The Role of the Dermis in the Induction of Neoplasia by Shope Papilloma Virus

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SUMMARY

Interactions of mesenchyme with epithelial cells are known to be responsible for the survival, proliferation, and differentiation of embryonic and adult epithelia of a variety of phenotypes. The importance of the dermis in the induction of neoplasia in rabbit epidermis infected with Shope papilloma virus was studied. Skin fragments were dissociated into epidermal and dermal components with trypsin, infected with Shope papilloma virus, and grafted to nondermal sites. The results were consistent with the view that little or no dermis is required for the development of typical Shope papillomas from Shope papillomas virus-infected epidermal cells.

INTRODUCTION

It is now well established that communicative interactions between phenotypically different cells are responsible for the elicitation of the capacity for continued proliferation and differentiation (8). Specifically in the case of skin, it has been shown that dermis can influence the spreading, orientation, proliferation, and keratinization of epidermis (11). In view of the importance of the dermis in the normal behavior of epidermis, it was of interest to determine whether dermis is required for the induction of neoplasia in autografts of rabbit skin infected with SPV. For this purpose, chips of skin taken from the ears of rabbits were split into epidermal and dermal components with trypsin. Various combinations of epidermis (both in sheets and cell suspensions) and viable or freeze-killed dermis were either SPV-infected or noninfected and grafted to nondermal sites. Observations on the papilomatous transformation of these grafts suggested that the presence of little or no dermis is required for the induction of neoplasia in this system.

MATERIALS AND METHODS

Animals. Giant Checker and New Zealand White rabbits of both sexes were housed in individual cages and fed Purina chow supplemented with fresh kale.

Virus. An approximately 10% extract of cottontail papilloma tissue (tumors obtained from Earl Johnson, Rago, Kansas) was prepared. Two pools of virus (stored at -70°) were used in the experiments. These preparations were infectious at a dilution of 10^4.

Preparation of Autografts. All grafts were obtained from dorsal ear skin. The skin was shaved with a razor blade, washed several times with 70% ethanol, and air dried. After a light coating of yellow petrolatum USP was applied, split-thickness sheets of skin approximately 0.5 x 5 x 7 mm were sliced off with a stainless steel safety razor blade.

Enzyme Dissociations. The technique was a modification of a previously described method (4). Split-thickness grafts floating epidermis up were incubated for 1 hr at 37° in 0.25% trypsin (1/250, Difco Laboratories, Detroit, Mich.) in BSS. Control grafts of undissociated skin were incubated in BSS alone. After enzyme digestion, the epidermis was mechanically detached from the dermis and either used as an intact sheet or further separated into single cells by scraping with a Teflon policeman during a brief soak in a solution of buffered citrate-NaCl solution (1 part 4% sodium citrate in 0.89% NaCl solution to 4 parts calcium-magnesium-free phosphate-buffered NaCl solution). Cell clumps were eliminated by drawing the suspension through a sterile tuft of cotton. Equal numbers of cells were used in the control and SPV-infected grafts.

Infection with Virus. Fragments of undissociated skin, epidermal sheets, and epidermal cell suspensions were washed in several changes of BSS. Grafts to be infected were incubated for 1 hr at 37° in a 10^4 dilution of virus suspension in BSS. Control grafts were incubated in BSS alone. The epidermal cell suspensions were centrifuged onto sheets of sterile tea bag paper (6 x 6 mm) to facilitate subsequent grafting manipulations.

Grafting Procedure. Rabbits were anesthetized i.v. with Nembutal and were given an i.m. injection of penicillin and streptomycin. Grafts were placed upon either the panniculus carnosus or the lumbodorsal fascia. The fragments were pinned to the subjacent tissue at 2 points with small hooks bent from fine stainless-steel wire. The grafts were kept from contact with overlying skin and muscle by suturing a dish-
shaped plastic cover, about 3.2 cm in diameter and 0.3 cm deep, over them (Fig. 1). These covers were cut from the cups of Model FB-24 "Disposotrays" purchased from Linbro Chemical Co., New Haven, Conn. After 3 weeks of growth, the animals were killed with chloroform. The grafts were removed and fixed in Bouin’s solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

RESULTS

Grafts on the Panniculus Carnosus. The incidence of papillomatous and epidermal cysts developing in grafts placed on the panniculus carnosus is recorded in Table 1. Epidermis alone formed keratinizing epidermal cysts in the single case tested. In the presence of SPV, epidermis alone developed into papillomatous cysts in 5 of 6 cases. Epidermis in the presence of dermis also responded to SPV infection with papilloma formation. The proximity of dermis to the outer layer of the panniculus carnosus raised the possibility that small numbers of dermal cells adherent to the panniculus might participate in the SPV-epidermal interactions. For this purpose, an additional series of grafts were placed on the lumbodorsal fascia. Further, a plastic cover prevented any part of the graft from coming into contact with the panniculus (Fig. 1).

<table>
<thead>
<tr>
<th>Type of graft</th>
<th>No. of total grafts</th>
<th>No. of papilloma cysts</th>
<th>No. of epidermal cysts</th>
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<tr>
<td>Epidermis</td>
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Table 1

Incidence of papillomatous and epidermal cysts in grafts of the panniculus carnosus

Dermal role in normal interaction

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<th>No. of papilloma cysts</th>
<th>No. of epidermal cysts</th>
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Dermal role in neoplastic interaction

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Dermal susceptibility

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<th>No. of epidermal cysts</th>
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<td>Dermis, SPV</td>
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<td>1</td>
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<tr>
<td>Frozen dermis, SPV</td>
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</table>

Table 2

Incidence of papillomatous and epidermal cysts in grafts to the lumbodorsal fascia

To determine whether a continuing but transitory effect of the dermis on the "pure" epidermal grafts was responsible for their ability to form papillomas when SPV-infected, we performed the following experiment. In 11 rabbits, epidermal cell suspensions were grafted to the lumbodorsal fascia and permitted to develop in situ for 3 weeks. At the end of this time, the epidermal grafts were surgically exposed and infected with SPV by needle inoculation. The incisions were closed and the grafts were permitted to grow for an additional 3 weeks. At the conclusion of this period, the animals were killed and the grafts were sectioned. Four of the 11 grafts contained no surviving epidermis. The remaining grafts were very hyperplastic, resembling papillomas but did not completely fulfill the inflexible criteria used for this diagnosis. The fact that 2 typical papillomas developed after SPV infection of long-established epidermal grafts indicates that a transitory, short-term effect of dermis on the epidermal cells does not exist.

Susceptibility of Epidermal Sheets and Cell Suspensions to SPV. The epidermal component in these experiments was grafted both as an intact sheet and as a clump-free cell suspension (5 to 15 million cells/graft). Approximately equal fragments alone indicates the presence of viable epidermis in the hair follicles remaining in the dermis. The development of a papilloma in 1 of 4 such dermal grafts which had been frozen and thawed 5 times clearly demonstrates survival of epidermal cells despite this drastic treatment. Freezing, therefore, cannot be expected to be totally effective in destroying dermal mesenchymal cells.
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tions of both types of grafts were used in all phases of these studies. The development of both epidermal and papillomatous cysts did not depend upon the state of the epidermal graft. Sheets and cell suspensions were equivalent in their behavior. There was no reformation of hair follicles, rete pegs, or sebaceous glands in any epidermal grafts without dermis in heterotopic sites.

DISCUSSION

In this study, transplants of epidermis infected with SPV almost invariably became papillomatous. There was no evidence of this phenomenon on the physical presence of dermis or on transitory dermal influences. The papillomas which developed in transplants of epidermis alone were in every way representative of the typical morphology of this tumor and identical with the controls, with the exception that tumors resulting from epidermal-dermal recombinants were sometimes larger than the tumors which developed from epidermis alone. This result may be explained by the presence of the increment of epidermal cells within hair follicles of the dermal fragment. That these residual hair follicles alone can generate papillomas was shown in the results of Table 2.

It is possible that the "pure" epidermal grafts used in this study actually contained some dermal fibroblasts. However, in our experience and that of Billingham, microscopic sections through the epidermal sheets have never shown adherent fibroblasts. Therefore, the dermal contaminant, if it does exist, must be a very small percentage of the cells grafted.

The results of the present study have direct relevance to some basic questions in both the biology of skin differentiation and related problems in neoplasia. It is well known that the differentiation of epithelium is the result of its interactions with mesenchyme. The degree of specificity of this interaction is quite variable. For example, only salivary gland mesenchyme can initiate morphogenesis of salivary gland epithelium (7), but in the thymus (3) and pancreas (12) nonspecific mesenchymal components will suffice. For epidermis, it has been shown that isolated rat epidermal cells placed beneath the renal capsule or in skeletal muscle will form nests of keratinizing epidermis. In the latter site, there was even some formation of dermal papillae and hair follicles (6). In the mouse, sheets of pure epidermis placed on the lumbodorsal fascia form epidermal cysts (1). Hair follicles and sebaceous glands are not regenerated. Clearly, the results of both the present report and the previous studies support the conclusion that mesenchyme elicited at a variety of nondermal sites can interact with epidermal cells to permit survival, proliferation, limited formation of skin appendages and even neoplastic transformation.

In contrast, another study indicated that isolated normal epidermis and the epithelium of basal cell carcinomas of humans would not survive in vivo in the absence of contact with dermis (10). Other investigators (2, 5, 9) have reported failure of tumors to develop in transplants of isolated mouse epidermis which was previously treated with methylcholanthrene. These latter experiments are difficult to interpret for the transplantation technique in this system interferes with the development of tumors in carcinogen-treated full-thickness skin.

There has been only one other study on the influence of epitheliomesenchymal interactions on tumor production by an oncogenic virus. It was concluded that polyoma virus could not induce the neoplastic transformation of mouse salivary gland epithelium in the absence of salivary gland mesenchyme (7). The apparent contradiction between this result and the one reported here can be resolved when the marked specificity of the normal epithelial-mesenchymal interaction in the salivary gland is compared to the lack of apparent specificity in skin. In the case of salivary gland, both normal and neoplastic epithelium remains dependent upon histotypic mesenchyme for survival and growth. However, in the case of skin, a variety of mesenchymal types will support the survival and proliferation of both normal and SPV-transformed epidermal cells.

The results of the current experiments indicate that the conditions which are sufficient for implanted epidermal cells to differentiate and form a keratinizing squamous epithelium without epidermal appendages are also sufficient for their transformation and growth as papilloma cells. No more complex or specific environment is required.

REFERENCES

Fig. 1. Schematic diagram illustrating anatomic relationships in placement of grafts on the lumbodorsal fascia.

Fig. 2. Portion of the wall of an epidermal cyst developing from a suspension of epidermal cells planted on the lumbodorsal fascia after centrifugation onto a fragment of tea bag paper. Note the abundant proliferation of mesenchymal cells and the reformation of germinal, spinous, keratohyalin, and keratinized layers. S, strands of tea bag paper. H & E, X 150.

Fig. 3. Portion of the wall of a papillomatous cyst developing from an epidermal cell suspension, treated except for SPV infection, in the same manner as the graft in Fig. 2. Materials in both Fig. 2 and Fig. 3 are from the same rabbit. A typical Shope papilloma is present. The epithelium is hyperplastic and hyperkeratotic. There is a marked acanthosis with an exaggerated intrusion of dermal papillae into the epithelium. H & E, X 150.

Fig. 4. Epidermal cyst developing from epidermal cell suspension grafted to the lumbodorsal fascia. Identical with Fig. 2, except from a different rabbit. H & E, X 150.

Fig. 5. Papillomatous cyst developing from an SPV-infected epidermal cell suspension grafted to the lumbodorsal fascia. Same rabbit as Fig. 4. H & E, X 150.

Fig. 6. Papillomatous cyst developing from an SPV-infected epidermal sheet grafted to the lumbodorsal fascia. F, lumbodorsal fascia. H & E, X 14.

Fig. 7. Same rabbit as Fig. 6. Papillomatous cyst which developed from an SPV-infected epidermal sheet combined with dermis and grafted to the lumbodorsal fascia. Note that the papilloma development is more extensive than in Fig. 6. F, lumbodorsal fascia. H & E, X 14.
Plastic cover

Skin

Panniculus carnosus

Lumbodorsal fascia

Sacrospinalis muscle

Graft

1

2

3

4

5

978

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