Induction of Blood-borne Metastases by Tumor Transplantation in the Tail of Mice*

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It is well known that the incidence of blood-borne metastases is influenced by various factors and by several experimental procedures. Some factors exert their action directly on the primary growth, most likely by modifying the tumor-host relationships and favoring the entrance of tumor cells into the bloodstream. Massage (14, 17, 19, 31), local roentgen ray radiation of tumor (9, 15, 16, 18, 34), and incision of the tumor (8, 23, 28) have been demonstrated to act directly on the primary growth.

Other factors exert their action on the "take" of tumor emboli, i.e., they apparently favor the growth of tumor emboli at their site of arrest, rather than influence the entrance of tumor cells from the primary tumor into the bloodstream. Total-body roentgen ray radiation (6, 10), trypan-blue injection of the host (11, 26), repeated bleeding (28), and cortisone (2, 5, 22, 25) have been demonstrated to act on the host, rather than on the primary tumor, enhancing the "take" of the released tumor emboli.

Numerous other factors have been shown to influence the incidence of metastases through yet unknown mechanisms. Of these, we can mention the duration of tumor growth (7, 12, 13, 21, 29, 31, 35), size of the tumor (13, 33), injection of tumor autolysate fraction into the host (4), ACTH (24), pregnancy (33), underfeeding of tumor-bearing animals (30), and heredity (27).

In spite of the fact that the mechanism of action of some factors has been identified, the study of blood-borne metastases is often hindered by the difficulty of separating the two stages (invasion of blood vessels and local growth at the site of arrest), and therefore of determining whether a given experimental procedure is affecting the primary growth or the host, or both. The present experimental procedure is based on the behavior of a transplantable tumor which produced no metastases to distant organs when it was implanted in the subcutaneous tissue of the trunk; however, when transplanted into the subcutaneous tissue of the tail, it yielded numerous metastases to the lungs. Such a behavior and the possibility of removing the primary growth by amputation of the tail at various intervals seemed to offer a simple procedure for studying the mechanism of blood-borne metastases in its different phases.

MATERIALS AND METHODS

Mice of the DBA/2 strain, obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine, were used. At the beginning of the experiment the animals were from 10 to 12 weeks old. They were kept in plastic cages and fed Rockland mouse diet and water ad libitum.

The tumor used throughout this experiment is a transplantable tumor which originated in this laboratory. The tumor had been induced in the subcutaneous tissue of a DBA/2 mouse by injection of 2 mg. of methylcholanthrene dissolved in 0.3 cc. of tri-n-caprylin and had been transplanted for several generations before its use in the present experiment. Histologically, the tumor (hereby designated as Gl-46) is a fibrosarcoma, which, when transplanted into the subcutaneous tissue of the abdomen or lumbar region, takes in 100 per cent of DBA/2 mice, becomes visible in 12-15 days, and grows steadily and usually kills the host 6-7 weeks after the implant. Blood-borne metastases in the lungs or other organs were never observed during the routine transplants.

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The tumor tissue (Gl-46) was ground in saline, the number of cells/ml was counted, and a variable amount of suspension was injected into DBA/2 mice divided into six groups:

Group 1.—Fifty mice, male and female, were given injections of $1 \times 10^6$ cells (0.3 ml. of suspension) per each mouse, by use of a 22-gauge needle, in the subcutaneous tissue of the right lower quadrant of the abdomen. This site has been selected for the present experiment, though, in
especially during the first attempts, some of the injections were made directly into the vein of the tail. The tumor suspension contained $6 \times 10^6$ cells/cc, so that only $\frac{1}{4}$ ml. of the suspension was injected, to avoid excessive deformation of the structures. In a few instances, especially during the first attempts, some of the injections were made directly into the vein of the tail. Such an occurrence can be easily recognized, because the plunger of the syringe does not oppose any resistance to the pushing finger of the injector, and the animal shows obvious signs of distress and very often succumbs. Such animals were discarded from the experiment.

Group 3.—Thirty mice, male and female, were given injections following the procedure described for Group 2. Two weeks after the first appearance of a visible growth, the tail was amputated proximally to the tumor in the following manner: The tail was clamped as near as possible to the root and thereafter removed by simply cutting with a pair of scissors. The procedure was rapid and reduced bleeding to a minimum.

Group 4.—Forty mice, male and female, were given injections following the procedure described for Group 2. One week after implantation, and before any grossly visible tumor growth appeared, the tail was amputated as near as possible to the root, following the technic described for Group 3.

Group 5.—Fifteen mice, male and female, were given implants in the subcutaneous tissue of the tail by a modification of the trocar method. A small piece of tumor tissue was inserted underneath the skin of the tail, between the dorsal vein and the right lateral vessel.

Group 6.—Fifteen mice, male and female, were given injections of $1 \times 10^6$ cells (0.3 ml. of suspension) per each mouse, with a 22-gauge needle, in the subcutaneous tissue of the right lower quadrant of the abdomen. In this particular group, the tumor was taken from the tail of a mouse that had metastases in the lungs.

All animals were numbered by ear clipping. The time of appearance of the primary growth and its course were charted individually.

Some of the animals died early in the experiment and were discarded. Other animals died 4-6 weeks after the implant, and all the survivors were killed 7 weeks after the implant.

The tumor size was determined by the method described by Zeldman et al. (35). A careful autopsy of each animal was performed; the lungs were removed and fixed in formalin, and the number of eventual metastases counted after fixation. Sections from the primary growth and from each pulmonary lobe of every animal were taken. The sections were stained with hematoxylin and eosin, with the Weigert method for elastic fibers, and were examined microscopically.

RESULTS

The results are shown in Table 1. It appears that the implant of tumor Gl-46 into the subcutaneous tissue of the tail induces blood-borne metastases. No metastases could be discovered in mice carrying the same tumor in the subcutaneous tissue of the abdomen.

It is also worth noticing the absence of metastases in Group 6, in which the mice were injected in the subcutaneous tissue of the right lower quadrant of the abdomen. The tumor used for this group had been taken from the tail of a mouse of Group 2, which had a total of seven pulmonary metastases. On the other hand, metastases were present in one-third of the animals of Group 3 (tail implant) in which the tail had been amputated 2 weeks after the appearance of a visible primary growth. The trocar method (Group 5) is far more tedious than the simple injection and gives identical results.

The average size of the primary tumor was definitely greater in mice carrying the tumor in the subcutaneous region of the abdomen than in those given implants in the tail.

The average duration of the primary growth (interval between the appearance of a grossly visible tumor and the death of the animal) did not differ significantly in Groups 1, 2, 5, or 6. Group 3, in which the tumor was removed by amputation of the tail 2 weeks after its first appearance, shows, of course, the shortest average duration of the primary growth.

Table 2 refers only to mice given implants in the subcutaneous tissue of the tail (Groups 2, 3, and 5), and shows a breakdown of the figures according to sex, duration of the primary growth, and size of the primary growth.

The number of metastases in each mouse ranged from one to thirteen; however, of 47 animals with metastases, 32 had only one or two metastatic nodules. The size of metastases varied from that of a pin head to 3 mm. in diameter, but the majority, 115 out of 131, were about 1 mm. in diameter. As Table 2 shows, the incidence of metastases is significantly higher in males than in females, both in number of animals with metastases and in the number of metastases. The figures related to the duration of the growth and to the size of the primary growth do not appear to be significant when the number of animals with metastases is taken into consideration, probably due to the relatively scarce number of animals. When the total number of metastases is considered, the figures, though not highly significant, show an increase with the increasing duration of growth, while the largest primary growths (more than 2 ml. in size) have the highest number of metastatic nodules.

Histopathology.—As we mentioned above, Gl-46 is, histologically, a fibrosarcoma. When implanted in the tail, tumor Gl-46 grows in the subcutaneous tissue, where it replaces the muscle bundles and invades the veins. In early stages, one side of the tail, usually the ventral side, is spared by the invading growth (Fig. 1), but in later stages the entire circumference of the tail is involved.
with destruction of the central ossicles. In many instances, the tumor cells were found actively proliferating in the lumen of the large tail veins.

Sections taken from tumors growing into the subcutaneous tissue of the abdomen showed invasion of venules and capillaries, though in no instance was invasion of large vessels noticed. The microscopic examination of the lungs confirmed the gross diagnosis concerning the absence or presence of metastases. In five instances, however, been released only a few days before the death of the animal.

**DISCUSSION**

In the present experiment, a transplantable sarcoma (GL-46) that does not produce blood-borne metastases when implanted into the subcutaneous tissue of the abdomen yielded numerous pulmonary metastases when transplanted into the subcutaneous tissue of the tail. The possibility that the procedure might have damaged the blood ves-

**TABLE 1**

**INCIDENCE OF METASTASES IN DBA MICE TRANSPLANTED WITH TUMOR GL-46**

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Site of transplant</th>
<th>No. animals</th>
<th>No. animals with metastases in the lungs</th>
<th>Av. size of the primary growth (ml.)</th>
<th>Av. duration of the primary growth (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Abdomen, subcutaneous</td>
<td>46</td>
<td>0</td>
<td>4.1</td>
<td>24.2</td>
</tr>
<tr>
<td>2</td>
<td>Tail, subcutaneous</td>
<td>64</td>
<td>23</td>
<td>1.8</td>
<td>26.9</td>
</tr>
<tr>
<td>3</td>
<td>Tail, subcutaneous*</td>
<td>39</td>
<td>12</td>
<td>1.1</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>Tail, subcutaneous†</td>
<td>32</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Tail, trocar method</td>
<td>16</td>
<td>7</td>
<td>2</td>
<td>25.8</td>
</tr>
<tr>
<td>6</td>
<td>Abdomen, subcutaneous‡</td>
<td>15</td>
<td>0</td>
<td>4.6</td>
<td>25.1</td>
</tr>
</tbody>
</table>

* The tail was amputated, proximally to the tumor, 2 weeks after the appearance of the primary growth.
† The tail was amputated 1 week after the transplant, before any grossly visible growth appeared.
‡ The tumor used for this transplant was taken from the tail of a mouse of Group 8 that had pulmonary metastases.

**TABLE 2**

**INCIDENCE OF PULMONARY METASTASES IN DBA/MICE TRANSPLANTED IN THE SUBCUTANEOUS TISSUE OF THE TAIL WITH TUMOR GL-46**

<table>
<thead>
<tr>
<th>SEX</th>
<th>TOTAL Male</th>
<th>Female</th>
<th>No. animals</th>
<th>No. animals with metastases</th>
<th>No. metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>106</td>
<td>52</td>
<td>44</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>28</td>
<td>19</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>131</td>
<td>65</td>
<td>38</td>
<td>28</td>
<td>12</td>
</tr>
</tbody>
</table>

* In 34 mice the size of the primary growth was not estimated.

The microscopic picture of metastases did not offer any peculiar feature (Figs. 2, 3) and seemed to follow the pattern of the histogenesis of metastases described by Baserga and Saffiotti (1) using tumor T150. In three animals of Group 2, intravascular tumor emboli, not extending beyond the endothelial wall, were found in the pulmonary arterioles. These three animals had died 29, 37, and 38 days after the tumor was implanted in the tail. The embolic tumor cells maintained their staining capacity and did not show any sign of degeneration or regression (Fig. 4). Using tumor T150, Baserga and Saffiotti (1) were able to demonstrate that such a finding represents an early stage in the development of blood-borne metastases. It is, therefore, reasonable to assume that such emboli had sels of the tail, thus favoring the direct entrance of tumor cells into the circulation, has been taken into consideration; metastases from the tail occurred also when the trocar method was used (Group 5). Furthermore, if the tail was amputated before any grossly visible growth appeared, no metastases could be detected (Group 4), thus indicating that metastases were actually due to the tumor growing in the tail. On the other hand, pulmonary metastases were found in twelve out of 26 animals when the tail was amputated 2 weeks after the appearance of a grossly visible tumor in the tail.

This experiment emphasizes the importance of the site of the primary growth in determining the production of metastases. In the literature, reports concerning the influence of the site of transplant on the incidence of metastases are scarce. The tail, as a site of transplantation, had already been used in 1925 by Boone (3), who used a metastasizing sar-
sarcoma of the mouse and showed that the tail implant increased the incidence of metastases. In 1933, Mazzacuva (20) showed that the Galliera sarcoma, which rarely metastasized when transplanted into the subcutaneous tissue, yielded a high percentage of metastases when implanted into the peritoneal cavity. Recently, Goldie and co-workers (18), using three different tumors and the technic of intra-peritoneal inoculation of organ brei, were able to detect metastatic tumor cells (not actual metastases) in different organs of tumor-bearing animals and to show that the incidence of metastatic tumor cells depended, among other factors, upon the site of growth of the primary tumor.

It is possible that such a phenomenon is due more to local anatomical conditions than to a change in the biology of the tumor, as evidenced by the negative results obtained in Group 6. Such a result seems to support the statement of Willis (32) that “the size of the vein invaded by a neoplasm is of great importance for the development of metastases,” and indirectly confirms that the number of embolic cells is an important factor in the establishment of a metastasis (35).

The experiment may offer a simple and valuable technic for studying the mechanism of metastases:

1. It affords the possibility of obtaining metastases from otherwise nonmetastasizing tumors, thus overcoming a difficulty which is often found in animal tumors (23).

2. It permits the removal of the primary growth with a simple procedure that reduces to a minimum trauma and bleeding, both being factors that influence the local growth of metastases (29).

3. Once the primary growth has been removed, it is possible to study and modify the third phase of blood-borne metastases, the so-called “local growth” (32), without acting upon the primary growth and without the disadvantages of the method of direct injection of tumor cells into the circulation (32). In particular, such a procedure provides a method for studying separately the factors that influence the penetration of tumor cells inside the blood vessels (direct action upon the primary growth) and those that favor or inhibit the “take” of the metastatic tumor emboli. The technic has been already adopted in this laboratory for an experiment the results of which will be published separately.¹

SUMMARY

Tumor Gl-46, a transplantable sarcoma which does not metastasize when implanted into the subcutaneous tissue of the trunk, yielded a high percentage of blood-borne metastases when transplanted into the subcutaneous tissue of the tail. This finding emphasizes the importance of the site of the primary growth in the spread of tumors by blood stream. Its significance and its value as a simple technic for the study of the mechanism of metastases are discussed.

ACKNOWLEDGMENTS

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Fig. 1.—Transverse section of the tail of a DBA/2 mouse implanted with tumor Gl-46, 3 weeks after the implant. The tumor (to the left) is growing into the subcutaneous tissue but has not yet invaded the bone (to the right). X12.

Fig. 2.—Metastasis in the lung of a DBA/2 mouse from Gl-46 implanted into the tail. Intra- and extravascular growth of the tumor cell embolus. X160.
FIG. 3.—Large metastasis in the lung of a DBA/2 mouse from Gl-46 implanted into the tail. Compression of the pulmonary parenchyma. ×600.

FIG. 4.—Intravascular tumor cell embolus in a pulmonary artery of a DBA/2 mouse implanted into the tail with Gl-46, 37 days after the implant. No extravascular spread. ×160.
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