H.T. 37, a Transplantable Mouse Tumor Originating after Heterologous Tumor Transplantation*

CLAIRE KLAUSNER† AND VICTOR RICHARDS‡

(Department of Surgery, Stanford University School of Medicine, San Francisco 15, California)

SUMMARY

H.T. 37 is a new tumor in Swiss Webster mice. It is a squamous-cell carcinoma growing subcutaneously and intraperitoneally. This neoplasm originated after implantation of the tumor from a human epidermoid carcinoma of the lung into pregnant Swiss Webster mice. Intra-embryonic injections of this human lung carcinoma into the fetuses were unsuccessful, but the tumor “took” in two pregnant females and grew progressively in both. This tumor has proved serially transplantable in subsequent generations of mice. Initially the percentage of “takes” was less than 15 per cent, but now in the 44th generation of transplants the tumor “takes” are over 90 per cent. Chromosomal studies of the initial tumor and transplants were not possible, but the histology of the tumor in subsequent transplants has remained relatively unchanged. Chromosomal studies, by Doctors Biesele and Hauschka, of the 14th and 24th generation transplants have disclosed the cells to be mouse rather than human. Either a human tumor has become adapted to a mouse host by changing its genetic structure, or a new tumor has been induced in the mouse by an unknown agent contained in the original human explant.

In April, 1958, studies on a human lung tumor growing in untreated Swiss Webster mice were reported (3). The purpose of this paper is to describe our present findings regarding H.T. 37 and to complete previous publications (4, 11) which might be misleading without these final observations. Microscopic findings alone suggested this to be a human tumor, but present chromosome studies established it as a murine tumor. H.T. 37 is a new tumor growing in the 44th generation of Swiss Webster mice and has been transplanted under strictly aseptic conditions. This tumor might be of interest to other investigators, since Klein suggests in his most recent review on tumor transplantation (6) that the recently derived spontaneous or induced tumors seem preferable to long established tumors in transplantation studies.

Cancerous tissue, despite its inherent growth potential, is normally rejected by the new host on homologous or heterologous transplantation. Toolan (12) in 1953 produced tolerance for human cancer transplanted into mice by suppressing the immune rejection response of the host. She employed total-body irradiation and continued cortisone treatment in laboratory animals and demonstrated the possibility of obtaining permanently transplantable human tumors (13). Medawar (9) in 1953 introduced into the field of homologous tissue transplantation the important concept of acquired tolerance. He demonstrated that an embryo is unable to respond to injected antigens by an immune reaction and that failure of reaction against this same antigen continues over into later life. Tolerance for a tissue homograft results when an animal has been confronted in fetal life with cells taken from its future donor. This specific inhibition of response by acquainting or challenging the fetus with an antigen to which it will later be exposed again and to which it will remain tolerant in later life has been called “actively acquired tolerance.”

In 1956 the authors began to employ the concept of “acquired tolerance” in an effort to induce
human cancer to grow heterologously in Swiss Webster mice. Human cancer growth could not be obtained by intra-embryonic injection of human cancer cells, but the attempt to establish a new epidermoid cancer in normal, untreated Swiss Webster mice was successful. This tumor, called H.T. 37, is a transplantable mouse epidermoid carcinoma, growing subcutaneously and peritoneally on implantation. It appears to have been induced by an initial heterologous human transplantation.

**MATERIALS AND METHODS**

Swiss Webster mice, originally obtained from the Rockefeller Foundation through the California State Public Health Department, were used for these experiments. This strain of mice has been used extensively in Stanford Hospital for chemotherapy studies. The incidence of spontaneous tumors has been less than 1—2 per cent, and occurred in animals over 12 months of age. Epidermoid tumors have not been reported.

The experimental animals were all 6—8 weeks of age. No pretreatment or conditioning was employed. The pregnant animals were in the 15th to 19th day of gestation.

The sources of tumors for implantations were fresh, aseptic surgical specimens from the Stanford University operating rooms. As soon as the neoplasm was removed from the patient a portion was immediately minced with fine scissors into fragments small enough to be implanted into the fetuses of pregnant mice. The anesthetized mouse was subjected to laparotomy, the fetuses were exposed, and the minced viable tumor fragments were injected into the exposed fetuses. Concurrently, fragments of tumor tissue were implanted subcutaneously in the axilla or groin of the pregnant mother. The tumor fragments were introduced through the open laparotomy incision by tumor forceps. The laparotomy incision was closed, and the mouse was allowed to recover, awaiting the birth of the litter. In each instance some of the initial tumor specimen was quick-frozen, stored in a deep freeze at $-75^\circ$ C., and used for later implantation into the surviving members of the litter.

In the transplantations into subsequent generations of untreated animals both pregnant and nonpregnant animals were employed. Fresh tumor was obtained from the previous generation host and was implanted subcutaneously by syringe. Photographs and histological studies were made in the usual fashion for each successful implant.

**RESULTS AND DISCUSSION**

The nature of the 80 different human tumors employed, the failure of intra-embryonic injection to bestow tolerance, the effect of repeated pregnancies on tumor “takes,” and the failure of all tumors except H.T. 37 to remain serially transplantable have been previously reported (4, 11). The present report deals with the origin and further studies on H.T. 37.

H.T. 37 appeared as a tumor “take” in the axillary implant of two pregnant mice and grew progressively in both. The implants were derived from an epidermoid carcinoma of the bronchus of a male patient, 55 years old. The implanted tumor has proved serially transplantable into all subsequent generations of Swiss Webster mice. It behaves in a cancerous fashion, grows continuously in size, independent of host nutrition, eventually ulcerates or kills the animal, and occasionally produces systemic metastases.

Table 1 demonstrates the general transplantation characteristics of the tumor. Initially the percentage of tumor “takes” on transplantation was low, and regression in tumor growths would occur. However, after the 10th generation the percentage of tumor “takes” reached 85 per cent, and tumor regressions no longer occurred. The latent period prior to a palpable tumor has been shortened from 3—4 weeks to 5 days. In the 44th generation the tumor is growing vigorously and seems firmly established as a new tumor in this strain of Swiss Webster mice.

The tumor has maintained quite closely the histological appearance of the initial human transplant. Photomicrographs of the original squamous-cell carcinoma of the bronchus from the patient (Figs. 1 and 2) demonstrate the sharply outlined nuclei, prominent clumps of chromatin, and abundant eosinophilic cytoplasm. With fairly well defined margins. Representative photomicrographs of the 9th generation transplant show a similar cytological picture (Figs. 3—8).

In attempts to obtain growth of the tumor in the ascitic form, peritoneal implants thus far have grown in the solid state and have killed the animals without producing ascitic fluid.

It was our initial hope that tumor H.T. 37, being of human origin, would retain certain human properties. However, when Korngold tested the tumor antigens from the 14th passage with four antihuman tumor sera, he obtained negative results with each. Double-blind independent chromosome studies on the 14th generation by Biesele and on the 24th passage by Hauschka, revealed exclusively murine cytology.
Biesel stated: "The chromosomes appeared to be all telomitic, with no biarmed chromosomes as in the normal human complement. The chromosome number lay in the low 50's. The chromosome number of about 52 rather than the normal diploid 40 of the mouse suggested that tumor cells were being examined; the one-armed nature of the chromosomes suggested that the cells were of murine derivation rather than human."

The following quotation is from Hauschka's report: "Twenty gammas of colchicine was injected directly into the tissue prior to fixation of a cell suspension containing many intact metaphases. One hundred well-spread metaphases were surveyed. All of these had typical mouse chromosome sets with telocentric spindle fiber attachments. Ninety-eight per cent of the cells were hyperdiploid; 2 per cent were approximately tetraploid, but also mouse. Seven plates could be counted exactly as follows: 49, 50, 50, 51, 51, 51, 54 chromosomes. In 2 of these plates typical mouse X chromosomes were identifiable. Although this is an epithelial tumor, the cells are quite small and the chromosome plates are crowded. Nevertheless, I did not find a single cell which could even be suspected of being of human origin."

We feel that H.T. 37, now an epidermoid mouse tumor, originated from an initially successful heterotransplant or (b) was induced by an agent contained in the original human explant. In all reasonable probability we are not dealing with a spontaneous mouse tumor. The incidence of spontaneous tumor in this strain of mice is less than 1–2 per cent over a period of a year. Spontaneous tumors have never occurred in animals of this age group (6–8 weeks).

The spontaneous tumors in mice that have been carefully studied by Woglom (14) are adenoma, cystadenoma, adenoma malignum, papillary cystadenocarcinoma, adenocarcinoma, and cystadenocarcinoma. All spontaneous tumors have had a basic acinar structure, but our H.T. 37 has maintained the epidermoid character of the original human tumor. The tumor also grew initially in both of the pregnant animals in which it had been implanted. Mammary cancers have been induced in mice by Lacassagne (7) under the influence of estrogen and progesterone injections, but these tumors have been in the breast and have been entirely different from H.T. 37.

Pregnancy may have played a role in the initial response to human tumor transplant. Heslop, Krohn, and Sparrow (1) showed that rabbit skin homografts transplanted to recipients that are between days 20 and 24 of pregnancy survive about twice as long as do homografts transplanted into normal males or females, or, indeed, into animals very early or very late in pregnancy. However, in mice Medawar and Sparrow (10) were unable to confirm this effect of pregnancy on homografts. It is postulated that increased corticoid levels during some stages of pregnancy may permit this interaction.
creased survival time of homografts. We have studied the effect of repeated pregnancies on human tumor heterotransplants (6) and have found that pregnant animals initially accept heterotransplants far more frequently than do control animals but that the tumors have not been serially transplantable. The histological appearance of these transplants did not at all resemble those of the original human tumors, and they showed almost uniformly a glandular structure. In many instances they had a lymphoid character.

Iversen (2) has recently reported on the intraperitoneal implantation of fifteen different human exudates containing tumor cells into mice from a subline of the Bagg strain. His animals were cortisone-treated. In five of the experiments the inoculated tumor cells proliferated in treated animals, and in three of the experiments ascites tumors could be serially transplanted. A real adaptation of the human cells was successful in only one experiment in which the ascites tumor, after two passages in hormone-treated mice, could be grown in untreated animals. Chromosome studies showed that the adapted ascites tumor contained cells with definite murine characteristics, whereas the cells before adaptation had human chromosome types, as had the cells in the other two nonadapted ascites tumors. Levan (8) also has reported on chromosome studies of some human tumors grown in vivo and in vitro. An original human tumor was taken from a treated rat host for tissue culture. Therefore, traces of rat stroma were probably present. After 6 months in vitro, samples of the cells were implanted into the chick chorioallantoic membrane. A tumor formed that was histologically identical in type with the original tumor, and human cells prevailed. Later, however, conditions changed so that the rat stroma cells, always present in tissue culture, overgrew the human cells. When chromosome analyses were made, all mitoses were of the rat type and, furthermore, were characteristic of malignant rather than normal tissue. Implantation of these cells into cortisone-treated irradiated rats and normal rats resulted in a rat fibrosarcoma, quite different in type from the original human tumor. These observations show that, when attempts to grow a human tumor in mice or rats are apparently successful, what really has happened in every case is the substitution of a mouse or rat tumor for the tumor of human origin. However, the adaptation of the mouse to an oncogenic agent is a distinct possibility.

An even more interesting explanation of the present tumor is that it represents a squamous-cell carcinoma induced by an agent contained in the original human explant. Grace, Mirand, and DiPaolo have recently induced multiple tumors in over 100 mice with cell-free material from various human malignant tissues, including a melanoma. 1

The initially low percentage of "takes" in the first few serial passages, followed by the high percentage of "takes" in the generations beyond the tenth, suggests that adaptation to the tumor agent still had to occur in the mouse. Either the tumor-inducing agent or the host underwent an adaptation.

REFERENCES

1 T. S. Hauschka, Surgical Forum (in press).
FIG. 1.—Squamous carcinoma of bronchus: original human tumor. X150.

FIG. 2.—Same as Figure 1. X620. Sharply outlined nuclei, prominent clumps of chromatin, and abundant eosinophilic cytoplasm with fairly well defined margins.

FIG. 3.—Transplant 3. X1000. Mitotic figure, left upper. Cytological picture not significantly altered by transplantation.

FIG. 4.—Transplant 5. X1000.

FIG. 5.—Transplant 9. X180. Histological pattern, after nine transplantations, retains its epidermoid characteristics.

FIG. 6.—Transplant 9. X900. Cytological picture not significantly altered by 9 transplantations.

FIG. 7.—Metastatic tumor in lung. X130. Infiltrative growth in the center.

FIG. 8.—Same as Figure 7 X1100.
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