Europ. J. Cancer Vol. 17, No. 6, pp. 651–658, 1981
Printed in Great Britain

0014-2964/81/060651-07 $02.00/0

A Murine Ovarian Tumor With Unique Metastasizing Capacity*

GIULIO ALESSANDRI, RAFFAELLA GIAVAZZI, PAOLO FALAUTANO, FEDERICO SPREAFICO, SILVIO GARATTINI and ALBERTO MANTOVANI†

Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157 *Milan, Italy*

Abstract—*A transplanted murine ovarian tumor* (M5076/73A, M5, *of* C57Bl/6 *origin*) *with unique metastatic potential is described. After i.m. or s.c. inoculation of the* M5 *ovarian tumor, spontaneous metastases were consistently observed in liver, spleen, kidney, ovary and uterus, but lung lesions were not usually found. Even after i.v. inoculation the tumor showed a selective tropism for the abdominal organs mentioned. When* M5 *ovarian carcinoma cells were injected i.p., the tumor grew as scattered lesions in the abdomen and resulted in carcinomatous ascites. Surgical excision of i.m. tumors as early as* 5 days *after implantation failed to protect mice against metastases, suggesting early widespread dissemination from the primary lesion. The tumor was poorly immunogenic as assessed by* in vivo *immunization with irradiated cells and by concomitant immunity experiments.*

INTRODUCTION

THE CAPACITY of tumor cells to disseminate to and grow at different anatomical sites is a crucial characteristic in malignancy, but the mechanisms involved in metastasis formation remain largely to be defined [1–4]. Appropriate models of cancer cell dissemination and metastasis are indispensable if any progress is to be made in this area. There are now a number of well characterized transplanted murine tumors spontaneously metastasizing to the lungs through the hematogenous route [5–7]. Inoculation of tumor cells at specific anatomical sites such as the tibia [8], testicle [9, 10] and pinna of the ear [2] results in lymph node metastasis, and transplanted murine carcinomas spreading to regional lymph nodes and lungs have been characterized [11–13].

In the present paper, we describe a transplanted murine ovarian tumor with a unique pattern of metastasization. After s.c. or i.m. inoculation of the tumor, secondary deposits were consistently observed at various visceral organs (liver, spleen, kidney, ovary and uterus) but usually there were no lung lesions.

MATERIALS AND METHODS

Mice

Female C57Bl/6 mice (6–8 weeks old) were obtained from Charles River, Calco, Italy.

Tumor

The M5076/73A (M5) ovarian tumor (Fig. 1) was obtained through the courtesy of Dr. A. Bogden (Mason Res. Inst., Worcester, MA). The tumor, histologically an anaplastic carcinoma, arose spontaneously in one C57Bl/6 mouse in Dr. W. F. Dunning's laboratory, Papanicolao Cancer Research Inst., Miami, FL. The tumor was maintained by s.c. passage with trocars every 3–4 weeks and was used at its 2nd–5th passage in this laboratory.

Growth

Tumors obtained 2–3 weeks after implantation were minced with scissors and disaggregated by exposure to trypsin (0.3%) in basal medium Eagle (BME) for 45 min at 37°C. The cells were washed twice with 50 ml BME and finally resuspended in BME. Graded numbers of tumor cells (10^3–10^6 in

Accepted 5 January 1981.
*Supported by Grant R01-CA-12764 from the National Cancer Institute, U.S.A., and by Contract 78.02.792.96 from Consiglio Nazionale delle Ricerche, Rome, Italy.
†To whom correspondence should be addressed.

0.05 ml) were injected i.m. in the right hind thigh. The mice were palpated every other day for tumor appearance and as soon as this was detectable, tumor diameters were measured with calipers. Diameters were converted to approximate tumor mass by the formula:

$$\frac{\text{length} \times (\text{width})^2}{2}$$

[14].

Metastases

The mice were examined at death to evaluate spontaneous metastases, after i.m. and s.c. inoculation of 10^5 cells, respectively. The number and weight of secondaries was measured as previously described [15]. To assess artificial colony formation, graded tumor cell numbers (10^2–10^5) were injected i.v. in 0.5 ml BME. Organs were inspected for metastases at death or 20 days after tumor cell inoculation. Formalin-fixed organs were examined histologically after staining with hematoxylin–eosin.

Surgery

The growing tumors were surgically removed by the amputating the tumor-bearing limbs. Controls were sham operated.

Immunogenicity

In an attempt to immunize mice against the M5 tumor, mice were injected with 10^7–2×10^7 irradiated (10,000 rads) tumor cells and challenged with live carcinoma cells (3–10^5) one week later. Immunogenicity was also assessed in concomitant immunity experiments. Mice were inoculated with 10^5 cells s.c. and, 15 days later, they were injected with 5×10^4 cells contralaterally. Control mice were normal non-tumor bearing animals. The incidence of tumor takes and the weight of the lesions were determined at different times after the second challenge.

RESULTS

When mice were injected with graded numbers of M5 ovarian tumor cells (10^3–10^6) all inoculated animals showed a palpable tumor, latency being related to the inoculum size (Table 1, Fig. 2). Survival time was also related to the inoculum size, ranging from 51 to 33 days for 10^3 and 10^6 cells, respectively. Examination of mice at autopsy revealed visceral metastases in all mice (Table 1). Metastases were most often observed in the liver, in terms of both incidence of animals with secondaries at this anatomical site and number of lesions per organ, but secondaries were also consistently observed in spleen, ovary, uterus and kidney. Lungs, on the other hand, were usually unaffected by the M5 ovarian tumor (Table 1).

The results presented in Table 1 were observed after i.m. inoculation of M5 ovarian tumor cells, but the pattern of metastasization was similar after s.c. injection of tumor cell suspensions (Table 2) or tumor fragments (unpublished data). Even after i.v. inoculation, the M5 ovarian tumor appeared to express a selective tropism for abdominal visceral organs; no lung lesions were found after injection of 10^3 cells. After larger i.v. cell inocula (10^5), a diffuse tumor cell infiltrate was observed in lungs at death, but this did not usually result in discrete gross metastases. These diffuse infiltrates were observed late (beyond day 12–15) after i.v. inoculation of 10^5 cells. Interestingly enough, after i.p. inoculation, the M5 ovarian tumor grew as scattered diffuse lesions in the abdomen, and resulted in carcinomatous ascites (Table 2).

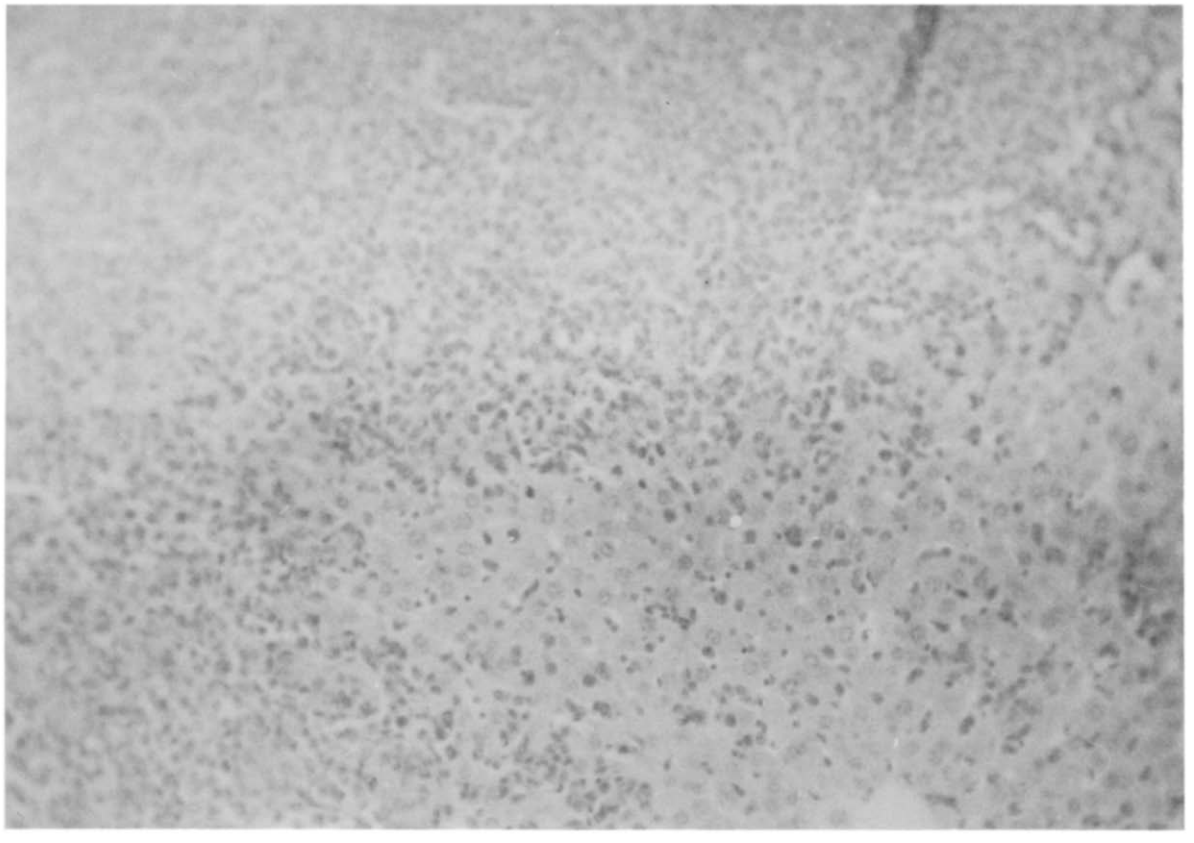
In an effort to assess whether metastasization in the M5 tumor model was an early event, the growing tumor was surgically excised at different times after transplantation (Table 3). Surgery as early as 5 days after tumor inoculation failed to cure all mice, death occurring as a consequence of disseminated disease. Survival time was significantly prolonged only with surgical excision on day 5; this therapeutic approach had no effect if carried out later.

To further characterize the M5 ovarian tumor model, attempts were made in a series of experiments to immunize hosts against the tumor. As shown in Table 4, immunization with irradiated M5 cells did not protect mice against subsequent challenge with viable tumor cells. A similar lack of detectable immunogenicity was observed in concomitant immunity experiments (Table 5).

DISCUSSION

Among transplanted murine tumors, the M5 ovarian neoplasm appears to have a unique metastatic potential. After i.m. or s.c. inoculation of the tumor, spontaneous metastases were observed in liver, spleen, ovary, uterus and kidney, lung lesions being extremely rare. Surprisingly, the same pattern of metastasis occurred after i.v. injection of low numbers (10^3) of M5 cells. I.v. inoculation of larger numbers of tumor cells resulted in a diffuse microscopic tumor infiltrate in the lungs, but not in gross, discrete lung colonies, further pointing to this tumor's scarce ability to form colonies in this organ. Spontaneous

(a)



(b)

Fig. 1.