C57BL/6 mice.

After the fifth passage, direct transplantation from the C57BL/6 subline to A strain mice resulted in 100 per cent takes and death with large tumors. In addition, at the eighteenth passage, transplantation was made into groups of eight mice each of A, BALB/c, and C3H normal, adult mice (both sexes). These latter homotransplants grew progressively; 75 per cent of the BALB/c, 75 per cent of the C3H, and 88 per cent of the A mice died with large tumors.

The continued maintenance of the sarcoma graft in C57BL/6 mice does not obliterate its potential ability for successful homotransplantation to the BALB/c, C3H, and A strain mice.

TABLE 2

#### OUTSTRAIN TESTS FROM THE BALB/C SUBLINE

Passage no. in BALB/c	A mice (per cent)	C3H mice (per cent)	C57BL/6 mice (per cent)
9	100 (12/12)*		
22	100 (10/10)	100 (10/10)	
28	100 (8/8)	100 (8/8)	50 (4/8)
33	100 (8/8)	0 (0/8)	0 (0/8)
44	100 (8/8)	75 (6/8)	25 (2/8)
50	100 (8/8)	75 (6/8)	0 (0/7)

\* Per cent died with tumor (number of deaths/total implanted).

A summary of the growth of Sarcoma I/Mo and the fate of the three inbred strain hosts is given in Table 3.

#### SERIAL TRANSPLANTATION OF SARCOMA I/MO IN A STRAIN MICE FROM THE C3H AND BALB/C TUMOR SUBLINES

In view of the findings that Sarcoma I was altered in its strain specificity, as evidenced by continuous growth in the other inbred strains discussed, the problem of whether the Sarcoma I/Mo would regain its A strain specificity on continued serial passages in A strain mice was investigated.

Two separate sublimes were used as the source of tumor origin: Sarcoma I/Mo from the 25th pas-

sage in the C3H subline, and the same tumor from the 33d passage in the BALB/c subline.

*Passage from the C3H subline origin.*—The tumor was directly implanted from the 25th C3H passage into normal A mice and carried in serial transplants for seventeen passages. In total, 144 mice (eight to nine mice per passage) over a 6-month period were implanted, and all died with large tumors. At the 5th passage in A strain mice, the tumor was implanted into ten C3H mice, and four of these died with large tumors. In six mice the tumor grew to about 1 cm. and regressed. At the 11th passage in A strain mice, the tumor was again implanted into eight C3H, to eight C57BL/6, and eight BALB/c mice, respectively. There was no evidence of “take” or growth in any of these latter implants at this time. Necropsy of the animals of the three strains after 60 days revealed no gross abnormality, and there was no evidence of the subcutaneous implant.

BALB/c subline passage resulted in further adaptive changes evidenced by the acquisition of the capacity to grow progressively in a significant number of normal C57BL/6 mice. Recovery of A strain specificity from the BALB/c subline requires more resident passages than the recovery of A strain specificity from the C3H subline. At the time of discontinuance, the sarcoma still retained a growth potential for BALB/c mice when transplanted from the serial passages in A mice.

#### CONTROL OF SARCOMA I FROM A STRAIN MICE

Throughout the 26-month period of the above reported studies, at regular intervals of 4–6 weeks, Sarcoma I, routinely carried in serial transplant passage in A/Jax or A/He mice, was directly implanted into eight normal adult mice, both sexes, of C3H, BALB/c, and C57BL/6 mice. Twenty such implants were made into each respective strain. In all 160 mice of each strain, the Sar-

TABLE 3  
HOMOTRANSPLANTATION IN SERIAL PASSAGE OF ALTERED SARCOMA I  
(SARCOMA I/MO) IN NORMAL ADULT MICE

Mouse strain	Total no. of passages	Time (months)	Total no. of mice	Mice dead with large tumors		Mice with tumors regressed	
				(No.)	(per cent)	(No.)	(per cent)
C3H	55	24	533	492	(92.3)	41	(7.7)
BALB/c	50*	20	457	423	(92.6)	34	(7.4)
C57BL/6	13*	4	126	109	(86.5)	17	(13.5)
	25†	12	238	146	(61.2)	92	(38.8)

\* Subline originating from sixth passage of C3H mice.

† Subline originating from 28th passage of BALB/c mice.

The sarcoma had reverted to its original A strain specificity upon residence in A mice after five serial transplant passages.

*Passages from the BALB/c subline origin.*—The tumor was directly implanted from the 33d BALB/c passage into normal A mice and serially transplanted for 21 passages. In total, 173 mice over a 7-month period (eight to ten mice per passage) were consecutively implanted, and all died with large tumors. At the thirteenth and twentieth passages, respectively, the tumor was returned to eight mice each of the C3H, BALB/c, and C57BL/6 strains. After the thirteenth passage in A mice, the tumor regressed in all C57BL/6 mice and regressed in 50 per cent of the C3H and BALB/c mice, the remainder dying with large tumors. The findings after the twentieth passage in A mice were: regression in all the C3H and C57BL/6 mice and, in the BALB/c mice, regression in five (64 per cent) and death with large tumor in three (36 per cent) mice.

This latter finding appears consistent with the finding that residence of the sarcoma in the

sarcoma I graft failed to take; in a few it became palpable but regressed within 10 days. No evidence of graft implant or other abnormality was found in these mice at necropsy 60 days after tumor implantation.

#### SUMMARY

1. The reversibility of the alteration in the strain specificity of Sarcoma I, induced by passage through actively immunized C57BL/6 mice, has been studied.

2. The altered Sarcoma I (Sarcoma I/Mo) was serially transplanted during a 2-year period in normal adult mice, both sexes, in 533 C3H, 457 BALB/c, and 364 C57BL/6 mice.

3. In each of the strains studied the adapted Sarcoma I/Mo established itself in a relatively constant “take” ratio, i.e., 92.3 per cent in C3H, 92.6 per cent in BALB/c, and 73.8 per cent in C57BL/6.

4. Sarcoma I/Mo continued to change when resident as a homotransplant, as evidenced by the acquisition of capacity to “take” in C57BL/6

mice only after residence in C3H and in BALB/c serial passages.

5. The acquired homotransplantability was reversible: A strain specificity was gradually regained when the Sarcoma I/Mo was returned to and carried in serial passages in A strain mice.

6. Given a changing, adverse, but nonlethal environment a mouse cancer graft, Sarcoma I, was induced to acquire several new compatibilities.

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