Effect of the Source of the Mouse Mammary Tumor Agent (MTA) upon Neutralization of the Agent with Antisera*

JOHN J. BITTNER AND DAVID T. IMAGAWA†

(Departments of Physiology, Division of Cancer Biology, and of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis 14, Minn.)

One of the primary inciting causes of mammary cancer in mice (3), the mammary tumor agent (MTA), has the properties of an infectious agent or virus (3—7), and numerous investigations have been concerned with the antigenic characteristics of the agent (1, 2, 7—16). Because of various observations which have been obtained following the use of different immunological technics (complement fixation, precipitation, and neutralization tests), Dmochowski and Passey (7) have expressed the opinion that, serologically, the mammary tumor agent might be similar to material occurring in normal tissues of mice.

In 1944 Andervont and Bryan (1) showed that antiserum against mouse mammary carcinoma containing the MTA, made in rabbits following intraperitoneal injection, would neutralize the agent in vivo and in vitro. However, mammary tumors developed in mice when serum prepared against the Brown-Pearce rabbit carcinoma and normal rabbit serum were tested. The observations obtained with the use of mouse cancerous tissue were confirmed by Green et al. (10) in experiments in which both rabbits and rats were employed for the production of antiserum. The anticancer serum from immunized rabbits did not prevent the development of mammary cancer in females of the cancerous CSH stock which had obtained the MTA from birth and were from 26 to 46 days of age when they were treated (5).

This report concerns neutralization studies with the use of sera, prepared either in rabbits or in guinea pigs, against mouse tissues containing or free of the MTA. The effects of these sera on the agent in vivo and in vitro were determined by the subsequent development of mammary cancer in the experimental animals.

MATERIALS AND METHODS

The mouse tissues employed for the preparation of antiserum in rabbits and guinea pigs were: (a) transplanted mammary cancer which had originated spontaneously in a mouse of the cancerous A stock and (b) normal mammary glands either from AZF hybrids (A females × Z [C3H] males) with the MTA or from AzZbF1 hybrids (Ax females × Zb males) without the MTA (5). The preparation of the antiserum has been described previously (14).

The agent-free test animals used for assay purposes were called ZBC hybrids and were obtained by mating AzZbF1 or ZbAxF1 females with Zb males (5). Details regarding the animals at the time of treatment are given in the account of each experiment.

Details regarding the source of the MTA and the method of preparing the agent-suspensions are listed below, by experiment.

Neutralization in vitro with rabbit antisera against an agent-extract prepared from A stock transplanted mammary tumors (Table 1).—Tissue (5.5 gm.) from several transplanted mammary tumors, indigenous to the cancerous A stock, was ground in a mortar, 50 ml. of saline (0.9 per cent NaCl) was added, and the suspension was homogenized in a Potter-Elvehjem glass homogenizer. The material was stored for 30 minutes in the refrigerator and cleared by spinning at 8,000 r.p.m. for 30 minutes. The supernatant was recentrifuged for 90 minutes at 18,000 g, after which the centrifugate was resuspended in saline to give a total volume comparable to 10 times that of the original tumor weight. This was centrifuged in a clinical centrifuge at 800 r.p.m. for 15 minutes, and the supernatant was stored in the refrigerator until used, approximately 30 minutes later.

An equal volume of normal serum, antiserum, or saline was mixed with the final supernatant of the 10 per cent mammary tumor agent suspension (A stock). The mixtures were in-

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† Present address: Departments of Pediatrics and Infectious Diseases, University of California, School of Medicine, Los Angeles 24, Calif.

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Neutralization in vitro with rabbit antisera against an agent-extract prepared from normal lactating mammary glands from females of the cancerous A stock (Table 2).—Dissected normal lactating mammary glands from several females of the cancerous A stock were forced through a tissue press, ground in a mortar, and extracted with saline to give a 20 per cent suspension. The material was centrifuged for 10 minutes at approximately 2,500 r.p.m., and the supernatant was diluted so that the injection of 1 ml. contained the agent obtained from $2 \times 10^4$ gm. equivalents of tissues. The agent-suspension was injected into mice which, except for the saline-agent-controls, had received 0.25 ml. of either antisera or normal rabbit serum on the two preceding days. The ZBC test animals were 22–26 days of age.

Neutralization in vitro with guinea pig antisera against an agent-extract prepared from AZF1 transplanted mammary tumors, No. 8415 after twelve passages in AxZbF1 hybrids (Table 3).—Tissue from several transplanted mammary tumors, AZF1 No. 8415 after twelve passages, was ground in a mortar, 80 ml. of saline was added, the suspension was homogenized and centrifuged at 8,000 r.p.m. for 30 minutes. The supernatant was spun for 15 minutes at 10,000 r.p.m. (Spinco) and the final supernatant at 40,000 r.p.m. (140,000 x g) for 1 hour. The sediment was resuspended in saline (30 ml.) and centrifuged for 10 minutes at 10,000 r.p.m. Saline was added to the final supernatant to make a total volume of 100 ml. (a dilution equivalent to approximately ten times the original tumor weight); this was used after being stored in the refrigerator for 1 hour. To each tube containing 9 ml. of the MTA suspension, derived from the AZF1 transplanted mammary tumors, an equal volume of either antiserum or saline was added. The mixtures were kept at room temperature for 2 hours, after which interval 0.5 ml. was injected intraperitoneally into litter-mate 22–24-day-old mice.

Neutralization in vivo with guinea pig antisera against an agent-extract prepared from AZF1 transplanted mammary tumors, No. 8415 after 31 passages in AxZbF1 hybrids (Table 4).—Tumor tissue (0.9 gm.) from eight animals was ground with sand, and distilled water was added to give a 10 per cent suspension. This was cleared at 2,500 r.p.m. for 10 minutes, and the supernatant was diluted so that the administration of 0.5 ml. contained the amount of the MTA from $2 \times 10^4$ gm. equivalents of tissue. (Other animals, not included in Table 4, received $10^2$ gm. equivalents.)

Neutralization in vivo with rabbit antisera against an agent-extract prepared from AZF1 transplanted mammary tumors, No. 8415, after 31 passages in AxZbF1 hybrids (Table 5).—Dissected normal lactating mammary glands from several females of the cancerous A stock were forced through a tissue press, ground in a mortar, and extracted with saline to give a 20 per cent suspension. The material was centrifuged for 10 minutes at approximately 2,500 r.p.m., and the supernatant was diluted so that the injection of 1 ml. contained the agent obtained from $2 \times 10^4$ gm. equivalents of tissues. The agent-suspension was injected into mice which, except for the saline-agent-controls, had received 0.25 ml. of either antisera or normal rabbit serum on the two preceding days. The ZBC test animals were 22–26 days of age.

Thus, in the experiments conducted in vitro, a mixture of the antiserum and agent-suspension, after standing at room temperature for a period of 2 hours, was administered to agent-free test ani-
mals. In the studies performed in vivo, information was obtained on the induction of passive immunity following the injection of samples of antisera into the test animals 2 days previous to the injection of the extracts containing the MTA.

RESULTS AND DISCUSSION

As controls for the animals which were tested for the neutralization of the mammary tumor agent (MTA), the incidences of mammary tumors was evident, was seen between the incidences of Groups B and D (Table 1), where the statistical difference was equal to 1.8 X S.E. Other comparisons, where there might be some questions of significance, are also presented in the tables.¹

Where the sera from rabbits were assayed, the agent-suspensions were made from either transplanted mammary tumors or normal mammary glands from donors of the cancerous A stock. The antisera from rabbits neutralized the agent in vitro (Table 1) and conferred passive immunity (Table 2) when materials representative of the same A stock were used as antigens, while the serum prepared against the agent-containing normal mammary glands of the AZF₁ hybrids showed no effect upon the MTA from the cancerous A stock in either study.

In the other two experiments, sera from guinea pigs were tested, and it was determined that the antiserum prepared against transplanted mammary cancer was effective in vitro (Table 1) and conferred passive immunity (Table 2) when materials representative of the same A stock were used as antigens, while the serum prepared against the agent-containing normal mammary glands of the AZF₁ hybrids showed no effect upon the MTA from the cancerous A stock in either study.

The greatest variation, except where neutralization was evident, was seen between the incidences of Groups B and D (Table 1), where the statistical difference was equal to 1.8 X S.E. Other comparisons, where there might be some questions of significance, are also presented in the tables.¹

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¹The differences between the incidences of mammary cancer involve variations between proportions rather than differences between means. It is, therefore, safer to require an observed difference between proportions to be three times its standard error before assuming significance.

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**TABLE 3**

Neutralization Effects in Vivo of Antisera Produced in Guinea Pigs for the MTA Derived from the Twelfth-Passage Transplanted Tumor, AZF₁ No. 8415

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Antiserum Samples from Guinea Pigs</th>
<th>No. MICE</th>
<th>PER CENT Cancer</th>
<th>AV. AGE Cancer (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Normal guinea pig serum</td>
<td>47</td>
<td>79</td>
<td>381</td>
</tr>
<tr>
<td>B</td>
<td>Distilled water suspension</td>
<td>48</td>
<td>79</td>
<td>385</td>
</tr>
<tr>
<td>C</td>
<td>Normal mammary glands without MTA (AxZbF₁ hybrids)</td>
<td>39</td>
<td>92</td>
<td>389</td>
</tr>
<tr>
<td>D</td>
<td>Normal mammary glands with MTA (AZF₁ hybrids)</td>
<td>47</td>
<td>9</td>
<td>469</td>
</tr>
<tr>
<td>E</td>
<td>Transplanted mammary cancer (A stock)</td>
<td>46</td>
<td>86</td>
<td>423</td>
</tr>
</tbody>
</table>

Statistical significance of difference between groups: A and C = 1.9 X S.E.; D and E = 3.9 X S.E.; B and E = 4.1 X S.E.

**TABLE 4**

Neutralization Effects in Vivo of Antisera Produced in Guinea Pigs for the MTA Derived from the 31st-Passage Transplanted Tumor, AZF₁ No. 8415

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Antiserum Samples from Guinea Pigs</th>
<th>No. MICE</th>
<th>PER CENT Cancer</th>
<th>AV. AGE Cancer (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Normal guinea pig serum</td>
<td>36</td>
<td>47</td>
<td>425</td>
</tr>
<tr>
<td>B</td>
<td>Distilled water suspension</td>
<td>36</td>
<td>42</td>
<td>428</td>
</tr>
<tr>
<td>C</td>
<td>Normal mammary glands without MTA (AxZbF₁ hybrids)</td>
<td>37</td>
<td>43</td>
<td>419</td>
</tr>
<tr>
<td>D</td>
<td>Normal mammary glands with MTA (AZF₁ hybrids)</td>
<td>36</td>
<td>3</td>
<td>496</td>
</tr>
<tr>
<td>E</td>
<td>Transplanted mammary cancer (A stock)</td>
<td>33</td>
<td>39</td>
<td>412</td>
</tr>
</tbody>
</table>

The differences between the incidences of mammary cancer involve variations between proportions rather than differences between means. It is, therefore, safer to require an observed difference between proportions to be three times its standard error before assuming significance.
mary tumors from the A stock was effective in neutralizing the agent from an AZF; transplanted tumor after it had been continued for twelve passages (Table 3), but the same antiserum gave no protection against the tumor when it had been transplanted for 31 passages (Table 4). However, the guinea pig serum elicited against the normal AZF; mammary glands, containing the MTA, prevented the development of mammary cancer in most mice which received, in tests conducted in vitro and in vivo, the agent-suspension of tissues of the twelfth and 31st-passage AZF; transplanted tumors.

There was a decrease in the tumor-inducing activity of the extracts containing the agents of tissues of the twelfth- (Table 3) and 31st- (Table 4) passage transplants of the AZF; tumor No. 8415. However, the observations are consistent within each study, and no significant difference was seen among the incidences of mammary cancer in mice injected with the untreated agent-extract, the extract mixed with antiserum characteristic of normal mammary glands free of the MTA, or the extract mixed with normal guinea pig serum.

Two groups of females of the C stock, between the ages of 77 and 188 days, also were treated with the extract from the 31st-passage AZF; tumors. In the animals which received the amount of the agent from either $2 \times 10^{-2}$ or $10^{-2}$ gm. equivalents of tissue, the respective cancer incidences were 22 and 0 per cent. Several females of each group were mated, and it was noted that some noncancerous C females had cancerous offspring.

Other studies (unpublished) may be cited in which the 24th-passage transplants of the AZF; tumor No. 8415 were assayed. Test animals which received the amount of the agent from $10^{-1}$ gm. equivalent of tissue showed an incidence of 100 per cent, as contrasted with an incidence of 76 per cent in females when $10^{-2}$ gm. equivalent was administered; the respective average cancer ages were 370 and 416 days. Thus, the decrease in the activity of the MTA in the transplanted AZF; tumor No. 8415 probably occurred after the 24th passage in agent-free AxZbF; hybrids.

In confirmation of previous observations (1, 10), it was determined that the sera elicited in rabbits and guinea pigs against transplanted mammary tumors neutralized the MTA, in vivo and in vitro, and also that the serum prepared against normal mammary glands containing the agent was as effective as the antitumor serum. However, these antisera may neutralize the agent from the same inbred strain or hybrid generation, but not necessarily when the source of the agent-containing tissues differed to the extent found between mother and hybrid offspring. Also, antigenically, the characteristics of the agent from the maternal stock may be more like those of the agent of the first-generation progeny than the reciprocal. Theoretically, the same agent would be transferred from the females of the cancerous A stock to their AZF; offspring, yet the antisera prepared against the agent-containing mammary glands of the hybrids failed to neutralize the MTA from the cancerous A stock but were active against the agent from the hybrids. This might imply that the host contributes some material which becomes incorporated within the agent-particles and which plays some role in determining the immunochemical properties of the MTA.

For the present, it would appear advisable not to speculate further upon the significance of these findings and to delay any attempt to interpret the data until they have been confirmed and extended in future studies.

**SUMMARY**

Antisera prepared in rabbits and guinea pigs against mouse tissue possessing or free of the mammary tumor agent (MTA) were investigated for their neutralizing effects, in vivo and in vitro, upon the MTA.

In none of the experiments was a significant difference observed in the incidences of mammary cancer in mice which received: (a) the agent-suspension prepared with saline or distilled water, (b) the agent-suspension mixed with normal rabbit or guinea pig serum, or (c) the same agent-suspension mixed with antisera produced against normal mammary glands without the MTA.

In comparable experiments it was observed that antisera prepared against normal mammary glands with the MTA were as effective in neutralizing the agent as were sera elicited against cancerous tissues containing the MTA.

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 F; 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.

The interpretation of these data should await confirmation in future studies.
REFERENCES


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