Characterization of Two Highly Metastatic Variants of Lewis Lung Carcinoma with Different Organ Specificities

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ABSTRACT

The biological properties and metastasis of two sublines of the Lewis lung carcinoma (3LL) which have maintained a stable pattern of organ-selective metastasis have been studied. Subline M-3LL, a lung-specific variant which originated from a lung metastasis of the parent line, metastasized only to the lung following injection of 10^5-10^6 tumor cells i.v. or s.c. Lymphatic metastases of this tumor were rarely detected.

Subline H-3LL, which was developed from a rare, spontaneous hepatic metastasis of the parent line metastasized primarily to the liver, but pulmonary metastases have also been observed. While it grew at local s.c. sites, this tumor metastasized to the regional lymph nodes draining the tumor site, as determined by histology and by bioassay of the lymph nodes following their grafting into new recipient animals. Histologically, the two lines were indistinguishable with the exception of a higher incidence of giant cells detected in tissue sections and culture monolayers of the liver-colonizing variant H-3LL. Ten clones derived from each of the variant lines were expanded in vitro and inoculated i.v. While none of the ten clones derived from line M-3LL gave rise to extrapolmonary metastasis, nine of ten clones derived from Tumor H-3LL gave rise to hepatic metastasis. Highly metastatic clones selected from each tumor were subsequently used to study the patterns of distribution and arrest of radiolabeled tumor cells following their inoculation i.v. No correlation could be found between the initial distribution of the radiolabeled tumor cells and the organ selectivity eventually noted in the site of the metastases.

INTRODUCTION

The phenomenon of organ-specific metastasis, namely the preferential metastasis of some neoplastic cells to selected secondary sites, has been recognized for many years through the study of human and experimental tumors (3). The mechanisms which control target organ selection by the tumor cells are, however, poorly understood.

Nonspecific factors such as physical proximity of the tumor to a target organ and/or favorable hemodynamic forces (the mechanistic view) (4) have been proposed by some. Others have suggested that specific host and/or tumor-dependent factors determine the site of secondary growth. Among these factors, cell-cell adhesion between the tumor cells and the capillary endothelium (5) or parenchymal cells (6, 7) of a target organ as well as organ-specific microenvironmental factors (8) have been proposed. The available experimental and clinical data suggest that organ specificity may not be determined by a single host or tumor cell property but rather that it is influenced by an interplay of several of these mechanisms (3, 9).

One of the problems which has contributed to the slow progress to date in elucidating the cellular and molecular basis of these mechanisms has been the scarcity of appropriate experimental tumor models. Studies of organ-specific metastasis have had to rely mainly on the analysis of: (a) tumor lines which were of independent origin and displayed different organ-colonizing potentials in vivo (10) or (b) variant sublines derived from a common tumor and selected for organ-specific metastasis following repeated systemic or intraorgan injection of high doses of the parent line (11-13). In the former, the study of tumor and host factors related to the organotropic behavior of the tumor cells is complicated due to the fact that the tumor lines under study are likely to differ in a variety of tissue and tumor-specific properties unrelated to their patterns of organ specificity. In the latter, on the other hand, tumor cell survival and growth in the target organs may be the result of systemic or intraorgan inoculation of high doses of tumor cells. These conditions may overwhelm and therefore mask local resistant mechanisms operative in the target organ (14).

In this paper, a murine tumor model of organ-specific metastasis is described which was developed from two metastases of the Lewis lung carcinoma which arose spontaneously in the lung and liver of mice bearing local s.c. tumors. These sublines have maintained stable affinities to their respective secondary organs, their patterns of dissemination being independent of the route of tumor inoculation. The stability of the related phenotypes is suggested by our cumulative experience with the tumors over a period of 2 yr and by cloning experiments. In addition we found that the tumor lines differed in their potential for lymphatic metastasis. We suggest that this tumor model offers a unique opportunity to analyze host and tumor-related factors which determine the organ-colonizing potentials of metastasizing tumor cells.

MATERIALS AND METHODS

Animals. For all experiments, 8- to 16-wk-old female C57BL/6 mice were used. They were supplied by Charles River Canada (Montreal, Canada).

Tumor Lines. Line M-3LL was derived from a pulmonary metastasis isolated from an animal bearing the syngeneic parent 3LL(15) as a s.c. tumor in the axillary region. The metastatic nodule was again implanted s.c. in the axillary region of a new recipient giving rise to a new tumor. Thereafter the line was maintained by consecutive s.c. implantations of pulmonary metastases in the axillary or flank region. The tumor cells used in the experiments described were the product of seven or more consecutive nodule implantations.

Line H-3LL was established by the s.c. grafting of a rare spontaneous hepatic metastasis detected in a mouse bearing a 3LL tumor s.c. in the axillary region. Following implantation of this nodule into a recipient animal, it gave rise to a local tumor. The tumor was resected, tumor cells were dispersed by enzymatic digestion with trypsin (14), and 5 x 10^5 cells were injected into two recipient mice. Numerous hepatic metastases were detected in these mice 27 days later when tumors measured 1.5 cm in diameter. The nodules were again implanted into new mice, and as was the case for line M-3LL, line H-3LL was thereafter propagated by consecutive implantation of hepatic nodules 1. The abbreviations used are: 3LL, Lewis lung carcinoma; FdUrd, 5-fluoro-deoxyuridine; [125I]dUrd; 5-[125I]iodo-2-deoxyuridine; PBS-EDTA, phosphate-buffered saline with 0.02% EDTA; RPMI-FCS, RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco, Burlington, Canada), 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (1 M solution), 2 x 10^-4 M glutamine, and 0.001% gentamicin sulfate.

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2 Medical Research Council of Canada Scholar.
derived from tumor-bearing mice in the axillary region or the flank. For the experiments described, tumor cells from the third implant generation and onward were utilized.

**Maintenance of Tumor Lines.** Single cell suspensions of tumor cells were obtained from solid s.c. tumors by digestion with 0.02% trypsin in Ca2+- and Mg2+-free PBS-EDTA as we described elsewhere (16). Dispersed viable cells were enumerated using trypan blue dye exclusion. If required, cells were then cultured in RPMI-FCS. The cells were maintained in vitro as monolayer cultures for periods not exceeding 2 wk prior to their use in the experiments described. Single cell suspensions of the adherent tumor cells were obtained by incubating the cell monolayer for 5–10 min at 37°C with PBS-EDTA. Enzymatic digestion with 0.02% trypsin was subsequently applied only if required.

**Tumor Cell Cloning.** Tumor cells dispersed from solid tumors were cultured in vitro in tissue culture flasks (Falcon; 75-cm² growth area) for 2 wk prior to cloning. Monolayers were fed with fresh medium 24 h prior to assay and then dispersed as described above, resuspended in RPMI-FCS medium, and seeded in 96-well microtiter plates (Falcon; Microtest III) at a concentration calculated to deliver 0.7 cells/well (16). Colonies appeared within 10–14 days later. They were expanded in tissue culture flasks (Corning; 25-cm² growth area) and maintained in culture for several weeks (2–3 passages) prior to their injection s.c. or i.v.

**Histology.** Tissue to be analyzed was fixed in either Bouin's fixative (lung) or 10% phosphate-buffered formalin (liver, lymph nodes, tumor). Five-μm paraffin sections were prepared and stained with hematoxylin and eosin (17).

**Evaluation of Tumor Growth.** Tumors growing s.c. were measured with calipers. The mean tumor diameter for individual tumors was calculated from measurements in two planes at right angles. To determine mean tumor diameter for a group of mice, the sum of the individual measurements was divided by the number of tumor-bearing mice.

**Evaluation of Metastasis.** Animals were routinely inspected for metastases when sacrificed. All organs were routinely screened, although metastatic foci were normally detected only in the lung and/or the liver. Lung foci were enumerated following immersion of the lungs in Bouin's fixative for 24 h. Liver colonies were enumerated immediately following removal of the organs. Livers were subsequently fixed in 10% phosphate-buffered formalin for further analyses. Mean diameter of the colonies was determined as described by Wexler (18).

**Grafting of Lymph Nodes.** Axillary and/or brachial lymph nodes were removed aseptically from tumor-bearing mice 15–40 days following the s.c. injection of 2 × 10⁷ tumor cells or the implantation of a solid in vivo tumor as described above. The resultant monolayers were cultured in vitro in tissue culture flasks (Falcon; 75-cm² growth area) and maintained in vitro as monolayer cultures for periods not exceeding 2 wk prior to cloning. Cell monolayers were then analyzed in a gamma counter. The score of ≥100 was given when the nodules were too numerous for an exact count.

**RESULTS**

Organ colonizing patterns of variant lines M-3LL and H-3LL were analyzed 16 days following the i.v. inoculation of tumor cells. Pulmonary and hepatic metastases were monitored by measurement of [125I]dUrd uptake (21) and by counting macroscopic nodules in these organs. The results shown in Table 1 indicate that both tumor lines could colonize the lung following i.v. injection of tumor cells. Hepatic metastases, however, developed only in mice inoculated with Tumor H-3LL. The absence of microscopic hepatic metastases in mice inoculated with Tumor M-3LL was confirmed by histology.

Light microscopy analysis of tissue sections of Tumors M-3LL and H-3LL was undertaken to assess the degree of morphological homology between the variant lines. As can be seen in Fig. 1, the majority of cells of both tumor variants retained the characteristic morphology of the parent line (Fig. 1, a–c) originally classified as a “poorly differentiated epidermoid carcinoma” (15). An increased incidence of multi- and uninucleated giant cells was, however, seen in tissue sections of Tumor H-3LL (Fig. 1, c and d) and in pulmonary and hepatic metastases of this tumor. Large atypical cells were also observed in tissue culture monolayers of Tumor H-3LL (Fig. 1, f and g) but not in those of Tumor M-3LL (Fig. 1e).

The metastatic potential of individual clones derived from variant lines was assessed following the i.v. injection of ten clonal lines derived from each variant. Results are shown in Tables 2 and 3. As can be seen in Table 2, all of the clones derived from Tumor M-3LL gave rise to lung colonies, while none gave rise to hepatic metastases. In contrast, nine of ten clones derived from Tumor H-3LL gave rise to hepatic metastases, although a heterogeneity was noted in the number of colonies produced by the individual clones. Lung metastases were also detected; however, no correlation could be seen between the potentials of these clones to colonize the lungs and the livers.

The highly metastatic clonal lines H-59 and M-27 were subsequently selected for further analysis. Growth rates of these sublines were compared following the s.c. injection of 10⁵ tumor cells into recipient animals. As evidenced in Fig. 2, there were no significant differences between the incidence or rate of growth of the tumors in the two experimental groups.

Subsequently, the patterns of spontaneous metastasis of these sublines were studied. Local tumors were derived by either s.c. injection of dispersed tumor cells or by the implantation of:

### Table 1 Experimental metastasis of Tumor 3LL sublines

<table>
<thead>
<tr>
<th>Organ examined</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor injected</td>
<td>No. of nodules/animal*</td>
<td>cpm (10⁻³)/animal</td>
</tr>
<tr>
<td>H-3LL</td>
<td>68 (44–102)⁴</td>
<td>18 (9.1–19)</td>
</tr>
<tr>
<td>M-3LL</td>
<td>0</td>
<td>3 (2.8–4.4)</td>
</tr>
<tr>
<td>Nil</td>
<td>2.5 (2.2–2.8)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Results are expressed as median of counts in seven animals.

* Numbers in parentheses, range.

⁴ The score of ≥100 was given when the nodules were too numerous for an exact count.
Fig. 1. Light microscopic appearance of Tumor 3LL and its highly metastatic variants. Cross-sections derived from the following s.c. tumors are shown: a, the parent Lewis lung carcinoma line; b, lung-specific subline M-3LL; and c, liver-homing variant H-3LL. H & E, × 500. A large multinucleated cell (arrow) and a second large cell with a central nucleus and clear cytoplasm (two arrows) typically seen in sections of Tumor H-3LL can be seen (c). A similar cell can also be seen (d, arrow) in a second section of H-3LL. Periodic acid-Schiff reagent, × 500. One-wk-old tissue culture monolayers of M-3LL (e) and H-3LL (f) are also shown. × 425. Arrowheads in f show two large atypical cells seen characteristically in cultures of H-3LL. The same cells can be seen at a higher magnification in g, × 700.
Table 2  Blood-borne metastasis of cloned sublines derived from Tumor M-3LL

Five animals in each group were given injections in the tail vein of 2.5 x 10^5 - 5 x 10^5 tumor cells. Animals were sacrificed 21-22 days later, and metastatic nodules in the lungs were enumerated following fixation in Bouin's fixative. Livers were screened for metastases immediately following removal and then fixed in 10% phosphate-buffered formalin for histology.

<table>
<thead>
<tr>
<th>Clone injected</th>
<th>No. of metastases/lung</th>
<th>Mean diameter/ nodule (µm)</th>
<th>No. of metastases/liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>2 (0-7)</td>
<td>69.4</td>
<td>Nil</td>
</tr>
<tr>
<td>M-17</td>
<td>5 (0-7)</td>
<td>62.5</td>
<td>Nil</td>
</tr>
<tr>
<td>M-87</td>
<td>11 (3-44)</td>
<td>109.1</td>
<td>Nil</td>
</tr>
<tr>
<td>M-42</td>
<td>16 (1-29)</td>
<td>103.7</td>
<td>Nil</td>
</tr>
<tr>
<td>M-24</td>
<td>21 (18-64)</td>
<td>87.6</td>
<td>Nil</td>
</tr>
<tr>
<td>M-19</td>
<td>44 (22-97)</td>
<td>79.5</td>
<td>Nil</td>
</tr>
<tr>
<td>M-96</td>
<td>57 (20-70)</td>
<td>78.1</td>
<td>Nil</td>
</tr>
<tr>
<td>M-27</td>
<td>85 (78-100)</td>
<td>88.2</td>
<td>Nil</td>
</tr>
<tr>
<td>M-20</td>
<td>95 (45-200)</td>
<td>76</td>
<td>Nil</td>
</tr>
<tr>
<td>M-31</td>
<td>100 (89-100)</td>
<td>66.5</td>
<td>Nil</td>
</tr>
</tbody>
</table>

* Median of five organs.
+ Numbers in parentheses, range.

Fig. 2. Growth curves of clonal lines M-27 and H-59. Five animals in each group received a s.c. inoculation of 10^5 tumor cells. All animals developed tumors by Day 14. O, Tumor M-27; △, Tumor H-59.

Table 3  Blood-borne metastasis of cloned sublines of Tumor H-3LL

In each group, three to five female C57BL/6 mice were given injections i.v. (tail vein) of 5 x 10^5 tumor cells. Animals were sacrificed 19 days later, and metastatic nodules were counted. Lung metastases were estimated following fixation in Bouin's fixative.

<table>
<thead>
<tr>
<th>Clone injected</th>
<th>No. of metastases/liver</th>
<th>Mean nodule diameter (µm)</th>
<th>Pulmonary metastases*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-66</td>
<td>0</td>
<td>100</td>
<td>++</td>
</tr>
<tr>
<td>H-158</td>
<td>5 (2-7)</td>
<td>108</td>
<td>+++</td>
</tr>
<tr>
<td>H-C7</td>
<td>5 (2-8)</td>
<td>57</td>
<td>++</td>
</tr>
<tr>
<td>H-B4</td>
<td>5 (2-9)</td>
<td>108</td>
<td>+++</td>
</tr>
<tr>
<td>H-17</td>
<td>10 (2-15)</td>
<td>50</td>
<td>++</td>
</tr>
<tr>
<td>H-F4</td>
<td>11 (10-16)</td>
<td>108</td>
<td>+++</td>
</tr>
<tr>
<td>H-G9</td>
<td>15 (12-17)</td>
<td>71</td>
<td>++</td>
</tr>
<tr>
<td>H-59</td>
<td>44 (29-58)</td>
<td>59</td>
<td>+++</td>
</tr>
<tr>
<td>H-15</td>
<td>48 (47-49)</td>
<td>124</td>
<td>++</td>
</tr>
<tr>
<td>H-75</td>
<td>67 (43-107)</td>
<td>86</td>
<td>++</td>
</tr>
</tbody>
</table>

* Median of three to five animals.
+ Numbers in parentheses, range.

In an attempt to determine whether different potentials to arrest in the vasculature of the lung and liver regulated the organ-specific patterns of metastasis noted in the present tumor model, [125I]dUrd-labeled cells of H-59 and M-27 were injected i.v., and their organ distribution was assessed by monitoring the retention of [125I]dUrd in the different organs for up to 24 h later. Five mice in each experimental group were sacrificed 20 days after tumor inoculation, and metastases in their lungs and livers were enumerated. Results of two representative experiments are shown in Table 6. As can be seen, the proportions of tumor cells initially arrested and subsequently retained in the lungs and livers of the inoculated animals within 24 h after injection were comparable for both tumor lines. Significant differences were found, however, between the two groups examined 20 days later. While hepatic metastases were only found in mice inoculated with Tumor H-59, the incidence of pulmonary metastases was higher (P < 0.04) in mice inoculated with Tumor M-27.
ORGAN-SELECTIVE METASTASIS OF LEWIS LUNG CARCINOMA VARIANTS

Table 4 Incidence of spontaneous metastases in animals bearing Tumors H-59 and M-27

Animals bearing H-59 tumors were sacrificed 30 days following the s.c. injection of $2 \times 10^8$ dispersed tumor cells or the implantation of hepatic nodules derived from tumor-bearing mice. Animals bearing M-27 tumors were sacrificed 26–46 days following the s.c. injection of $10^7-5 \times 10^7$ dispersed tumor cells or the implantation of metastatic pulmonary nodules.

<table>
<thead>
<tr>
<th>Source of tumors</th>
<th>Tumor size (cm)</th>
<th>Pulmonary metastases $^a$</th>
<th>Hepatic metastases $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-59 $^c$</td>
<td>M-27 $^d$</td>
<td>H-59 $^e$</td>
</tr>
<tr>
<td>Implants of metastases</td>
<td>2.3 ± 0.2</td>
<td>1.96 ± 0.3</td>
<td>13 (10-17) $^g$</td>
</tr>
<tr>
<td>Dispersed tumor cells</td>
<td>2.2 ± 0.2</td>
<td>1.7 ± 0.9</td>
<td>74 (14-111)</td>
</tr>
</tbody>
</table>

$^a$ Results are expressed as median. Pulmonary metastases were enumerated following fixation of the lungs in Bouin's fixative, while hepatic metastases were enumerated immediately following removal of the organs.

$^b$ Seven animals were analyzed in each group with H-59 tumors and five in each group with M-27 tumors.

$^c$ Mean ± SD.

$^d$ Numbers in parentheses, range.

$^e$ The difference between this group and that implanted with pulmonary nodules of Tumor M-27 or given injections of dispersed tumor cells of line H-59 was statistically significant ($P < 0.05$).

DISCUSSION

In this paper, we have described a new experimental model of organ-selective metastasis. We have shown that tumor line M-3LL as well as ten clonal sublines which were derived from it were lung specific, while Tumor H-3LL and its clonal sublines metastasized to the liver. In addition we found that animals bearing local tumors of H-3LL origin had a significantly higher incidence of lymphatic metastases than animals bearing tumors of line M-3LL origin.

Morphologically, the tumors derived from both lines appeared similar with the exception of a higher incidence of giant cells seen in tissue sections and culture monolayers of Tumor H-3LL. Subsequently we studied the metastasis of the two highly metastatic clonal lines H-59 and M-27 derived from Tumors H-3LL and M-3LL, respectively. We found that these clonal lines displayed the organ selectivity characteristic of their parental lines. We also noted that pulmonary but not hepatic metastasis of clone H-59 could be considerably reduced when animals were implanted with isolated liver nodules rather than given injections of dispersed tumor cells.

Using $^{125}$I-dUrd-labeled tumor cells we noted further that the arrest and retention of tumor cells in the livers and lungs as measured by $^{125}$I uptake for 24 h after cell inoculation i.v. were comparable for both lines. This was despite significant differences in the numbers of hepatic and pulmonary metastases which eventually developed in such animals.

The different patterns of metastasis of the tumor lines and our finding that they home to their respective target organs regardless of the site of tumor inoculation (i.e., s.c. in the axillary region or flank, or tail vein) argue that, in the present tumor model, specific tumor cell properties and/or altered host responses rather than anatomical or so-called "mechanistic" factors determine the secondary sites of tumor growth. Our results also suggest that the site of metastases formation may be related to the route of tumor dissemination.

At present, this relationship between the target organ specificity and the different routes of dissemination apparently utilized by the tumor cells (i.e., lymphatic or hematogeneous) is...
Fig. 4. Lymphatic metastasis of Tumor H-59. a, axillary lymph nodes and liver of a mouse which was given injection of 10^6 H-59 cells s.c. in the axillary region and sacrificed when mean tumor diameter measured 2.4 cm. Black arrow points to the axillary lymph node which was draining the tumor site. Metastasis is also apparent in the contralateral node and in the inguinal lymph node (white arrow). b, low-power view (x 72) of a cross-section of a regional (axillary) lymph node derived from animal bearing Tumor H-59 (2.0 cm). Extensive infiltration of the node by tumor cells (light staining areas) is evident. c, high-power (x 400) view of the same section showing clusters of lymphocytes (top left) surrounded by tumor cells.

**Table 5** Incidence of metastases in regional lymph nodes of tumor-bearing mice as measured by s.c. implantation of the nodes into new recipient animals

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>Size of tumors at time of lymph node excision (cm)</th>
<th>Incidence of tumors from implants of regional lymph nodes</th>
<th>No. of days prior to appearance of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-3LL</td>
<td>1.56 ± 0.43*</td>
<td>3/8*</td>
<td>21 ± 4.3*</td>
</tr>
<tr>
<td>M-27</td>
<td>1.58 ± 0.19</td>
<td>0/5*</td>
<td>1.1 ± 0.6*</td>
</tr>
<tr>
<td>H-59</td>
<td>1.61 ± 0.37</td>
<td>11/13</td>
<td>10.3 ± 3</td>
</tr>
</tbody>
</table>

* Mean ± SD.

* Significantly lower than animals bearing H-59 tumors (P < 0.025).

* P<0.01 (t test).

**Table 6** Retention of [³H]Urd-labeled tumor cells and incidence of metastases in lungs and livers of i.v. inoculated mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>Isotope recovery (% of injected total) at</th>
<th>No. of metastases*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>20 min</td>
<td>4 h</td>
</tr>
<tr>
<td>M-27</td>
<td>74 ± 10^6</td>
<td>9.6 ± 2.9</td>
</tr>
<tr>
<td>H-59</td>
<td>80 ± 1.6</td>
<td>5.3 ± 1.1</td>
</tr>
<tr>
<td>Liver</td>
<td>9 ± 0.6</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>M-27</td>
<td>9.2 ± 1.5</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>H-59</td>
<td>17 ± 1.6</td>
<td>3.2 ± 0.7</td>
</tr>
</tbody>
</table>

* Four mice in each group were sacrificed 20 days after inoculation and metastases were enumerated. Results are expressed as median.

* Significantly lower than animals bearing H-59 tumors (P < 0.025).

* P<0.01 (t test).

* Numbers in parentheses, range.

unclear. Evidence from other tumor models suggests that hematogenously disseminating tumor cells tend to metastasize preferentially to the lung, whereas lymphogenously disseminating cells generally appear to have a wider range of secondary (and possibly tertiary) sites (23). In our model, tumor deposits of line H-3LL have frequently been observed throughout the lymphatic system, including the regional nodes located contralaterally to the tumor (Fig. 4) and the mesenteric lymph nodes. However, spontaneous metastases in organs other than the lung and to a lesser extent the lung (i.e., kidney, spleen, heart, and brain) have rarely been seen. Moreover, the tumor cells homed to their respective target organs following the direct inoculation of the tumor cells i.v., suggesting that the formation of liver metastases did not depend on tumor dissemination via the regional lymph nodes. Alternatively, it is conceivable that, in the present tumor system, the same tumor cell properties which are required for the survival and spread of the tumor through the lymphatics are also essential for tumor cell growth in the liver. A relative insensitivity of the tumor cell to a host resistance mechanism present in both sites (e.g., resident macrophages) (24) could, for example, account for the selective ability of Tumor H-3LL to metastasize to these organs.

The difference in the ability of the tumor lines to metastasize to regional nodes may suggest that the host immune response influences the metastatic patterns of the tumors. The following lines of evidence argue against such a role. (a) Tumor M-3LL and, in particular, its subline M-27 are highly metastatic to the lung. Several studies with highly metastatic clones of the Lewis lung carcinoma have shown them to be poorly immunogenic (21, 25) and relatively resistant to natural killer cells (26). (b) In preliminary studies (not shown), we found that, in athymic nude mice, the rate of growth of local M-27 tumors and the incidence of metastases were decreased rather than enhanced.
Metastasis in the nude mice was again confined to the lung. These findings suggest that, in the present model, T-cells do not play a major role in the organ specificity of metastasis. We are currently investigating the importance in this model of other tumor-inhibitory mechanisms, in particular those mediated by macrophage-like cells (i.e., Kupffer cells, sinus histiocytes).

Recently a variant of Tumor 3LL which was selected by repeated passage of the tumor cells through the spleen has been shown to metastasize to the liver. Natural resistance mechanisms have subsequently been implicated in the increased metastatic potential of this line (13). Gorelik et al. (27) and we (26) have previously described the metastatic behavior of a subline of Tumor 3LL selected for resistance to splenic natural killer-like cells. Although an increased potential for pulmonary metastasis was observed (26), we rarely observed extrahepatic metastases in the tumor-bearing mice (not published). This suggests that resistance to natural killer-like cells may be only one of several requirements for formation of hepatic metastases. Injection of the tumor cells directly into the spleen as was described in the above-mentioned report (13) may circumvent some of the other requirements, allowing for metastases formation in the liver.

Our findings following the i.v. inoculation of [3H]dUrd-labeled tumor cells derived from highly metastatic clonal lines of Tumors H-3LL and M-3LL suggest that, in the present model, the observed target organ selectivity is not related to different patterns of tumor cell arrest and distribution measurable immediately following tumor inoculation as has been shown to be the case in other tumor models (28). The results point instead to the importance of other, later mechanisms which may be organ specific and may determine whether tumor cells could survive and proliferate in the microenvironment of a particular organ. Similar findings were also described by Hart (3) for organ-specific Tumors M5076 (liver) and B16-F10 (lung). Recent studies reported by Tarin et al. (8) have shown that soluble factors released by liver fragments in vitro were cytotoxic to some tumors and that this correlated with the potential of these tumors to metastasize to the liver. In another communication by Mandick and Berger (29), similar conclusions were reached using a somewhat different in vitro assay. In our tumor model, recent results have shown that tumor cells derived from the liver-homing subline H-59 were significantly (3- to 4-fold) more adhesive to monolayers of cultured hepatocytes than tumor cells derived from the lung-specific subline M-27. This is in accordance with findings reported in other tumor systems (6, 30). The relationship between the increased binding of the tumor cells to the hepatocytes and their ability to survive and proliferate in the liver is the subject of current investigations in our laboratory.

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REFERENCES

Characterization of Two Highly Metastatic Variants of Lewis Lung Carcinoma with Different Organ Specificities

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