Attempts To Induce Immunity against Mammary Adenocarcinoma in Inbred Mice

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INTRODUCTION

The literature on "tumor immunity" has been reviewed by Spencer (14), Wogulum (18), and, more recently, by Haushka (9). Most of the investigations were made with tumors transplanted either into random-bred rats or into strains of mice alien to the strain in which the neoplasm first occurred. In several instances, however, immunity was produced against tumors which arose in inbred strains of rats (10, 11) and mice (6, 12).

In the experiments by Lewis et al. (10, 11), immunity to uniformly progressive tumors of inbred rats was produced following autolysis of the tumor as a result of surgical ligation of its blood supply or by its strangulation. These results and those of Gross (6, 7) were explained by the assumption that the tumor had mutated during repeated transplantations (9, 18). It was of interest to repeat the ligation experiments of Lewis et al. with strain-specific tumors in inbred mice. Accordingly, a series of experiments was carried out with mammary adenocarcinomas of C3H/He mice. After this work had been completed, a paper by Fardon and Prince (2) appeared describing similar experiments in which mammary carcinoma dbdB was studied in DBA/1 mice.

MATERIALS AND METHODS

CSH (Heston subline) and C57Bl/6 mice, received from the Jackson Memorial Laboratory, and ZBC mice,1 obtained from the surplus stock of Dr. J. J. Bittner, were used in the experiments. Spontaneous mammary adenocarcinomas 108 and 21 arose in breeding females of the C3H/He strain; they were transplanted at approximately 3-week intervals in C3H/He mice. The Z tumor was obtained from a breeding female of the Z strain from Dr. Bittner; it was carried in ZBC mice. These tumors were of the third to seventh transplant generation at the time the present experiments were begun. Adenocarcinoma E 0771 and CSH-BA, obtained from the Jackson Memorial Laboratory, were used in other experiments; these were carried by transplantation in C57Bl/6 and C3H/He mice, respectively. All the tumors grew progressively in the mice specified, killing the hosts in from 4 to 7 weeks.

1 ZBC mice are produced by mating mice of the A strain and Dr. Bittner's CSH (called Z strain) to produce F1 hybrids, and mating F1 females to Z males.

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These tumors were chosen because they differ in their characteristics of transplantability: Spontaneous adenocarcinomas 108, 21, and adenocarcinoma Z are specific; the former grow progressively upon transplantation only in C3H/He mice and the latter only in Z or Z back cross mice (ZBC). In repeated trials in successive transplant generations, in which the C3H/He tumors were implanted into ZBC mice or in which the Z tumor was implanted into C3H/He mice, there have been no exceptions to this observation. In our experience, adenocarcinoma CSH-BA grows progressively in all C3H/He mice when injected subcutaneously or intra- dermally as tumor cell suspensions by trocar. It grows progressively in all ZBC mice when implanted by trocar. It was observed that a high rate of tumor regression occurred in ZBC mice which had been given subcutaneous injections of saline suspensions of cells of adenocarcinoma CSH-BA. This occurred following the injection of fresh suspensions or sterile, incubated cell suspensions. Most ZBC mice in which the tumor regressed failed to grow progressive tumors when reimplanted by trocar. Injection of mice with incubated cell suspensions was, therefore, employed in certain experiments as a means of inducing immunity in ZBC mice.

Adenocarcinoma CSH-BA growing in C3H/He mice was removed aseptically and finely minced with scissors. After the addition of a small quantity of saline, the tumor cell suspension was transferred to a sterile glass tissue grinder in which the fragments were mashed by gentle trituration. More saline was then added to make a 10 per cent suspension on a wet weight basis. Mice were injected with 0.2 ml. of freshly prepared suspensions or those which had been incubated in a water bath at 37° C. for 1.5 hours. Progressive tumors grew in approximately one-third of the ZBC mice which had been given subcutaneous injections of saline suspensions of cells of adenocarcinoma CSH-BA. This occurred following the injection of fresh suspensions or sterile, incubated cell suspensions. Most ZBC mice in which the tumor regressed failed to grow progressive tumors when reimplanted by trocar.

When the tumors reached 0.75–1.0 cm. in diameter, they were ligated. This was accomplished in lightly etherized mice by firmly, but not tightly, drawing a loop of surgical thread around the base of the tumor made accessible by stretching the skin and tumor outward from the muscles of the back. Eight and 24 hours later, additional progressively tighter loops were placed around the skin pocket in which the tumor was enclosed. This procedure was followed in an attempt to facilitate absorption of products of autolysis from the tumor. Within an hour after ligation, the muscle was retracted, and the skin turned to a deep purple color; this condition persisted for 24 hours. Drying of the tissue then began, which progressed until finally (within 4–5 days) the tumor disappeared. In some cases additional loops were necessary on the 3d or 4th day to complete the process. When the tumor invaded...
the muscle tissue, as happened in a few cases, it was not possible to stop its progressive growth.

In attempts to induce immunity against the chosen tumors, experimental mice were subjected to various procedures: (a) Progressively growing tumors in C3H/He and ZBC mice were destroyed by ligation. (b) ZBC mice were injected subcutaneously with incubated cell suspensions of adenocarcinoma C3H-BA and were kept under observation until spontaneous regression of the tumors occurred. (c) Saline cell suspensions of CSH-BA were also injected into the tails of C3H/He and ZBC mice, care being taken to miss the tail veins. No tumors developed in the latter.

Tumors which developed in C3H/He mice were destroyed by intermittent ligation of the tails above the tumor with soft copper wire (5). (d) Fragments of an autolyzed tumor (C3H-BA, growing in a C3H/He mouse) ligated 24 hours previously, were implanted by trocar into other C3H/He mice. None of these developed tumors as a result of this implantation. (e) In other experiments, 0.5 ml. of citrated blood obtained by heart puncture from C3H/He mice was injected intraperitoneally, and mammary tissue from nursing C3H/He mice was implanted subcutaneously by trocar into ZBC mice. All the mice were challenged for immunity by grafting fragments of the selected tumors subcutaneously by trocar on the opposite flank. These challenging implantations were made within 2–3 weeks following disappearance of ligated or spontaneously regressed tumors or other preparative treatments to which the mice were subjected. Mice in which the first challenge graft failed to grow were rechallenged after 3 weeks. ZBC mice found to be immune to tumor C3H-BA were rechallenged with Z tumor. All mice were observed for 2 months after this final challenge. Details and results of the experiments are shown in Table 1.

**DISCUSSION**

These results are in agreement with those recently described by Fardon and Prince (2), who were unable to induce immunity in DBA/1 mice by ligation of mammary carcinoma dbrbB. By the methods used, immunity has not been induced in C3H/He mice against spontaneous mammary adenocarcinoma 108 or adenocarcinoma C3H-BA, or in ZBC mice against a mammary carcinoma which arose in a mouse of Dr. Bittner's Z stock. Similarly, in experiments not recorded in Table 1, no immunity has been observed in C57B1/6 mice against adenocarcinoma 21. However, many ZBC mice were rendered immune to reimplantation of adenocarcinoma C3H-BA, which regularly grows progressively following implantation by trocar. This could be accomplished by a number of procedures including prior injection of blood or mammary tissue from C3H/He mice. Such mice, although immune to adenocarcinoma C3H-BA, were not immune to the adenocarcinoma Z, to which ZBC mice are genetically susceptible. It

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**Table 1**

**Effect of Pretreatment on Immunity of CSH or ZBC Mice to Reimplanted Tumors**

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>RECIPIENT MOUSE</th>
<th>PREPARATORY TREATMENT</th>
<th>TEST OF IMMUNITY</th>
<th>IMMUNE TO REIMPLANTED TUMOR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>108</td>
<td>ZBC</td>
<td>Tumor regressed (94)†</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>CSH</td>
<td>Tumor ligated (20)</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>Tumor failed to grow (17)</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>108</td>
<td>Z</td>
<td>Tumor failed to grow (10)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>108</td>
<td>CSH</td>
<td>Tail tumors ligated (18)</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>Blood from CSH injected (7)</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

*All mice implanted as controls in these tests died of progressive tumors.
† Figures in parentheses indicate number of mice pretreated.
197 tumors regressed spontaneously.
would appear that adenocarcinoma C3H-BA is a relatively nonspecific tumor, being transplantable to both C3H/He and ZBC mice, as contrasted with spontaneous tumors 108 and 21, which grow progressively only in C3H/He mice, and spontaneous tumor Z, which grows only in Z or in ZBC mice. In this respect adenocarcinoma C3H-BA resembles lymphosarcoma 6-C3H-ED, which grows progressively in several sublines of C3H mice and also in ZBC mice (5). It is probable, therefore, that the protection afforded ZBC mice against reimplantation of adenocarcinoma C3H-BA has its basis in genetic differences between the tumor and the recipient mice.

A similar explanation applies to the results which have been obtained with C3H mice which can be immunized against lymphosarcoma 6-C3H-ED by a number of procedures (3–5, 16), to those of Gross with mammary tumors in sublines of C3H mice (8), and probably also to those instances in which regression of tumors in rats has been induced by chemotherapeutic agents (1, 17). Another example of the same phenomenon is shown by the studies of Stoerk et al. (15), who found that immunity to the Murphy-Sturm lymphosarcoma of rats could be induced by injection of homologous lymphoid cells. Such results indicate that progressive growth of tumors upon transplantation is no assurance of genetic homogeneity between the graft and the susceptible host, and that immunogenetic manifestations may be observed following a variety of experimental procedures when tumors are transplanted into genetically dissimilar hosts or when tumors which have mutated are implanted into inbred animals of the strain of origin.

SUMMARY

Experiments are described in which attempts were made to induce immunity in C3H/He and in ZBC mice by strangulation of implanted strain-specific mammary carcinoma. C3H/He mice in which tumors were thus destroyed were not immune upon reimplantation with adenocarcinoma C3H-BA, nor with two other spontaneous mammary tumors which arose in this strain. Similarly, ZBC mice were not immune upon reimplantation with a mammary tumor which arose in the Z strain, nor were C57BI/6 mice immune to reimplantation of adenocarcinoma E 0771.

Induced autolysis and destruction of these strain-specific mammary adenocarcinomas growing in mice of the strain in which they arose spontaneously does not lead to immunity against the same strain-specific tumor.

REFERENCES

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