The Susceptibility of Fetal Rat Skin in Different Immunologic Environments to Neoplastic Induction with Shope Papilloma Virus

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SUMMARY

The susceptibility of fetal rat skin to neoplastic induction with Shope papilloma virus (SPV) was studied. While a high proportion of fetal rat skin grafts infected with SPV and transplanted to the cheek pouches of cortisone-treated hamsters became papillomatous, only a small proportion of similar grafts placed in syngeneic rats became transformed. Those papillomas which did develop in the fetal rat skin grafts in syngeneic hosts always regressed. The regression was preceded and accompanied by a lymphocytic infiltration. An explanation for these observations may be that rats are capable of recognizing a new antigen in syngeneic epidermal cells transformed by SPV, and of subsequently mounting an effective immunologic attack against the papilloma cells.

INTRODUCTION

Although most of the oncogenic DNA viruses can readily induce tumors in animals of widely variant species (7), the Shope papilloma virus (SPV) can initiate neoplastic alterations only in rabbits and hares (1). The demonstration by Greene (4) that embryonic rat skin would become papillomatous after infection with SPV and transplantation to the "immunologically privileged" sites of the brain or anterior chamber of the eye has suggested that the skin of the rat is, under certain circumstances, susceptible to SPV infection.

Despite Greene's demonstration, subsequent workers have been unable to induce papillomas with SPV in the skin of intact rats. The study reported here is a preliminary investigation of the nature of the resistance of rats to infection with SPV. The problem posed is whether the growth and histologic appearance of SPV-infected embryonic rat skin grafts might differ in immunologically privileged sites (2) from that obtained in syngeneic and immunocompetent hosts.

The methods used were a variation of a previously described technic in which adult animals received orthotopic and subcutaneous skin grafts which had been previously incubated with SPV or appropriate control solutions (5). Since adult or newborn rat skin could not be transformed by SPV in Greene's report, only syngeneic fetal rat skin was used as grafts in our study. The fate of these grafts in immunologically accessible sites of syngeneic hosts was compared with the behavior of similar grafts placed in the immunologically privileged site of the cheek pouches of cortisone-treated hamsters (2).

MATERIALS AND METHODS

Animals

Syngeneic rats (Lewis strain) were purchased from Microbiological Associates, Walkersville, Maryland. Young adults of either sex (10–12 weeks) and pregnant females (16–21 days) were used. Young, adult, random-bred golden Syrian hamsters were purchased from the Lakeview Hamstery, Lakeview, New Jersey.

Virus

A 10% (w/v) extract in phosphate buffer (0.0067 M, pH 7.0) was prepared from glycerinated, pooled papillomas purchased from Earl Johnson, Rago, Kansas. The virus was titrated by inoculation into the scarified skins of New Zealand white rabbits. The minimal infectious concentration was at a dilution of $10^{-3}$. Virus stocks were stored at $-70\degree C$ until used in an experiment.

Preparation of Grafts

Fetuses were removed aseptically from the uterine cornua. Full-thickness skin, from both ventral and dorsal aspects of the trunk, was stripped with forceps, washed free of blood with Hanks' solution, and cut into 2- to 3-mm squares. The skin fragments were then incubated in a Petri dish which contained a 1:4 dilution of virus in Hanks' solution. Control skin grafts were treated similarly except that the virus was not added. After incubation for 30 minutes at 36°C, the grafts were placed into the recipient animals.

Grafting to Hamsters

Under ether anesthesia, the hamster's cheek pouch was everted, pinned at three points to a corkboard, and washed with 70% ethanol. The ethanol was then wiped off with a sterile gauze pad. A 2-mm incision was made in the wall of the...
pouch, and fetal rat skin fragments were inserted with toothless forceps. Hamsters were given 3 mg of cortisone acetate (Cortone, Merck, Sharpe and Dohme Co., West Point, Pa.) subcutaneously at the time of grafting followed by 1 mg at biweekly intervals. Inspections of grafts were made weekly under ether anesthesia. Histologic sections were prepared from all control and virus-infected grafts. Tissues were fixed in Bouin’s fluid, embedded in paraaffin, and stained with hematoxylin and eosin.

Grafting to Rats

Orthotopic fetal skin grafts were closely fitted into the defect obtained by excising a 2- to 3-mm fragment of the recipient’s skin. A “Sheer Spot” (Johnson and Johnson, New Brunswick, N. J.) with vaseline-treated gauze pad was firmly placed over the graft. Subcutaneous grafts were inserted with forceps through a small incision on the lateral aspect of the dorsum. In all cases, virus-treated orthotopic and subcutaneous grafts were placed on one side of the rat; control grafts were placed on the opposite side. Occlusive dressings composed of 4 x 4 inch gauze pads and adhesive tape discouraged the animals from tampering with the grafts.

The dressings were removed 7—10 days after operation. After dressing removal, observations were made every four days until the animals were killed. Histologic sections were processed the same as the hamster tissues.

RESULTS

Rat Fetal Skin Grafts to the Hamster Cheek Pouch

Control grafts were placed in the eight cheek pouches of four hamsters. All grafts survived the period of observation (14 days). The histologic appearance of the controls was that of a normal epidermal cyst (Fig. 1). The hair follicles were well developed. There were no hyperplastic foci or acanthosis.

SPV-infected grafts were placed in the eight cheek pouches of four hamsters. Again, all grafts survived the period of observation. In contrast to the control grafts, six of the eight were markedly hyperplastic with acanthosis, pronounced thickening of the keratohyalin layer, prominence of keratohyalin granules, and marked hyperkeratosis (Fig. 2). The two grafts which showed no papillomatous changes were indistinguishable from the control grafts.

Rat Fetal Skin Grafts to Adult Rats

Eight separate experiments were performed. A different pregnant rat provided the pooled, fetal skin for each experiment. Forty recipient rats were used. A total of 65 control grafts were studied. These grafts showed only a transient hyperplasia which disappeared by 10—12 days. In the next few days, new hairs which were much more delicate and closer spaced than the hairs of the adjacent host skin appeared on the graft surface. Thereafter, the gross and microscopic appearance of the grafts was unremarkable (Fig. 3).

Only a small proportion of SPV-infected grafts showed changes similar to those seen in the virus-infected grafts in the hamster cheek pouch. Out of 191 SPV-infected grafts, only 26 (7.3%) became grossly papillomatous (Figs. 4—8). Except for cyst formation in the subcutaneous sites, microscopic changes were similar in both orthotopic and subcutaneous papillomatous grafts. These grafts showed pronounced focal hyperplasia, acanthosis, and many subepidermal keratin cysts.

The course of development of the few tumors was interesting. After the initial hyperemia and induration of the grafting procedure disappeared (10—12 days postoperation), minute foci of new induration and hyperemia emerged in less than 10% of the grafts (at 14—21 days). It must be emphasized that the entire graft was not uniformly affected. The tumors were highly localized and almost exclusively found at the periphery of the graft. The appearance was quite similar to that of early developing Shope papillomas in the rabbit.

Over the next week, the tumors reached a maximum size of 5 mm in height and 3 mm in diameter. Frequently some tumors became hyperkeratotic. From this point on, the lesions all progressively decreased in size until they were completely absent within 7—10 days after their initial appearance. Microscopic sections obtained during the regression invariably showed infiltrates of large and small lymphocytes about the epidermal hyperplastic foci and even within the papillomatous epithelium (Figs. 4—8). These mononuclear infiltrates were not found in any of the control sections. Furthermore, the localization of lymphocytes was highly specific and completely restricted to the papillomatous regions. The normal appearing portions of the graft epidermis were never affected (Fig. 6).

In each instance where papillomatous changes appeared in a graft, the tumor components eventually regressed. After regression was complete, the appearance of virus-treated grafts never differed from the controls through four months of weekly observations.

The incidence of papillomatous transformation in the infected grafts was not related to the age of the fetuses used as donor animals. There was also no correlation with age or sex of the recipient animals.

DISCUSSION

The results described in this study confirm and extend the findings of Greene (4) who first described the susceptibility of fetal rat skin to neoplastic induction with the SPV. He observed a comparatively high incidence of papillomatous transformation when SPV-infected rat fetal skin was grafted to an immunologically privileged site, i.e., brain or anterior chamber of the eye. Our studies have shown that SPV-infected fetal rat skin frequently becomes papillomatous when transplanted to the immunologically privileged hamster cheek pouch.

Greene also placed SPV-infected fetal skin grafts into the subcutaneous space of adult rats. He noted that here the papillomatous changes were less, and tended to be more limited in distribution, than in the brain transplants, but he did not describe regression of the papillomas. Furthermore, although not described in the text, Greene’s photomicrographs of these subcutaneous grafts show what appear to be subepidermal infiltrates of inflammatory cells at 28 days postgrafting, which is
Immunologic Effects on Fetal Rat Skin Carcinogenesis

within the time period in our experience when lymphocytic infiltration and regression of papillomas occurred.

In our studies, SPV-infected fetal rat skin placed in orthotopic or subcutaneous sites shows a low incidence of papillomatous transformation, and the papillomas invariably regress.

Further, the regression of papillomas arising in grafts of fetal rat skin infected with SPV is preceded and accompanied by lymphocytic infiltration. This is analogous to the findings in the spontaneous regression of papillomas in the natural host, the rabbit (3, 5, 6). The papilloma regressions in the rabbit are apparently the result of an immune mechanism directed against the autochthonous papilloma cell, for the incidence of regression is increased by vaccination with tumor (3) and decreased by treatment with methylprednisolone (6). In addition, regressor rabbits fail to develop tumors after reinoculation with autologous papilloma cells, but tumors do develop in nonregressors similarly inoculated (5).

While the association of lymphocytic infiltration and regression of papillomas in the rat system is highly suggestive, it certainly does not prove that the papilloma regression in the rat is the result of an immunologic mechanism.

One approach to the problem which might give indirect evidence would be the demonstration of an increased incidence of papillomas or a decreased incidence of regressions in animals which have been immunosuppressed (6). Experiments using methylprednisolone and an anti-rat lymphocyte serum are currently in progress.

If the regression of papillomas induced by the SPV in fetal rat skin can be shown to have an immunologic basis, it might be desirable to study the papilloma regression phenomenon in the rat rather than the rabbit for the following reasons: (a) This study suggests that rats are apparently more resistant to the continuing growth of SPV-induced tumors than rabbits, since about 2/3 rabbits tolerate papillomas indefinitely, but no rats are tolerant. (b) Syngeneic rats are available, but comparable rabbit strains are not. The use of syngeneic animals will permit greater uniformity in experimental response. In addition, the ability to transplant SPV-induced tumors or to transfer immune lymphocytes between syngeneic individuals would markedly broaden the spectrum of experimentation possible with the SPV.

REFERENCES


Fig. 1. Section of epidermal cyst derived from implant of normal fetal rat skin in a hamster cheek pouch 14 days after grafting. RE, rat epidermis; BE, buccal epithelium of hamster. H & E, × 100.

Fig. 2. Section of papillomatous cyst derived from Shope papilloma virus-infected implant of fetal rat skin in the hamster cheek pouch 14 days after grafting. RE, rat papillomatous epithelium; BE, buccal epithelium of hamster. H & E, × 100.

Fig. 3. Section of orthotopic graft of normal fetal rat skin to syngeneic adult rat. Arrows indicate junction between host and recipient tissue. Grafts were transplanted 25 days prior to section. H & E, × 100.

Fig. 4. Section obtained 25 days after grafting Shope papilloma virus-infected fetal rat skin to an orthotopic site in a syngeneic adult rat. The epidermis is thrown up into papillary projections which are hyperkeratotic. The subepidermal tissues are infiltrated by mononuclear leukocytes. Numerous subcutaneous keratin cysts are visible. H & E, × 70.

Fig. 5. Section obtained 25 days after grafting Shope papilloma virus-infected fetal rat skin to an orthotopic site in a syngeneic adult rat. There is a very intense lymphocytic infiltration about the deepest portions of the papillomatous epithelium. H & E, × 70.

Fig. 6. Shope papilloma virus-infected fetal rat skin graft 25 days after grafting. The field of view is deep in the dermal portions of the graft. A very hyperplastic hair follicle is heavily infiltrated by lymphocytes. Note that four adjacent follicles are not hyperplastic and have not attracted lymphocytes. H & E, × 250.

Fig. 7. Shope papilloma virus-infected fetal rat skin graft 25 days after grafting; a hyperplastic rete peg is sectioned longitudinally. Note that the lymphocytes not only surround the papillomatous epithelium but actually penetrate it. H & E, × 600.

Fig. 8. Shope papilloma virus-infected fetal rat skin graft 25 days after grafting. Lymphocytes in close proximity to papilloma cell nuclei; they are possibly intracytoplasmic. H & E, × 1560.

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