Immunity to Spontaneous and Methylcholanthrene-induced Tumors in Inbred Mice

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SUMMARY

Inbred C3H/HEN mice were shown to develop a specific immunity to a spontaneously arising adenocarcinoma as well as to a methylcholanthrene-induced fibrosarcoma when the tumors were allowed to grow temporarily in their hind limb. The resistance in the spontaneous carcinoma system was less than in the fibrosarcoma system. In each case the immunity produced was specific for the immunizing tumor, and no cross-immunity was observed. Animals challenged shortly after removal of their immunizing tumor and animals in which the immunizing tumor was not removed did not exhibit any resistance toward a second tumor transplant.

Whether other spontaneous mammary carcinomas and methylcholanthrene-induced sarcomas will behave similarly requires further investigation. The results reported in this paper may apply only to the specific tumors studied.

MATERIALS AND METHODS

An adenocarcinoma arising spontaneously in the breast of a female C3H/HEN mouse was passed through a Snell cytosieve (16) and injected into the thigh of 107 6-week-old female C3H/HEN mice. In 16 days, when the tumors were approximately 1 cm. in diameter, the tumor-bearing extremities were amputated with a direct cautery under pentobarbital anesthesia. One hundred and fifteen tumor-free mice were also subjected to hind-quarter amputation at this time and served as controls. The two groups were bled from the ophthalmic venous plexus 3 days after amputation for antibody studies, which will be reported later. Seven days after amputation one-half of each group was challenged with 1000 viable tumor cells from the first transplant generation of the adenocarcinoma, and the remaining one-half was given inoculations of 1000 viable cells from the third transplant generation of a methylcholanthrene-induced fibrosarcoma (see below). Each cell suspension was injected into a separate group of 30 untreated mice to ascertain the potency of the suspension.

A fibrosarcoma induced by 20-methylcholanthrene in the hind limb of a female C3H/HEN mouse was used in the second part of this study. The immunization procedure followed for this group of mice was essentially the same as that described for the spontaneous carcinoma with these exceptions: the first tumor cell inoculum used for immunization was obtained from the second transplant generation of the fibrosarcoma; as this tumor grew more rapidly amputation of the tumor-bearing extremities was performed on the 7th day; the mice were bled on the 3d and 9th days after amputation and were challenged 14 days after amputation with 1000 viable tumor cells from either the third transplant generation of the methylcholanthrene-induced fibrosarcoma or the first transplant generation of the spontaneous breast carcinoma.

In the third part of this study the primary tumor was allowed to remain in the host, which was subsequently challenged with an additional tumor transplant. One hundred and twenty female C3H/HEN mice were given injections of a concentrated cytosieve preparation from...
the third transplant generation of the fibrosarcoma, and the resulting tumor was allowed to grow for 14 days. Sixty of these animals then underwent a hind-quarter amputation to remove the tumor. Twenty hours later the entire group of 120 mice, as well as eighteen untreated controls, were given inoculations of 1000 viable tumor cells from the same transplant generation of the fibrosarcoma. Those animals in which the initial tumor had not been removed received their second transplant in the opposite thigh and were housed individually to provide optimum conditions for survival.

Tumor cell viability was determined by the dye exclusion technic, with 0.5 per cent trypan blue, and cells were counted in a hemocytometer. Cell suspensions were prepared in Hanks balanced salt solution without antibiotics.

RESULTS

Following injection of the challenge tumor cell inocula tumors became palpable in 2 weeks and grew progressively until the animals died or were sacrificed. After 7 weeks essentially no new tumors were discovered, and the final results were determined. These data are tabulated in Table 1. Only four animals developed recurrent tumors at the site of amputation, and these animals were excluded from the study.

Tumors resulting from transplants of the spontaneous adenocarcinoma appeared later and grew more slowly than did tumors resulting from transplants of the methylcholanthrene-induced fibrosarcoma. Moreover, the spontaneous adenocarcinoma produced less immunity in host mice than did the methylcholanthrene-induced fibrosarcoma.

Those animals in which the adenocarcinoma was used as the immunizing tumor showed no resistance to subsequent transplants of the fibrosarcoma, and those animals in which the fibrosarcoma had grown were not resistant to the carcinoma. With challenge inocula of 1000 viable tumor cells tumors developed in over 80 per cent of control animals and in those animals challenged with a tumor different from that used for their immunization.

In those animals given a challenge tumor cell inoculum without removal of the primary immunizing tumor no immunity to the second tumor transplant was demonstrated. Also, there was no significant resistance to a second tumor transplant in mice in which the immunizing tumor had been removed just 20 hours prior to injection of the challenge inoculum. There was a slight delay in the growth of the second tumor transplant in the amputated mice, but the number of animals developing tumor did not differ significantly from mice in which the primary tumors were not removed.

DISCUSSION

The existence of tumor-specific immunity to spontaneous tumors in mice has not as yet been generally accepted by workers in the field of tumor immunology. Hirsch (5) was able to demonstrate that inbred mice immunized with a spontaneous mammary tumor had prolonged survival times but eventually died of their tumor. The one brief report by Morton, in which a significant number of immunized animals was observed which did not develop tumors, appears to have been overlooked. In the present study, significant resistance ($\chi^2: P < .001$) to challenge inocula of 1000 viable tumor cells from a spontaneous breast carcinoma was exhibited by mice in which this tumor had previously grown. Furthermore, the resistance was specific for the immunizing tumor. Mice subjected to the temporary growth of the spontaneous carcinoma were not resistant to subsequent transplants of the methylcholanthrene-induced sarcoma, and mice immunized with the fibrosarcoma offered no resistance to the growth of the breast carcinoma. If the immunity demonstrated within this isologous system were simply due to an increase in nonspecific host resistance or to a manifestation of the homograft reaction, some degree of resistance should have been evidenced against the unrelated tumor. Therefore, tumor-specific antigens must have been present in this spontaneously arising breast carcinoma. However, until more data can be accumulated it should not be assumed that all spontaneous mammary carcinomas can induce an immune response in mice. The immunity observed in this study and the studies of Hirsch and Morton may be peculiar to the specific tumors and not a general phenomenon of spontaneous breast carcinoma in mice.

The spontaneous carcinoma produced less immunity than did the fibrosarcoma. This is as would be expected, from the difficulty a number of investigators have had in demonstrating immunity to this tumor (4, 14). However, the challenge inoculum from the spontaneous carcinoma

<table>
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<td><strong>PERCENTAGE OF ANIMALS DEVELOPING TUMORS</strong></td>
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| EXPERIMENTAL GROUPS | CHALLENGING TUMOR* |
| --- | --- | --- |
| | Spontaneous carcinoma | Fibrosarcoma |
| | Tumor/total mice | Per cent | Tumor/total mice | Per cent |
| **Exp. 1:** | | | | |
| Immunized with spontaneous carcinoma | 33/55 | 60 | 50/52 | 96 |
| Nonimmunized amputated controls | 49/55 | 89 | 60/63 | 95 |
| Untreated controls | 26/30 | 87 | 24/29 | 83 |
| **Exp. 2:** | | | | |
| Immunized with fibrosarcoma | 55/59 | 93 | 4/52 | 8 |
| Nonimmunized amputated controls | 53/55 | 95 | 51/55 | 93 |
| Untreated controls | 25/25 | 100 | 29/31 | 94 |
| **Exp. 3:** | | | | |
| Immunized with fibrosarcoma | | | | |
| a) amputated 20 hr. prior to challenge | 54/58 | 93 |
| b) no amputation prior to or after challenge | 58/60 | 97 |
| Untreated controls | 16/18 | 89 |

* In each experiment the same tumor cell suspension used to challenge the immunized animals was used in the control groups.
was given 7 days after removal of the primary tumor, whereas the fibrosarcoma was transplanted 14 days after the immunizing tumor was removed. The differences in these time intervals may account, in part, for the lesser degree of immunity produced by the spontaneous adenocarcinoma.

In the present study, no cross-resistance between the spontaneous carcinoma and the methylcholanthrene-induced sarcoma could be deduced. Consequently, there appears to be no common tumor antigens shared by these tumors which would inhibit the growth of subsequent tumor transplants. Prehn (12—14), working with a variety of sarcomas induced by cyclic hydrocarbons, was unable to demonstrate any convincing resistance to tumor transplants that differed from the immunizing tumor. Conversely, in some viral-induced tumor systems animals which are immunized against the virus are resistant to all types of tumors induced by that virus (3, 7, 15). However, if the animals are immunized by tumor transplants that do not contain demonstrable virus, they become resistant only to that specific tumor and not to other tumors induced by the same virus (3, 15). It would appear that the tumor cells per se contain specific antigens, but in some instances these antigens may react with antibodies formed against a viral antigen.

The degree of immunity produced by the temporary growth of a tumor was greatest when the mice received their second or challenge transplant several days after their primary growth had been removed. No immunity could be demonstrated if the primary tumor was not removed, or if the animals were not allowed sufficient time, after removal of the tumor, to develop some resistance prior to receiving the second transplant. In isolated instances in man removal of a primary malignant tumor has altered host resistance, and subsequent spontaneous regression of distant metastases has occurred (6, 9). Everson and Cole (1) reported 47 cases culled from the world literature in which spontaneous regression of malignant tumors had been documented. Many of these patients had undergone partial or complete removal of their primary tumor before regression occurred. Whether the presence of a primary tumor suppresses the immune response, as is suggested by this animal study and by the few reported cases in man, is an intriguing problem in tumor immunology. Possibly the increase in host resistance to subsequent tumor transplants is associated with some substances partially or wholly inactivated by the presence of the large primary tumor mass. Conceivably these substances are bound to the primary growth and the animal requires a finite time after removal of the tumor to re-accumulate these substances in sufficient quantity to resist a second tumor cell inoculum.

REFERENCES

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