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The Shedding of Viable Circulating Tumor Cells by Pulmonary Metastases in Mice

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RESECTION of pulmonary metastatic lesions has in some patients been followed by long-term survival free of disease,[1, 9] thus mitigating the sentence of "incurable" for a fortunate few. The decision to intervene surgically in these patients, and the timing of such intervention are difficult issues. Restraint and a period of observation is more likely for an initial lesion indicating widespread metastatic dissemination. Another consideration is the possibility that a pulmonary metastasis may itself give rise to other metastases. This question has not to our knowledge been convincingly answered, even in experimental animal tumor systems, and is the focus of this study.

In a primary tumor-bearing animal with pulmonary metastatic lesions, the origin of any further metastases, whether from the primary or the initial metastases, is uncertain. Thus the question of whether metastases do metastasize does not lend itself readily to investigation. If venous drainage from pulmonary metastatic lesions in an animal otherwise free of tumor, when injected into the systemic venous system of identical syngeneic animals resulted in the formation of pulmonary tumor growths in these recipients a partial answer would be provided. That pulmonary metastases do shed viable circulating tumor cells would be established and the formation of pulmonary embolic tumor growths in recipients, identical to pulmonary metastases in the host, would be suggestive that pulmonary metastases in turn metastasize.

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Materials and Methods

Three host-tumor systems were studied: T241 sarcoma of Lewis in C57BL/6N mice, mammary adenocarcinoma in C3H/HeN mice, and S91 melanoma in CDF_1 mice. The T241 is a methylcholanthrene induced sarcoma, and the latter two are spontaneous tumors. All have been carried for years in this laboratory by animal transplantation. The metastatic behavior of all three has been established by previous study and the tumors metastasize selectively to the lungs during a period of time covered in this study.[4] All mice were female, 6–8 weeks old, and fed Purina chow.

TABLE 1. *Incidence of Pulmonary Metastases in Donor Mice*

Strain	No. of Donor Mice	No. with Metastases	No. of Metastatic Lesions (range)
C57 BL/6N	20	20 (100%)	5 to TLC*
C3H/HeN	30	28 (93%)	1 to TLC
CDF_1	25	25 (100%)	All TLC

* TLC = Too large and numerous to count.

Primary tumors were established by inoculation subcutaneously in the thigh of 0.05 ml. of a uniform cytosieve tumor-cell suspension. Twenty C57BL/6N mice, 30 C3H/HeN, and 25 CDF_1 mice were thus inoculated. The primary tumors were then allowed to grow for the period of time determined by previous study,[4] in which pulmonary metastases would form in virtually all mice yet the primary tumor would still be completely removable by amputation. This interval between inoculation and amputation was 9 days for the C57BL/6N mice, 13 days for the C3H/HeN, and 21 days for the CDF_1 strain. Amputation was performed with a cutting cautery. A period of time was then allowed for the maximal enlargement of pulmonary metastases which avoided deaths due to the tumors. This interval was 4 weeks for the C57BL/6N and C3H/HeN mice and 6 weeks for the CDF_1 strain. There was 0.6 cc. of blood drawn from the left ventricle of these mice under direct vision, using a #27 needle and heparinized syringe. This was the maximal quantity which could generally be obtained. The blood was immediately injected into the tail vein of an identical syngeneic mouse. After a 4-week interval recipient mice were sacrificed, and the lungs were examined for pulmonary embolic tumor growths. This was done macroscopically using India ink-Fekete's solution differential staining of normal lung tissue and tumor for the C57BL/6N-fibrosarcoma and C3H/HeN-mammary adenocarcinoma systems as previously described.[8] The darkly pigmented S91 melanomas could be counted directly without ink staining of the normal lung tissue.

Pulmonary tumor growths of recipient mice were compared histologically with the primary tumors and pulmonary metastases of the donor mice in all three systems.

Results

All mice inoculated in the thigh developed primary tumors. There was no mortality from amputation and no recurrence of tumor at the amputation site. Metastatic deposits were confined to the lungs in donor mice.

In primary tumor-bearing donor mice, 100% of both the C57BL/6N and the CDF_1 and 93% of the C3H/HeN mice had pulmonary metastatic lesions at the time pulmonary venous blood was drawn from the left heart (Table 1). The pulmonary metastatic spread was further quantitated. Among 20 C57BL/6N donor mice, 18 had metastases too numerous and confluent to count, one had eight metastatic deposits, and one had five. In 30 C3H/HeN donor mice, eight had lesions too confluent to count, five had between six and ten metastases, 15 mice had between one and five lesions, and two were free of pulmonary metastases. All 25 CDF_1 donor mice had lung metastases too numerous and confluent to count. Thus the great majority of donor mice had a large amount of metastatic tumor in their lungs.

In recipient mice receiving tail vein injections, 40% of the C57BL/6N, 20% of

TABLE 2. *Incidence of Pulmonary Embolic Tumor Growths in Recipient Mice*

Strain	No. of Recipient Mice	No. with Pulm. Embol. Tumor Growths	No. of P.E.T.G./ms. (range)
C57 BL/6N	20	8 (40%)	1 to TLC*
C3H/HeN	30	6 (20%)	1 to 4
CDF_1	25	4 (16%)	1 to 9

* TLC = Too large and numerous to count.

the C3H/HeN, and 16% of the CDF_1 mice developed pulmonary embolic tumor growths (Table 2). Of eight C57BL/6N mice with pulmonary embolic tumors, three had lesions too numerous and confluent to count, one had five lesions, one had three lesions, and three had one lesion each. Among the six C3H/HeN mice, two had four lesions, two had two lesions, and two had one lesion each. Of the four recipient CDF_1 mice with lesions, one had nine lesions and three had one lesion each. Thus while many recipient mice had solitary embolic tumor growths, the majority had multiple lesions and in a few the lesions were too numerous and confluent to count.

On histological review, the pulmonary embolic tumor growths of recipient mice were identical to the metastatic and primary tumor growths of donor mice in all three tumor systems.

Discussion

Pulmonary embolic tumor growths in recipient mice of all three tumor-host systems establishes that pulmonary metastases shed viable circulating tumor cells. Alternate sources of such cells, other metastatic lesions or residual primary tumor, were evident in none of the donor mice. The fact that circulating tumor cells have not been detectable in the blood 5–6 hours following intravenous injection,[2, 7] makes the primary tumors, removed weeks before, an unlikely source of tumor cells in pulmonary venous drainage in the present study.

The formation of metastatic lesions does not necessarily follow from the presence of circulating tumor cells. Clinical studies of circulating tumor cells have shown no correlation between detection of circulating tumor cells and metastatic spread.[3] In the present study, however, formation of pulmonary lesions in a syngeneic host establishes the viability and metastatic potential of circulating tumor cells. It is equally clear that this metastatic potential might not have found expression by formation of metastases in donor mice due to host resistance induced by previous tumor exposure. Methylcholanthrene induced sarcomas have been shown to be immunogenic and previous exposure to protect against a second tumor inoculum.[6] Whether tumor immunity modifies or influences spontaneous metastatic spread is not established. With the shedding of viable tumor cells from pulmonary metastatic lesions established, however, and the potential of these cells to form pulmonary tumor growths similar to metastases demonstrated it appears that pulmonary metastases can metastasize.

In a consideration of the management of solitary pulmonary metastases in patients, suggestions arising from the results of this study must be examined against the obvious host differences involved. The behavior of transplanted tumors in animal hosts may differ importantly from that of autochthonous tumors in patients.[5] Nevertheless, the demonstrated ability of pulmonary metastases to shed viable circulating tumor cells would preclude a prolonged period of observation of pulmonary lesions. Further metastatic spread from such a metastatic deposit is distinctly possible.

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