ABSTRACT

The arrest and retention patterns in lung tissues of [125I]-5-iododeoxyuridine-labeled Lewis lung carcinoma cells injected into the systemic circulation of tumor-bearing (TB) mice with defined metastatic status and non-tumor-bearing mice were determined. The presence of overt lung metastases did not significantly alter the arrest or subsequent rates of release of cancer cells compared with non-tumor-bearing controls. However, the percentage of cancer cells retained in the 3-week TB animals was significantly greater than that in the 1-week TB mice. In 3-week TB animals, 4 times as many cancer cells were arrested in the "noninvolved" lung tissues than in the metastases. The relative vascularities of the metastases and lung tissues were assessed following injection of [55Cr]-labeled erythrocytes, and from these and published data it is suggested that major factors determining the differential distribution of cancer cells are the relative areas of microvascular endothelium and blood flow per g in the lungs and metastases.

INTRODUCTION

During the natural process of hematogenous metastasis, cancer cells must be delivered to organs already containing metastases, yet this situation has been largely neglected in most experimental studies. In this communication, we have therefore determined whether the presence of metastases within an organ modifies the retention of viable cancer cells in that same organ by injecting radiolabeled cancer cells i.v. into TB animals with metastatic status defined in terms of the mean numbers of overt (>0.25 mm diameter) pulmonary metastases and comparing the arrest and rates of release with NTB controls.

MATERIALS AND METHODS

Tumor

In all experiments, Lewis lung 3LL carcinoma was used in 6- to 8-week-old syngeneic male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) by s.c. inoculation of 10^6 viable 3LL cells (>95% viable; trypan blue exclusion) into the right inguinal region.

Preparation of 3LL Single-Cell Suspensions

Aliquots of nonnecrotic s.c. 3LL tumor were minced and stirred with 10 ml 0.25% protease (type VI; Pronase P; Sigma Chemical Co., St. Louis, Mo.) in HBSS and 12 drops (25-gauge needle) 0.02% DNase (Sigma) in HBSS for 15 min at 37°C, in an atmosphere of 5% CO2 in air. Tumor fragments were allowed to settle, the supernatant was drawn off, and the remaining tumor was retreated with fresh enzyme solutions. The supernatant was filtered through 200 wire mesh (Buffalo Wire Works Co.), centrifuged, washed twice with 10 ml RPMI 1640 plus 10% FCS plus 6 drops of DNase, and resuspended in RPMI 1640 plus 10% FCS. The cell suspension from the second incubation was treated similarly. Finally, the 2 suspensions were filtered and resuspended at a concentration of 10^6 viable 3LL cells/0.1 ml Dulbecco's phosphate-buffered saline (pH 7.4).

Isotope Labeling

Cancer Cells. 3LL cell suspensions were isotopically labeled with [125I]dUrd (Amersham, Arlington Heights, III.). This label has been shown previously to be incorporated efficiently into the DNA of tumor cells and to remain bound until cell death, after which the labeled DNA is rapidly deiodinated and excreted with little reutilization by other cells (12). [125I]dUrd (1.5 μCi) was added to 5 to 6 x 10^7 3LL cells/50 ml RPMI 1640 plus 10% FCS plus 0.1% penicillin-streptomycin, and the cell suspension was incubated in T-flasks overnight at 37°C. The cells were detached using 0.25% trypsin plus 0.25% EDTA in HBSS, washed 3 times with 12 drops of DNase and 10 ml of PBS-CMF, and finally resuspended in regular Dulbecco's phosphate-buffered saline at a concentration of 10^6 viable 3LL cells/0.1 ml. Cells labeled in this manner retain their tumorigenic properties, although some cell death results from the use of higher concentrations of radioisotope. Under the present labeling conditions, its seems unlikely that the clonogenicity of the labeled cells would be affected (3), inasmuch as our experiments extend only over a 24-hr period.

Erythrocytes. Blood was obtained by cardiac puncture from 2 anesthetized mice, collected in 20 ml cold PBS-CMF, and centrifuged at 4°C. The pellet was washed 3 times in cold PBS-CMF and resuspended in HBSS, 20% (v/v) Na235CrO4 (New England Nuclear, Boston, Mass.) was added at 50 μCi/10 ml suspension, incubated in a water bath at 37°C for 60 min, and washed 3 times with cold PBS-CMF. The suspension was adjusted to a final erythrocyte concentration of 2 x 10^6/0.1 ml HBSS.

Injection of Radiolabeled Cells

Cancer Cells. In one series of experiments, 10^6 [125I]dUrd-labeled 3LL cells were injected into the lateral tail veins of NTB and 1-, 2- and 3-week TB mice. Aliquots (0.1 ml) of the injected suspension were counted to obtain the radioactivity associated with 10^6 3LL cells. At 5 min (t0), 60 min (t60), 120 min (t120), 180 min (t180), 240 min (t240), and 24 hr (t24) following these injections, animals were killed under chloroform, and the lungs were inflated in situ with 1 ml 0.15% India ink. The lungs were placed in 70% ethanol and γ-counted, the ethanol was then changed 3 times over 3 days, and the specimens were recounted. This procedure removes radioactivity not associated with intact cells at the time of fixation (4).

Erythrocytes. In a second series of experiments, 2 x 10^6 [55Cr]-labeled erythrocytes were injected into the lateral tail veins of NTB and 3-week TB mice. Animals were killed 5 min after injections, and ligatures were placed around the descending aorta and the pulmonary vessels, near the base of the heart. The lungs were separated from the
heart and aorta, placed in 10% buffered neutral formalin, and γ-counted for 10 min.

Assessment of Tumor Burden

In all experiments, primary tumor volumes were measured by volume displacement, and the number of overt (>0.25 mm) spontaneous lung metastases were counted under a stereoscopic dissecting microscope (×15). Metastases were dissected from the lung tissue, and each component was blotted dry, weighed, and separately γ-counted. The efficiency of the dissections was monitored by examination of hematoxylin- and eosin-stained sections (Fig. 1).

The majority of the overt metastases in the 1- and 2-week TB groups were between 0.25 and 1.0 mm in diameter. It was not technically possible to dissect such small metastases free from the lung tissues, and therefore the weights of the metastatic growth in these groups could not be determined; data are given for the 3-week TB animals only.

RESULTS

Metastatic Load. The numbers of overt (>0.25 mm) spontaneous lung metastases at 1, 2, and 3 weeks following s.c. inoculation of 10^6 3LL cells are given in Table 1. The percentages of experimental animals with metastases were 10, 77, and 100%, respectively, and the mean number of metastases per animal also increased. At 1 week, there was a mean of 1.5 ± 0.5 (S.E.) metastases, which increased 5.5 and 51 times at 2 and 3 weeks, respectively, in groups consisting entirely of animals with metastases.

Arrest and Retention of Radiolabeled 3LL cells. Tail vein injections of radiolabeled 3LL cells were given to NTB and TB mice at zero time (t0). Ten-min γ-counts were made on the lungs excised from animals killed at the specified times, as summarized in Chart 1 and expressed as a percentage of the dose given at t0 in individual experiments (2.5 to 7.5 x 10^6 counts/10 min/10^6 3LL cells).

In all lungs, there was a small (<5.0%) consistent loss of radioactivity on alcohol extraction. Similar percentages (0.8 > p > 0.3) of injected 3LL cells were arrested initially in the lungs of NTB and TB mice, and thereafter they were gradually released. Despite the significant differences between the mean numbers of metastases in all of the 1-, 2-, and 3-week TB mice (0.01 > p > 0.001), the percentage retention of 3LL cells at the specified times in these groups compared with NTB controls were not significantly different, except between the 1-week TB mice and the controls after 60 min (0.02 > p > 0.01) and 240 min (0.05 > p > 0.02). However, the percentages of 3LL cells retained at the specified times (excluding 24 hr) in the 1-week TB animals were significantly higher than those in the 3-week TB animals (Chart 1).

The liver γ-counts corresponding to the lung counts are summarized in Table 2. It is shown that, in all groups a small percentage of the initial injected dose of radiolabeled cancer cells are released from the lungs and are temporarily arrested and retained in the liver with peak values between 120 and 180 min after injection. These percentages are not significantly different in the 4 experimental groups, although there is an approximate 1-hr delay in attainment of peak values in the 3-week tumor bearers. Comparison of Table 2 and Chart 1 shows

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean primary tumor volume (ml)</th>
<th>Mean no. of metastases in all animals</th>
<th>Median no. of metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>No. of ani-</td>
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<td></td>
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<td>es</td>
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<tr>
<td>1-wk TB</td>
<td>0.09 ± 0.01 * (41)</td>
<td>4/41</td>
<td>0.15 ± 0.08 (a) (41)</td>
</tr>
<tr>
<td>2-wk TB</td>
<td>0.78 ± 0.04 (66)</td>
<td>51/66</td>
<td>6.44 ± 1.47 (b) (66)</td>
</tr>
<tr>
<td>3-wk TB</td>
<td>2.59 ± 0.17 (58)</td>
<td>58/55</td>
<td>76.84 ± 5.38 (58)</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

Numbers in parentheses, number of animals.

By t tests: a versus b, 0.01 > p > 0.001; c versus d, 0.2 > p > 0.3; d versus e, p < 0.001.

Numbers in parentheses, range.

Chart 1. Retention of radiolabeled 3LL cells in the lungs at specified times after tail vein injections in TB (●) and NTB (○) mice. All counts are given as percentages of the administered dose at t0. Bars, S.E.
that most of the released radioactivity does not go to the liver. Of those 3LL cells in the liver, the majority are nonviable on the basis of loss of radioactivity following alcohol extraction except in the case of 24-hr specimens taken from 24 hr NTB animals and 1-week tumor bearers.

The differential distribution of radiolabeled 3LL cells in the dissected metastases and the lung tissues proper is expressed in absolute γ-counts [percentage of dose at t₀ (Table 3)]. Results from the 1- and 2-week TB groups were excluded because of the technical problems discussed in "Materials and Methods." More cancer cells were arrested (t₀, p < 0.001) and retained (t₁₀₀, t₁₂₀, T₁₂₀, 0.02 > p > 0.001) in the lung tissues of the 3-week TB group than in the metastases; this relationship held when the data were expressed on a weight basis (mean lung tissue weight, 0.15 ± 0.01 g; mean metastasis weight, 0.19 ± 0.01 g) when more cancer cells per 0.1 g were arrested (t₀, 0.01 > p > 0.001) and retained (t₁₀₀, t₁₂₀, t₁₂₀, 0.01 > p > 0.001) in the lung tissues (Chart 2).

The lungs from NTB animals arrested more cancer cells (t₀, 0.01 > p > 0.001) than did those from TB mice, but when the data were normalized on a weight basis (NTB mean lung weight, 0.24 ± 0.01 g; 3-week TB mean lung weight, 0.15 ± 0.01 g), there was no significant difference (t₀, 0.2 > p > 0.1) between them.

Trapping of ⁵¹Cr-labeled Erythrocytes. Estimates of the comparative vascularity of the lung tissues to the metastases in the 3-week TB group showed a significantly higher vascularity (p < 0.001) than did those from the 3-week TB mice, but when the data were expressed on a weight basis, the 2 were not significantly different (p = 0.18). ⁵¹Cr counts were normalized on a weight basis (NTB mean lung tissue weight, 0.15 ± 0.01 g; mean metastasis weight, 0.19 ± 0.01 g) when more cancer cells per 0.1 g were arrested (t₀, 0.01 > p > 0.001) and retained (t₁₀₀, t₁₂₀, t₁₂₀, 0.01 > p > 0.001) in the lung tissues (Chart 2).

DISCUSSION

Metastasis is an ongoing process in which not only uninvolved organs but also those bearing metastases must continuously receive waves of cancer cells from generating sites elsewhere in the body. In this communication, we report the effects of lung metastases from s.c. Lewis lung tumors in mice on the arrest and retention patterns of circulating radiolabeled cancer cells in the metastases themselves and in metastasis- and non-metastasis-bearing lung tissues. It is of course possible that the results with other types of cancers will differ from those given here.

The results given in Table 1 show that, associated with the increase in "primary" tumor volume over 1 to 3 weeks, there is a sharp progressive increase in both the percentages of animals with overt pulmonary metastases (10, 77, 100%) and the mean numbers of pulmonary metastases per animal (0.15, 6, 77). In spite of these considerable differences in the metastatic status of the lungs, no statistically significant differences were detected between the 3 experimental groups themselves or these groups and NTB controls with respect to either initial radiolabeled cancer cell arrest in the lungs as a whole or their subsequent rates of release (Chart 1). The major significant differences were the higher numbers of viable cells, expressed as percentages of the injected dose, retained in the lungs of the 3-week compared with the 1-week tumor bearers 60, 120, 180, and 240 min after injection.

Following release of temporarily arrested cancer cells in the lungs, some are redistributed to other organs, notably the liver (24), and most of the noncellular ¹²⁵I activity is found in the urine, feces, and thyroid (12). In the present experiments, our major focus has been on the retention of viable cells in the lungs rather than the fate of released cells. For this reason, the degree of radiolabeling was limited (3), and counts made on other organs, except for the liver, were too close to background to permit meaningful comments on redistribution. As shown in Table 2, the percentages of loss after alcohol extraction of radiolabeled 3LL cells from 3-week TB and NTB mice were made on animals killed 5 min after receiving tail vein injections of ⁵¹Cr-labeled erythrocytes. The results, expressed as percentage of the dose given at t₀ (600,000 counts/10 min), are given in Table 3. The percentage of retention of radiolabeled 3LL cells at specified times in lung tissues and metastases from 3-week TB mice and NTB animals is shown in Table 3.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>NTB</th>
<th>1-week TB</th>
<th>2-week TB</th>
<th>3-week TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₀</td>
<td>1.54 ± 0.18² (7)²</td>
<td>73.31 ± 2.22</td>
<td>2.70 ± 0.4 (7)</td>
<td>73.61 ± 2.46</td>
</tr>
<tr>
<td>t₁₀₀</td>
<td>4.16 ± 0.72 (9)</td>
<td>69.5 ± 1.03</td>
<td>4.29 ± 0.60 (6)</td>
<td>70.03 ± 2.45</td>
</tr>
<tr>
<td>t₁₂₀</td>
<td>6.08 ± 1.28 (9)</td>
<td>88.65 ± 1.19</td>
<td>7.20 ± 0.63 (6)</td>
<td>71.46 ± 1.62</td>
</tr>
<tr>
<td>t₁₅₀</td>
<td>5.81 ± 0.76 (9)</td>
<td>89.66 ± 2.35</td>
<td>6.74 ± 0.40 (6)</td>
<td>64.07 ± 3.42</td>
</tr>
<tr>
<td>t₁₇₀</td>
<td>5.92 ± 0.44 (9)</td>
<td>56.56 ± 3.09</td>
<td>4.07 ± 0.75 (7)</td>
<td>65.16 ± 4.60</td>
</tr>
<tr>
<td>t₁₉₀</td>
<td>0.24 ± 0.02 (9)</td>
<td>33.39 ± 7.68</td>
<td>0.22 ± 0.03 (5)</td>
<td>33.01 ± 4.88</td>
</tr>
</tbody>
</table>

² Mean ± S.E.
² Numbers in parentheses, number of animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>t₀</th>
<th>t₁₀₀</th>
<th>t₁₂₀</th>
<th>t₁₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-wk TB (lungs)</td>
<td>71.75 ± 5.62² (8)²</td>
<td>43.92 ± 4.48 (8)</td>
<td>55.03 ± 6.66 (9)</td>
<td>31.99 ± 4.38 (7)</td>
</tr>
<tr>
<td>3-wk TB (metastases)</td>
<td>15.99 ± 5.10 (8)</td>
<td>23.54 ± 6.96 (8)</td>
<td>13.36 ± 3.69 (9)</td>
<td>14.66 ± 3.90 (7)</td>
</tr>
<tr>
<td>NTB</td>
<td>96.77 ± 5.2 (7)</td>
<td>74.98 ± 5.04 (9)</td>
<td>58.42 ± 7.33 (9)</td>
<td>40.23 ± 5.89 (9)</td>
</tr>
</tbody>
</table>

² Mean ± S.E.
² Numbers in parentheses, number of animals.
susceptibility of different cancer cells to NK cells. Both lethal and nonlethal injury may promote cell detachment (22), and although we are unaware of direct evidence that the different lung retention patterns of 3LL cells are in fact due to NK cell-induced injury as distinct from that mediated by other mechanisms (24), the role of NK cells in this phase of metastasis is worthy of attention.

We next examined the differential distribution of radiolabeled cancer cells in the metastases and the lung tissues proper. Absolute counts of radioactivity in animals with 3-week tumors revealed that, over the period of 5 to 240 min after injection, 3 to 9 times more cancer cells were retained in the lung tissues than in the metastases. When the counts were expressed as a percentage of the dose of radiolabeled cells retained per 0.1 g of tissue, as shown in Chart 2, these proportions are maintained.

Five min after the injection of radiolabeled cancer cells into animals bearing 3-week tumors, 88% of the dose was present in the lungs as viable cells, of which 72% was in the lungs proper and 16% was in the metastases, at a ratio of 4.5:1. When these percentages were normalized on the basis of weights of residual lung tissue and metastases, cell retention was 52.3 ± 6.6 and 15.4 ± 7.1% per 0.1 g, respectively, corresponding to a ratio of 3.4:1 (0.01 > p > 0.001). Five min after injection, the normal lungs from non-tumor bearers gave counts corresponding to 39.8 ± 2.2% per 0.1 g, compared with the 52.3 ± 6.6% in the lung tissues of tumor-bearing lungs; these percentages are not significantly different (0.2 > p > 0.1) and indicate that the arrest properties of the lung tissues are not altered in the presence of overt metastases.

Approximate estimates of the comparative vascularity of lung tissues and metastases were made by counting trapped, i.v.-injected 51Cr-labeled erythrocytes after ligature and fixation. The ratio of 5-min counts, expressed as a percentage of injected dose, between lungs and metastases was 4.6:1 compared with 1.3:1 on a percentage g basis; the ratio of 4.5:1 between the percentages of injected cancer cells arrested in the lung tissues and metastases, as described above, corresponds remarkably well with the 4.6:1 ratio of blood values and indicates that the access of blood-borne cancer cells to both lung tissues and metastases depends on their comparative vascularity rather than weight.

In considering differences between the lung metastases in 1- and 3-week tumor bearers, we were able to assess metastasis vascularity only in the 3-week animals because those in the 1-week group were too small. One possibility to at least partially account for local differences in vascularity in and around the metastases is that, in the 1-week animals, neovascularization would not have occurred in contrast to the 3-week group (8). Thus, in the latter group of animals, recruitment of new capillaries might well have provided additional microvas-

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**Arrest and Retention of Cancer Cells by Metastases**

Table 2, the majority of cells temporarily retained in the liver after temporary arrest in the lungs were nonviable, but no consistent significant differences were detected between the 1-, 2-, and 3-week tumor bearers and NTB animals. In the case of the lungs of 1- and 3-week tumor bearers, the differences between counts representing total cellular retention before alcohol extraction (Chart 1) were not due to differential killing, because the percentage of viability of the retained cells was consistent (>95%). Therefore, because the percentages of dead cells in the lung and liver (a major organ of redistribution) are similar in the 1- and 3-week TB groups of animals, it seems likely that the differences between them represent differential retention rather than differential survival per se.

It has been shown that host sensitization to tumors may be associated with altered retention patterns of cancer cells in the lungs (7, 16, 18, 27) which are immunospecific (28). However, the differences between the 1- and 3-week tumor bearers are unlikely to be due to such immunological mechanisms because no significant differences were observed between the 3-week tumor bearers and the NTB controls; in addition, the Lewis lung tumor is very weakly immunogenic because sensitization with this tumor does not induce rejection (13) but merely elicits a weak serological response of a nonspecific nature.

The decreased cell retention in the lungs of the 1-week compared with the 3-week tumor bearers could at first sight be ascribed to a decrease in NK cell activity induced by the greater tumor burden in the latter group of mice, since it has been shown that elimination of radiolabeled RBL-5 lymphoma cells injected i.v. from the lungs of various strains of mice is increased in mice with high (spleen) NK cell activity as assessed by in vitro assay (17). Talmadge et al. (20) have also shown that the numbers of surviving B16 melanoma cells retained in the lungs of mice after i.v. injection was increased in NK cell-deficient beige mice. However, as emphasized by Riccardi et al. (17) among others, NK cell activity does not completely account for the rates of loss of radiolabeled cells from the lungs, because they correlate poorly with the in vitro

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**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>% of 51Cr-labeled erythrocytes trapped</th>
<th>% of 51Cr-labeled erythrocytes trapped/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTB</td>
<td>7.7 ± 0.80* (9)</td>
<td>34.86 ± 4.02 (9)</td>
</tr>
<tr>
<td>Lung, tissue (3-wk TB)</td>
<td>4.60 ± 0.53 (12)</td>
<td>18.68 ± 1.81 (12)</td>
</tr>
<tr>
<td>Metastases (3-wk TB)</td>
<td>1.00 ± 0.19  (12)</td>
<td>14.49 ± 2.41 (12)</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

Numbers in parentheses, number of animals.

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*D. Glaves, Department of Experimental Pathology, Roswell Park Memorial Institute, personal communication.*
cular endothelial surface to promote cell arrest.

Five min after the i.v. injection of single cancer cells into rodents, they are seen arrested in the pulmonary microvasculature, particularly the arterioles and capillaries (2, 21), from whence most of them escape over a period of 24 hr. The arrest phase may be assessed by lung counts in animals killed within 5 min after i.v. injection, and the secondary release phase may be assessed later. Two factors influencing the numbers of cancer cells arrested in a vascular bed are the surface area of vascular endothelium available for adhesive interactions and the numbers of cells delivered (=blood flow). In the present experiments, following injections of radiolabeled erythrocytes, 1 ml of circulating blood had an activity of 300,000 counts/10 min, and the counts in the lung tissues and metastases corresponded to blood volumes of 0.09 and 0.02 ml, respectively; in these experiments, the mean residual lung weights were 0.24 and 0.11 g for the metastases; thus, 38% of the lung weight was contributed by blood compared with 18% in the case of the tumors. Our estimate of 18% closely agrees with the value of 17% given by Hilmas and Gillette (11). In the present studies, the large pulmonary vessels were ligated and removed before y-counts were made on the lungs; therefore, a high proportion of the counts represent blood trapped in the lung microvasculature and, since the tumor vasculature also consists largely of capillary-sized vessels, the results therefore suggest on a per g basis that the lung tissues present up to twice as much endothelial surface to circulating cancer cells as do the metastases. Data collated by Peterson (15) show that the blood flow in a number of different small, nonnecrotic tumors approximates to 0.14 ml/min/g. Pulmonary blood flow in mice is given as 0.25 ml/min (9); pairs of lungs used by us have a mean weight of 0.25 g, corresponding to pulmonary blood flows of 1.0 ml/min/g giving ratios of blood flow per g between lungs and tumors of approximately 7:1. Therefore, if comparisons of cancer cell arrest could be made solely in terms of relative vascular endothelial surface area and blood flow-dependent cancer cell delivery, it would be expected that lung tissue will arrest approximately (2/1 x 7/1 =) 14 times as many cancer cells per g squared as pulmonary metastases; our observed results show that a mean of 4 times as many radiolabeled cells are arrested per g of lungs compared with our estimate of (\sqrt{14}) = 3.7/g.

Parenthetically, it is also of interest that, when radiolabeled Walker 256 cancer cells were injected into the portal veins of rats bearing liver tumors, approximately 4 times more cancer cells were arrested in the liver than in the tumors on a per g basis (23).

In an adult human male, where the total pulmonary metastatic burden is often small compared with the weight of unaffected lung, the relative capillary blood flows will be 5400 ml/min for the lung (1) compared with 0.14 ml/min/g (i.e., 38,571: y) for nonnecrotic tumors of total weight g (14). A lung capillary blood volume of 100 ml (19) in capillaries with diameters of 10 \mu m corresponds to a minimal capillary endothelial surface area of 4 \times 10^6 sq cm. In a tumor weighing g grams, a capillary blood volume of 0.17 y/ml (11) corresponds to a surface area of 680 y/sq cm, giving a calculated lung to tumor capillary surface area ratio of 588:y. If the present considerations of Lewis lung tumors are relevant to the situation in humans, then the relative chances of circulating cancer cells being arrested in the capillary beds of lung tissue and metastases would be (38,571/y \times 588/y =) 23 \times 10^6:y^2 (i.e., approximately 4796:y). If the development of tumors in the 2 types of sites is proportional to the numbers of cancer cells originally arrested in them (28), then it can be appreciated why metastasis to metastases is probably a rare event (5, 6, 10).

ACKNOWLEDGMENTS

We wish to thank D. Graham and D. Lombardo for their technical assistance.

REFERENCES


Fig. 1. Pulmonary metastases dissected from 3-week 3LL s.c. TB mice; margins are free of "noninvolved" lung tissue. H & E. Scale, 1 mm in 0.1-mm divisions.
Arrest and Retention of Circulating Cancer Cells in the Lungs of Animals with Defined Metastatic Status

Leonard Weiss and Pamela M. Ward


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