Studies on Transplantation and Behavior of Hamster Melanoma*

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SUMMARY

The behavior of hamster melanoma after homologous transplantation to different sites has been studied. The relationship between transplanted tumor size, gross metastases, and circulating tumor cells was recorded. The variability of the heterotransplants, depending on the site of transfer and recipient animal, was noted. The following observations were made:

The hamster melanoma was readily transplantable to all attempted sites in the hamster. There was some tendency toward sarcomatous transformation upon homologous transplantation of the hamster melanoma. In hamsters bearing malignant melanoma transplants, circulating tumor cells in the blood stream could be detected only after gross metastases had appeared.

Heterologous transplantation was accomplished in the subcutaneous space of Swiss mice, peritoneal cavity of the guinea pig fetus, and the anterior chamber of guinea pigs' eyes.

The first report of a methylicholanthrene-induced transplantable melanotic tumor in a Syrian golden hamster was published by Shubik and associates (14). Spontaneous hamster melanomas have been reported by Fortner and Allen (1) and Greene (5). Shubik found that no metastases occurred even when the tumors attained a size of 4 cm. in diameter. Fortner and Allen, and Greene reported diffuse metastases occurring after homologous transplantation of the spontaneous hamster melanoma.

The present report deals with the behavior of hamster melanoma upon homologous transfer to various sites in the hamster and upon heterologous transfer to other animals.

MATERIALS AND METHODS

The hamster melanoma used in these investigations arose spontaneously in the flank of a 2½-year-old male Syrian golden hamster and was supplied through the courtesy of Dr. Harry S. N. Greene of the Department of Pathology, Yale University.

The animals used for the homologous transfers were Syrian golden hamsters. No conditioning measures were employed. With rare exceptions, the tumor implants grew in all the animals irrespective of site of transplantation. For the heterologous transplantation studies the following animals and sites were used: Swiss mice—brain, subcutaneous space, and peritoneal cavity; stock guinea pigs—anterior chamber of the eye, brain, peritoneal cavity of guinea pig's fetus; stock rabbits—anterior chamber of the eye.

Some of the animals were conditioned to reduce their resistance to the hamster melanoma transplant. Mice were given 2 mg. of cortisone acetate subcutaneously at the time of implantation of tumors and on alternate days for four injections. Guinea pigs were treated similarly, except that each dose of cortisone acetate was increased to 4 mg. Splenectomy was performed in some of the host guinea pigs. The melanoma was incubated in recipient guinea pig serum for 30 minutes at body temperature and prior to anterior chamber transplantation in some of the guinea pigs. Various combinations of splenectomy, incubation in recipient guinea pig serum, and cortisone were tried before attempting heterologous anterior chamber trans-
plants of melanoma to guinea pigs. Suspensions of melanoma cells were also injected into the peritoneal cavity of fetuses of guinea pigs after exposing the gravid uterus through an abdominal incision.

The technics of transplantation were similar to those described by Greene (2) and Lutz et al. (9). The tumors were implanted by trocar, with fragments of tumor measuring 1–2 mm. in diameter.

RESULTS

Homologous subcutaneous transplants to the flank, inner thigh, cheek pouch, and intraperitoneally were performed to determine the frequency and site of resultant metastases (Table 1). In these series, post-mortem studies revealed that, after intraperitoneal transplantation, there were extensive serosal and visceral growths in all seven animals, with metastases to both axillae in one animal. The results of flank transplants in 27 animals were as follows: seventeen animals with axillary node metastases, eleven animals with mediastinal node metastases, two animals with inguinal node metastases, eighteen animals with pulmonary metastases, and two animals with involvement of kidney, diaphragm, and heart. Flank transplants produced axillary node metastasis in 2 months with pulmonary metastases developing in the 3d month. After subcutaneous inner thigh transplants, the hamster melanoma metastasized to the regional nodes in three animals and to the axillary and mediastinal nodes and the lung in one animal. Cheek pouch transplants were accompanied by mediastinal node involvement in one animal and regional cervical node involvement in two animals.

The transplanted tumor is usually pigmented and varies in appearance from coal black to grayish brown. The pigment content tended to be increased in the metastases, especially in the lungs and mediastinal nodes. There was no discernible correlation between the pigment content of the tumor and the degree of malignancy, as takes and deaths from metastases were the usual outcome after transplantation. All the animals died within 4–5 months of diffuse metastases. Histologically, the appearance of the hamster melanoma as obtained showed melanoma cells arranged in alveolar groupings with abundance of pigment (Fig. 1). Metastases were usually arranged in solid masses of cells without the usual whorled pattern. Some of the melanotic tumor transplants showed sarcomatous changes (Fig. 2).

Heterologous transplants of the hamster melanoma to the anterior chamber of the guinea pigs’ eyes were attempted following treatment by either administration of cortisone or splenectomy of the host, incubation of tumor in recipient guinea pig serum, or combinations of the above methods. Anterior chamber transplants of the hamster melanoma were carried out in 88 guinea pigs, and satisfactory tumor growth occurred in only two, one in the control and the other in a splenectomized guinea pig. Subsequent passage to hamsters and guinea pigs resulted in failure when the growth in the control guinea pig was used. One hundred percent transplantability in the hamsters but no takes in the guinea pigs occurred when the growth from the splenectomized guinea pig was used. Microscopic sections revealed the transplanted melanoma in the anterior chamber of the guinea pig to be composed of cells arranged in sheets and whorls, with some melanin pigment and scanty stroma similar to the parent hamster melanoma (Fig. 3). The subsequent passage of the anterior chamber growth from the splenectomized guinea pig grew
progressively in the recipient hamsters; all died of diffuse metastases.

Heterologous transplantation of the melanoma to brains of Swiss mice and guinea pigs resulted in no takes. The melanoma did not survive transplantation to the anterior chamber of the rabbits' eyes nor in the peritoneal cavity of the Swiss mice. Intraperitoneal transplants of the hamster melanoma in the guinea pigs resulted in small pigmented nodular implants on serosal surface of the stomach, omentum, small intestine, and its mesentery. Microscopic sections of the nodules revealed no active growth or proliferation but large areas of necrosis and melanin-filled macrophages. Subcutaneous transfer of melanoma in Swiss mice resulted in four takes in the controls and two takes in the cortisone-treated animals. Subsequent transplants failed when hamsters and Swiss mice were used as host animals. Microscopically, there were viable melanoma cells with giant-cell reaction and a sarcomatous appearance (Fig. 4). Suspensions of the melanoma cells were injected intraperitoneally into thirteen fetuses of six pregnant guinea pigs after exposure of the gravid uterus through an abdominal incision. Three animals aborted in the immediate postoperative period. Out of the four living offspring, one animal had a 1.5 X 1 cm. growth in the mesentery of the small intestine and died 59 days after injection of the melanoma cells (Fig. 5). Histological appearance of this growth showed anaplastic cells without any specific pattern (Fig. 6).

**DISCUSSION**

Homologous transplantation of the hamster melanoma showed that this neoplasm spreads at first by direct permeation into the surrounding tissues, then by lymphatics to regional lymph nodes, and later by blood-borne metastases. This mode of spread is similar to that in the human malignant melanoma. When blood-borne metastases occur they are usually widespread, invariably involving the lungs and occasionally liver, kidney, and other viscera.

With the technic described by Sandberg and Moore (13) of concentrating circulating tumor cells, an attempt was made to correlate the number of melanoma cells in the peripheral circulation with the age, size of tumor, and number of metastases (Chart 1). Papanicolaou stains of tumor imprints of the hamster melanoma were made and used for comparison in identifying circulating tumor cells (17). Circulating tumor cells were found in most of the hamsters after 34 days of tumor growth. When the melanoma attained a size of 4 cm. or greater, melanoma cells were found in the peripheral circulation. Out of the eight hamsters found to have positive blood smears, seven had gross metastases. The number of circulating tumor cells was directly proportional to the size of the tumor and the frequency of metastases.

The capacity of this hamster melanoma to undergo sarcomatous transformation is unique, and our findings generally follow those described by Greene (5).

The fact that normal animals possess a defense mechanism against heterologously and, in some instances, homologously transplanted tissues is well known, although the precise mechanism is obscure. The importance of lymphocyte response in the immune mechanism of host resistance has been known since the work of Murphy (10–12) and Loeb (8). Murphy concluded that lymphocytes were concerned with maintaining the defenses of host against foreign tissue which he lowered by depleting lymphocytes with irradiation. Further studies elaborating this work have been reported by other workers (7, 15, 16). Greene has emphasized the correlation between clinically demonstrable metastases and the heterotransplantability of cancers (4) and reported that metastasizing or metastatic cancers transplant successfully (3).

In the heterologous transplantation of the hamster melanoma into the guinea pigs' eye only the
growth in the splenectomized guinea pig survived subsequent transfer to hamsters. Splenectomy may have an adverse effect on the reticuloendothelial system and antibody production. The transplants in the subcutaneous space of Swiss mice resulted in no takes when transferred to hamsters and Swiss mice. The fact that hamster melanoma can grow for one generation in the splenectomized guinea pig and, upon retransplantation into the hamster, continue to metastasize and kill the animal supports the concept that the intrinsic biological qualities of the tumor have not been altered by the heterologous anterior chamber transplantation. Greene (5) reported that only the growths of the hamster melanoma in the subcutaneous space of the mice could be successfully transferred to the anterior chamber of the guinea pig. The capacity of the hamster melanoma to produce melanin pigment was not altered by the heterologous anterior chamber transplant in the guinea pig. This suggests that the cells continue to maintain their host relationship and are able to utilize the hormonal and enzymatic system of the host in producing the melanin pigment. The observation tends to confirm Greene’s contention that “anterior chamber transplants are part of the new host, subject to the stimuli and inhibition that regulate and integrate the organism” (6).

The variability of the heterotransplants depending on the site of transfer and the recipient animal was also an interesting one. The anterior chamber transplant in guinea pig maintained essentially the same appearance of the parent hamster melanoma. The subcutaneous transplant in Swiss mice had a sarcomatous picture, and the intraperitoneal growth in guinea pig (transfer done in utero) had an anaplastic appearance.

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