Promotion of Pulmonary Metastasis in Mice by Bleomycin-induced Endothelial Injury

F. W. Orr, I. Y. R. Adamson, and L. Young

From the Department of Pathology, University of Manitoba, Winnipeg, Manitoba, R3E 0W3, Canada

ABSTRACT

The passage of circulating tumor cells across the vascular wall is an important step in the evolution of cancer metastases. Since tumor cells attach preferentially to subendothelial matrix at sites of endothelial injury and retraction in vitro, we have used an established in vivo model of pulmonary endothelial damage to examine the effects of endothelial injury on the localization and metastasis of circulating tumor cells in vivo. C57BL/6 mice were given a single i.v. dose of bleomycin (120 mg/kg) or multiple i.p. injections (10 mg/kg, twice weekly for 6 wk). Five days after the single injection or 4 days after the last i.p. injection, 2 x 10^6 [131I]iododeoxyuridine-labeled fibrosarcoma cells or unlabeled cells were injected i.v. Two to 8 times as many labeled cells were found in the lungs of bleomycin-treated animals after 24 h. Two and 3 wk after injecting unlabeled fibrosarcoma cells, 1.4 to 5 times more metastatic lung colonies were counted in bleomycin-treated animals than in controls. Morphometric analysis of histological sections demonstrated that the percentage of lung area occupied by tumor in bleomycin-treated animals was between 4 and 16 times that of controls. Analysis of bronchoalveolar lavage fluids demonstrated 5-fold increases of total protein content and leakage of previously injected [125I]-labeled albumin, indicating increased endothelial permeability. Electron microscopic examination of lungs of bleomycin-treated mice demonstrated endothelial retraction with exposure of the underlying basement membrane. Electron microscopy of [3H]thymidine-labeled tumor cells, located by autoradiography, demonstrated their attachment to exposed basal lamina. Data from these experiments in vivo support the hypothesis that endothelial damage can facilitate the metastasis of circulating tumor cells.

INTRODUCTION

The intravascular transit of malignant tumor cells constitutes an important step in the evolution of distant metastases. Subsequently the development of tumors in extravascular tissues depends upon the arrest of these cells, their exit from the circulation by crossing the barriers formed by endothelium and basement membrane, and their growth in the extravascular environment. Although it seems possible that these events might occur at random in normal tissue, there is considerable evidence for the existence of a number of controlling mechanisms, some of which are host related. Important examples include tumor cell interactions with organ-specific endothelial determinants (1), the formation of intravascular platelet-fibrin thrombi and/or tumor cell aggregates (2-4), the ability of tumor cells to degrade extracellular basement membranes (5-7), and the responses of tumor cells to locally generated chemoattractants (8-11).

In the case of lung metastasis, there is experimental evidence that pulmonary X-irradiation is followed by enhanced metastasis of i.v. injected tumor cells (12, 13). Administration of cytotoxic drugs or exposure to high concentrations of oxygen has been reported to have a similar effect on the metastasis of i.v. injected tumor cells (14-19) or on the spontaneous metastasis of solid tumors (20). Tumor cells attach to extracellular matrix components in preference to endothelial surfaces (7, 21), and it seems possible that the effects observed in these models of lung metastasis could be the consequence of endothelial damage with exposure of the underlying basement membrane. However, the observations reported to date have not defined the relationship between metastatic enhancement and the pathological nature of the lung injury.

In a recent in vitro study using various cytotoxic drugs including bleomycin, Nicolson and Custead (22) showed that endothelial cell retraction with exposure of subendothelial matrix was associated with enhanced tumor cell binding. In the present study we have examined metastatic tumor enhancement in vivo in mice receiving bleomycin treatment. It is well established that acute and chronic bleomycin-induced lung injury is characterized by initial damage to the pulmonary endothelium followed by a reproducible sequence of cellular events, the timing of which depends on whether acute or chronic drug regimens are used (23, 24). We now report a correlation between endothelial damage and the enhancement of tumor cell localization with metastatic growth.

MATERIALS AND METHODS

Animals. Female C57BL/6 mice (10 to 15 wk old weighing approximately 20 g) were maintained according to the University of Manitoba's guide for the care and use of laboratory animals.

Bleomycin. Bleomycin (Blenoxane) was kindly provided by Bristol-Myers Canada, Inc. Previous studies using a different strain of mouse indicated that pulmonary damage occurred as early as 5 days following a single i.v. injection of bleomycin (120 mg/kg) (23). Initial experiments were carried out to determine an effective drug dose in C57BL/6 mice using various single injected doses. Subsequently two schedules of administration were used. Acute treatment consisted of a single i.v. (tail vein) injection of bleomycin (120 mg/kg in 0.2 ml saline). Then as the single dose of bleomycin required to produce endothelial injury in C57BL/6 mice was found to be similar to doses that were effective in Swiss Webster mice, a schedule for chronic administration was also based upon the previous dose response data (23). This “chronic” treatment consisted of i.p. injections of bleomycin (10 mg/kg in 0.2 ml saline) given twice weekly for 6 wk. Chronic treatment was included in our protocols because the dosage and frequency of drug administration are more analogous to regimens used in clinical practice. Animals from control
groups received 0.2 ml injections of sterile saline i.v. or i.p.

Fibrosarcoma Cell Line. A previously described syngeneic, methylcholanthrene-induced fibrosarcoma with a propensity to metastasize to the lung (25) was stored in liquid nitrogen after selecting cells from the tenth metastatic passage, and experiments were performed with cultured cells from frozen stocks or from s.c. tumors grown directly from metastatic cells. Cells were cultured in Medium 199 supplemented with 10% fetal bovine serum, penicillin (100 units/ml), fungisone (0.25 μg/ml), and streptomycin (100 μg/ml) (Grand Island Biological Co., Chagrin Falls, OH). This line was free of mycoplasma contamination judged by cell culture in a mycoplasma-supporting broth (Mycomycin-TC; Hana Media, Inc., Berkeley, CA) and by the absence of fluorescent staining with 4,6-diamido-2-phenylindole.

Tumor Cell Localization. Fibrosarcoma cells in the logarithmic phase of growth were cultured for 24 h in the presence of [131I]iododeoxuridine (0.5 μCi/ml medium; specific activity, 900 to 1500 μCi/mg). After 3 washes in culture medium, 2 × 10^5 cells suspended in serum-free medium were injected via the tail vein into animals from various treatment groups. Tumor cells were killed 5 days after acute bleomycin treatment and 3 or 4 days after the last dose of chronic bleomycin. Tumor cells were injected at the same times after saline in the control groups. In most experiments, animals were killed 24 h after injection of labeled cells in others, mice were killed at 15 min, 4 h, and 24 h to examine localization within the first day. Ten to 12 animals from each group were killed at each time. The lungs were excised, fixed in 3 changes of ethanol, and radioactivity was counted in a gamma counter. The percentage of injected tumor cells present in the lungs was calculated from the total number of counts obtained in lung samples compared with the number of counts obtained in noninjected samples of 2 × 10^5 tumor cells. In some experiments, liver, kidneys, and spleen were also removed, fixed, and counted.

To identify tumor cells in the lungs, fibrosarcoma cells were labeled by three 2-h pulses of [3H]thymidine (0.1 μCi/ml; specific activity, 2.0 Ci/mmol). Cells (2 × 10^5) in serum-free medium were injected into mice given acute and chronic bleomycin treatments. The mice were killed 24 h later by i.p. Nembutal and lungs were fixed by i.t. injection of 4% buffered glutaraldehyde and processed for light and electron microscopy. Autoradiographs were prepared on a 0.5-μm plastic section using Kodak NTB2 emulsion and a 2-wk exposure. Tumor cells were identified by the silver grains over the nuclei using light microscopy. Subsequently, the next section from tissue blocks showing labeled tumor cells was cut for examination by electron microscopy to study the relationship of the tumor cells to the endothelium.

Pulmonary Metastasis. To examine the effect of bleomycin treatment on pulmonary metastasis, 2 × 10^5 unlabeled fibrosarcoma cells were injected i.v. in treatment groups and controls 5 days after acute injection of bleomycin or 4 days after the last dose of chronic bleomycin treatment. Animals from each group were killed 14 days after being given injections and grossly visible metastatic tumors were counted under the dissecting microscope after i.t. injection of Bouin’s solution (26). In some experiments, histological sections were prepared from these lungs after the numbers of tumors and grossly visible metastases were counted under the dissecting microscope. The percentage of lung area occupied by metastatic tumor was determined by morphometric analysis, using a Microplan II Image Analysis System (Laboratory Computer Systems Inc., Cambridge, MA).

Analysis of Bleomycin-Induced Endothelial Injury. Animals from each treatment group were given i.v. injections of 0.4 μCi 125I-labeled albumin (specific activity, 1.28 Ci/μg) 1 h before they were killed by i.p. Nembutal. A tracheotomy was performed and the lungs were lavaged 3 times with a total volume of 2 ml sterile 0.9% NaCl. On each lavage fluid, the end of the drug treatment period and tissue was prepared for electron microscopy to assess lung injury.

RESULTS

Bleomycin Dose Response. Our initial objective was to find a reasonable dose of bleomycin that would have a measurable experimental effect without great mortality. Preliminary experiments confirmed previous dose response data (23) for a drug dose that would induce endothelial injury in C57BL/6 mice. Localization of radiolabeled tumor cells in the lung was examined 5 days after single i.v. injections of bleomycin decreasing 10-fold from 120 down to 0.12 mg/kg. In 2 experiments, only the highest dose level induced a significant increase in tumor cell localization 24 h after cell injection (Table 1). At lower doses, the values were not different from saline controls. Based on this experiment, we chose 2 schemes of drug administration for further study: (a) acute bleomycin, with tumor cells injected 5 days after a single i.v. dose of bleomycin (120 mg/kg); and (b) chronic bleomycin, with tumor cells injected 4 days after twice weekly i.p. bleomycin (10 mg/kg for 6 wk for a total dose of 120 mg/kg). With acute bleomycin (120 mg/kg) we had <10% mortality in 5 days and even up to 2 wk later for mice with tumors (see below). No mortality was observed in animals treated with the chronic regimen followed up to 10 wk.

Tumor Localization and Metastasis. Localization of radiolabeled tumor cells was examined during a 24-h period after acute bleomycin treatment. Significantly greater numbers of i.v. injected tumor cells were retained in the lungs of bleomycin-treated animals than in the lungs of controls. Differences were not seen 15 min after injection but were apparent after 4 h and again after 24 h when 3 to 8 times as many tumor cells could be detected in treatment groups (Table 1 and Table 2, Experiment A). Twenty-four h after injection, more cells were also found in the liver and kidneys of treated animals (Table 2, Experiment B). The differences in these organs were small, however, which may be a reflection of the kinetics of bleomycin clearance, with maximum damage to these organs having occurred before the injection of tumor cells at 5 days (27). Greater numbers of i.v. injected cells were also retained in the lungs of animals treated with chronic bleomycin but the magnitude was less than after single-dose treatment and no differences were found in the localization of cells in the liver, kidney or spleen (Table 2, Experiment C).

Animals that received acute or chronic bleomycin or saline treatment before being given injections of unlabeled tumor cells were examined for metastases. We found that animals given

<table>
<thead>
<tr>
<th>Dose of bleomycin (mg/kg)</th>
<th>% of cells located in lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>5.4 ± 7.0*</td>
</tr>
<tr>
<td>12</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>1.2</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>0.12</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>Saline control</td>
<td>3.5 ± 0.4</td>
</tr>
</tbody>
</table>

* Mean ± SE.

Table 1

Effect of acute bleomycin treatment dose on the localization of [131I]iododeoxuridine-labeled fibrosarcoma cells in the lung

A single i.v. injection of bleomycin (120 mg/kg) was given 5 days prior to i.v. injection of the fibrosarcoma cells, and measurements were made 24 h later.

<table>
<thead>
<tr>
<th>Dose of bleomycin (mg/kg)</th>
<th>% of cells located in lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>5.4 ± 7.0*</td>
</tr>
<tr>
<td>12</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>1.2</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>0.12</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>Saline control</td>
<td>3.5 ± 0.4</td>
</tr>
</tbody>
</table>

* Mean ± SE.

P < 0.05 by Mann-Whitney U test.
BLEOMYCIN-PROMOTED LUNG METASTASIS

Table 2
Localization of i.v. injected [131I]iododeoxyuridine-labeled fibrosarcoma cells after acute and chronic bleomycin treatment

<table>
<thead>
<tr>
<th>Organ</th>
<th>Time after cell injection</th>
<th>% of cells injected</th>
<th>Bleomycin</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>15 min</td>
<td>58.2 ± 2.4</td>
<td>63.0 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>4 h</td>
<td>32.7 ± 4.0</td>
<td>18.9 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>24 h</td>
<td>17.7 ± 5.4</td>
<td>2.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Experiment B, injection of cells 5 days after acute bleomycin</td>
<td>Lung 24 h</td>
<td>11.6 ± 4.2</td>
<td>2.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>24 h</td>
<td>0.5 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>24 h</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>24 h</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Experiment C, injection of cells after chronic bleomycin</td>
<td>Lung 24 h</td>
<td>3.9 ± 1.5</td>
<td>1.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>24 h</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>24 h</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>24 h</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE.  
p < 0.05 by Mann-Whitney U test.

Table 3
Development of grossly visible pulmonary metastases 14 days after i.v. injection of fibrosarcoma cells into bleomycin-treated C57BL/6 mice and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice/group</th>
<th>No. of pulmonary metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleomycin</td>
<td>11</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>223–500</td>
</tr>
</tbody>
</table>

* Unlabeled fibrosarcoma cells (2 × 10^6) were injected i.v. 5 days following a single i.v. injection of bleomycin (120 mg/kg).

Table 4
Percentage of lung area occupied by metastatic tumor 14 days after i.v. injection of fibrosarcoma cells

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice/group</th>
<th>Median</th>
<th>Range</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleomycin</td>
<td>11</td>
<td>6.4a</td>
<td>0.7–14.9</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>0.4</td>
<td>0.0–8.7</td>
<td>1.4 ± 0.7</td>
</tr>
</tbody>
</table>

* Unlabeled fibrosarcoma cells (2 × 10^6) were injected i.v. 5 days following acute bleomycin treatment and 4 days after chronic treatment.

Table 5
Effects of bleomycin treatment (using 5 to 6 animals/group) on the composition of bronchoalveolar lavage fluids

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Acute treatment</th>
<th>Chronic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleomycin</td>
<td>Control</td>
<td>Bleomycin</td>
</tr>
<tr>
<td>% of total i.v. dose × 10^4</td>
<td>4.3 ± 0.3</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>Total protein (mg/ml)</td>
<td>1.9 ± 0.5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Total cell content × 10^4</td>
<td>16.2 ± 2.3</td>
<td>13.9 ± 3.3</td>
</tr>
<tr>
<td>Macrophages × 10^6</td>
<td>15.5 ± 2.1</td>
<td>13.4 ± 3.0</td>
</tr>
<tr>
<td>Neutrophils × 10^6</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Chemotactic activity (tumor cells/high-powered field)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean ± SE.  
p < 0.05 by Mann-Whitney U test.

Many grossly visible tumors were large and appeared confluent in the bleomycin groups whereas tumors detected in the saline groups were very small (Fig. 1). As additional quantitation of metastatic tumor growth from random paraffin sections, the percentage of lung occupied by tumor was determined using a Microplan II digitizing tablet. Metastatic tumors occupied a greater percentage of lung in the bleomycin-treated mice compared to saline controls as measured in 5 sections/animal (Table 4). Chronic low-dose bleomycin induced a similar increase in grossly visible tumors after 14 days when more metastases were counted in the bleomycin-treated group (Table 3, Experiment B). Tumors at the pleura of these mice were also much larger than those seen in the saline controls. After acute or chronic bleomycin treatment, we did not find other than occasional extrapulmonary metastases in the treatment or control groups (data not shown), even though the experiments with [131I]iododeoxyuridine-labeled cells had demonstrated localization of small numbers of tumor cells in the liver, spleen, and kidney.

Induction of Lung Injury by Bleomycin Treatment. The injurious effect of bleomycin treatment on the blood-air barrier was demonstrated by examination of bronchoalveolar lavage fluids from animals treated with both acute and chronic bleomycin (Table 5). In both groups, bronchoalveolar lavage fluids contained increased quantities of i.v. injected 125I-labeled albumin and protein even though the fluids were free of erythrocytes. The total cell content of the fluids was increased, significantly so in animals given chronic treatment, and this was due to increased numbers of pulmonary macrophages without change in the numbers of neutrophils. Chemotactic activity by tumor cells was not detected in these fluids.

Cell injury in the lung was confirmed by electron microscopy. Acute bleomycin treatment resulted in focal areas of endothelial cell swelling and some cell necrosis (Fig. 2); other pulmonary cells appeared normal. Similar morphological changes in endothelium were seen after chronic administration of bleomycin (not shown).

Tumor Cells and Lung Injury. When [3H]thymidine-labeled tumor cells were injected into the lungs, they could be located subsequently in autoradiographs. More labeled cells were seen in bleomycin-treated animals and they were usually wedged in...
capillaries or small venules (Fig. 3). By cutting the next section of such a tissue block for electron microscopy, the tumor cells could be identified. The majority of tumor cells were found at areas of endothelial necrosis where they were attached to basement membrane and small fibrin clots (Figs. 4 and 5).

**DISCUSSION**

Bleomycin is a commonly used chemotherapeutic agent which clinically has advantages over other drugs because it is not considered to have major effects on the bone marrow or on immunocompetence (28); however, pulmonary fibrosis is a common side effect. Similarly the administration to mice of bleomycin by i.v. or i.p. routes fails to affect humoral or cell-mediated immunity (29) but can result in pulmonary fibrosis. Serial studies have shown that the initial event is damage to the vascular endothelium of the lung (23). As early as 5 days after a single dose, endothelial damage occurs and this observation has been confirmed in the present study using a different strain of mice in which only pulmonary endothelial cells were injured at the times examined after acute or chronic drug administration. This morphological evidence of endothelial injury was confirmed by the increased leakage of radiolabeled albumin from blood to alveoli and by the increased protein content of bronchoalveolar lavage fluids of mice treated with bleomycin as compared to saline controls.

Ultrastructural examination of bleomycin-induced lung injury showed swelling and disruption of many endothelial cells in capillaries and larger vessels. In some areas necrosis of endothelial cells left segments of denuded basement membrane. It was at these sites that tumor cells were preferentially attached, sometimes at areas that also incorporated fibrin, although it is noteworthy that fibrin deposits were not seen at a comparable level of endothelial injury when tumor cells were not present. By initially locating labeled tumor cells by light microscopic autoradiography, we were able to identify positively each tumor cell on the following serial section cut for electron microscopy. Using this procedure, we have demonstrated tumor cell adherence to extracellular matrix and fibrin at areas of endothelial injury and retraction. Although endothelial damage occurred in all sizes of vessels, tumor cell attachment was observed only in capillaries, a finding that may be related to the size of the tumor cell, the slow passage through these small vessels, or perhaps to specific endothelial surface determinants. The relationship between tumor cell attachment to damaged endothelium and metastatic growth in vivo supports the recent observations of Nicholson and Custead (22) who showed tumor cell binding to extracellular matrix at areas of endothelial cell retraction in vitro.

We and others have shown that the generation of extravascular chemoattractants can influence the localization and metastasis of intravascular tumor cells (8–11). In a previous study we found that acute inflammatory reactions are accompanied by the generation of such chemoattractants (9). In the lung, acute inflammation was elicited by i.t. injection of carbon particles and it was shown that bronchoalveolar lavage fluids contained chemotactic activities for cells from the same syngeneic fibrosarcoma line as used here. When tumor cells were injected i.v. into animals with pneumonitis, more cells were retained in the lung and more tumors grew than in animals without inflammation. The generation of chemotactic factors correlated with the presence of polymorphonuclear leukocytes in the fluids and not with the increase in macrophage numbers (10). In the present study we were unable to detect chemotactic factors in the bronchoalveolar lavage fluids after acute or chronic bleomycin and no increase in polymorphonuclear neutrophils was observed. This suggests that the increased tumor cell adherence and metastatic growth seen after bleomycin cannot be explained by generation of inflammatory-derived chemotactic factors. It is also improbable that immunosuppression could account for the increased incidence of pulmonary metastasis in bleomycin-treated animals since single or total doses of bleomycin, greater than those used in this study, fail to affect humoral or cell-mediated immunity in the mouse (29). While the fibrosarcoma used in these studies may itself be immunosuppressive (30), the same cells were administered to test and control animals. In bleomycin-treated animals, enhanced tumor cell localization was found only 24 h after the i.v. injection of the tumor cells, a time before humoral and cellular immune mechanisms would be expected to operate. In addition, recent data indicate that these fibrosarcoma cells are insensitive to natural cytotoxic- or natural killer-mediated cell lysis in vitro (31).

Tumor cell localization and metastatic growth in the lung was dramatically enhanced following the single large dose of bleomycin. However, a much smaller dose, which by itself gave no such effect, produced changes in metastatic properties of injected tumor cells when injected twice/wk over a 6-wk period. Other studies have indicated that the drug is preferentially retained in the lung (23) and it appears that the accumulated drug from multiple small doses also causes lung damage and increased metastatic tumors. This chronic experiment may have some clinical relevance since it follows the usual pattern of chemotherapy.

**REFERENCES**


BLEOMYCIN-PROMOTED LUNG METASTASIS

13. Hirata, H., and Tanaka, K. Artificial metastases and decrease of fibrinolysis in
    the nude mouse lung after hemithoracic irradiation. Clin. Exp. Metastasis, 2:

    drugs on the incidence of pulmonary metastasis in mice. Cancer Res., 37:

15. Milas, L., Malenic, B., and Allegretti, N. Enhancement of artificial lung metas-
    tases in mice caused by cyclophosphamide. I. Participation of impairment of

16. Moore, J. V., and Dixon, B. Metastasis of a transplantable mammary tumour
    in rats treated with cyclophosphamide and/or irradiation. Br. J. Cancer, 35:
    221-226, 1977.

17. Steel, G. G., and Adams, K. Enhancement by cytotoxic agents of artificial

18. Stewart, R. A. N., and Hacker, M. P. Enhancement of B16 melanoma pulmo-
    nary colonization in mice following bleomycin or hyperoxia. Proc. Am. Assoc.

    Fuzy, M. Enhancement by drugs of metastatic lung nodule formation after

20. Poupon, M. F., Pauwels, C., Jasmin, C., Antoine, E., Lascaux, V., and Rosa,
    B. Amplified pulmonary metastases of a rat rhabdomyosarcoma in response

    preferentially to the extracellular matrix underlying vascular endothelial cells.

22. Nicolson, G. L., and Custead, S. E. Effects of chemotherapeutic drugs on
    platelet and metastatic tumor cell-endothelial cell interactions as a model for

23. Adamson, I. Y. R., and Bowden, D. H. The pathogenesis of bleomycin induced


25. Orr, F. W., Varani, J., Delikatny, J., Jain, N., and Ward, P. A. Comparison of
    the chemotactic responsiveness of two fibrosarcoma subpopulations of differ-

26. Fidler, I. J. General considerations for studies of experimental cancer metas-

    Microbiological assay of bleomycin, inactivation, tissue distribution and clear-

28. Lahane, D. E., Hurd, E., and Lane, M. The effect of bleomycin on immunocom-

29. Dlugi, A. M., Robie, K. M., and Mitchell, M. S. Failure of bleomycin to affect
    1974.

30. Lovett, E. J., Dickinson, R. W., and Varani, J. Metastatic variants from a
    methylcholanthrene induced syngeneic murine fibrosarcoma produce immu-
    nosuppression proportional to the metastatic potential of the variant. In: K.

31. Hiserodt, J. C., Laybourn, K., and Varani, J. Laminin inhibits the recognition of
    tumor target cells by murine natural killer (NK) and natural cytotoxic (NC)
Fig. 1. Paraffin sections of lungs of 2 mice. A, after acute bleomycin; B, after saline. Both animals were given injections of tumor cells 5 days later and were then killed at 2 wk. Many more and larger tumors are seen in mice given bleomycin injections. H & E, x 6.

Fig. 2. Electron micrograph showing endothelial injury in the lung 5 days after acute bleomycin. A, capillary endothelial cell (arrows) is swollen and edematous; B, endothelium is sloughed off (arrows) leaving denuded basement membrane. Epithelial cells appear normal. ALV, alveolus; L, capillary lumen. x 10,500.

Fig. 3. Autoradiograph, 0.5 μm section. The mouse received a single dose of i.v. bleomycin. Five days later [3H]thymidine-labeled tumor cells were injected i.v. and the animal then was killed after 24 h. Silver grains are seen over the nuclei of large cells in a pulmonary capillary. Toluidine blue, x 850.
Fig. 4. Electron micrograph of the lung section shown in Fig. 3. A large tumor cell completely fills the capillary and appears to be attached to the wall (arrow). EP, alveolar epithelial cell. x 11,500.

Fig. 5. High-power electron micrograph of image pinpointed by the arrow in Fig. 4. The type 1 epithelial cell (EP) is normal; the capillary endothelium shows a break (arrows) where the tumor cell is adherent to the basal lamina and dense fibrin (F). x 33,000.
Promotion of Pulmonary Metastasis in Mice by Bleomycin-induced Endothelial Injury

F. W. Orr, I. Y. R. Adamson and L. Young