Metastatic Behavior of a Murine Reticulum Cell Sarcoma Exhibiting Organ-specific Growth

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ABSTRACT

The metastatic properties of the M5076 tumor, a reticulum cell sarcoma of ovarian origin, were examined. This tumor metastasizes preferentially to the peritoneal viscera (liver, ovaries, spleen, and kidneys) regardless of the site or route of tumor cell injection. Subcutaneous tumor growth followed by metastasizes preferentially to the peritoneal viscera (liver, ovarian, and renal tumor colonies were formed after i.v. injection of tumor cells. Radiolabeled tumor cells were used to study the arrest, distribution, and survival of tumor cells injected i.v. These tumor cells were rapidly arrested in the lungs and were retained there for 3 to 4 days. They then slowly detached, recirculated, and were arrested in the liver, where they subsequently developed into tumor nodules. These results strongly support the "soil-seed" hypothesis of metastatic growth and demonstrate that long-term retention of tumor cells in an organ need not result in the formation of a clinically obvious tumor nodule.

INTRODUCTION

In the accompanying paper, we have described and characterized a murine tumor of spontaneous origin, identified by functional and ultrastructural studies as a reticulum cell sarcoma of macrophage origin (26). Here, we report on the metastatic patterns, distribution, and fate of cells from the M5076 reticulum cell sarcoma transplanted by different routes into syngeneic C57BL/6 mice and show that this tumor line selectively colonizes in the peritoneal viscera.

The organ-specific nature of metastatic spread is well recognized, and studies of several animal tumor systems have shown that the anatomical location of secondary tumor deposits is determined by both host and tumor cell properties (9). One approach to analyzing the properties of tumor cells responsible for such organ-specific growth has been the selection of variant lines from a parent tumor that colonizes in particular organs (1, 2, 8, 22, 24, 27). Most metastatic solid tumors in rodents, however, metastasize to the lungs, regardless of the route of tumor injection (9). Because they exhibit selective patterns of metastatic growth that may exclude the lungs, it has been proposed that murine reticuloendothelial tumors may serve as good models for investigating organ-specific metastasis (5, 18, 19).

We are using the M5076 tumor line in an attempt to determine factors responsible for specific patterns of metastatic spread. The localization, retention, and survival of a relatively large number of neoplastic cells in the lungs do not result in the formation of grossly obvious tumor foci by the time the animals die of metastases of the abdominal viscera. These results suggest that host organ factors, other than specific cell-to-cell interactions that determine the initial arrest of circulating tumor cells, may contribute to the phenomena of tumor dormancy and site-specific patterns of metastasis.

MATERIALS AND METHODS

Mice. Specific-pathogen-free mice of the inbred strain C57BL/6 were obtained from the Frederick Cancer Research Center's Animal Production Area. Within a single experiment, all mice were age matched. Unless otherwise noted, only female mice were used.

Tumor Cells. The murine tumor M5076 arose as a spontaneous tumor in the ovary of a C57BL/6 mouse in the laboratory of Dr. W. F. Dunning of the Papanicolaou Research Institute in Miami, Fla. We obtained the tumor from Dr. D. P. Griswold of the Southern Research Institute, Birmingham, Ala. The tumor was frozen and supplied to us in the 136th serial passage in syngeneic mice. The cultured tumor used in these experiments was examined for and found free of Mycoplasma and the following murine viruses: reovirus type 3, pneumonia virus of mice, K virus, Theiler's virus, Sendai virus, minute virus of mice, mouse adenovirus, mouse hepatitis virus, lymphocytic choriomeningitis virus, ectromelia virus, and lactate dehydrogenase virus (Microbiological Associates, Inc., Walkersville, Md.)

Culture Conditions. The M5076 cells were grown suspended in Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 17% equine serum (Flow Laboratories, Inc., Rockville, Md.), 2 mM glutamine, 1 mM sodium pyruvate, and 50 μg gentamycin per ml. The cells were split 1:2, subcultured weekly, and incubated at 37° in a humidified atmosphere of air and 5% CO2.

To establish primary cultures, ascites-passaged tumor cells were aseptically removed from tumor-bearing mice with peritoneal washes of serum-free medium, washed twice in serum-free medium, and cultured in 100-mm x 20-mm tissue culture plates (Falcon) where the cells grew in suspension.

Experimental Metastasis Formation. Cells were harvested from mid-log-phase cultures by removing aliquots of the cell suspension and washed twice in Hanks' balanced salt solution. Viability was assessed by trypan blue exclusion, and only suspensions with >90% viability were used. The tumor cell suspensions were adjusted to the required number of viable cells per ml and injected into mice via the lateral tail vein in a volume of 0.2 ml Hanks' balanced salt solution. The animals were killed 21 days later; the organs were removed, rinsed in...
were performed to determine whether tumor cells lodged in pulmonary tissue remained viable and capable of proliferation in spite of their failure to form clinically apparent metastases. Tumor cells were harvested as described above. Animals given injections of radiolabeled tumor cells show the uniformity of labeled cells that remain viable in vitro (>90%). Cultures in mid-log growth were labeled using 0.3 μCi [125I]iodo-2'-deoxyuridine (IUdR) per ml for 72 hr. This method results in uniformly labeled cells that remain viable in vitro (>90%). Animals given injections of radiolabeled tumor cells show the same qualitative and quantitative patterns of metastasis at necropsy as those animals given injections of control untreated cells.

Labeled cells were harvested and washed as described above. The suspension was diluted to give 50,000 single cells/0.2 ml, the inoculum dose per mouse. Triplicate inocula were prepared, and their radioactivity was monitored to determine the activity per cell. Mice were given injections in the tail vein, and 5 mice from each group were killed at intervals ranging from 5 min to 21 days. Lungs, liver, spleen, kidneys, ovaries, and 0.2 ml of blood were collected from each mouse. The organs were washed for 72 hr in 3 changes of 70% ethanol to remove ethanol-soluble free [125I]. The radioactivity for each washed organ was counted using a well-type NaI γ counter. The number of originally injected tumor cells viable in the organs at the time of death was calculated from the activity per cell ratio to the representative inoculum samples and corrected for decay (7, 10). The number of tumor colonies on Day 21 after i.v. injection was determined with the aid of a dissecting microscope.

Bioassay for Presence of Tumor Cells. Two experiments were performed to determine whether tumor cells lodged in pulmonary tissue remained viable and capable of proliferation in spite of their failure to form clinically apparent metastases. In the first experiment, portions of lungs (1 cu mm) were removed from mice bearing i.m. primary tumors and transplanted into the mammary fat pad of normal recipients. In the second experiment, 50,000 viable M5076 cells were injected i.v. into mice; at 4, 7, and 14 days after injections, these animals were killed, and the lungs and livers were removed and dissected into 1-cu mm portions and implanted in the mammary fat pads of groups of normal mice. Recipient animals were monitored for tumor growth at the site of organ fragment implantation and were examined for the presence of spontaneous metastases at necropsy.

RESULTS

Gross Pathology and Spontaneous Metastasis. At the time of necropsy, tumor foci were grossly obvious as small (0.5- to 1-mm diameter), discrete, grayish-white nodules. The extent and pattern of metastasis depended on the anatomical site of the primary tumor growth.

Mice inoculated with 10,000 to 50,000 viable tumor cells in the posterior mammary fat pad developed a palpable tumor approximately 14 days after tumor cell inoculation. The primary tumor commonly involved the musculature of the abdominal wall and frequently gave rise to the formation of hemorrhagic ascites. Metastasis from this implantation site probably occurs both by direct extension and by hematogenous spread, as organ metastases observed in mice that displayed ascites formation were similar to those following i.p. injection of tumor cells. Discrete neoplastic lesions were scattered throughout the mesentery and omentum and were found in the liver, spleen, ovaries, pancreas, and occasionally the kidneys. Tumor cells were also seen in the mesenteric and lumbar lymph nodules. In many animals, tumors growing s.c. in the flank did not display the usual extensive invasion of the abdominal wall. Metastases in such animals were generally restricted to the liver, spleen, and ovaries, suggesting that the spread occurred by the hematogenous route (Table 1).

Tumors implanted s.c. in the scapular region or in the external pinna (of the ear) and i.m. in the footpad gave similar patterns of metastatic spread, with hepatic, splenic, and ovarian involvement. The number of spontaneous hepatic nodules formed after the injection of 5 × 10⁴ M5076 cells into the dorsal scapular region was lower in male than in female recipients (p < 0.001, Mann Whitney U test). No differences in spontaneous metastasis from other primary sites were detected between male and female mice (Table 1).

Tumor cells inoculated into the external ear grew more slowly and had longer induction times than tumors at the other s.c. sites (Table 2). Male and female mice exhibited similar quantitative and qualitative patterns of spontaneous metastasis. Tumor growth was slower in the footpad of one of the hind legs than at any other site and resulted in fewer spontaneous metastases than those found at other anatomical sites (Table 2).

Histological Appearance. Tumors growing at both primary and metastatic sites were composed of sheets of randomly orientated, highly pleomorphic cells (Figs. 1 to 6). Tumor cells exhibited a high nucleus-cytoplasm ratio, and mitotic figures were plentiful. The nuclei were irregular in shape and size, had multiple prominent nucleoli, and showed a slight degree of chromatin margination. The neoplastic tissue appeared well vascularized, was unencapsulated, and had a minimal inflammatory cell component.

Metastasis Formation following i.v. Injection of M5076 Tumor Cells. M5076 tumor cells injected i.v. produced metastatic patterns similar to those seen following s.c. or i.m. implantation; no tumor nodule formation in the lungs was grossly evident. Significant numbers of hepatic nodules (median, 24; range, 4 to 109) were formed following the injection of as few as 5000 viable cells. Most metastatic nodules resulting from i.v. injection of M5076 cells were located in the liver, but occasionally an animal developed a few (1 to 3) lung nodules (Table 3), especially when a large number of cells were injected. No differences were detected between the number of hepatic tumors formed in male and female mice following injection of equal numbers of tumor cells; but male mice fre-
Consequently developed renal tumors, whereas female mice did not.

Quantitative Analysis of Tumor Cell Distribution and Survival. Results of a representative experiment are summarized in Table 4. By 5 min after i.v. injection, practically all \(^{125}\)IUDR-labeled M5076 tumor cells were arrested in the lungs and remained there at high levels for the next 6 hr. A gradual decline in cells retained in the lungs was accompanied by a gradual increase in cells arrested in the liver. Thus, by 4 days after injection, more radiolabeled tumor cells were in the liver than in the lungs (Chart 1). Cell-associated radioactivity in the visceral organs (spleen, kidney, and liver) peaked at 15 min, 4 hr, and 2 days. These “peaks” and “troughs” were reproducible in several experiments (Chart 1) and presumably are indicative of recirculation of tumor cells (7). The gradual shift in cell retention patterns is evident from the fact that, 60 min after i.v. injection, 87% of injected cells were in the lungs, 3.8% in the liver, and 0.12% in the kidneys. By 1 day after i.v. injection, 32% of radiolabeled tumor cells were in the lungs, 7.8% in the liver, and 1% in the kidneys.

Tumorigenicity of M5076 Cells Retained in the Lungs. The absence of visible lung metastases was not associated with the complete destruction of tumor cells at this site. In 2 experiments in which lung fragments were implanted into the mammary fat pads, tumors grew in the majority of recipient animals. Portions of lungs were removed from 13 animals 41 days after they had been given i.m. (footpad) injections of M5076 cells (see above). These animals displayed the described metastatic pattern and had no grossly apparent lung tumors. However, 11 of 13 animals that received lung grafts from these tumor-bearing animals developed tumors at the implantation site. Furthermore, the 11 mice that developed tumors at the implant site were necropsied 28 days after organ transplantation, and all animals

Table 1

| Experiment | (s.c. injection into mammary fat pad) | 50,000 | 6 | F | 0.74 ± 0.02 | 31 | 143 (7->300) | 157 (0->300) | 0/6; ovaries, 6/6; uterus, 2/6; pancreas, 3/6; ascites, 2/6; mesentery, 4/6 |
| Experiment | (s.c. injection into mammary fat pad) | 50,000 | 7 | F | 0.26 ± 0.16 | 31 | 140 (0->300) | 0/5; ovaries, 3/5; uterus, 0/5; pancreas, 3/5; ascites, 3/5 |
| Experiment | (s.c. injection into mammary fat pad) | 10,000 | 5 | F | 0.26 ± 0.16 | 31 | 127 (3->300) | 0/3; ovaries, 3/6; uterus, 6/9; pancreas, 9/9; ascites, 9/9; mesentery, 7/9 |

Table 2

| Experiment 1 (s.c. injection into mammary fat pad) | 50,000 | 6 | F | 0.74 ± 0.02 | 31 | 143 (7->300) | 157 (0->300) | 0/6; ovaries, 6/6; uterus, 2/6; pancreas, 3/6; ascites, 2/6; mesentery, 4/6 |
| Experiment 2 (s.c. injection into mammary fat pad) | 50,000 | 7 | F | 0.26 ± 0.16 | 31 | 140 (0->300) | 0/5; ovaries, 3/5; uterus, 0/5; pancreas, 3/5; ascites, 3/5 |
| Experiment 3 (s.c. injection into dorsal scapular region) | 50,000 | 11 | F | 1.14 ± 0.2 | 30 | 150 (0->300) | 0/6; ovaries, 3/6; uterus, 0/6; pancreas, 2/6; ascites, 1/6; mesentery, 1/6 |

* The tumors were measured, using a vernier caliper, in 2 diameters at right angles to one another. The average tumor volume was calculated from the equation: volume = 0.4 x a x b², where a is the major axis and b the minor axis. Average tumor size (cu cm) ± S.E.

Numbers in parentheses, range.

Table 3

<table>
<thead>
<tr>
<th>Organ preference in metastatic growth</th>
<th>Spleen</th>
<th>Ovaries</th>
<th>Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 (s.c. injection into mammary fat pad)</td>
<td>50,000</td>
<td>11</td>
<td>F</td>
</tr>
<tr>
<td>Experiment 2 (s.c. injection into mammary fat pad)</td>
<td>50,000</td>
<td>14</td>
<td>M</td>
</tr>
<tr>
<td>Experiment 3 (s.c. injection into dorsal scapular region)</td>
<td>12,500</td>
<td>20</td>
<td>F</td>
</tr>
</tbody>
</table>

* The tumors were measured, using a vernier caliper, in 2 diameters at right angles to one another. The average tumor volume was calculated from the equation: volume = 0.4 x a x b², where a is the major axis and b the minor axis. Average tumor size (cu cm) ± S.E. at day of necropsy.

Numbers in parentheses, range.

Table 4

<table>
<thead>
<tr>
<th>No of cells injected</th>
<th>No of animals</th>
<th>Sex</th>
<th>Tumor size</th>
<th>Reoxygenation</th>
<th>Necropsy day</th>
<th>No of hepatic nodules (median)</th>
<th>Incidence of extrahepatic metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 (s.c. injection into external ear)</td>
<td>25,000</td>
<td>15</td>
<td>F</td>
<td>0.28 ± 0.07</td>
<td>30</td>
<td>20 (0-120)</td>
<td>0/15</td>
</tr>
<tr>
<td>Experiment 2 (s.c. injection into footpad)</td>
<td>25,000</td>
<td>8</td>
<td>F</td>
<td>0.06 ± 0.01</td>
<td>42</td>
<td>8 (0-200)</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* The tumors were measured, using a vernier caliper, in 2 diameters at right angles to one another. The average tumor volume was calculated from the equation: volume = 0.4 x a x b², where a is the major axis and b the minor axis. Average tumor size (cu cm) ± S.E. at day of necropsy.

Numbers in parentheses, range.

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Table 3
Formation of gross metastases following i.v. injection of M5076 tumor cells into syngeneic C57BL/6 mice

<table>
<thead>
<tr>
<th>No. of cells injected</th>
<th>No. of animals</th>
<th>Sex</th>
<th>No. of pulmonary nodules (median)</th>
<th>No. of hepatic nodules (median)</th>
<th>Incidence of extrahepatic experimental metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100,000</td>
<td>10</td>
<td>F</td>
<td>0 (0–3)*</td>
<td>237 (111–&gt;300)*</td>
<td>5/10</td>
</tr>
<tr>
<td>50,000</td>
<td>9</td>
<td>F</td>
<td>0 (0–2)</td>
<td>140 (55–273)</td>
<td>2/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td>10</td>
<td>F</td>
<td>0 (0)</td>
<td>30 (10–116)</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,000</td>
<td>10</td>
<td>F</td>
<td>0 (0)</td>
<td>16 (4–36)</td>
<td>0/10</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50,000</td>
<td>8</td>
<td>F</td>
<td>0 (0–2)</td>
<td>164 (69–223)</td>
<td>2/8</td>
</tr>
<tr>
<td>25,000</td>
<td>10</td>
<td>F</td>
<td>0 (0–1)</td>
<td>104 (98–130)</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td>9</td>
<td>F</td>
<td>0 (0)</td>
<td>48 (29–107)</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,000</td>
<td>10</td>
<td>F</td>
<td>0 (0)</td>
<td>24 (9–44)</td>
<td>0/10</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25,000</td>
<td>10</td>
<td>M</td>
<td>0 (1–6)</td>
<td>117 (43–7300)</td>
<td>5/10</td>
</tr>
<tr>
<td>5,000</td>
<td>10</td>
<td>M</td>
<td>0 (0)</td>
<td>32 (12–109)</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, range.

Table 4
Arrest, distribution, and survival of 50,000 [15I]IUdR-labeled M5076 tumor cells injected i.v. into syngeneic C57BL/6 mice

<table>
<thead>
<tr>
<th>Time of death postinjection</th>
<th>No. of viable tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td>5 min</td>
<td>47,414 ± 3,500^b</td>
</tr>
<tr>
<td>15 min</td>
<td>45,491 ± 3,599^b</td>
</tr>
<tr>
<td>1 hr</td>
<td>40,390 ± 3,179</td>
</tr>
<tr>
<td>2 hr</td>
<td>35,707 ± 8,880</td>
</tr>
<tr>
<td>4 hr</td>
<td>29,797 ± 4,700</td>
</tr>
<tr>
<td>6 hr</td>
<td>20,841 ± 3,000</td>
</tr>
<tr>
<td>24 hr</td>
<td>9,357 ± 1,544</td>
</tr>
<tr>
<td>48 hr</td>
<td>1,498 ± 1,101</td>
</tr>
<tr>
<td>72 hr</td>
<td>332 ± 37</td>
</tr>
</tbody>
</table>

^ Mean ± S.D. (5 mice/time interval).

b 0.2 ml blood.

had spontaneous metastases involving the liver and spleen but no grossly obvious pulmonary nodules.

In the second experiment, mice were given i.v. injections of 5 × 10^4 viable M5076 cells, and at 4, 7, and 10 days after the injection, portions of the lungs and livers from 2 mice were grafted into the mammary fat pads of recipient animals (10 animals/organ/time point). By 20 days after organ implantation, all recipient animals had developed palpable tumors at the implant site, and at necropsy similar spontaneous metastatic patterns were exhibited in mice bearing either lung or liver implants. Again, the liver and spleen bore multiple, discrete metastatic foci, but the lung appeared grossly to be uninvolved.

DISCUSSION

The tendency for tumors of a certain histological type to metastasize to particular organs has been well documented in clinical oncology and in experimental animal tumor systems (4, 9, 14, 19). Mechanistic explanations of such organ-specific metastatic patterns fall into 2 broad categories. The first, exemplified by the views of Ewing (6), supposes that the arrest and subsequent development of circulating tumor cells into metastatic growths is attributable to hemodynamic factors alone. The second hypothesis, first proposed by Paget in the late 1800s (17), is rather more broad in concept and states...
that tumor cells that reach the circulation are distributed to all
organs but only develop in those organs where the microenvi-
ronment is favorable for tumor cell growth. This "seed and
tumor" hypothesis suggested that metastatic growths are a con-
sequence of both host and tumor factors and has been sup-
ported by recent experimental evidence (12, 14, 20, 21).
Distribution studies using radiolabeled tumor cells have shown
that initial arrest of circulating cells neither correlates with nor
predicts subsequent development of tumor foci (7, 10, 11),
whereas host organs appear to play some role in determining
metastatic development (12, 14).

The M5076 tumor line used in these studies, a reticulum cell
sarcoma of histiocytic origin, exhibits organ-specific patterns
of metastasis consistent with the "seed and soil" hypothesis
(17). Regardless of the route or site of tumor cell inoculation,
metastasis occurs predominantly in the liver and spleen. This
pattern of growth has been observed by other workers using
this tumor (15). Other peritoneal organs may be involved, such
as the ovaries, when a sufficiently high number of tumor cells
are injected or when extensive peritoneal involvement occurs
after direct spread. However, even following the i.v.
injection of large numbers of tumor cells, pulmonary nodules are rarely
formed. Similar patterns of metastatic spread have been re-
ported for other murine tumors of histiocytic or lymphoid origin
(3-5, 18, 19, 25).

The failure of the M5076 tumor line to form pulmonary tumor
nodules in the majority of animals given i.v. injections of these
tumor cells is particularly interesting in view of the studies
performed with the [125I]IUDR-labeled neoplastic cells and the
"bioassay" experiments. The i.v. injection of such cells was
followed by their rapid initial arrest and retention in the lungs.
These arrested cells were slowly released and then trapped
and retained by the liver such that by 3 to 4 days postinjection
this organ is the site of the majority of viable tumor cells.
However, the long-term retention of viable tumor cells in the
lungs is greater than that seen when the B16-F10 cells (with
high lung-colonizing potential) are injected into similar syngene-
ic mice (10). From these data, it would appear that long-term
retention of tumor cells in the lungs does not correlate with the
formation of pulmonary lesions. The lung appears not to "proc-
ess" M5076 tumor cells so that recirculating cells die rapidly
after release, as has been proposed in other tumor systems
(28), as implantation of lungs removed from mice given i.v.
injections of tumor cells resulted in tumor growth at the implant
site and the subsequent formation of spontaneous metastases.
Indeed, as is apparent from the organ implant experiments, the
lungs do not act purely as a filter to remove i.v.-injected tumor
cells. Spontaneously metastasizing M5076 cells localize to the
lungs and are arrested and held in a viable state. However,
either tumor cell replication at this site is diminished compared
to other anatomical sites or tumor cell division is matched by
organ (13, 16, 23, 29). This concept of metastatic patterns
being determined by selective adhesion need not be universal
and tends to be countermanded by data derived from radiola-
beled tumor cell distribution studies (3, 7, 10, 11) and by the
demonstration that tumor cells can pass through organ capil-
lar beds (30). Certainly, it seems unlikely that such a mecha-
nism is operating in the experiments reported here. Both in vivo
(Chart 1) and in vitro (Chart 2) M5076 tumor cells readily adhere
to pulmonary cells. Moreover, the fluctuating levels of liver-asso-
ciated radiolabeled tumor cells that are obtained (Chart 1)
suggest that such cells are not "held fast" but are capable of
recirculation. Also, from the bioassay data, it is obvious that
viable cells are found in lung tissue as a result of spontaneous
metastasis from a distant site.

The reasons M5076 cells do not form overt pulmonary me-
tastases are still unclear. Metastatic growth of these tumor
cells does not occur in the lungs of immunosuppressed mice
(adult thymectomy and 450 R X-irradiation),
suggesting that host-specific immunity per se does not play a major role in
preventing pulmonary tumor growth. However, we cannot rule
out the possibility that M5076 cells are particularly susceptible
to destruction by lung-associated defense cells such as mac-
rophages. Indeed, recent work has suggested that the deple-
tion of host macrophage levels may lead to pulmonary tumor
growth of M5076 (15).

It also seems unlikely that oxygenation of lung tissue is
responsible for limiting the development of tumor foci. M5076
tumor cells failed to grow in lungs implanted in the thigh muscle
of syngeneic mice, although in the same experiments, the lung-
seeking B16 melanoma was capable of growing both in the in
situ and ectopic lung (12).

In summary, it appears that the metastatic behavior of the
M5076 tumor line is strongly supportive of the organ-microen-
vironment requirement as suggested by the "seed and soil"
hypothesis (16). Mere arrest and retention of tumor cells in a
specific organ is not necessarily followed by growth and tumor
development. We are currently attempting to elucidate further
the mechanisms involved in producing the selective patterns of
metastatic spread reported here and feel that the M5076 tumor
offers possibilities as a model system for studying selective
patterns of metastatic spread and tumor dormancy.

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technical assistance of Pat Donovan.

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1933

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1928.
Figs. 1 to 6. Histological appearance of tumor foci resulting from the injection of M5076 tumor cells into syngeneic C57BL/6 mice. Animals were given i.m. injections of 5 × 10^6 cells, 0.1 ml inoculum volume, in the hind limb and killed 31 days later. Fig. 1, primary tumor growing in skeletal muscle; Fig. 2, metastatic foci in liver; Figs. 3 and 4, renal tumor nodules; Figs. 5 and 6, tumor foci in ovaries. The tumors are highly invasive and are composed of sheets of randomly oriented cells. There are many mitotic figures, best seen in Fig. 5. H & E, Figs. 1, 2, 3, 5, and 6, × 110; Fig. 4, × 170.
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