Host-induced Changes in the Mouse Mammary Tumor Agent from Transplanted Tumors as Determined by Neutralization Studies*

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The mouse mammary tumor agent (MTA), normally transferred in the milk of females of cancerous strains, has the properties of an infectious agent or virus (1, 3–6). Antisera produced against agent-containing mouse mammary tissues, either normal or cancerous, in rabbits, rats, or guinea pigs have been found to neutralize the MTA in vivo and in vitro (1, 15, 24).

The studies to be reported (8) were initiated in an attempt to corroborate previously published data in which it was observed that (15):

"The neutralizing effects of the agent-antisera were dependent, in most tests, upon the source of the agent used to prepare the antiserum and agent-suspension.

"An antigenic difference was noted between the MTA of a cancerous strain (A stock) and that of the F1 hybrids.

"Evidence was obtained that the antigenic properties of the agent in a transplanted tumor may become altered with continued passage of the tumor in agent-free animals."

MATERIALS AND METHODS

The sources of the MTA antigens were transplanted mammary carcinomas that had originated in either the cancerous A or Z(C3H) stocks. Tumor No. 9607 developed in a breeding female of the A strain when the animal was 334 days of age, a representative of the 91st inbred generation and of the 69th successive generation to have the disease. The tumor from the Z stock, No. 9618, was observed in a member of the 88th inbred generation and of the 70th successive generation to have cancer when the breeder was 197 days of age.

These mammary tumors were transplanted by trocar in mice of the respective inbred strain and in reciprocal agent-free AxZbF1 (Ax X Zb) hybrids, as illustrated in Chart 1. In addition to the stock of origin, the tumors were designated according to the number of passages the tumors had been carried in the inbred or hybrid mice, as A in A, 1st passage; A in F1, 5th passage; Z in F1, 5th passage; etc. Tissues from the transplanted tumors were used to produce antisera in guinea pigs and/or for extracts that were tested by biological assay for the MTA and neutralization studies.

The test animals used to determine the tumor-inducing activity of the extracts and the neutralization of the agent were agent-free ZBC hybrids, as illustrated in Chart 1. In addition to the stock of origin, the tumors were designated according to the number of passages the tumors had been carried in the inbred or hybrid mice, as A in A, 1st passage; A in F1, 5th passage; Z in F1, 5th passage; etc. Tissues from the transplanted tumors were used to produce antisera in guinea pigs and/or for extracts that were tested by biological assay for the MTA and neutralization studies.

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obtained by crossing the reciprocal Ax × Zb F₁ females with Zb males, and having an incidence of mammary cancer in breeding females of less than 1 per cent (7). The mice were separated into two or three groups as they were weaned, and these litter-mate groups were given injections of different fractions in the same experiment. Each animal received 0.5 ml. intraperitoneally, and the animals were from 23 to 27 days of age.

Preparation of antigens and antisera.—To prepare the various antigens, non-necrotic tumor tissue was removed from donors under aseptic conditions, the tissue was finely minced with scissors, and 9 volumes of Hanks' balanced salt solution was added per gram of wet tissue to give a 10 per cent tissue suspension. The suspension was ground for 10 minutes in an ice-chilled Potter-Elvehjem homogenizer, and the homogenate was centrifuged for 30 minutes at room temperature at 1,500×g. The resulting supernate was removed carefully with frequent shakings, 0.5 ml. of each mixture was injected intraperitoneally into different groups of ZBC test animals. The results of five completed studies are to be discussed and the experiment number will correspond to that of the table in which the data are presented. The experimental animals were continued as breeders, and only noncancerous animals that survived for at least 300 days have been included. In describing the various experiments, details are given concerning the source of the transplanted tumor and the passage in either inbred or hybrid mice.

A statistical evaluation of the data is given in the summary of the five experiments (Table 6).¹

**RESULTS**

**EXPERIMENT 1 (Table 1)**

**MTA Tumor Extract:** Tumor #9618, 3d passage in Z mice.

**Guinea Pig Antisera:** A tumor #9607, 1st passage in Z mice, and Z tumor #9618, 1st passage in Z mice.

The final supernatant of the tumor extract was diluted so that the administration of 0.5 ml. contained the material extracted from 2×10⁻² gm. eq. of tissue, and, when this was mixed with an

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**TABLE 1**

<table>
<thead>
<tr>
<th>ANTI-SERUM</th>
<th>ANTI-SERUM DILUTION</th>
<th>SOURCE OF MTA</th>
<th>NO.</th>
<th>PER CENT WITH CANCER</th>
<th>AVERAGE AGE IN DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A in A, 1st</td>
<td>Undiluted</td>
<td>Z in Z, 3d</td>
<td>21</td>
<td>10</td>
<td>445</td>
</tr>
<tr>
<td>A in A, 1st</td>
<td>1:10</td>
<td>Z in Z, 3d</td>
<td>20</td>
<td>15</td>
<td>510</td>
</tr>
<tr>
<td>A in A, 1st</td>
<td>1:50</td>
<td>Z in Z, 3d</td>
<td>14</td>
<td>71</td>
<td>254</td>
</tr>
<tr>
<td>Z in Z, 1st</td>
<td>Undiluted</td>
<td>Z in Z, 3d</td>
<td>19</td>
<td>0</td>
<td>640</td>
</tr>
<tr>
<td>Z in Z, 1st</td>
<td>1:10</td>
<td>Z in Z, 3d</td>
<td>21</td>
<td>5</td>
<td>617</td>
</tr>
<tr>
<td>Z in Z, 1st</td>
<td>1:50</td>
<td>Z in Z, 3d</td>
<td>25</td>
<td>65</td>
<td>366</td>
</tr>
<tr>
<td>N.G.P.S.</td>
<td>Hanks' solution</td>
<td>Z in Z, 3d</td>
<td>20</td>
<td>75</td>
<td>361</td>
</tr>
<tr>
<td>Saline</td>
<td>Extract undiluted</td>
<td>Z in Z, 3d</td>
<td>21</td>
<td>5</td>
<td>328</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z in Z, 3d</td>
<td>12</td>
<td>85</td>
<td>345</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z in Z, 3d</td>
<td>17</td>
<td>65</td>
<td>370</td>
</tr>
</tbody>
</table>
equal volume of N.G.P.S., Hanks' solution, or saline, each mouse then would receive $10^{-3}$ gm. eq. As may be seen from the data given in Table 1, the four groups of controls showed approximately the same incidences and average ages, although the lowest incidence, as well as the latest average age, was observed in the mice which received the undiluted agent-supernatant.

The neutralizing effects of antisera elicited against the 1st-passage tumors of the A in A and Z in Z transplants were similar on the extract of the 3rd-passage transplants of the Z tumor in Z mice when used either undiluted or at a dilution of 1:10. Both antisera were without effect when diluted to 1:50 (with Hanks') upon the MTA in the extracts.

The incidence may be due to the small number of mice involved.

**EXPERIMENT 3 (TABLE 3)**

**MTA Tumor Extract:** A tumor #9607, 12th passage in A mice, and A tumor #9607, 12th passage in AxZbF₁ hybrids.

**Guinea Pig Antisera:** A tumor #9607, 5th passage in A mice, and A tumor #9607, 5th passage in AxZbF₁ hybrids.

The supernatants, following centrifugation for 20 minutes, were diluted with distilled water to a concentration so that, with the addition of an equal volume of normal guinea serum, 0.5 ml. of the mixture would contain the amount of the MTA from $10^{-2}$ gm. eq. of tumor tissue.

### TABLE 2

**NEUTRALIZATION STUDIES ON THE MOUSE MAMMARY TUMOR AGENT (MTA)**

Effects of antisera produced in guinea pigs against the MTA obtained from A tumor transplanted in A mice, 5th passage, and A tumor in F₁ mice, 5th passage, upon the agent-extract of the A tumor, 10th passage in F₁ mice.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Antiserum dilution</th>
<th>Source of MTA</th>
<th>No.</th>
<th>Percent with cancer</th>
<th>Average age in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A in A, 5th</td>
<td>Undiluted</td>
<td>A in F₁, 10th</td>
<td>14</td>
<td>7</td>
<td>403</td>
</tr>
<tr>
<td>A in A, 5th</td>
<td>1:10</td>
<td>A in F₁, 10th</td>
<td>7</td>
<td>14</td>
<td>442</td>
</tr>
<tr>
<td>A in A, 5th</td>
<td>1:50</td>
<td>A in F₁, 10th</td>
<td>13</td>
<td>54</td>
<td>386</td>
</tr>
<tr>
<td>A in F₁, 5th</td>
<td>Undiluted</td>
<td>A in F₁, 10th</td>
<td>14</td>
<td>0</td>
<td>750</td>
</tr>
<tr>
<td>A in F₁, 5th</td>
<td>1:10</td>
<td>A in F₁, 10th</td>
<td>9</td>
<td>54</td>
<td>333</td>
</tr>
<tr>
<td>A in F₁, 5th</td>
<td>1:50</td>
<td>A in F₁, 10th</td>
<td>13</td>
<td>54</td>
<td>369</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>A in F₁, 10th</td>
<td>10</td>
<td>40</td>
<td>509</td>
</tr>
</tbody>
</table>

**EXPERIMENT 2 (TABLE 2)**

**MTA Tumor Extract:** A tumor #9607, 10th passage in AxZbF₁ hybrids.

**Guinea Pig Antisera:** A tumor #9607, 5th passage in A mice, and A tumor #9607, 5th passage in AxZbF₁ hybrids.

The supernatant was diluted with saline so that 0.5 ml. contained the agent from $2 \times 10^{-2}$ gm. eq.; and, when this was mixed with an equal volume of saline or antiserum, the amount of the agent administered in 0.5 ml. would be equal to $10^{-2}$ gm. eq. Only ten control animals were maintained, and they gave a tumor incidence of 40 per cent.

Undiluted antisera produced against the 5th-passage transplants of the A tumor grown in F₁ mice neutralized the MTA obtained from the 10th-passage transplants of the A tumor in F₁ hybrids, while the A tumor in A antiserum did not significantly affect the activity of the agent from the same tumor. In this case, lack of statistical significance may be due to the small number of mice involved.

The antiserum produced against the 5th-passage A tumor in A mice was effective against the agent in the extract of the A tumor in A mice but not against the agent obtained from the 12th-passage of the A tumor in F₁ hybrids. The antiserum elicited against the 5th-passage A tumor in F₁ mice neutralized the agent from the 12th passage of the same tumor in F₁ hosts, but the same undiluted antiserum, A tumor in F₁ of the 5th passage, did not significantly alter the tumor-inducing activity of the MTA obtained from the 12th-passage tumor in A mice.

Both antisera proved to be inactive when diluted 1:10 and tested against the agents obtained from the 12th-passage tumors of A in A and A in F₁. These incidences in test animals were comparable to those obtained following the administration of the mixtures of normal serum with the extracts containing the MTA of each tumor (Table 3).
**EXPERIMENT 4 (TABLE 4)**

**MTA Tumor Extract:** A tumor #9607, 14th passage in A mice, and A tumor #9607, 14th passage in AxZbF1 hybrids.

**Guinea Pig Antisera:** A tumor #9607, 5th passage in A mice, and A tumor #9607, 5th passage in AxZbF1 hybrids.

Following centrifugation of the 10 per cent tumor suspensions for 20 minutes, the supernatants were not diluted. Equal amounts of the tumor preparation and antiserum, etc., were mixed, and after standing at room temperature for 2 hours 0.5 ml. of the mixture was injected into groups of ZBC mice.

As may be seen from the data presented in Table 4, no significant differences in tumor incidence were noted when the tumor supernatants were mixed with antiserum diluted 1:10 or when distilled water was added. Of possible significance may be the difference in average cancer ages for the animals that received the tumor extracts prepared from the A in A vs. the A in F1 mice, not shown in this experiment.

### TABLE 3

**Neutralization Studies on the Mouse Mammary Tumor Agent (MTA)**

Effects of antisera produced in guinea pigs against the MTA obtained from A tumor transplanted in A mice, 5th passage, and A tumor in F1 mice, 5th passage, upon the agent-extract of the A tumor, 12th passage in A and 12th passage in F1 mice.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Antiserum Dilution</th>
<th>Source of MTA</th>
<th>No.</th>
<th>Per cent With Cancer</th>
<th>Average Age in Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A in A, 5th</td>
<td>Undiluted</td>
<td>A in A, 12th</td>
<td>17</td>
<td>12</td>
<td>584</td>
</tr>
<tr>
<td>A in A, 5th</td>
<td>1:10</td>
<td>A in A, 12th</td>
<td>17</td>
<td>65</td>
<td>655</td>
</tr>
<tr>
<td>A in F1, 5th</td>
<td>Undiluted</td>
<td>A in A, 12th</td>
<td>18</td>
<td>61</td>
<td>489</td>
</tr>
<tr>
<td>A in F1, 5th</td>
<td>1:10</td>
<td>A in A, 12th</td>
<td>14</td>
<td>64</td>
<td>341</td>
</tr>
<tr>
<td>N.G.P.S.</td>
<td></td>
<td>A in A, 12th</td>
<td>18</td>
<td>50</td>
<td>422</td>
</tr>
<tr>
<td>A in A, 5th</td>
<td>Undiluted</td>
<td>A in F1, 12th</td>
<td>18</td>
<td>33</td>
<td>439</td>
</tr>
<tr>
<td>A in A, 5th</td>
<td>1:10</td>
<td>A in F1, 12th</td>
<td>18</td>
<td>67</td>
<td>450</td>
</tr>
<tr>
<td>A in F1, 5th</td>
<td>Undiluted</td>
<td>A in F1, 12th</td>
<td>17</td>
<td>18</td>
<td>443</td>
</tr>
<tr>
<td>A in F1, 5th</td>
<td>1:10</td>
<td>A in F1, 12th</td>
<td>18</td>
<td>72</td>
<td>415</td>
</tr>
<tr>
<td>N. G. P. S.</td>
<td></td>
<td>A in F1, 12th</td>
<td>18</td>
<td>67</td>
<td>402</td>
</tr>
</tbody>
</table>

### TABLE 4

**Neutralization Studies on the Mouse Mammary Tumor Agent (MTA)**

Effects of antisera produced in guinea pigs against the MTA obtained from A tumor transplanted in A mice, 6th passage, and A tumor in F1 mice, 5th passage, upon the agent-extract of the A tumor, 14th passage in A and 14th passage in F1 mice.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Antiserum Dilution</th>
<th>Source of MTA</th>
<th>No.</th>
<th>Per cent With Cancer</th>
<th>Average Age in Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A in A, 5th</td>
<td>Undiluted</td>
<td>A in A, 14th</td>
<td>17</td>
<td>6</td>
<td>387</td>
</tr>
<tr>
<td>A in A, 5th</td>
<td>1:10</td>
<td>A in A, 14th</td>
<td>19</td>
<td>74</td>
<td>666</td>
</tr>
<tr>
<td>A in F1, 5th</td>
<td>Undiluted</td>
<td>A in A, 14th</td>
<td>15</td>
<td>60</td>
<td>382</td>
</tr>
<tr>
<td>A in F1, 5th</td>
<td>1:10</td>
<td>A in A, 14th</td>
<td>17</td>
<td>76</td>
<td>375</td>
</tr>
<tr>
<td>Distilled H2O</td>
<td></td>
<td>A in A, 14th</td>
<td>21</td>
<td>76</td>
<td>386</td>
</tr>
<tr>
<td>A in A, 5th</td>
<td>Undiluted</td>
<td>A in F1, 14th</td>
<td>17</td>
<td>0</td>
<td>646</td>
</tr>
<tr>
<td>A in A, 5th</td>
<td>1:10</td>
<td>A in F1, 14th</td>
<td>14</td>
<td>86</td>
<td>646</td>
</tr>
<tr>
<td>A in F1, 5th</td>
<td>Undiluted</td>
<td>A in F1, 14th</td>
<td>18</td>
<td>33</td>
<td>418</td>
</tr>
<tr>
<td>A in F1, 5th</td>
<td>1:10</td>
<td>A in F1, 14th</td>
<td>18</td>
<td>72</td>
<td>425</td>
</tr>
<tr>
<td>Distilled H2O</td>
<td></td>
<td>A in F1, 14th</td>
<td>20</td>
<td>65</td>
<td>423</td>
</tr>
</tbody>
</table>
only for the controls but when antisera diluted 1:10 were mixed with the extracts.

The antiserum made against the A tumor in A mice of the 5th passage neutralized the MTA in extracts of the 14th-passage A tumor in A mice and also the same passage in F1 hybrids. The antiserum produced against the 5th-passage tumors of A in F1 hybrids did not give protection of statistical significance against the MTA or the 14th passage of the same tumor in either A or F1 hosts.

The data for Experiments 3 and 4, testing the same antisera against the MTA from comparable transplanted tumors, are very similar, except for variations which could result from the small numbers of mice in the various groups.

As might be expected from the data obtained in previous experiments, antiserum produced against the 5th-passage Z tumors in Z mice was active in neutralizing the MTA in extracts of the 11th-passage Z tumors in F1 hybrids, while the tumor antiserum of the 5th-passage A tumor in A mice proved to be inactive. Low incidences of tumors were observed in test animals which received the tumor extract mixed with antiserum elicited against either the 5th-passage tumors of Z in F1 or A in F1 hybrids. The data are very suggestive of a protective effect, but they were not of statistical significance, possibly because of the small numbers in the different groups as well as the low incidence in the controls in this particular passage of the transplanted tumor.

**DISCUSSION**

Observations have been presented in five separate experiments to give information upon the neutralization of the mammary tumor agent (MTA) by guinea pig antisera elicited against transplanted mammary carcinomas. The data obtained following the use of undiluted sera are summarized in Table 6; several other studies are under observation.

The same technic has been employed for the preparation of extracts of either normal or neoplastic tissues for biological assay of the tumor-inducing activity of the MTA, and it should be
kept in mind that many factors influence the final incidence and tumor age in the test animals (7–12, 14). These include the genetic constitution of the assay animals, age of the mice at the time of administration of the extracts, strain of the source material, concentration, etc. In a considerable number of assays it has been determined that the concentration of the agent-containing extracts need not be the determining factor, since a single injection containing $10^{-3}$ gm. equivalents of material was found to produce a higher incidence and earlier cancer age than was observed in litter-mates.

It has been reported (15) that antisera produced which received five injections of extracts of the same tumor tissue over a period of 5 days when the extract was diluted only tenfold (10).

Considerable variation was also detected in the activity of the agent(s) derived from spontaneous tumors that arose in mice of the same inbred stock, even those from litter-mates (9). In testing transplantable tumors, a decrease in the activity of the agent might be observed following repeated passage of the tumors (9).

Thus, the variations encountered in the present series of studies are similar to those seen in other biological assays for the mammary tumor agent, especially when small numbers of test animals were used. Although approximately 800 experimental mice survived in these tests for an adequate period of time to be included in the tabulations, certain of these studies involved ten groups of mice of about twenty females each, in which the age difference was no greater than 4 days. Now that information has been secured about the action of the various antisera at different concentrations, fewer groups need be given injections, and this will permit more animals to be included in each assay when undiluted antisera are being tested in future neutralization studies.

It has been reported (15) that antisera produced

<table>
<thead>
<tr>
<th>TUMOR SOURCE</th>
<th>Untreated controls</th>
<th>Effect of antiserum</th>
<th>P VALUES*</th>
<th>Effect of antiserum</th>
<th>P VALUES*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.</td>
<td>No.</td>
<td>Cancer</td>
<td>(per cent)</td>
<td>Produced against:</td>
<td>No.</td>
</tr>
<tr>
<td>3</td>
<td>A in A, 12th</td>
<td>18</td>
<td>50</td>
<td>A in A, 5th</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>A in A, 14th</td>
<td>21</td>
<td>76</td>
<td>A in A, 5th</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>A in F1, 10th</td>
<td>10</td>
<td>40</td>
<td>A in A, 5th</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>A in F1, 12th</td>
<td>18</td>
<td>67</td>
<td>A in A, 5th</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>A in F1, 14th</td>
<td>20</td>
<td>65</td>
<td>A in A, 5th</td>
<td>17</td>
</tr>
<tr>
<td>1</td>
<td>Z in Z, 3d</td>
<td>20</td>
<td>75</td>
<td>Z in Z, 1st</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>Z in F1, 11th</td>
<td>19</td>
<td>47</td>
<td>Z in Z, 5th</td>
<td>19</td>
</tr>
</tbody>
</table>

* These probability values were obtained by comparing the respective treatment effect with the untreated control. The values were calculated, using the averaged angular transformation proposed by Freeman and Tukey (92). Both treated groups were compared with the control group in each experiment, using Tukey's procedure (16) for testing, simultaneously, differences between all possible pairs of means in an experiment. In each experiment the simultaneous P values are quoted if P < 0.06. Where P > 0.05, this is indicated by a dash (—).
from the maternal A stock did not prevent the development of mammary tumors when it was mixed with the agent-containing extract of the altered F1 tumors. Prior to this change in activity of the agent in the transplanted F1 tumors, antisera produced not only against the F1 agent but also against the MTA from mice of the inbred A stock were found to neutralize the agent from the F1 tumors. No neutralizing effects could be demonstrated following the testing of antisera produced against agent-free AxZbF1 tissues upon the agent(s) from either the A strain or AZF1 hybrids.

The present series of studies was designed to ascertain whether the MTA propagated and transferred in the cells of transplanted mammary carcinoma might become altered following passage in agent-free reciprocal F1 hybrids as opposed to the agent from tumors transplanted and inoculated in members of the inbred strain in which the tumors arose spontaneously. If so, the agent present in the tumor from hybrid host should become antigenically distinct from that obtained from the inbred host.

It was found that by the time mammary tumors which arose spontaneously in members of inbred cancerous stocks had been transplanted in agent-free F1 hybrids for five passages, the antisera produced in guinea pigs against the tumors from F1 hybrids would neutralize the MTA present in an extract of the tumors from F1 hosts, but not the agent obtained from the same mammary tumors inoculated only in mice of the inbred stock. However, the antisera elicited against the agent of the original mammary tumor transplanted in inbred mice would inactivate the agent from the same mammary tumor regardless of whether the tumor had been transplanted in inbred or hybrid animals, although in some instances the data are suggestive rather than of statistical significance.

Further investigations must be completed to determine the possible antigenic relationship of the agents present in the original spontaneous mammary tumors from mice of different cancerous stocks and the agents in later transplants in mice of these inbred stocks. In one experiment the antisera representative of the first-passage A tumor in A mice was found to neutralize the agent from the third-passage Z tumor in Z mice. One possible explanation for this activity was that the agent-containing supernatant of the Z tumor was used at a higher dilution than in any other experiment. In another study still under observation, antisera produced against the 24th passage of the A tumor in A mice did not reduce the tumor-inducing activity of the agent from the 2d passage of another Z tumor in Z mice, #10172. However, this same A in A tumor antiserum was active against the MTA in the extract of the 2d passage of the A tumor in A mice, #10192; also the Z tumor in Z mice antiserum was active when tested against the MTA of the Z tumor in Z mice, #10172. These preliminary data would suggest that the agent(s) from different tumors of the same inbred stocks are similar and may be neutralized by the same tumor antisera.

Observations from another preliminary study indicate that, while the MTA present in the Z tumor following inoculation in AxZbF1 mice had become antigenically different from the agent extracted from the Z tumor transplanted in Z mice, after the Z tumor from F1 hosts was again passed in Z mice for several passages, the agent in the tumors had regained the antigenic characteristics of the agent in the Z tumor from Z mice.

Other data demonstrate that antisera capable of neutralizing the agent(s) must be administered before the mice become infected with the MTA to protect against the subsequent development of mammary cancer.

During the past few years many interesting theories have been advanced which may have significance for the cancer problem. Host-induced changes in bacterial viruses have been reported by several workers (2, 28–31). In reviewing the possible relationship of viruses to cancer, Stanley (32, 33) wrote that it might be advisable to revise the generally accepted definition of a virus to include nucleic acids; also, under certain conditions viruses may act as genes and genes as viruses. A few pertinent publications on the genetic concept of cancer also may be cited (25–27, 34).

While the observations concerning the antigenic properties of the MTA in hosts other than mice from this laboratory (13, 15) have been consistent, neutralizing antibodies have not been demonstrated in the serum of mice, even those immunized against the agent (19–21, 23). Also, foreign particulate substances have not been identified in mice of cancerous stocks by physical examination and correlated with biological assays to demonstrate tumor-inducing activity when mammary tumors from virus-free mice were studied as controls, as pointed out by Dmochowski (19).

In considering the problem of mammary cancer in mice, it is probable that Dmochowski has had more experience in using a combination of biological, immunochemical, and biophysical technics than any other investigator. As early as 1953, Dmochowski (17) intimated the possibility that the MTA could be a cytoplasmic agent, dependent
upon the action of genes, and that the agent could have arisen by the transformation of a normal cell constituent. Such a theory would "stress the necessity of looking at the agent not as an entity in itself, but as an entity among the physiologically coordinated nuclear and cytoplasmic units which comprise the cell" (18). A similar theory had been advanced earlier by Heston (25).

The data presented in this report on neutralization studies of the MTA would suggest that some normal tissue component(s) contributed by the host had influenced the antigenic characteristics of the agent, in accord with the observations reported previously (15). It would seem expedient to delay any interpretation and discussion of the possible significance of the results until the many theories on mammary carcinogenesis, including the nature and the role of the MTA in its etiology, have been elucidated.

SUMMARY

Spontaneous mammary cancers from breeders of the A and Z(C3H) cancerous stocks were transplanted in mice of the respective inbred strains and reciprocal agent-free AxZbF1 hybrids. Antisera were prepared against the tumors in guinea pigs after they had been carried for one, five, and more passages in the various hosts and tested in neutralization studies on the mammary tumor agent (MTA) present in extracts of the transplanted tumors.

The antisera produced against the MTA from tumors grown in inbred mice neutralized the agent in extracts of the same tumors inoculated in either inbred or hybrid animals, while the antisera elicited against the tumors from F1 hosts were active against the agent from the same tumors transplanted in F1 hybrids but not against the agent from the same tumor carried in inbred animals.

In one experiment, the antisera produced against the A tumor transplanted in F1 hybrids for five passages reduced the tumor-inducing activity of the MTA obtained from the Z tumor of the 11th passage also from F1 hosts, while the A in A tumor antiserum was inactive.

The experimental data indicate that the antigenic characteristics of the mouse mammary tumor agent may be influenced by the incorporation of normal tissue component(s) of the host in the virus particles.

REFERENCES


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