A HISTOLOGICAL STUDY OF HETEROLOGOUS TUMOR GRAFTS

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This paper records three series of experiments upon closely related factors in tumor immunity: (a) a study of the relation between the local tissue reaction and the heteroplastic tumor graft, (b) the effect of removal of the spleen upon the growth of heterologous tumor, (c) a study of the immunizing power of one heterologous tumor upon the growth of a second heterologous tumor of different type, and the possible influence of splenectomy upon such immunity.

The presence of lymphocytes, often in large numbers, in the tissues surrounding human tumors has long been observed by pathologists, many of whom have interpreted the condition as an effort on the part of the body to combat the growth of tumor cells. Da Fano (1), the first investigator definitely to correlate the presence of lymphocytes with the immunity to transplanted tumors, explained this immunity in terms of lymphocytic activity, the plasma cell, also, being considered as a factor. He offered no explanation regarding the manner in which these cells influence the growth of the tumor graft. Da Fano’s suggestion has been supported by other investigators, and especially by Murphy (2), who has noted that the presence of large numbers of lymphocytes in the region of a tumor is frequently followed by cessation of growth, and believes that the two phenomena are causally related.

In a study of homeoplastic epithelial grafts, Leo Loeb (3) has expressed the additional view that the destruction of these grafts is partly due to the action of the lymphocytes which pene-
trate them and cause the death of their cells. This action is aided by the presence of dense fibrous material which grows into the grafts, separating them into small islands, and by continued growth gradually compressing and perhaps eventually destroying the cells. It is Loeb's opinion that these two agents may act either independently of each other, or together.

A study of heteroplastic tumor grafts of several different strains was undertaken in the hope of shedding some light on the histological relationship between the tumor and its surrounding leukocytes and connective tissue, and also to determine whether this relationship remains constant regardless of the strain of tumor used for the inoculations. Although Bashford and Russell, in an investigation similar to the one recorded in this paper, have ascribed the death of the graft to the cytotoxic and cytolytic action of the body juices, it is hardly probable that they would have their conclusions regarded as otherwise than purely inferential, as there is no form of cell degeneration characteristic of an attack by cytotoxins or cytolysins. Since a histological study shows the results rather than the cause of a degeneration, only the conditions of the tissues that may lead to the death of the graft and not the ultimate causes of cell death are considered in this paper.

Two carcinomata and two sarcomata were selected for the experiment. Of the carcinomata, mouse tumor 5 shows a rapid rate of growth in the mouse, in contrast to the very slow growth of mouse tumor 58, the other carcinoma employed. Mouse sarcoma 180 and rat sarcoma 7 proliferate rapidly in the mouse and the rat respectively; the former grows progressively, the latter regresses at a comparatively early period. These tumors were inoculated into the subcutaneous tissues by needle, in the usual dose of 0.003 gram.1 From ten to fourteen days after inoculation, the grafts and their surrounding tissues were excised and submitted to microscopical examination (serial

1 In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.
sections), attention being focused on the stroma reaction, the relative number of lymphocytes, the degree of fibrosis, the vascularity of the surrounding tissues, and the condition of the graft. The results of this examination are arranged and tabulated below (table 1).

Mouse carcinoma 5 was inoculated into 25 rats, and the grafts were removed on the twelfth and thirteenth days after inoculation. Upon removal, 68 per cent of the grafts of this tumor were degenerated. In the degenerated grafts, lymphocytic infiltration and fibrosis existed in about equal degree, though one or the other might predominate in the individual graft. In the grafts in which the tumor was growing, or in which the cells persisted in good histological condition (38 per cent), lymphocytes were scarce and fibrosis was almost absent. These results are capable of two interpretations; (a) either fibrosis and lymphocytic infiltration were preliminary to the death of the graft, or (b) fibrosis and lymphocytic infiltration was a replacement process, occurring after the death of the graft.

Mouse carcinoma 58 was inoculated into 14 rats, and the grafts were removed on the twelfth day after introduction. This tumor in foreign hosts calls forth a tissue reaction of very low grade, characterized by a relative scarcity of lymphocytes, a slight connective tissue response, and a poor blood supply to the surrounding tissues and the graft. While the histologically apparent causes leading up to the death of the graft may possibly be found in the presence of lymphocytes and the fibrous changes, slight though these reactions are, the scanty blood supply appears to be the more important factor. The study shows that a slowly growing tumor, when introduced into an alien host, causes a relatively slight response by the tissues of that animal, and that the fate of the graft is not determined simply by the relative number of lymphocytes and the degree of fibrosis.

Mouse sarcoma 180 was inoculated into 16 rats, the grafts being removed on the tenth and twelfth days of growth. Of the two growing tumors in this series, one was surrounded by but few lymphocytes, while the other was surrounded by a
large number of these elements. In several of the rats in which the tumors failed to grow, lymphocytes were few in number about the degenerated graft. In these animals, fibrous changes about the degenerated tumor were slight.

Rat sarcoma 7 was inoculated into 21 mice, the grafts being removed ten, thirteen, and fourteen days after inoculation. This tumor produces in twelve to fourteen days a very mild reaction, characterized by a small number of lymphocytes in the immediate neighborhood of the graft, and an absence, or only a slight grade, of fibrosis. The tissue about the graft was very vascular and generally edematous. About the bloodvessels in this tissue, at a distance from the graft, the lymphocytes were more abundant. It seems scarcely permissible to attribute the death of the graft to the action of the lymphocytes, unless it be conceived that the lymphocytes exert their lethal action at a distance. In one animal, in which the graft was much degenerated, showing but few surviving sarcoma cells, no lymphocytes could be detected in the tissues or in the immediate vicinity of the tumor. In the majority of the other animals, the extent of degeneration of the tumor cells can not be explained by the number of lymphoid cells, as they were few, even when degeneration was marked. Neither can death of the graft be referred to the activity of the fibrous tissue, for in cases where degeneration of the graft was extensive or even complete, fibrosis was absent or only beginning. A more plausible explanation of the death of the graft, based solely upon the microscopical picture and excluding cytolyis for the reasons given in another paragraph, is found in the disturbance of nutrition due to the edema of the tissues, and consequent interference with the blood supply.

In a further series of inoculations, the following results were obtained. Rat tumor 7, which was inoculated into 24 mice, produced at the end of fourteen days 5 growing tumors, the largest measuring 3 by 8 mm., and 9 degenerated tumors with surviving tumor cells. Mouse tumor 180 was inoculated into 109 young, and very young rats, and gave rise on the fourteenth day after inoculation to 9 tumors, the largest being 7 by 11 mm.
The same strain of tumor was inoculated into 200 older rats, of which 7 had tumors on the thirteenth day, the largest measuring 6 by 10 mm.; on the twenty-first day this tumor measured 5 by 7 mm. There was no evidence that young rats, two to six weeks old, offer a better soil for heteroplastic tumors than do animals three to five months old. The grafts did not grow so well in sick as in healthy animals.

The following conclusions may be drawn from this study. While the causes leading to death of heteroplastic grafts may, perhaps, be attributed to the combined action of lymphocytes and connective tissue, one or the other exerting the predominating action, they are not always determined by the number of lymphocytes or the degree of fibrosis. With certain tumors (sarcoma), death of the graft is not due to fibrosis and cannot be attributed to the lymphocytes, unless it be assumed that these cells act through a distance by the production of some toxic agent (cytolysin or cytotoxin) inimical to the tumor cells. Tumors introduced into animals of foreign species elicit at first a reaction of very much the same character as that produced in homologous animals. Ehrlich has shown that certain tumors survive for only about a week in the tissues of an animal of alien species and until recently this fact was held to be applicable to heterologous grafts in general. W. E. Bullock (4), however, has demonstrated that they can be kept alive in foreign tissues for nearly three weeks, and that if removed and transplanted into other animals of the same foreign species before regression sets in, can be propagated for several generations. Bullock's observation confirms a previous observation of Lewin (5), who was able to grow a rat sarcoma in a mouse for three weeks. Tumor strains show wide differences in the character of their growth in foreign hosts (see table 1), and Bullock's successful results may be explained by the choice of tumor used for the inoculation.

In another connection (6), we have demonstrated that removal of the spleen has no effect upon the fate of a homologous graft. Since the publication of that article, Murphy and Morton (2) have asserted that heterologous tumor grafts grow better
<table>
<thead>
<tr>
<th>TUMOR</th>
<th>STROMA REACTION</th>
<th>LYMPHOCYTES</th>
<th>FIBROSIS</th>
<th>VASCULARITY</th>
<th>CONDITION OF GRAFT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>Mouse carcinoma 5</td>
<td>Positive 32</td>
<td>Few 20</td>
<td>Absent 8</td>
<td>Moderate</td>
<td>Growing 16</td>
</tr>
<tr>
<td></td>
<td>Negative 68</td>
<td>Moderate 48</td>
<td>Slight 12</td>
<td>Moderate</td>
<td>Persistent 16</td>
</tr>
<tr>
<td></td>
<td>Positive 7</td>
<td>Few 21</td>
<td>Slight 86</td>
<td>Poor</td>
<td>Growing 7</td>
</tr>
<tr>
<td>Mouse carcinoma 58</td>
<td>Positive 79</td>
<td>Moderate 70</td>
<td>Moderate 14</td>
<td></td>
<td>Degenerated 93</td>
</tr>
<tr>
<td></td>
<td>Negative 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse sarcoma 180</td>
<td>Positive 12</td>
<td>Few 19</td>
<td>Slight 25</td>
<td>Moderate</td>
<td>Growing 12</td>
</tr>
<tr>
<td></td>
<td>Positive? 6</td>
<td>Moderate 6</td>
<td>Moderate 25</td>
<td></td>
<td>Degenerated 88</td>
</tr>
<tr>
<td></td>
<td>Negative? 82</td>
<td>Abundant 75</td>
<td>Extensive 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat sarcoma 7</td>
<td>Positive 28</td>
<td>Very few or none</td>
<td>Branch</td>
<td>Absent 67</td>
<td>Growing 28</td>
</tr>
<tr>
<td></td>
<td>Positive? 43</td>
<td>none 62</td>
<td>Slight 33</td>
<td>Marked</td>
<td>Persistent 43</td>
</tr>
<tr>
<td></td>
<td>Negative? 29</td>
<td>Few 24</td>
<td></td>
<td></td>
<td>Degenerated 20</td>
</tr>
</tbody>
</table>

In classifying the stroma reaction as "positive" or "negative," it may be maintained that the time interval after inoculation is (10–14 days) in some animals long enough to permit a stroma reaction occurring and subsiding before examination. The truth of this possibility cannot be denied.
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and survive for a longer period of time in animals which have been exposed to x-rays, attributing this to damaged lymphoid tissues. With the idea of impairing the lymphocytic processes of the body, the spleens were removed from 132 animals either before or after inoculation with alien cancer. One hundred and fifty-six normal animals served as controls. The tumors used were mouse carcinomata 5 and 58, mouse sarcoma 180, and rat sarcoma 7. From twelve to fourteen days after inoculation, the tumor grafts were removed and studied microscopic-

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>NUMBER OF ANIMALS</th>
<th>TIME OF SPLEEN REMOVAL</th>
<th>GRAFT EXCHANGED IN DAYS</th>
<th>NUMBER OF TAKES</th>
<th>NUMBER SHOWING GROWTH</th>
<th>MAXIMUM SIZE OF TUMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>7</td>
<td>6 days post inoculation</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>0.5 by 1 mm.</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Not removed</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>22</td>
<td>5 and 6 days post inoculation</td>
<td>12 and 13</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>19</td>
<td>Not removed</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>47</td>
<td>1 day before inoculation</td>
<td>12 and 13</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>56</td>
<td>1 day before inoculation</td>
<td>10, 12, 13</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>60</td>
<td>Not removed</td>
<td>10, 12, 13</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

As shown in table 2, the spleen-free group gave a total of five growing tumors, and 16 growths more or less degenerated, but containing living tumor cells. In the control group, there were three growing nodules and seven degenerated ones with a few living cells. The growths averaged larger in the spleen-free than in the control animals, and the largest tumor (measuring 10 by 12 mm. on the twelfth day) was found in a spleen-free animal. Since these results are within the bounds of individual variations, it may be concluded that removal of the spleen has no appreciable influence upon the receptivity of an
animal for heteroplastic grafts, and that the growth of these grafts is not favored thereby.

Russell, Da Fano, and Bashford and Russell have shown that one inoculation of a heterologous tumor induces an immunity in the host toward a subsequent graft of the same strain of heterologous tumor. Bashford and Russell have specifically stated that this immunity is one directed against the foreign tumor merely as a foreign tissue and not in any sense as a neoplasm. We repeated these experiments, using splenectomized as well as normal animals, and various types of heterologous tumor for the second inoculation.

Twenty-one spleen-free and thirty-two control rats which had been inoculated with mouse tumor 180 (a sarcoma), and in which the tumors had disappeared, were reinoculated with mouse carcinoma 5. The results were negative.

Twenty-seven spleen-free and 34 control mice which bore no tumors after inoculation with rat sarcoma 7, were reinoculated twenty-one days later with the Flexner-Jobling carcinoma. No growing tumors were observed after eleven days, but a few of the grafts contained persisting cancer cells. Ninety-six rats inoculated with mouse sarcoma 180, 6 of which had tumors measuring 4 by 4 mm. or over, were reinoculated with the Ehrlich mouse sarcoma twenty-three days subsequent to the first inoculation. One had a growing tumor 6 by 9 mm. twelve days after inoculation and 4 by 6 mm. a week later. This tumor was found in an animal which had produced a growing tumor on the first inoculation with tumor 180. One hundred and eighty normal rats unsuccessfully inoculated with mouse sarcoma 180 were reinoculated with mouse sarcoma 37. Twenty-five bore tumors (see Chart 1) which in twelve days reached their maximum size and gradually receded thereafter; the largest tumor attained the size of 11 by 16 mm. The longest period of time in which cancer cells persisted in the tissues of an alien species was fifty-four days. This occurred after the inoculation of an emulsion of rat carcinoma 9 into a mouse. Figure 1 illustrates the persisting carcinoma cells; figures 2 and 3 show for comparison a larger field of tumor 9
taken from a stock series, and a section of mouse breast. It will be noted that the tumor cells are in better condition than the stroma, the latter showing marked hyaline degeneration.

Bashford and Russell, as a result of their investigations, consider immunity to heterologous tumors as a manifestation of immunity to a foreign tissue and not to tumor, and according to their viewpoint, we should expect immunity to a second inoculation of heterologous tumor irrespective of the type of tumor used. The results of a second inoculation of heteroplastic tumor do, in fact, agree with the results recorded by the investigators quoted when sarcoma inoculation is followed by carcinoma inoculation, but they do not appear to harmonize when sarcoma inoculation is followed by sarcoma inoculation. Several explanations may be given for the diver-
gent results; they may be due to a difference in the growth energy of the two tumors at the time that they were used; or to a difficulty in producing an immunity against these sarcomas (7); or finally, it is possible that the dosage of the initial inoculation (0.003 gram) was insufficient to cause immunity against the second inoculation.

**SUMMARY**

While the death of some heteroplastic tumor grafts may, perhaps, be attributed to the action of lymphocytes and connective tissue, there is no histological proof that it is always determined by these factors.

Tumors elicit in foreign species a reaction of much the same character as that produced in homologous animals.

Removal of the spleen has no influence upon the receptivity of an animal toward heteroplastic tumor grafts.

Splenectomy does not favor the growth of heteroplastic tumor grafts.

One inoculation of a heterologous tumor does not always render an animal immune to the temporary growth of a subsequent heterologous graft.

**REFERENCES**


**PLATE 1**

**Fig. 1. Graft of Rat Tumor 9, Persisting in Mouse Tissue for 54 Days.**

X 250
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PLATE 1

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PLATE 2

Fig. 2. Normal Growth of Rat Tumor 9, for Comparison with Figure 1. 
$\times 250$
PLATE 3

FIG. 3. NORMAL MOUSE BREAST.  \( \times 250 \)