Effect of Size and/or Rate of Growth of a Transplantable Mouse Adenocarcinoma on Lung Metastasis Production*

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The factors responsible for the metastatic spread of malignant tumors are not as yet completely known. According to Zeidman et al. (4), the number of metastatic nodules found in the lungs is directly proportional to the number of embolic cells detached from the primary growth. This was demonstrated in mice by injecting intravenously different tumor cell concentrations and, after a given length of time, counting the number of tumorous nodules found in the lungs. They also found a direct proportionality between the time during which the tumor stays in the host and the number of metastases produced by such a tumor. However, they were not able to demonstrate any relationship between the final size of the primary tumor and the number of metastases. Wood et al. (3) repeated the same experiments under the same experimental conditions, but used a larger number of mice and were able to show a good correlation between the final size of the primary growth and the number of metastatic tumors in the lungs: the larger the primary tumor, the more metastases were found.

Since the time required for the development of a new tumor when mammary tumors in mice are transplanted into the subcutaneous tissue is dependent, at least in part, on the size of the donor tumor (2) and since metastases are essentially autotransplants of embolic tumor cells detached from the primary growth, the determination of the time at which this process takes place and its correlation, if any, with a corresponding size of the primary tumor are of particular interest.

With the use of mice bearing a transplanted tumor, an experiment was designed which consisted of the removal of the primary growth at different time intervals after its inoculation and, 45 days later, the determination of the incidence of lung metastases in the various groups. In doing so, it was thought that it might be possible to find out with some degree of accuracy the time at which metastatic tumor cells are detached to invade the general circulation and to see whether that time bears any relationship to the size of the primary tumor when removed.

MATERIALS AND METHODS

Young female mice of the ZBC stock were used as recipient hosts. Mice of this strain are able to grow tumors from the Z (CSH) strain. The tumor used was a transplantable adenocarcinoma of the mammary gland which arose spontaneously in a Z (CSH) breeding female and has been maintained in this laboratory for 52 successive passages into ZBC mice.

Two types of experiments were performed. In one, the tumor was inoculated as a 5 per cent tumor cell suspension in saline and injected subcutaneously into the right side of the body, in an amount of 0.5 cc/mouse. As soon as the tumors began to appear, the animals were divided into four different experimental groups. In the first group the tumors were surgically removed when their sizes ranged from 0.60 to 0.79 cm., as determined by caliper measurements. In the second group the primary growths were removed when they reached sizes ranging from 0.80 to 0.99 cm. In the third group the tumors were extirpated when they had attained sizes ranging from 1.00 to 1.40 cm. Finally, in the fourth group the tumors were removed when their sizes ranged from 1.21 to 1.40 cm. in diameter. After the operation mice showing recurrence of the tumor due to an incomplete removal were discarded. The rest were kept alive for 45 days and then sacrificed, the lungs dissected out, and the presence of tumor nodules determined under a dissecting microscope (1.3 X 10 diameters). The tumorous nature of the nodules was confirmed histologically.

To facilitate the removal of the primary tumor, the tumor was transplanted in the second experi-
ment into the subcutaneous tissue of the tail. In one group, the donor tumor used for transplantation had a mean diameter of 1.15 cm. In another group, the same tumor was used when it attained a mean size of 0.60 cm. In both instances, a tumor cell suspension was prepared at an 8 per cent concentration in saline and injected into the subcutaneous tissue of the tail in an amount of 0.05 cc/mouse, following the technical procedure described by Baserga et al. (1). At different intervals after the inoculation in both experiments, groups of mice bearing tumors of different sizes were submitted to removal of the primary tumor by amputation of the tail without anesthesia; 45 days later the animals were sacrificed, the lungs dissected out, and the incidence of lung metastases determined as before.

**TABLE 1**

**INCIDENCE OF LUNG METASTASES IN MICE BEARING A TRANSPLANTED MAMMARY ADENOCARCINOMA, WHICH WAS REMOVED AT DIFFERENT TIMES AFTER THE INOCULATION**

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Mean size of the primary tumor when removed (cm.)</th>
<th>No. mice</th>
<th>Incidence of lung metastases* (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-15</td>
<td>0.60-0.79</td>
<td>34</td>
<td>24</td>
</tr>
<tr>
<td>15-18</td>
<td>0.80-0.99</td>
<td>28</td>
<td>58</td>
</tr>
<tr>
<td>18-20</td>
<td>1.00-1.20</td>
<td>24</td>
<td>71</td>
</tr>
<tr>
<td>20-22</td>
<td>1.20-1.40</td>
<td>17</td>
<td>88</td>
</tr>
</tbody>
</table>

*At 45 days after removal of primary tumor.

**RESULTS**

**Subcutaneous implantation into the right side of the body.**—The results of this experiment are shown in Table 1. As can be seen in the group of mice in which the primary tumor was removed when its size ranged from 0.60 to 0.79 cm. in diameter, eight out of 34 (24 per cent) had metastatic tumors in the lung when examined 45 days later. In the second group, in which the primary tumor was removed when its size ranged from 0.80 to 0.99 cm., fifteen out of 26 animals (58 per cent) had lung tumors. In the third group, in which the primary tumors were removed when they had reached a size ranging from 1.00 to 1.20 cm., lung metastases were observed in seventeen out of 24 mice (71 per cent). Finally in the group in which the primary growths were removed when their sizes ranged from 1.21 to 1.40 cm., fifteen out of seventeen animals (88 per cent) had developed metastatic tumors in the lungs.

**Subcutaneous implantation in the tail.**—The results obtained in both experiments listed in Table 2 (series 1 and 2) showed qualitatively the same results. In fact, there was a progressive increase in the incidence of lung metastases with a corresponding increase in the size of the primary tumors. However, by comparing the results obtained in both series, it seems that, at any given time after the inoculation, the incidence of lung metastases was higher in the group of animals bearing rapidly growing transplants from the large donor tumor than in the group bearing transplants from the small tumor.

**DISCUSSION**

The experiments described seem to indicate that there is a relationship between the size of the primary tumor and the incidence of lung metastases. Both experiments, i.e., the one in which the tumor was transplanted into the subcutaneous tissue of the body and the one in which the tumor was inoculated into the subcutaneous tissue of the tail, gave qualitatively the same results: the larger the tumor when removed, the higher the incidence of metastases found. These findings seem to agree with those reported by Wood et al. (8), who demonstrated a direct correlation between the final size of the primary tumor and the number of metastases.
ferent rates of growth are compared (Table 2, series 1 and 2) seem to indicate that the rate of growth of the primary tumor, rather than the number of days during which the tumor remains in the host, determines the time at which the metastatic cells are released from the tumor. As can be seen in the table, it seems clear that at any given time after the inoculation the incidence of lung metastases was greater in the group of animals bearing fast-growing tumors (series 2) than in the group of mice bearing slow-growing tumors (series 1).

SUMMARY

By surgically removing the primary tumor at different intervals after its inoculation and determining 45 days later the incidence of lung metastases in the operated mice, the following results were obtained:

1. The incidence of lung metastases was related to the size of the primary tumor at the time of its removal. The larger the tumor when removed, the higher the incidence of lung metastases found.

2. Fast-growing tumors produced metastases earlier than slow-growing ones, in spite of the fact that the latter stayed longer in the host than the fast-growing tumors.

REFERENCES


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