Normal keratinized mucosa transplants in nude mice

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Two types of normal keratinized mucosa were transplanted to subcutaneous sites of nude mice of two different strains. 24 intact specimens of clinically normal human palatal mucosa were transplanted to nude mice of the strain nu/nu NC. The transplants were recovered after 42 d with a recovery rate of 96 %. Moreover, 22 intact specimens of normal rat forestomach mucosa were transplanted to nude mice of the strain nu/nu BALB/c/BOM. These transplants were recovered after 21 d with a recovery rate of 63 %. The histologic features of the transplants were essentially the same as those of the original tissues. However, epithelial outgrowths from the transplants differed with respect to the pattern of keratinization. The outgrowths of human palatal mucosa transplants were essentially unkeratinized, while the outgrowths of the rat forestomach transplants showed continued keratinization.

Key-words: Oral mucosa; histology; epithelial-mesenchymal interaction

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A model for studying tissue interactions in human oral mucosa *in vivo* utilizing transplantation of intact and recombined human tissue to nude mice, has recently been described (4, 5, 8). By this method, unkeratinized human buccal mucosa has been shown to maintain essentially the preexisting histological features after transplantation and growth in the mice (5).

One of the most common pathological epithelial changes of the oral mucosa is hyperkeratosises. An alteration in the process of keratinization seems to be an essential feature in the pathogenesis of such lesions. For the study of epithelial-mesenchymal interactions in these lesions utilizing the above mentioned model, it is necessary to demonstrate that normal intact keratinized mucosa retains normal histologic features after transplantation to nude mice. One such normally keratinized human oral epithelium is that of the palatal mucosa (10). Another epithelium which has been the subject of studies of experimental carcinogenesis and epithelial-mesenchymal tissue interactions is that of the mucosa of the rat and mouse forestomach (3, 6). As a basis for future

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studies of tissue interactions during experimental carcinogenesis of rat forestomach mucosa transplants in nude mice it is necessary to demonstrate that normal forestomach mucosa after transplantation to the nude mouse retains normal histologic features.

So the purpose of the present investigation was to examine the histologic features of mucosa of the human palate and of the rat forestomach after transplantation to nude mice.

MATERIAL AND METHODS

Tissue of clinically normal human palatal mucosa was obtained from four non-smoking patients (1 male, 3 females) undergoing surgical treatment involving the palatal mucosa. The age of the patients ranged from 12 to 15 years. The tissue was divided into 24 specimens (size: approximately 3 x 3 x 1 mm^3) and transplanted to subcutaneous sites in the flank region of 24 nude mice (nu/nu NC – the progeny of a 4th backcross to the NC strain) of both sexes and seven weeks of age. Moreover, 22 intact specimens of normal rat forestomach mucosa were obtained from 11 rats (Wistar, random bred, 4 months of age); these biopsies were transplanted to subcutaneous sites in the flank region of 22 nude mice (nu/nu BALB/c/BOM – 12th backcross of a gene transfer to BALB/c/BOM) of both sexes, eight weeks of age. Prior to transplantation, a part of the tissues was immediately fixed in 10 % neutral formalin and prepared for histologic examination. Tissue preparation, housing of the mice and transplantation procedures have been described previously (4).

The human palatal mucosa transplants were recovered after 42 d and the rat forestomach transplants after 21 d. Both types of transplants were fixed in 10 % neutral formalin and processed for histological examination, which was carried out according to a previous

Table 1. *Histologic epithelial characteristics of control palatal mucosa and palatal mucosa transplants and their outgrowths*

	Number of specimens				Number of epithelial cell layers	
	with rete ridges	with unkeratinized epithelium	with single cell keratinization	with parakeratinized epithelium	thinnest parts	thickest parts
Control palatal mucosa N = 4	4	0	0	4	Range: 4–7 Mean: 5.8 s.e.: 0.5	Range: 24–29 Mean: 26.0 s.e.: 1.1
Palatal mucosa transplant N = 23	23	1	0	22	Range: 3–21 Mean: 8.0 s.e.: 9	Range: 11–32 Mean: 20.6 s.e.: 1.2
Outgrowths of palatal mucosa transplant N = 13	0	13*)	1*)	0	Range: 2–4 Mean: 3.0 s.e.: 0.2	Range: 3–7 Mean: 4.9 s.e.: 0.3

*) In one outgrowth the unkeratinized epithelium showed the presence of scattered keratinized single cells

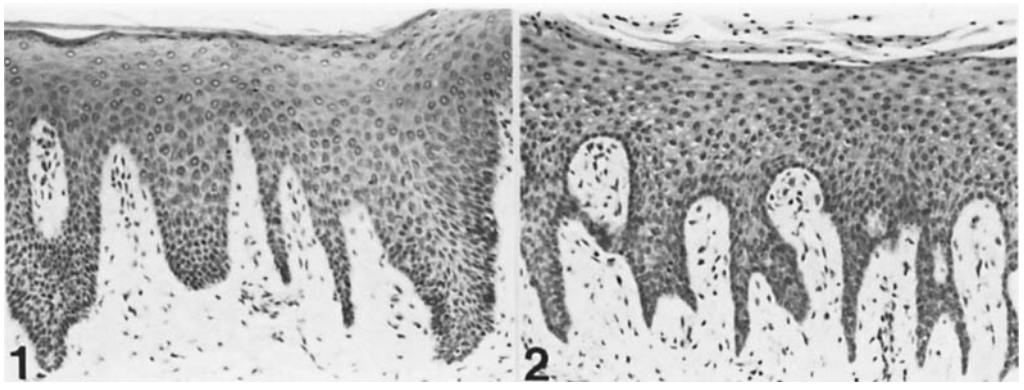

Fig. 1. Normal parakeratinized palatal mucosa. H & E, X 110.

Fig. 2. Transplant of normal human palatal mucosa. Note the parakeratinization and the rete ridges. H & E, X 110.

description (5). The examination included registration of the number of epithelial cell layers, keratinization pattern and presence or absence of epithelial rete ridges.

RESULTS

Human palatal mucosa

23 (96 %) of 24 transplants were recovered with a vital epithelium and connective tissue (Fig. 2). One transplant was lost due to total necrosis. In five cases, only parts of the transplanted connective tissue showed the presence of blood vessels with erythrocytes. The thickness of the connective tissue in these cases exceeded 0.9 mm and a considerable portion of the overlying epithelium showed necrosis. In all 23 successfully recovered specimens, the epithelium overlying transplanted connective tissue presented rete ridges (Table 1). 22 transplants showed parakeratinization, the keratinization in one case presenting only flattened superficial cells and keratohyalin granules. This transplant and the transplant with unkeratinized epithelium were characterized by the presence of excess connective tissue. The presence of rete ridges and keratinization of the transplants did not differ from that of the control specimens (Table 1, Fig. 1).

The numbers of epithelial cell layers of transplants and control specimens are also shown in Table 1. The ranges of the transplants were greater than those of the controls. Furthermore, the mean number of epithelial cell layers in the thinnest areas of the transplants was slightly more than the controls, whereas a decrease in the mean number of cell layers was seen in the thickest areas. The presence of scattered single neutrophils was seen in the connective tissue of 3 of the 23 transplants.

Outgrowths of stratified squamous epithelium from the transplants (Fig. 3) were seen in 13 cases (57 %); in 7 cases (30 %) the outgrowths formed cysts. The outgrowths, all formed over what appeared to be murine connective tissue, never presented rete ridges. In contrast to the parent transplants, they were always unkeratinized. One case, however, presented single cell keratinization (Table 1). Moreover, the outgrowths were always thinner than the epithelium of the transplants, and occasional flattened basal cells were observed.

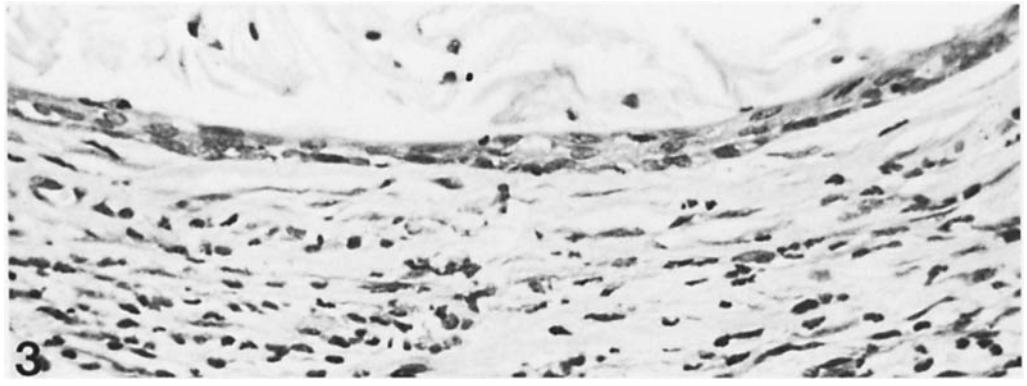

Fig. 3. Outgrowth from transplant of normal human palatal mucosa. The epithelium shows lack of keratinization and rete ridges and is only 3 – 4 cell layers thick. H & E, X 350.

Rat forestomach

15 (68 %) of 22 transplants were successfully recovered. Seven transplants were lost: two cases due to death of the animal, one case due to total necrosis of transplant and four cases due to unsuccessful identification of transplants. The subepithelial connective tissue of the transplants showed the presence of blood vessels with erythrocytes. A smooth muscle layer (lamina muscularis mucosae) was seen immediately under the subepithelial connective tissue and appeared vital. As seen in Table 2 the histologic characteristics of the transplanted epithelium are very similar to those of the controls (Figs. 4 & 5). The epithelium of the controls had a wavy appearance, the surface of which was characterized by the presence of keratin spines. Both the wavy appearance of the epithelium and the keratin spines were lacking in most transplants, which most often demonstrated a smooth surface. Finally, scattered single neutrophils were seen in the connective tissue of one transplant.

Outgrowths of stratified squamous epithelium from the transplants (Fig. 6) were seen in 6 cases (40 %), five of which (33 %) formed cysts. The outgrowths were always thinner than the epithelium from which they originated, but they always retained an orthokeratinized pattern. Occasionally flattened basal cells were seen. The outgrowths were formed on what appeared to be murine connective tissue, but in some cases isolated fusiform smooth muscle-like cells were seen in the subepithelial region (Fig. 6).

DISCUSSION

In the present study two different types of normal keratinized mucosa were transplanted to nude mice of two different strains using identical techniques. The rat forestomach mucosa was transplanted to nu/nu BALB/c/BOM mice with a successful recovery rate of 68 %, which is the same as that obtained previously with human oral mucosa transplants in the same strain of mice (4,5). The human palatal mucosa was transplanted to nu/nu NC mice. The rate of successful recovery was 96 %, which is a considerable improvement compared to the previously obtained recovery rates in studies of shorter duration. None of the present mice suffered from the waisting syndrome (11) and the nu/nu NC strain thus appears to be a promising host for future experimental *in vivo* studies of mucosa transplants.

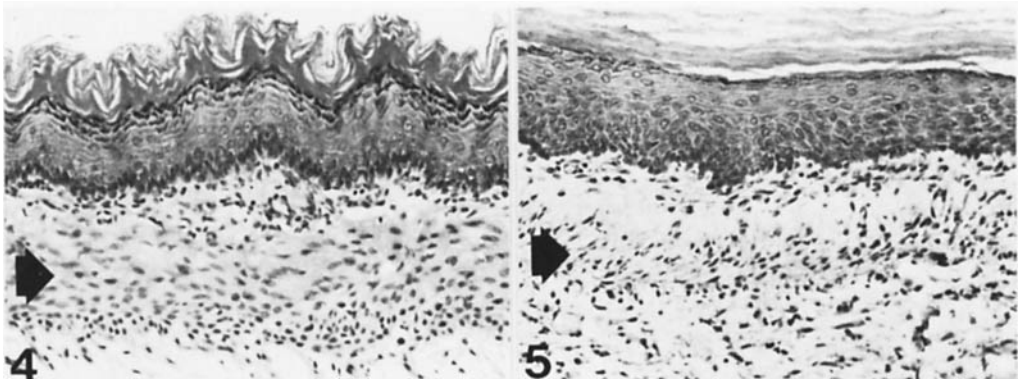

Fig. 4. Normal orthokeratinized rat forestomach mucosa. Note the wavy appearance of the epithelium and the keratin spines. Between the epithelium and lamina muscularis mucosae (arrow) is a border of connective tissue. H & E, X 140.

Fig. 5. Transplant of normal rat forestomach mucosa. Arrow indicates lamina muscularis mucosae. The wavy appearance of the epithelium and keratin spines are not present. Small irregular rete ridges are seen. H & E, X 140.

Table 2. *Histologic epithelial characteristics of control rat forestomach mucosa and rat forestomach mucosa transplants and their outgrowths*

	Number of specimens		Number of epithelial cell layers	
	with rete ridges	with orthokeratinized epithelium	thinnest parts	thickest parts
Control rat forestomach mucosa N = 15	15	15	Range: 3–5 Mean: 4.5 s.e.: 0.2	Range: 7–11 Mean: 9.1 s.e.: 0.3
Rat forestomach mucosa transplant N = 15	11	15	Range: 4–9 Mean: 5.7 s.e.: 0.5	Range: 6–14 Mean: 9.4 s.e.: 0.7
Outgrowths of rat forestomach mucosa transplant N = 6	0	6	Range: 4–6 Mean: 4.7 s.e.: 0.3	Range: 4–9 Mean: 6.0 s.e.: 0.7

Both types of mucosa transplants showed histological features essentially similar to those of the respective control tissues. The ranges in number of epithelial cell layers of the human palatal epithelium were increased within the transplants. This may partly be due to varying thickness of transplanted connective tissue. The lack of keratin spines and wavy appearance of the epithelium in the rat forestomach mucosa transplants may be due to lack of tension in lamina muscularis mucosae.

It is interesting that the outgrowths

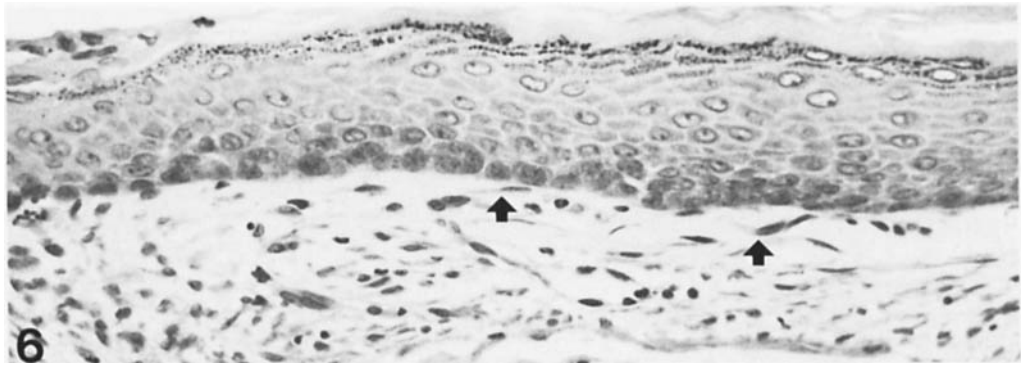

Fig. 6. Outgrowth from transplant of normal rat forestomach. Keratinization is retained. Note the isolated fusiform, smooth muscle-like cells (arrows) immediately subjacent to the epithelium. H & E, X 350.

from the two types of transplanted tissue were different with respect to the pattern of keratinization. The outgrowths of the human palatal mucosa transplants were essentially unkeratinized, whereas the outgrowths of the rat forestomach transplants showed continued keratinization although the post-transplantation period of these transplants was shorter. The difference in keratinization pattern of the two types of outgrowths may be due to: 1. lack of terminal differentiation in the outgrowths of human epithelium, 2. outgrowths of rat connective tissue underlying the rat epithelial outgrowths, whereas the human epithelial outgrowths were not underlined by human connective tissue, 3. The keratinization pattern of the human epithelium may depend upon a message from the subepithelial connective tissue, whereas such a dependence possibly does not exist with the rat epithelium.

It is not known whether the present human outgrowths are terminally differentiated or whether they are in a situation comparable to that seen in wound healing of the epidermis and of hard palate epithelium of guinea pigs (1, 2). However, with shorter post-transplantation periods, outgrowths of keratinized human oral leukoplakia transplants in nude mice have been shown to keratinize (4). With the methods employed, it is not possible to determine the origin of the subepithelial tissue. The finding of isolated fusiform cells in the subepithelial connective tissue of the forestomach mucosa outgrowths suggests the existence of smooth muscle cells of rat origin in that area. In a study of the outgrowths of autografts of tongue mucosa and skin in the rat uterus, the epithelial outgrowths formed on uterine connective tissue continued to keratinize (7 9). The authors concluded that a site specific connective tissue was not necessary to maintain keratinization. This tissue, however, appeared necessary for the expression of the original tissue architecture. In the present study both epithelial keratinization and architecture were altered in the palatal mucosa outgrowths which did not present rete ridges and showed a marked decrease in epithelial thickness. Future studies with extended post-transplantation periods are needed to throw light upon the reason for the epithelial changes of the outgrowths.

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REFERENCES

1. Andersen, L. Quantitative analysis of epithelial changes during wound healing in palatal mucosa of guinea pigs. Cell Tiss. Res. 1978, 193, 231 - 246
2. Christophers, E. Kinetic aspects of epidermal healing. In: Maibach, H.I. & Rovee, D.T. eds. Epidermal wound healing. Year Book Med. Publ. Inc., Chicago 1972, pp. 53 - 69
3. Fukamachi, H., Mizuno, T. & Takayama, S. Epithelial-mesenchymal interactions in differentiation of stomach epithelium in fetal mice. Anat. Embryol. 1979, 157, 151 - 160
4. Holmstrup, P., Dabelsteen, E. & Roed-Petersen, B. Oral leukoplakia transplanted to nude mice. Scand. J. Dent. Res. 1979, 89, in press
5. Holmstrup, P., Stoltze, K., Bretlau, P. & Dabelsteen, E. Human buccal mucosa transplants in nude mice. Scand. J. Dent. Res. 1981, 89, 89 - 96
6. Koestner, A.W., Ruecker, F.A. & Koestner, A. Morphology and pathogenesis of tumors of the thymus and stomach in Sprague-Dawley rats following intragastric administration of methyl nitrosourea (MNU). Int. J. Cancer. 1977, 20, 418 - 426
7. Mackenzie, I.C. Spatial organization and tissue architecture in normal epithelia. In: Mackenzie, I.C., Dabelsteen, E. & Squier, C.A. eds. Oral premalignancy. University of Iowa Press, 1980, pp. 220 - 241
8. Mackenzie, I.C., Dabelsteen, E. & Roed-Petersen, B. A method for studying epithelial-mesenchymal interactions in human oral mucosal lesions. Scand. J. Dent. Res. 1979, 87, 234 - 243
9. Mackenzie, I.C. & Smith, M.I. Organization and differentiation of epithelia transplanted to the uterus. J. Dent. Res. Special issue B 1976, 55, 218
10. Müller, W. & Schroeder, H.E. Differentiation of the epithelium of the human hard palate. Cell. Tissue Res. 1980, 209, 295 - 313
11. Rygaard, J. Thymus. Thesis. F.A.D.L.s Forlag, Copenhagen 1973