Transplantability and Sex Steroid Hormone Responsiveness of Cervicovaginal Tumors Derived from Female BALB/cCrgl Mice Neonatally Treated with Ovarian Steroids

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ABSTRACT

Twenty-eight cervicovaginal tracts from approximately 2-year-old female BALB/cCrgl mice neonatally exposed to ovarian steroids were cut into small segments and transplanted into syngeneic hosts. Within six months, six of 28 host animals developed tumors. Three tumors were from progesterone-exposed mice, two were from estrogen-exposed mice, and one was from estrogen-progesterone-exposed mice. These tumors have been maintained by serial transplantation for approximately two years. The progesterone-induced tumors are mixed tumors with both squamous cell and glandular components. The estrogen-induced tumors are squamous cell carcinomas. The estrogen-progesterone-induced tumor was originally a squamous cell carcinoma, which now resembles a basal cell carcinoma. The other tumors have maintained their original morphological characteristics. All tumors have proven to be hormone independent. No control cervicovaginal tracts developed tumors after transplantation, even after 24 months in the host animals.

INTRODUCTION

In adult female mice given estrogen or androgen injections neonatally, the vaginal epithelium frequently develops lesions by 6 months of age or later (2, 9). Some of these lesions have histological features which resemble squamous cell carcinomas. Takasugi (8) transplanted the vaginas of mice treated neonatally with estrogen into syngeneic hosts. Two of 18 such transplants transformed into what Takasugi termed basal cell carcinomas. These tumors appeared anaplastic and bore little resemblance to the tissue of origin. However, when Dunn and Green (3) transplanted cervicovaginal tissue of mice neonatally treated with diethylstilbestrol into syngeneic hosts, tumors arose which did resemble epidermoid cancer.

Progesterone (100 \( \mu g \) daily for 5 days), when administered neonatally, induces hyperplastic lesions in both the vagina and cervix of adult mice (4, 5). These lesions have been shown to provide the bed for the development of carcinoma-like structures exhibiting both squamous cell and glandular features. The present study was aimed at examining the behavior of segments of the genital tract (vagina and cervix) of mice neonatally exposed to progesterone alone after transplantation into syngeneic hosts. In addition, segments of the genital tract of adult mice neonatally treated with estrogen, alone or in combination with progesterone, along with tissues from control mice, were examined after transplantation into syngeneic hosts.

MATERIALS AND METHODS

Female mice of the BALB/cCrgl strain were used. A total of 28 animals as transplant donors was randomly selected from larger groups of mice (Table 1) which had been treated neonatally with 5 or 20 \( \mu g \) 17\( \beta \)-estradiol, 100 \( \mu g \) progesterone, 5 \( \mu g \) 17\( \beta \)-estradiol plus 100 \( \mu g \) progesterone, or 20 \( \mu g \) 17\( \beta \)-estradiol plus 100 \( \mu g \) progesterone in 0.02 ml sesame oil (the vehicle) for 5 days beginning within 36 hr after birth (4). Control animals used as transplant donors were treated with the vehicle or received no treatment.

During the autopsy, the genital tracts (vagina and cervix of those mice selected) were removed and then cut sagittally into 2 halves; one-half of the genital tract was used for histological examination, and the other was used for transplantation. The half of the genital tract to be transplanted was then placed in Medium 199 containing 100 units of penicillin, 50 \( \mu g \) of streptomycin, and 5 \( \mu g \) of insulin per ml of medium for approximately 30 min to reduce the possibility of infection upon transplantation. The genital tract was then cut laterally into 4 segments (lower vagina, upper vagina, lower cervix, and upper cervix) either intact or trimmed to 2 to 3 cm. These were transplanted under the abdominal skin near the first and second pairs of mammary gland fat pads and near the fourth pair of mammary gland fat pads of intact female mice. Portions of genital tracts from control mice of approximately the same age were transplanted using the same procedure. When tumors arose in the host animals, they were cut into approximately 1-cm segments, placed in Medium 199 with antibiotics and insulin, and then transplanted into syngeneic hosts.

Hormonal responsiveness of the established tumor lines was tested by transplanting pieces of tumor into both intact and castrated male and female mice. The castrated female mice were separated into 3 groups, namely, those treated with estradiol and/or progesterone, those treated with sesame oil, and those untreated. The hormone-treated groups were given s.c. injections of either 7.5 \( \mu g \) 17\( \beta \)-estradiol or 150 \( \mu g \) progesterone or a combination of both in 0.02 ml of sesame oil (the vehicle) daily for a maximum period of 1 month. Tumor size was measured weekly with calipers by recording the major diameter of the tumor and then recording a second diameter perpendicular to the first. Hormone responsiveness was determined by comparing the average growth of the tumor after 1...
month in each castrated group with that in the untreated intact group. The nonpalpable growth pattern of the estradiol plus progesterone-induced tumor prevented measurement of its diameters to determine growth rate. Therefore, hormone responsiveness for the estradiol plus progesterone-induced tumor was determined by comparing the period of time between transplantation and death in the host animals of each group.

All tumor lines were tested for hormone responsiveness in their fourth transplant generation, with the exception of the estradiol plus progesterone-induced tumor, which was tested in its 14th transplant generation.

RESULTS

Upon histological examination of the remaining one-half of the genital tract of those mice neonatally exposed to steroids, 17 of 28 mice were found to have cervicovaginal lesions, one of which was a frank tumor (Fig. 5). In 6 of 28 host animals bearing genital tract segments from an equal number of neonatally steroid-treated animals, tumors developed. Table 1 summarizes the data on the tumors studied. All tumors were readily transplantable as solid fragments from host to a new recipient with a "take-rate" of 100%. No tumors developed in 20 host mice with transplanted genital tract segments from sesame oil-treated or untreated donors.

Tumor TJ-6271 (Fig. 1) was detected 2 months after transplantation of genital tract segments from a neonatally estrogen-treated mouse into syngeneic hosts. Histologically, it proved to be a mixed tumor with both squamous cell and glandular components. The original histology of the cervicovaginal lesion (determined from sections from the untransplanted half) was glandular in appearance. Tumors HAB-6292 and BV-6268 were detected 6 months after transplantation and were derived from genital tract segments from 2 other mice treated neonatally with progesterone. They were also mixed tumors. These cervicovaginal lesions initially were largely squamous cell types with some glandular areas.

TJ-6271 has been maintained as s.c. transplants for 2.4 years (14 transplant generations), whereas HAB-6292 and BV-6268 have been maintained for 2.0 years (8 transplant generations). All 3 tumors grow slowly with one-half of the tumor being filled with stagnant blood and necrotic tissue. Metastases have been found in the intestinal region. No changes have been noted in histological appearance or rate of growth of the tumors.

Two tumors, AK-6293 and LW-6214 (Figs. 2 and 3), were detected 5.5 months after transplantation of genital tract segments from 2 neonatally estrogenized mice into syngeneic hosts. Histologically, these tumors have only a squamous cell component which is similar in histology to the cervicovaginal lesions noted in the tissue of origin. Both tumors have maintained their histological pattern for 11 transplant generations. These tumors grow more slowly than did the progesterone-induced tumor lines, but they also metastasize to the intestinal region.

Tumor TJ-6196 (Fig. 4) was detected 2 months after transplantation of genital tract segments from a mouse neonatally treated with estradiol plus progesterone. The genital tract of this animal had a frank squamous cell carcinoma (Fig. 5). After 3 transplant generations, the character of the tumor changed from squamous cell pattern to one resembling a small cell carcinoma (Fig. 6). It has maintained this anaplastic histological pattern for 29 transplant generations. The tumor is highly invasive, metastasizing to both the liver and the intestinal region within 1 week.

The average diameter of the estrogen- and progesterone-induced tumors was approximately the same at the end of 1 month in both intact and castrated female and male hosts (Table 2). The average period of time before death occurred in estrogen plus progesterone-induced host groups was approximately 16.2 ± 0.8 (S.D.) days. The addition of estradiol or progesterone, alone or in combination, had no effect on the growth of tumors in castrated female hosts.

All tumor lines grew equally well in intact and castrated female and male hosts. The addition of estradiol plus progesterone, alone or in combination, had no effect on the ability of the tumors to grow in castrated female hosts.

DISCUSSION

It has been well established that neonatal steroid treatment can induce both persistent vaginal cornification and hyperplastic lesions of the cervicovaginal region (2, 9). However, the cervicovaginal lesions which have developed have been described as hyperplastic lesions and are rarely referred to as "preneoplastic" or "neoplastic," although a neoplastic significance was inferred. The first published reports on the ability of the genital tract of neonatally estrogen-treated mice to form tumors upon transplantation were those of Dunn and Green (3) and Takasugi (8). However, the tumors which developed in the experiment of Dunn and Green were not examined for histolog-
ical stability, hormone responsiveness, or serial transplantability. Those tumors which developed in experiment of Takasugi were studied for histological stability for 4 transplant generations, with the initial tumor (first transplant generation) bearing little resemblance to the tissue of origin. The results of the present investigation demonstrate that the hyperplastic lesions in the genital tract of neonatally steroid-treated mice may be either preneoplastic or neoplastic and that most of the tumors which do occur are histologically stable.

The preneoplastic nature of the cervicovaginal tract of neonatally estrogen-treated mice resembles that of the hyperplastic alveolar nodule in the genesis of mammary tumors of mammary tumor virus-infected mice (7). With regard to the mouse mammary tumor system, mammary tumor virus exerts its tumorigenic effect by initiation of the neoplastic transformation within the nodule cell population. A comparable situation might occur in the neonatal mouse genital tract. Neonatal steroid treatment of mice leads to persistently cornified and hyperplastic lesions produced tumors after transplantation, the histology of the lesions observed in neonatally estrogen-treated mice is predominantly squamous cell, whereas the cervicovaginal lesions in mice neonatally treated with progesterone have 2 components, namely, squamous cell and glandular. The cervicovaginal tumors which developed after transplantation of the hyperplastic lesions of the genital tract were histologically variable. However, the histology of the tumor did correlate with the histology of the hyperplastic area from which it arose. Although only 6 of 17 genital tracts with hyperplastic lesions produced tumors after transplantation, the frequency of tumors may depend on several factors, including the portion of the genital tract transplanted. It is also difficult to identify a hyperplastic lesion or a small tumor in a fresh preparation at autopsy. In the present experiment, random segments of the genital tract were transplanted, and it is possible that the lesions were not among those segments. In addition, as in the case of hyperplastic alveolar nodules in the mouse mammary tumor system, some cervicovaginal lesions may not give rise to tumors after transplantation.

All tumor lines which have been serially transplanted for over 2 years have maintained their original histology, with the exception of tumor LAJ-6195. This tumor changes its histological pattern after 3 serial transplantations (6 months), becoming anaplastic. Although some tumors arose from cervicovaginal tissue which was considered to be ovary dependent based on the absence of cornification of the vagina in ovariectomized neonatal steroid-treated mice, all tumor lines were found to be ovary independent.

Serial transplantation of experimentally induced carcinomas in laboratory animals has been difficult to maintain (10). The tumors tend to change morphologically and to show increasing anaplasia. Although there are some well-characterized transplantable carcinomas (1, 6, 10), to our knowledge there are no published reports of cervicovaginal tumor lines derived from neonatally steroid-treated animals.

The results presented herein support the idea that hyperplastic lesions in the cervicovaginal region of neonatally steroid-treated mice are potentially neoplastic, based upon the fact that when transplanted into host animals they may give rise to carcinomas. Furthermore, the study demonstrates the feasibility and possible value of transplantation of adult tissues of animals neonatally exposed to various agents.

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REFERENCES

Fig. 1. Histological appearance of Tumor TJ-6271 after first transplant generation. Presence of squamous cell and adenocarcinomatous features. H & E, x 100.

Fig. 2. Histological appearance of Tumor AK-6293 after first transplant generation. Presence of squamous cells. H & E, x 100.

Fig. 3. Histological appearance of Tumor LW-6214 after first transplant generation. Presence of squamous cells. H & E, x 100.

Fig. 4. Histological appearance of Tumor LJ-6195 after first transplant generation. Presence of squamous cells. H & E, x 100.

Fig. 5. Cervicovaginal tumor from a 21-month-old mouse treated neonatally with 5 μg 17β-estradiol + 100 μg progesterone. Squamous cell appearance of the tumor. H & E, x 100.

Fig. 6. Histological appearance of Tumor LJ-6195 from the tumor shown in Fig. 5 after 22 transplant generations. Anaplastic appearance of the tumor. H & E, x 100.
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