The Transplantability of Mammary Cancer in Mice Associated with the Source of the Mammary Tumor Milk Agent*

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The experiments on the transplantation of tumors by Little and Tyzzer (31), Strong (36), Strong and Little (40) and Little and Strong (30) resulted in the advancement of the genetic theory of transplantation. According to this hypothesis, susceptibility or non-susceptibility to grafts of tumor tissue is dependent upon the genetic relationship between the host inoculated and the tumor cell. The growth of leukemia (32, 34, 20), other types of tumors (see 27 and 28), and the retention of normal tissue, such as spleen (29, 12), may be explained upon the same basis.

Sex-linkage (39) and linkage with color genes has been indicated (30) and demonstrated (5-9). Several investigators (37, 38, 4, 18, 19) have detected mutations in the genetic constitution of the transplantable mammary tumors. Whereas multiple mammary tumors from a single animal have never required the same genetic make-up for progressive growth (39, 4, 18, 19, 5-9), animals inoculated with multiple grafts of the same tumor responded by either growing all or none (3). Gorer (21-23) has evidence that there may be a relationship between some of the genes necessary for the growth of some transplanted tumors and those which determine the presence of an antigen. The protective antibodies he considers to be iso-antibodies.

Spontaneous mammary cancer in mice usually results from the action of three primary factors (14). One of these is the mammary tumor milk agent (11) which because of its size, ability to propagate in the living cell, and its antigenic properties may be classified as a filterable agent or virus (see 14-16, 33, 2, for literature).

In various reciprocal crosses between high cancerous strains of mice different incidences have been noted in the hybrids depending upon the maternal stock. In some crosses these differences were noted when the mice were continued as breeders (10), in others only the virgin hybrids showed the variation (33, 16, 41, 17). How these data may be interpreted at this time is problematical. If the agents from two high cancerous strains might be considered to be the same, then they have different activities when obtained by hybrid mice with the same genetic constitution; if the agents are not the same, then the hybrids with identical susceptibilities for spontaneous mammary cancer, perhaps expressed in part through hormonal stimulation, produce different activity as determined by the incidence of mammary cancer and average cancer age. These in turn may be altered by changing the degree of hormonal stimulation (16, 17).

In this study we have investigated the transplantability of spontaneous mammary cancer primarily from the standpoint of the source of the milk agent.

MATERIALS AND METHODS

In Table I we have listed the mammary tumors which were transplanted, the strains in which they developed and the strains from which the animals obtained the milk agent. The tumors were inoculated subcutaneously by the trochar method.

The various strains of mice tested with implants of these tumors are too well known to require description and are given in the various tables. However, some lines of these strains had been nursed by females of other stocks so that they had obtained either the mammary tumor milk agent from the fostering mothers or the milk agent had been eliminated by the same process.
TABLE I: GIVES THE ORIGIN OF THE MAMMARY TUMORS WHICH WERE TRANSPLANTED AND THE SOURCE OF THE MILK AGENT

<table>
<thead>
<tr>
<th>Tumor inoculated</th>
<th>Stock of origin</th>
<th>Source of milk agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Az # 7666</td>
<td>A stock</td>
<td>Z (C3H) stock</td>
</tr>
<tr>
<td>Za # 7667</td>
<td>Z (C3H) stock</td>
<td>A stock</td>
</tr>
<tr>
<td>Za # 7727</td>
<td>Z (C3H) stock</td>
<td>A stock</td>
</tr>
<tr>
<td>AaZF1 # 7668</td>
<td>Z Φ X Z σ F1</td>
<td>A stock</td>
</tr>
<tr>
<td>ZaF1 # 7665</td>
<td>Z Φ X A σ F1</td>
<td>Z stock</td>
</tr>
<tr>
<td>D1 # 5687</td>
<td>D stock - line 2</td>
<td>D2 line</td>
</tr>
<tr>
<td>D1 # 5736</td>
<td>D stock - line 1</td>
<td>D1 line</td>
</tr>
<tr>
<td>D2 # 6086</td>
<td>D stock - line 2</td>
<td>D2 line</td>
</tr>
<tr>
<td>D2 # 6087</td>
<td>D stock - line 8</td>
<td>D2 line</td>
</tr>
<tr>
<td>D1 # 6088</td>
<td>D stock - line 1</td>
<td>D1 line</td>
</tr>
<tr>
<td>B1 # 7106</td>
<td>B stock - line 6</td>
<td>A stock</td>
</tr>
<tr>
<td>B4 # 7476</td>
<td>B stock - line 6</td>
<td>A stock</td>
</tr>
<tr>
<td>B4 # 7477</td>
<td>B stock - line 6</td>
<td>A stock</td>
</tr>
</tbody>
</table>

RESULTS

The results obtained following the inoculation of spontaneous mammary carcinoma from the A and Z (C3H) cancerous strains and their reciprocal hybrids are tabulated in Table II.

TABLE II: RESULTS OBTAINED FOLLOWING THE TRANSPLANTATION OF MAMMARY CANCER FROM THE A AND Z (C3H) STOCKS AND THEIR RECIPROCAL HYBRIDS (A TUMOR WITH Z AGENT, No. 7666: Z TUMOR WITH A AGENT, No. 7667; AND RECIPROCAL HYBRID TU~ORS WITH EITHER A, No. 7668; OR Z MILK AGENT, No. 7665)

<table>
<thead>
<tr>
<th>Stock inoculated</th>
<th>Genetics of hosts</th>
<th>Milk agent</th>
<th>Az # 7666</th>
<th>Z # 7667</th>
<th>AzZF1 # 7668</th>
<th>ZzAF1 # 7665</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa</td>
<td>A stock</td>
<td>A stock</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Az</td>
<td>A stock</td>
<td>Z stock</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Ax</td>
<td>A stock</td>
<td>None</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>15*</td>
</tr>
<tr>
<td>Za</td>
<td>Z stock</td>
<td>Z stock</td>
<td>0</td>
<td>6</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Zz</td>
<td>Z stock</td>
<td>Z stock</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>5*</td>
</tr>
<tr>
<td>Zb</td>
<td>Z stock</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>ZσAF1</td>
<td>Z Φ X Z σ A σ</td>
<td>A stock</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ZaAF1</td>
<td>Z Z σ X A σ</td>
<td>A stock</td>
<td>11</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>AxB σF1</td>
<td>A σ X Z σ B σ</td>
<td>None</td>
<td>33</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Z b x X σF1</td>
<td>Z σ X A σ σ</td>
<td>None</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>AaDFσ1</td>
<td>A Φ X D σ σ</td>
<td>A σ σ</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AaDFσ1</td>
<td>A Φ X D σ σ</td>
<td>A σ σ</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>ZσDFσ1</td>
<td>Z Φ X D σ σ</td>
<td>Z σ σ</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DσDFσ1</td>
<td>D Φ X Z σ σ</td>
<td>D σ σ</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DσDFσ1</td>
<td>D Φ X Z σ σ</td>
<td>D σ σ</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AxCeFσ1</td>
<td>A σ σ X C σ σ</td>
<td>None</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>ZbDFσ1</td>
<td>Z Φ X D σ σ</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
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</table>

* See text.

Tumor Az No. 7666 developed in a female of the A strain with the milk agent from the Z stock and when transplanted grew progressively, resulting in the death of the hosts, in all the animals of the A strain and in F1 hybrids which had one parent from the A strain. Whether or not these animals had the milk agent made little difference. One exceptional inoculation was noticed in that one of the six F1 hybrids between Z and D (dilute brown) strains was susceptible to the tumor from the A stock.

Only animals of the Z (C3H) strain or hybrids derived by mating Z mice to animals of other stocks responded by being susceptible to the tumor, No. 7667, which developed spontaneously in a female of that strain with the A milk agent. Nine mice of the Z stock, descended from 4 mothers of which 2 were litter mates, proved to be resistant but were found to be susceptible to another Za tumor, No. 7727. Ten Aa and 3 Ax mice, negative to tumor Za No. 7667, were reinoculated with the Az tumor, No. 7666 and all grew that tumor progressively.

The tumors from the reciprocal hybrids between the A and Z strains, AaZF1 No. 7668 with the milk agent from the A stock and ZzAF1 No. 7665 with the Z milk agent, failed to grow in any of the mice of the two parental stocks regardless of milk agent. They both grew progressively in all hybrids of the A X Z cross, in mice with or without the agent. A few hybrids that had one parent from either the A or the Z strain were inoculated and they were all resistant to the two hybrid tumors (Table II).

Several mammary tumors were transplanted which arose spontaneously in breeding females of the dilute brown stock. Several sublines of this stock were tested and the source of the lines were: sublines 1 or 12 and 2 or 212, G. W. Woolley, Bar Harbor, while the mice referred to as line 8 had been secured from S. G. Warner, Springville, N. Y. The results are given in Table III.

The mice of line 2 of the dilute brown stock and their F1 hybrids (D2 Χ Z stocks) showed progressive growth of grafts of the 2 tumors that arose in mice of that line but they were all resistant to 2 tumors from line 1 and 1 tumor from line 8. The 2 tumors from line 1 produced transplanted tumor in all but one mouse of that subline and their F1 hybrids. Six of the 10 mice from line 1 were susceptible to a tumor from a mouse of line 8 but none grew the tumors from line 2. Mice of line 8 were
susceptible to grafts of the tumor from line 8, resistant to the tumor from line 2, and 1 of 16 grew the tumor from line 1.

Four sublines of the C57 black or B stock were tested to implants of mammary tumors from that stock. They were, with the source of the original materials: line 4, W. L. Russell, Bar Harbor; line 6, E. Fekete, Bar Harbor; and lines B and D, S. G. Warner, Springville. The mammary tumors arose in mice of line 6 of the B stock which had the milk agent from the A stock. Tumor No. 7106 developed time these studies were completed, it has become quite evident that the growth of these mammary tumors was not associated with the presence of the milk agent. Also, had the action of the milk agent been primarily responsible for the growth of such tumors, multiple primary tumors from a single host would have been expected to have given identical results when transplanted. That is, the inoculation of animals of the generations where segregation of the determining genes takes place would be expected to grow either all or none of these primary tumors when they were grafted simultaneously. These results have never been realized in that two primary tumors from a single host have never been found which required identical genes for progressive growth (39, 4, 18, 19, 4-9).

In this report we have considered the transplantation of spontaneous mammary carcinoma which arose in mice of 2 inbred strains of mice after the animals had obtained the milk agent from the other stock by foster nursing. Previous studies (16, 17) showed that the milk agent from the two donor strains did not have the same activity (tumor incidence and average cancer age) in mice with the same genetic susceptibility for spontaneous mammary cancer. If the growth of these tumors, when transplanted, had been dependent entirely upon the presence and "type" of milk agent, the following results might have been obtained:

1. If the milk agent from the 2 stocks were the same, all animals with the agent would be expected to be susceptible while mice of the same genetic constitution but without the agent should be resistant.

2. If the milk agents from the A and Z strains were not identical, the tumor from the A stock with the Z agent would be expected to grow in all mice with the Z agent and the tumor from the Z stock with the A agent should give progressive growth in mice with the A agent.
3. The growth of tumors from the reciprocal hybrids would be dependent upon the presence of the milk agent and respond accordingly. The results showed, however, confirming the observations of others, that the transplantability of these mammary tumors was not dependent upon the presence of the milk agent. The tumors grew equally well in animals that lacked the agent as in those that had it and in mice possessing an agent from a different source (i.e., a different strain) as in animals with the agent from the same source as was present in the tumor. The reaction of the host was dependent upon the genetic relationship between the host and the tumor cell.

The application of these findings to the growth of spontaneous mammary cancer in mice would be entirely theoretical. If cancer develops as the result of a somatic mutation, as has been suggested by many investigators, any cell capable of becoming cancerous but with a genetic constitution incompatible with that of the host would not be expected to survive. For obvious reasons it would be impossible to obtain such data.

Although the mice of all of the sublines of the C57 black stock responded alike to grafts of the 3 tumors from that stock, this does not imply that they had the same susceptibility for spontaneous mammary cancer. Others have found that the incidence of spontaneous mammary cancer may range from approximately 10 per cent (1, 15) to 76 per cent (24) in mice of the various sublines of this strain when they have the milk agent. Mice of at least 2 of the lines used will develop mammary cancer if they possess the agent.

In the dilute brown stock different incidences of spontaneous mammary cancer are being obtained in mice of the various sublines. Thus, they may have different susceptibilities for the growth of transplanted mammary cancer as well as for spontaneous mammary cancer. It has never been suggested that these may be comparable.

SUMMARY

The growth of mammary cancer in mice, as determined by its transplantability, is dependent upon the genetic relationship of the host and the tumor cell and not the mammary tumor milk agent.

The theoretical application of these results to the growth of spontaneous mammary cancer was considered.

Genetic differences in sublines of the same stock to transplanted mammary tumors may or may not indicate genetic variations in the inherited susceptibility for spontaneous mammary cancer.

REFERENCES

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