Site-specific Metastasis of Mouse Melanomas and a Fibrosarcoma in the Brain or Meninges of Syngeneic Animals

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ABSTRACT

Different subpopulations of cells from two different murine melanomas (K-1735 and B16) and a fibrosarcoma (UV-2237) were injected into the internal carotid artery of syngeneic inbred mice. The common route of tumor cell injection, tumor lesions in the brain were unique to each tumor type and developed at different sites in the brain. Gross and histological examinations revealed that different subpopulations of cells derived from the K-1735 melanoma produced only parenchymal lesions, cells of the B16 melanoma produced lesions in the meninges and ventricles, and cells of the UV-2237 fibrosarcoma produced lesions throughout the brain. This site specificity for tumor growth was not due to the initial tumor cell arrest in the microvasculature of different regions-areas in the brain as evidenced by detailed studies with radiolabeled cells. The site specificity of this experimental brain metastasis was not random and correlated well with the clinical situation. The exact interactions of tumor cells with different microenvironments in the brain need further elucidation.

INTRODUCTION

The development of metastasis to the brain is often associated with the terminal stage of cancer. The treatment of choice for a single brain metastasis is surgery, but the prognosis is poor, with patients surviving a median of 4 to 6 mo. The addition of chemotherapy and radiotherapy somewhat increases the median survival time over 6 mo but rarely brings about complete cure (1–5).

In order to develop better therapies for cancer metastases in the brain, we must improve our understanding of the biology of these lesions, which necessitates the development of suitable in vivo models. The ideal experimental model for such studies should mimic the natural route of tumor cell dissemination, produce a high rate of lesions in the brain, and correlate with the clinical behavior of appropriate tumor systems. To allow sufficient time for studies on the progressive growth of brain cancer metastases, it is important that the predominant disease be in the brain and not in visceral organs where it would lead to early death.

We have recently developed a murine model for brain metastasis in which tumor lesions in the brain are produced by the injection of tumor cells into the internal carotid artery of syngeneic mice. In this model, the high incidence of brain lesions and the low incidence of fast-growing visceral lesions allow for studies on the establishment and growth of brain tumor foci-metastases (6).

This paper concerns investigations of brain lesions produced by cells of two different murine melanomas and a fibrosarcoma. We show that, despite the common route of injection, subpopulations of tumor cells from different syngeneic tumors produced lesions in different regions in the brain, such as the parenchyma or the meninges. These unique sites of growth were not due to initial tumor cell distribution but more likely to the proliferation of tumor cells in a suitable organ environment.

MATERIALS AND METHODS

Mice. Specific-pathogen-free female mice of the inbred strains, C3H/HeN (mammary tumor virus negative), C57BL/6, and C57BL/6 × C3H/HeN F1 (hereafter called B6C3F1) were purchased from the Animal Production Area, NCI-Frederick Cancer Research Facility (Frederick, MD). The mice were 6 to 8 wk old at the time of the experiments.

Tumor Cell Lines. The K-1735 melanoma syngeneic to C3H/HeN mice (7) was a gift from Dr. M. L. Kripke, M. D. Anderson Hospital and Tumor Institute, Houston, TX. We derived the K-1735 SW-1 line from a nonpigmented spontaneous lung metastasis, produced by the parent K-1735 melanoma growing s.c. The K-1735 SW-1 cells are highly metastatic and, in syngeneic mice, produce only melanotic metastases in the brain, amelanotic metastases in the heart and muscle, and melanotic or amelanotic metastases in the lung (8, 9). The K-1735 2B-2 line was isolated from a melanotic brain metastasis produced by cells of the K-1735 parental line subsequent to i.v. injection. K-1735 2B-2 cells are tumorigenic and metastatic. The tumorigenic nonmetastatic K-1735 clone 19 was isolated from the parental K-1735 line by a double dilution method (10, 11). The K-1735 M-2 line was established from a spontaneous lung metastasis. This line is highly tumorigenic and metastatic (12).

The highly invasive B16-BL6 melanoma (13) was derived from the B16-F10 line syngeneic to C57BL/6 mice (14). The highly metastatic UV-2237 MM line (15) was derived from the parental UV-2237 fibrosarcoma that had been induced in a female C3H/HeN mouse by chronic exposure to ultraviolet-B radiation (16).

All tumor cell lines were maintained in culture in Eagle's minimal essential medium supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, l-glutamine, 2-fold vitamin solution, and penicillin-streptomycin (Flow Laboratories, Rockville, MD). Cell cultures were maintained on plastic and incubated in 5% CO2–95% air at 37°C. Cultures were free of Mycoplasma and the following murine viruses: reovirus type 3; pneumonia virus; Sendai virus; minute virus; mouse adenovirus; mouse hepatitis virus; lymphocytic choriomeningitis virus; ectromelia virus; and lactate dehydrogenase virus (assayed for all by M. A. Bioproducts, Walkersville, MD).

For in vivo studies, tumor cells in exponential growth phase were harvested by a 1-min treatment with 0.25% trypsin-0.02% EDTA solution (w/v). The flask was tapped to detach the cells, supplemented with 5% fetal bovine serum, sodium pyruvate, essential medium supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, l-glutamine, 2-fold vitamin solution, and penicillin-streptomycin (Flow Laboratories, Rockville, MD). Cell cultures were maintained on plastic and incubated in 5% CO2–95% air at 37°C. Cultures were free of Mycoplasma and the following murine viruses: reovirus type 3; pneumonia virus; Sendai virus; minute virus; mouse adenovirus; mouse hepatitis virus; lymphocytic choriomeningitis virus; ectromelia virus; and lactate dehydrogenase virus (assayed for all by M. A. Bioproducts, Walkersville, MD).

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The abbreviations used are: HBSS, Hanks' balanced salt solution; IdUrd, iododeoxyuridine.

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iodine, and the skin was cut by a mediolateral incision. After blunt dissection, the trachea was exposed. The muscles were separated to expose the right common carotid artery, which was then separated from the vagal nerve. The artery was prepared for an injection distal to the point of division into the internal and external carotid arteries (Fig. 1). A ligature of 5-0 silk suture was placed in the distal part of the common carotid artery. A second ligature was placed and tied proximal to the injection site. The artery was nicked with a pair of microscissors, and a <30-gauge glass cannula was inserted into the lumen. To assure proper delivery, the cells were injected slowly, and the cannula was removed. The second ligature was tightened, and the skin was closed by sutures. To inject cells into the internal or external carotid artery, the glass cannula was threaded into the internal or external carotid artery, respectively. All intracarotid artery injections were always done under a dissecting microscope, and all required a glass cannula with a smaller than 30-gauge diameter. We prepared such cannulas from 1-mm-diameter glass capillary tubes that were heated and then stretched. The diameter could be varied by varying the amount of stretching, and each cannula could be used repeatedly. The cannula was fixed to a 1-ml plastic syringe by melting the hub around the cannula.

In Vitro Labeling of Melanoma Cells with [125I]IdUrd. K-1735 SW-1 and B16-BL6 cells, respectively, were seeded into 75-cm² tissue culture flasks at 1.5 × 10⁵ cells/flask in supplemented medium. Twenty-four h later, 0.3 µCi of [125I]IdUrd per ml was added (New England Nuclear, Boston, MA; 2000 mCi/µmol). Twenty-four h later, the cell monolayers were rinsed twice with an excess of Ca²⁺ and Mg²⁺-free HBSS to remove nonbound radiiodine. The cultures were then overlaid with a thin layer of 0.25% trypsin-0.02% EDTA solution for 1 min. The cell suspension was washed and resuspended in Ca²⁺- and Mg²⁺-free HBSS at a final concentration of 1 × 10⁵ cells per 0.1 ml, the inoculum volume per mouse.

Distribution and Fate of [125I]IdUrd-labeled Melanoma Cells after Carotid Artery Injection. Radioactively labeled cells were injected into the internal or external carotid arteries of mice at the dose of 1 × 10⁶ cells/mouse. For 5 and 60 min, groups of 3 mice each were killed. Brain, meninges, heart, lung, liver, spleen, kidney, the salivary gland, and the head from each mouse (taken at the 60-min time point) were placed into vials containing 70% ethanol. The ethanol was replaced daily for 3 days to remove all soluble 125I (17). Blood samples (0.5 ml/mouse) were placed directly into test tubes for radioactive monitoring. All sampled organs were monitored for radioactivity in a gamma counter (Tru Analytic, Elk Grove Village, IL). Triplicate tubes containing the inoculum dose were retained, and the radioactivity was determined at the same time as the sample organs. The mean counts in organs from each group of mice (n = 3) were expressed as the percentage of input counts. All measurements were corrected for radioactive decay.

**RESULTS**

Production of Brain Lesions in Syngeneic Mice. In the first set of studies, we determined the minimal number of tumor cells injected into the internal carotid artery necessary to consistently produce brain tumor foci. Groups of 3 or 4 syngeneic mice each were given injections with 1 × 10⁵, 1 × 10⁵, 1 × 10⁴, or 1 × 10³ K-1735 SW-1, K-1735 2B-2, K-1735 clone 19 cells (into C3H/HeN mice), B16-BL6 (into C57BL/6 mice), or UV-2237 MM fibrosarcoma cells (into B6C3F mice). The mice were observed daily and killed upon development of clinical symptoms of disease. Asymptomatic mice were killed 10 wk after the injection. Complete necropsies were performed and organs were processed for histological studies.

With all 3 tumor systems, some mice given injections of as few as 1000 cells developed tumor growth in the brain. All mice given injections of 1 × 10⁵ cells developed tumor lesions, and therefore this inoculum dose was used for all further studies.

K-1735 Melanoma Lines. In various experiments, a total of 167 C3H/HeN mice received injections of cells from 4 different lines of the K-1735 melanoma. The data summarized in Table 1 show that, regardless of their metastatic nature, all K-1735 melanoma cells injected into the internal carotid artery could produce brain tumor foci. For example, cells of the K-1735 clone 19 line, which are not metastatic from s.c. sites (10), were as efficient in producing brain lesions as were cells of the K-1735 SW-1 line or K-1735 M-2 line, which are highly metastatic from an s.c. tumor (9). Differences in the biological behavior among the three K-1735 melanoma lines were noted with regard to the time from intracarotid injection to morbidity (K-1735 M-2 < K-1735 SW-1 < K-1735 2B-2 < K-1735 clone 19) and the production of extracranial tumor growths in the lungs and heart (observed in mice given injections of K-1735 M-2, K1735 SW-1, or K-1735 2B-2 cells). Regardless of the cell line injected, the terminal stage of the disease was associated with the production of highly melanotic melanomas in the brain parenchyma, mainly in the right hemisphere, the side of injection (Fig. 2). The tumors were detected in the area of the middle cerebral artery and the anterior cerebral artery.

The nature and site of the melanoma lesions were confirmed by histological examination of 16 sections per brain. We studied a total of 137 brains. Clear evidence of tumor growth in the parenchyma was found in 23 of 137 mice (46 of 46, K-1735 SW-1; 35 of 42, K-1735 C-19; 20 of 24, K-1735 2B-2; 22 of 25, K-1735 M-2). In mice killed before terminal disease, we did...
Table 1 *The production of lesions in the brain of mice given injections of $1 \times 10^7$ tumor cells into the internal carotid artery*

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Tumorigenicity*</th>
<th>Gross pathology</th>
<th>Histology†</th>
<th>Extracranial lesions$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parenchyma</td>
<td>Meninges</td>
<td>Parenchyma</td>
</tr>
<tr>
<td>K-1735 SW-1</td>
<td>54/56</td>
<td>49/56</td>
<td>0/56</td>
<td>46/46</td>
</tr>
<tr>
<td>K-1735 C-19</td>
<td>40/47</td>
<td>38/47</td>
<td>0/47</td>
<td>35/42</td>
</tr>
<tr>
<td>K-1735 2B-2</td>
<td>35/39</td>
<td>33/39</td>
<td>0/39</td>
<td>20/24</td>
</tr>
<tr>
<td>B16BL-6</td>
<td>49/49</td>
<td>0/49</td>
<td>49/49</td>
<td>0/39</td>
</tr>
<tr>
<td>UV-2237MM</td>
<td>35/43</td>
<td>Not detected</td>
<td>35/43</td>
<td>35/43</td>
</tr>
</tbody>
</table>

* Number of positive mice (gross pathology and/or histology)/number of mice given injections.
† Number of positive mice/number of mice receiving injections. Tumor growth was either grossly evident or confirmed histologically.
$^a$ Sixteen sections/brain.

Fig. 2. Brain metastases produced by the injection of K-1735 melanoma cells into the internal carotid artery. a, gross pathology of lesions produced by K-1735 SW-1 cells; b, intraparenchymal growth of K-1735 SW-1 cells.

Fig. 3. Brain metastases produced by the injection of B16 melanoma cells into the internal carotid artery, a, gross pathology of meningeal melanoma. Note tumor growth in the meninges associated with the cranium. b, B16 melanoma cells proliferating in the meninges. Note tumor growth adjacent to the skull.

not find K-1735 melanoma growth in the meninges (Table 1). The clinical symptoms associated with K-1735 melanoma brain metastases were cachexia, listlessness, crouching position, and ruffled fur.

**B16-BL6 Melanoma.** In various experiments, 49 mice were given injections in the internal carotid artery with $1 \times 10^7$ cells. Morbidity followed rapidly, and by 2 to 3 wk, all 49 animals had reached terminal stages and were killed. Cerebral metastases of the B16-BL6 melanoma developed only in the ventricles and in the meninges (Fig. 3). Histological examination of 39 brains (16 sections/brain) confirmed this site specificity. We found growths only in the meninges and ventricles but not in the brain parenchyma. The major clinical symptoms in mice given injections of B16-BL6 cells were the protrusion of the eye bulb on the right, the side of tumor cell injection. Distant metastases in the lungs were found in 45 of 49 mice (Table 1).
UV-2237 MM Fibrosarcoma. Forty-three mice were given injections, and 35 developed tumor growths in the brain which caused death by 2 to 3 wk. The clinical symptoms associated with these brain lesions were mild, and many mice died without evidence of morbidity. Histological examination revealed cerebral tumor foci of the UV-2237 fibrosarcoma in the brain parenchyma, the ventricle, and in the leptomeninges (Fig. 4). Distant fibrosarcoma metastases were found in the lungs of 30 of 43 mice (Table 1).

Histological Study of the Pathogenesis of Brain Parenchyma and Meningeal Melanoma Metastasis. The above data conclude that K-1735 melanoma cells produce melanotic lesions in the brain parenchyma, whereas B16-BL6 cells produce melanotic lesions in the ventricles and meninges (Fig. 5). In the next set of experiments, we wished to examine the early events in the pathogenesis of brain tumor lesions. K-1735 SW-1 or B16-BL6 cells (1 × 10⁷/mouse) were injected into the internal carotid arteries of C3H/HeN and C57BL/6 mice, respectively. Groups of 3 to 5 mice each were killed 1, 7, 14, 21, and 24 days thereafter. Complete necropsies were performed, and the brain and meninges of each mouse were processed for histopathological examination.

For the K-1735 melanoma, the first clear evidence of tumor cell infiltration and growth in the brain parenchyma was found by 7 days after intracarotid injection. Tumor cells were found growing in small blood vessels with clear evidence for invasion into the parenchyma (Fig. 6, a and b). Tumor cell proliferation followed rapidly, grossly evident disease was detected 21 days after the injection, and by Day 24, all mice had developed terminal symptoms. Death was due to decompensated pressure within the brain caused by the tumor growth and surrounding edema.

The first histological evidence of B16-BL6 cells proliferating in the meninges was also found at 7 days after the intracarotid injection of B16-BL6 cells (Fig. 6c). By Day 14 after the injection, grossly evident disease was detected associated with the clinical symptom of the protrusion of the right eye bulb. By Day 18, all mice exhibited tumor growths in the meninges and the ventricle of the brain (Fig. 6d).

Organ Distribution of [¹²⁵I]IdUrd-labeled K-1735 SW-1 and B16-BL6 Cells. In the next set of studies, we investigated whether the pattern of growth in the brain unique to the K-1735 melanoma (brain parenchyma) and the B16-BL6 melanoma (ventricle, meninges) could have been due to a different pattern of initial tumor cell localization at different sites. To do so, we studied the organ distribution and fate of [¹²⁵I]IdUrd-labeled K-1735 SW-1 and B16-BL6 cells after injection into the right internal or right external carotid arteries. Mice were given injections of 1 × 10⁷ radiolabeled cells, and groups of 3 mice each were killed 5 or 60 min later. The percentage of injected viable radioactive cells retained in the brain, meninges, several visceral organs, and the blood is shown in Tables 2 and 3. When examining these data, it is important to remember that the internal carotid artery primarily supplies blood to the brain tissue and not to the meninges, whereas the external carotid artery primarily supplies blood to the meninges and not to the brain (18).

Five min after the injection of radiolabeled melanoma cells into the internal carotid artery, 64.4% of the K-1735 SW-1 and 37% of the B16-BL6 cells were found in the brain, and 1 h after, the values were 52% and 26%, respectively (Tables 2 and 3). In contrast, after the injection of K-1735 or B16 cells into the external carotid artery, less than 0.1% and 0.5%, respectively, of the cells were found in the brain. In general, the arrest of the B16-BL6 tumor cells in the brain (Table 3) was significantly lower than that observed for the K-1735 SW-1 cells (Table 2). The B16-BL6 cells probably pass more rapidly through the cerebral circulation than do the K-1735 melanoma cells. After external carotid artery injection, very few tumor cells arrested in the brain or the meninges. The slightly higher number of tumor cells in the meninges after internal carotid artery injection may be due to reflux into the external carotid artery during injection and to recirculation via the internal-external anastomoses (18). It is also important to note that only the meninges lining the upper surface of the skull with dura mater were monitored. Technical reasons prevented monitoring of meninges lining the base of the brain.

In the next experiment (Table 4), mice were given injections of K-1735 SW-1 cells into the internal or external carotid arteries. After 60 min, groups of 3 mice each were killed. The head with all musculature, salivary glands, and skin, which are supplied with blood by branches of the external carotid artery and one branch of the internal carotid artery (18), was monitored for radioactivity (Table 4). Subsequent to injection, the head and associated structures (devoid of the brain) contained 37% and 86% of the cells injected into the internal or external carotid artery, respectively. The addition of all cpm monitored from the head and brain yielded a near 100% recovery of the injected cells.

DISCUSSION

Despite the increased interest in biological studies of cancer metastasis, data on experimental cerebral metastasis are rare.
Subsequent to i.v., s.c., or foot pad injection, most murine tumors do not produce brain metastases (19–21). Two methods have been proposed to facilitate the studies of experimental brain metastasis: the selection of subpopulations of metastatic cells with ability to produce brain metastases (22–25); and the introduction of tumor cells into the left heart (26), common carotid artery (27, 28), and internal carotid artery (6). The selection of tumor cells capable of giving rise to brain metastases (22, 23, 25) is a tedious procedure and, therefore, is not applicable for cells isolated from surgical specimens of human neoplasms. The injection of cells into the left heart (26) is easy to accomplish but produces lesions in most visceral organs, where rapid proliferation of tumor cells leads to the death of the animal. For these reasons, we developed the procedure of injecting neoplastic cells into the internal carotid artery, which supplies the brain parenchyma. In our present studies, we used cells from different mouse neoplasms. Despite the consistent route of injection, cells of the various lines of K-1735 melanoma produced lesions in the brain parenchyma (151 of 167 mice receiving injections), the B16-BL6 cells produced lesions in the meninges and ventricle (49 of 49 mice receiving injections), and the UV-2237 MM fibrosarcoma cells grew in all sites of the brain (35 of 43 mice receiving injections). The unique patterns of experimental brain metastases were, therefore, due to properties of the injected tumor cells.

Clinical observations of human cancer have suggested that certain neoplasms produce metastasis to specific organ sites, independent of vascular anatomy and number of cells delivered (29–32). Interest in the factors that determine the pattern of metastasis was already enunciated by Paget in 1889 (33). He hypothesized that the process of metastasis was not due to chance but, rather, that specific tumor cells had an affinity for the environment provided by certain organs. Metastases resulted only when the tumor cells (seeds) and the organs (soil) were matched (33). In 1928, Ewing suggested that metastasis occurs merely as a consequence of mechanical factors of the circulation (34). Neither mechanism, however, is mutually exclusive (35–37).

Studies using experimental animal tumor systems have demonstrated that the production of cancer metastases in specific organ sites is determined by both host factors and tumor cell properties (14, 16, 38). In different animal tumor systems, tumor cells with unique organ specificity for metastasis have been isolated (14, 15, 19–25, 29, 38–40). This preferential organ metastasis is not simply due to the ability of tumor cells to reach the capillary bed of an organ. Studies on the distribution and fate of hematogenously disseminated radiolabeled tumor cells reveal that tumor cells can reach the microvasculature of many organs, but the growth of the arrested cells into clinically relevant metastases occurs in only some (17, 19, 21, 40).

Metastasis to the central nervous system occurs in 22% of patients with soft tissue sarcoma (41). These metastases do not show predilection for growth in a particular tissue site in the brain. Similarly, the UV-2237 fibrosarcoma cells produced multifocal growths in the parenchyma, leptomeninges, and ventricle. In the brain parenchyma, the lesions were small and surrounded blood vessels.

Malignant melanoma will produce metastases in the brain of 40 to 50% of patients (1, 41), which is the highest incidence of brain metastasis for any one tumor system. Of these brain metastases, 49% are intraparenchymal, 22% are leptomeningeal, and 32% are dural (41). Thus, in clinical disease in different patients, malignant melanoma can metastasize to different sites in the brain. The results with the two different mouse melanomas provide evidence that growth in particular regions in the brain is not random. All the K-1735 tumor cell lines (regardless of metastatic potential) produced growths in the parenchyma, whereas the B16-BL6 melanoma cells grew exclusively in the meninges and the ventricles. This unique pattern of growth could not be simply due to the circulatory system of the brain, although the functional deficit of the blood-brain barrier in the choroid plexus (42, 43) might explain the seeding of the B16-BL6 cells in this region. Whether the B16 melanoma cells cannot overcome the blood-brain barrier is still unclear as is the lack of seeding of the K-1735 cells in the choroid plexus.

Tumor cells that enter the carotid artery can reach both the brain parenchyma (internal carotid artery) or the skin, musculature, salivary glands, and meninges (external carotid artery).
Fig. 6. The pathogenesis of different melanoma brain metastases. a, K-1735 melanoma cells proliferating in a blood vessel 7 days after injection into the internal carotid artery; b, K-1735 melanoma cells proliferating in the brain parenchyma 14 days after injection into the internal carotid artery; c, B16BL-6 melanoma cells in a cortical vessel 7 days after injection into the internal carotid artery; d, growth of B16BL-6 cells in the choroid plexus 14 days after their injection into the internal carotid artery.

Table 2 Distribution of [3H]dUrd-labeled K-1735 SW-1 melanoma injected into the internal or external carotid arteries

<table>
<thead>
<tr>
<th>Organ</th>
<th>Internal carotid artery</th>
<th>External carotid artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Brain</td>
<td>64.4%</td>
<td>52.0%</td>
</tr>
<tr>
<td>Meninges</td>
<td>2.0%</td>
<td>1.2%</td>
</tr>
<tr>
<td>Blood</td>
<td>1.2%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Heart</td>
<td>0.4%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Lung</td>
<td>26.5%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Liver</td>
<td>1.3%</td>
<td>17.0%</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.0%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.9%</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

* Percentage of viable cells was calculated by comparison to cpm in the inoculum dose of 1 x 10⁶ cells. The data are derived from 3 independent samples. Variation from the mean did not exceed 15%.

Table 3 Distribution of [125I]dUrd-labeled B16-BL6 melanoma cells injected into the internal or external carotid arteries

<table>
<thead>
<tr>
<th>Organ</th>
<th>Internal carotid artery</th>
<th>External carotid artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Brain</td>
<td>37.0%</td>
<td>26.0%</td>
</tr>
<tr>
<td>Meninges</td>
<td>2.0%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Blood</td>
<td>2.7%</td>
<td>8.8%</td>
</tr>
<tr>
<td>Heart</td>
<td>0.6%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Lung</td>
<td>16.6%</td>
<td>15.9%</td>
</tr>
<tr>
<td>Liver</td>
<td>2.3%</td>
<td>9.6%</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.1%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.1%</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

* Percentage of viable cells was calculated by comparison to cpm in the inoculum dose of 1 x 10⁶ cells. The data are derived from 3 independent samples. Variation from the mean did not exceed 15%.

The introduction of the K-1735 cells into the internal carotid artery allows these cells to reach the junction of the grey and white matter in the brain (1). The growing K-1735 tumor can reach the surface of the brain, but the cells do not cross the pia mater. The growth of the B16-BL6 cells in the dura mater and the ventricles could occur subsequent to the entry of cells into the choroid plexus and the meningeal artery. Also tumor cells that circulate in the liquor can reach the leptomeninges (1, 41).

The use of tumor cells whose DNA is labeled with [125I]dUrd allows exact determinations of the organ distribution and fate of the cells (17). We used this technique and, subsequent to their injection into the internal carotid artery, found most cells in the brain. More K-1735 cells than B16-BL6 cells arrested in the brain. For both the melanomas, only a few cells reached the meninges. The K-1735 cells failed to proliferate at this site; the
B16-BL6 cells grew rapidly. These data suggest that initial tumor cell arrest in the microvasculature of different areas in the brain did not necessarily predict or correlate with eventual development of metastasis.

The site specificity of the experimental brain metastases for the tumors we studied was not random, a specificity that correlates well with the clinical situation. The effects of the "soil" on tumor cell growth in metastatic sites (33) could well involve interaction of tumor cells with endothelial cells (44-46), reaction of tumor cells to unique short distance growth factors liberated by the microenvironment (47), or many other possibilities (48). These are now being investigated.

**REFERENCES**


44. Kramer, R. H., and Nicolson, G. L. Interactions of tumor cells with vascular endothelial cells (44-46), reaction of tumor cells to unique short distance growth factors liberated by the microenvironment (47), or many other possibilities (48). These are now being investigated.
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