

Antigenic Properties of Methylcholanthrene-induced Tumors in Mice of the Strain of Origin

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Immunity has been produced against transplantable tumors which arose in inbred mice by Gross (3) and in inbred rats by Lewis *et al.* (6, 7). These results have been explained on the basis either that the tumor has mutated during repeated transplantation, or that immunogenetic differences among the inbred mice have developed (4, 8). In the experiments of Lewis *et al.*, immunity to uniformly progressive tumors of inbred rats developed following autolysis of the tumor (6, 7). Fardon and Prince (1), using this technic, failed to demonstrate immunity against spontaneous mammary carcinoma dbr/B in DBA/1 mice, and we (2) have failed to demonstrate immunity against spontaneous mammary carcinoma of C3H-He mice following ligation of the tumor. We have continued these studies with spontaneous mammary carcinoma and with sarcomas induced by methylcholanthrene in C3H-He mice and have obtained results which indicated that these two types of tumors differ in their capacity to alter the susceptibility of mice to reimplantation after prior ligation.

MATERIALS AND METHODS

All experiments were done with C3H (Heston subline) mice obtained from the Jackson Memorial Laboratory. Young adults of both sexes were used. Four of the sarcomas (S215, S290-C1, S290-C2, S299-B2) arose 60-90 days following subcutaneous injection of 1 mg. of methylcholanthrene dissolved in 0.05 ml. of lard, and two (S303, S339) following subcutaneous implantation of a methylcholanthrene crystal. At the time when they were transplanted to new mice, they measured $1.0 \times 1.0 \times 0.8$ cm. Several of the tumors grew slowly on the first transplantation in C3H-He mice, but on subsequent transplantation all grew rapidly, reaching palpable size in 7 days and killing the mice in about 6-8 weeks. Histologically, the tumors appeared as fibrosarcomas with numerous mitotic figures. Routine passages were made in C3H-He mice, and in every instance the tumors

grew progressively. Three mammary carcinomas which arose in breeding females were also studied (AC 304, AC 304-1 and AC 322). All implantations were made with a 13-gauge trocar subcutaneously in the region of the right scapula. The tumor fragments introduced weighed 10-12 mg. All mice were palpated at least once a week to determine growth of the tumor and were observed for a period of 2 months following the last challenge implantation. When the transplanted tumors reached 0.75-1.0 cm. in the largest diameter, they were ligated. This was accomplished in lightly etherized mice by firmly, but not tightly, drawing a loop of surgical thread under the base of the tumor made accessible by stretching the skin and tumor outward from the muscles of the back. Twenty-four hours later, a tighter loop was placed around the skin pocket in which the tumor was enclosed. Tumors thus ligated usually dried up within a few days and disappeared leaving a well healed scar. Occasionally, additional loops were necessary on the 3d or 4th day to complete the process. When the tumor invaded the muscle tissue, as occasionally happened, it was not possible to stop its progressive growth. Mice were challenged for immunity by reimplantation of tumor on the opposite flank, at different intervals, 6 or 2 days before, on the same day, or 2-24 days following the beginning of the ligation procedure.

EXPERIMENTAL

Tumor tissue for transplantation and histological study was removed from the mouse in which it arose, by biopsy or after killing the animal, and was transplanted into groups of C3H-He mice. All developed progressive tumors, but, as was the case with tumors S215, S303, S339, and S299-B2, the rate of growth in the first transplant generation varied in the implanted mice. For this reason, most of the experiments were done with a tumor of the second or later generations in which the transplants grew at a more even rate in the recipient mice. The progressively growing tumors were

Received for publication February 9, 1953.

ligated when they had reached sufficient size, and the mice in which the tumors disappeared were challenged by reimplantation of tumor fragments. The results are shown in Table 1.

It is seen (Table 1) that C3H-He mice in which Sarcoma 215 was caused to regress as a result of ligation were immune when the tumor was reimplanted. This was observed in mice carrying tumors of all transplant generations at which tests were made. Immunity of the mice was manifest by failure of the implanted tumor to grow at all or by

primary carcinoma CA 108 which arose in a C3H-He breeding female (2). This tumor grew progressively and killed all the sarcoma-immune mice. Similar results have also been obtained with other mammary tumors implanted into mice immune to Sarcoma 339, 290-C1 and 290-C2.

In certain mice in which the challenge tumor implant failed to grow following tumor ligation, it was observed that complete destruction of the first tumor had not been accomplished, as shown by ultimate progressive growth under the area where

TABLE 1
IMMUNITY IN C3H-HE MICE FOLLOWING INDUCED REGRESSION OF SARCOMAS WHICH AROSE IN THIS STRAIN

Tumor	Transplant generation used for first graft	Tumor regressed following ligation	Transplant generation of tumor used for challenge	Mice in which tumor regressed immune upon reimplantation	Growth of tumor in untreated mice*
AC 304	1	6/7† (12)	2	0/6	6
AC 304-1	1	10/12 (14)	2	0/10	6
	2	9/9 (16)	3	0/9	6
AC 322	1	6/6 (14)	2	0/6	6
	2	6/6 (12)	2	0/6	6
S 215	2	8/8 (12)	2	7/8‡	6
	2§	9/9 (21)	5	2/9	6
	2§	6/6 (10)	5	3/6	6
	3	2/2 (10)	4	2/2	3
	4	8/9 (14)	5	5/8	6
	5	5/6 (6)	6	5/5	3
	7	6/6 (10)	9	6/6	5
S 339	2	12/15 (5)	2	11/12	6
S 299-B2	2	5/5 (19)	2	5/5	4
S 290-C1	1	4/7 (24)	1	4/4	4
S 290-C2	1	4/4 (6)	1	4/4	5
	2	6/8 (7)	2	6/6	4
S 303	2	6/6 (10)	2	5/6	6

* All controls died of progressive tumors.

† Numerator = Tumor regressed following ligation. Denominator = Number ligated.

‡ Numerator = No growth or regression of tumor. Denominator = Number of mice challenged.

§ The tumor used in these experiments was transplanted from very large, old tumors at the first and second transplant generation, and the mice were challenged with the same tumor which had been repeatedly transplanted at 10-day intervals.

Figures in parentheses represent days elapsed between beginning of ligation and challenge reimplantation of tumor.

regression of tiny hard palpable nodules. Regression occurred in eleven of the nineteen mice carrying the second transplant generation of tumor S215. The mice found to be immune in the first experiment were reimplanted after 1 month with tumor from the fifth passage generation; all failed to develop tumors. The results with the other methylcholanthrene-induced tumors are of a similar nature; significant numbers of mice in which tumors were ligated were immune when reimplanted with the same tumor. The four mice shown to be immune to S290-C2 were rechallenged 1 month after the first test with tumor of the first transplant generation and again failed to develop tumors, while five normal control mice died of progressive tumors. Ten mice shown to be immune to S215 were challenged with a spontaneous mam-

mary carcinoma CA 108 which arose in a C3H-He breeding female (2). This tumor grew progressively and killed all the sarcoma-immune mice. Similar results have also been obtained with other mammary tumors implanted into mice immune to Sarcoma 339, 290-C1 and 290-C2. In certain mice in which the challenge tumor implant failed to grow following tumor ligation, it was observed that complete destruction of the first tumor had not been accomplished, as shown by ultimate progressive growth under the area where

developed progressive growths, while eight implanted 2 days after ligation failed to develop tumors.

Mice in which mammary tumors AC 304, AC 304-1, and AC 322 were destroyed by ligation developed progressive tumors when reimplanted 12-16 days after ligation.

DISCUSSION

The results of the above experiments confirm and extend those previously reported (2), showing that destruction of spontaneous mammary carcinomas of C3H-He mice by ligation does not induce immunity to progressive growth of the same tumor subsequently implanted. They further show that ligation of methylcholanthrene-induced sarcomas which arose in this strain of mice regularly induced a state of immunity which prevented growth of the same tumors when fragments were later implanted. This was observed with six unselected tumors which arose in mice treated with carcinogen, and immunity was induced against all these tumors at either the first or second transplant generation. Gross (3) and Lewis *et al.* (6, 7) have previously reported on the development of immunity in inbred mice and rats with methylcholanthrene-induced tumors. Those results have been explained as being due either to mutation of the tumor during repeated transplantation over long periods of time or to immunogenetic differences in the strains of animals used (4, 8). In the present work immunity was demonstrated before repeated transplants had been made, and, therefore, the chance of mutation during passage was minimized. The possibility of heterogeneity in the mice of the C3H-He stock cannot be completely ruled out, but, if the stock is heterogeneous, this factor does not influence the response of the mice to transplanted mammary tumors, for, in repeated trials with such tumors, no instance of immunity resulted after ligation, whereas immunity usually followed ligation of methylcholanthrene sarcomas.

Further study will be required to explain the different behavior of the two types of tumors, and the possibility that the methylcholanthrene-induced sarcomas represent a mutated tissue immunologically distinguishable from that of the strain of mouse in which they arose, and that a comparable mutation is not a characteristic of the spontaneous mammary tumors of C3H-He mice we have studied, should be considered. Such an event is consistent with the somatic mutation theory proposed by Strong (9), who has pointed

out that carcinogen-induced tumors show evidence of mutation more rapidly than do spontaneous tumors (10). In this regard, attention should be called to the studies of Kirschbaum and Bittner (5), who described histological differences between methylcholanthrene-induced and spontaneous mammary tumors which arose in DBA mice.

Under the conditions of these experiments, the immunity induced by the ligated methylcholanthrene-induced sarcomas appears to be operative only in preventing progressive growth of tumor tissue implanted subsequent to ligation of the existing tumor; tumors implanted previously, and presumably established, were not adversely affected.

SUMMARY

Experiments are described in which it was shown that immunity of C3H-He mice resulted following ligation and atrophy of methylcholanthrene-induced sarcomas which arose in mice of this inbred strain. The results are discussed in relation to previously reported experiments in which C3H-He mice were not immunized against mammary carcinoma by similar methods.

ACKNOWLEDGMENTS

The author wishes to acknowledge the technical assistance of Mr. W. Morgan during the course of this work.

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