was further characterized as noninfective, nonantigenic for fluorescein-tagged antibody, and Feulgen positive. Furthermore, thymidine did not reverse the inhibitory effect of aminopterin on the maturation of the TT agent in McCoy cells(9) but did exert a reversal effect on aminopterin-inhibited adenovirus type 12(20). In view of these combined observations, it may be argued that significant synthesis of DNA occurs early in the developmental cycle. Therefore, we would expect to find incorporation of thymidine during all stages of the growth cycle if it occurred at all. Under the conditions of this study, no significant incorporation of thymidine was noted by either the agent of psittacosis or trachoma.

Summary. Significant incorporation of tritiated thymidine into cytoplasmic inclusion bodies of either the TT strain of psittacosis or the T'ang strain of trachoma could not be demonstrated by autoradiographic technics during any stage of their developmental cycles in the McCoy cell line.

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Viable Tumor Cells in Blood and Lungs of Mice with Metastasizing and Non-Metastasizing Transplantable Tumors. (28530)

HUMBERTO GARCIA, GABRIEL GASIC AND TATIANA BAYDAK
(Introduced by A. Schatz)
Department of Oncology, School of Medicine, University of Chile, Santiago

Blood-borne metastasis requires at least 2 separate processes: (a) release of a single cell or group of cells into the blood, *i.e.*, embolism, and (b) establishment and proliferation of emboli in a distant organ, *i.e.*, colonization(1). An embolus does not necessarily mean that metastasis will occur(2), since tumor emboli are frequently found in necropsies of cancer patients lacking visible metastases(3). Furthermore, examination of systemic blood has not shown any direct correlation between embolism and colonization(4,5,6,7). Finally, tumors metastasize preferentially to certain sites(8) that may receive relatively few emboli, like metastases in the adrenal in cases of lung cancer(9). Such considerations indicate that the frequency with which metastases develop is not

necessarily related to the number of emboli. Autopsy observations and experimental work support the belief that most tumor emboli are destroyed in the blood(10). Presumably, embolism occurs naturally, frequently, and quite regularly in cancer. Metastasis, however, depends on the defense reaction of the organism, the number of emboli formed, the frequency with which tumor cells are released, and the characteristics of these cells.

Since transplantable tumors are most frequently used for experiments on metastases (11), such tumors were employed in the present studies to determine the magnitude and frequency of embolism.

Methods and materials. Three non-metastasizing and 3 metastasizing tumors were selected from mice in this laboratory. The former tumors did not produce macroscopic metastases during the natural life span of the host. Autopsy of tumor-bearing mice included examination of lungs under the dissecting microscope, but serial histological sections were not prepared. When metastases occurred, they were macroscopically discernible in the lung surface. Host survival and tumor growth rate were about the same with all tumors, although tumor size varied somewhat at the time of natural death.

The metastasizing tumors were K7, K9, and T150. The first 2 originated in 1952 in this laboratory in strain C3H female mice, were histologically mammary carcinomas, and had been transplanted for more than 200 generations in the strain of origin. For the K7 and K9 tumors, the incidence of lung metastases was 71 and 100%, respectively. The T150 bladder carcinoma originated in C57 mice at the Wistar Institute, Philadelphia, and had been maintained in this laboratory for more than 160 generations in Lp mice. This host is genetically related to the strain of origin. The T150 tumor produces lung metastases in all mice in which it is implanted(12).

Non-metastasizing tumors included the mammary carcinomas Sa and Sb, which originated in 1952 in this laboratory in strain DBA female mice. After more than 140 generations in DBA mice, the tumor cells appear undifferentiated and spindle-shaped (13). The other non-metastasizing tumor was the fibrosarcoma GL/46 induced by methylcholanthrene in a DBA mouse at the Department of Oncology, Chicago Medical School. GL/46 does not ordinarily metastasize when implanted subcutaneously in the flank. However, metastases do occur if it is implanted in the tail of the mouse(14).

All tumors were maintained and bioassayed for viable cells in inbred C3H, Lp, and DBA strain mice originally obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. These animals were kept in plastic cages, with wood shavings, fed Cía. Molinera San Cristóbal mouse pellets, and given water *ad libitum*. Males and females were used indiscriminately, except that "donors" were 9 to 11 weeks old and "recipients" 4 to 6 weeks.

For tumor transplantation one part by weight of tumor was homogenized in 2 volumes of saline in a glass tissue grinder. Streptomycin (1.0 mg) and terramycin (1.0 mg) were added to each 0.2 ml of homogenate. The "Standard dose" of tumor inoculation was 0.2 ml of homogenate, equivalent to about 80×10^6 cells. This inoculum, administered subcutaneously in the left flank, resulted in 100% takes.

The bioassay technic to determine viable tumor cells in blood has been described by Gasic *et al.*(15). With light ether anesthesia, the thorax of the tumor-bearing mouse was opened through the sternum, and 0.6 ml of blood withdrawn from the right ventricle by a tuberculin syringe with a No. 22 needle. This blood was then immediately inoculated intraperitoneally in a "recipient" mouse. The lung method has been described by Goldie *et al.*(16). Lungs, aseptically removed without trachea and major bronchi, were finely lacerated by scissors in a sterile petri dish. One-tenth ml of the resulting brei was taken up in a tuberculin syringe with 0.1 ml of saline containing terramycin (1.0 mg) and streptomycin (1.0 mg), and then immediately inoculated intraperitoneally in a "recipient" mouse. For controls, plasma was obtained by centrifugating heparinized blood from metastasizing tumor-bearing mice, and inocu-

TABLE I. Development of Peritoneal Tumors in Mice Inoculated with Blood or Lung Homogenate from Mice with Transplantable Tumors.

Tumor	Donors with metastases, %	Mean life span, days	% of intraperitoneal tumors from Lung inocula No. of mice	%	Blood inocula No. of mice	%
K9	100	28	20	100	20	25
K7	71	30	10	100	12	50
T-150	100	21	15	100	12	66
Sa	0	35	14	79	15	7
GL/46	0	25	24	88	15	53
Sb	0	30	16	94	20	0

lated intraperitoneally in "recipients" of the same strain.

Results and discussion. "Donor" mice were implanted with tumors, and assayed after 20 days for viable tumor cells in blood and lungs. "Recipient" mice were observed until natural death, or killed when moribund. Negative groups were sacrificed after 60 days' observation. Autopsies were performed on all animals, and macroscopic lesions were studied histologically. The number of animals in each group is shown in Table I.

Peritoneal tumors were revealed by enlarged abdomens. Abundant hemorrhagic ascitic fluid was found in the peritoneal cavity, together with soft, necrotic tumor masses. Microscopically these showed accumulation of tumor cells with little supporting matrix and few blood vessels (Fig. 1 and 2). Necrosis was conspicuous.

All recipients of lung tissue from metastasizing tumor-bearing mice developed intraperitoneal tumors. Twenty-five percent of the recipients of blood from metastasizing K9-bearing mice developed tumors. The corresponding values for K7 and T150 were 50% and 66%, respectively.

With lung tissue homogenate, 79%, 88%, and 94% of the recipients developed intraperitoneal tumors when inoculated from non-metastasizing tumor-bearing mice with Sa, GL/46, and Sb, respectively. Two groups of recipients of blood from non-metastasizing tumor-bearing mice developed intraperitoneal tumors: 7% of the recipients corresponding to Sa and 53% of the recipients corresponding to GL/46. Recipients of blood from mice bearing the Sb tumor did not develop intraperitoneal tumors.

Recipients of cell-free plasma from metastasizing tumor-bearing mice did not develop tumors. On the basis of the negative results obtained with cell-free plasma and results from other studies(6,12,16), the peritoneal growth here observed was ascribed to viable tumor cells in the inocula. Cells sufficiently numerous to elicit tumors in the peritoneal

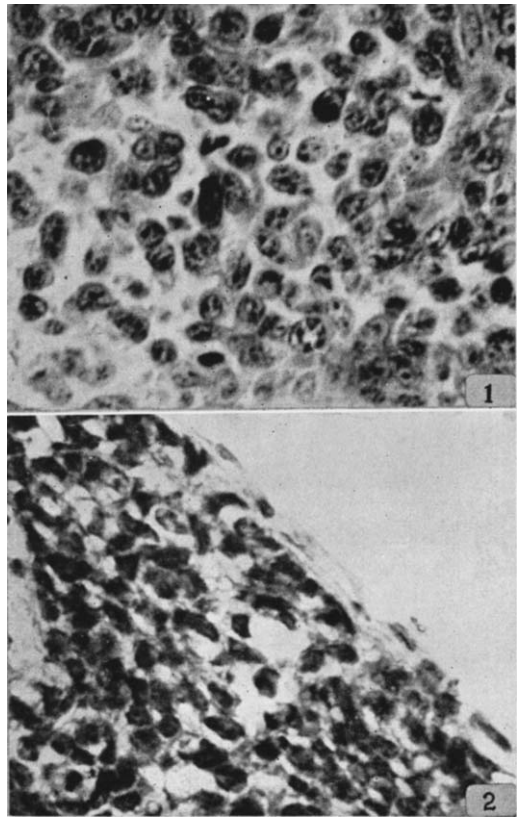

PLATE I. Peritoneal tumors in mice inoculated with blood (Fig. 1) or lung homogenate (Fig. 2) from mice with non-metastasizing transplantable tumors.

cavity of recipients were present in the lungs of host animals for all 3 non-metastasizing tumors. These results, therefore, establish the occurrence of embolism for the non-metastasizing tumors employed in these experiments.

The number of takes in the peritoneum was smaller for non-metastasizing than metastasizing tumors when lung tissue was inoculated. Since a minimal cell concentration is necessary for tumor initiation(17), this may indicate that fewer cells are released by non-metastasizing tumors. This difference could explain lack of metastasis in the former group. However, it may also be due to more active destruction of tumor cells in the lung.

Tumor cells are released into the circulating blood sporadically(7). Consequently, the capacity of a blood inoculum to elicit peritoneal growth in recipient mice will vary from time to time. Such sporadic embolism could explain the lack of correlation between the number of peritoneal takes and metastasizing potentiality, when blood was used, as well as the negative results obtained with blood from Sb tumor-bearing mice.

Summary. Experiments were conducted to study blood-borne tumor emboli for non-metastasizing, transplantable mouse tumors. Susceptible mice were inoculated with blood and lung brei from non-metastasizing, transplantable tumor-bearing animals. Intraperitoneal tumor growth resulting from viable tumor cells in the inocula was obtained from lung tissue in the 3 non-metastasizing tumors Sa, Sb, and GL/46; and from blood in the case of Sa and GL/46. The same procedure was followed with blood and lung brei from metastasizing, transplantable tumor-bearing mice. Here, viable tumor cells were demonstrated in the blood and lung tissue of hosts for all tumors used. That metastases did not appear in hosts of Sa, Sb and GL/46 is therefore not due to absence of embolism, but to other factors which are discussed.

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