Expression of Basement Membrane Proteins and Interstitial Collagens in Dermal Papillae of Human Hair Follicles

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The expression of basement membrane molecules and interstitial collagens in human hair follicle mesenchyme was studied by immunohistochemical staining of tissue sections and of cells cultured from dermal papillae. Type I and type III collagens were found in the dermal sheath and in the dermal papilla throughout the hair cycle. Laminin and type IV collagen were expressed at the outer root sheath basement membrane and in the extracellular matrix of the dermal papilla of anagen and catagen follicles. In telogen follicles, where the volume of the dermal papilla extracellular matrix is much reduced, outline staining of dermal papilla cells for laminin and type IV collagen was still apparent. Staining for bullous pemphigoid antigen was also seen at the outer root sheath basement membrane extending to the lower tip of the hair bulb. In anagen follicles, there was no staining for bullous pemphigoid antigen at the interface between hair bulb epithelium and the dermal papilla and no staining within the dermal papilla. However, linear staining for bullous pemphigoid antigen became continuous around hair follicle epithelium during catagen and telogen. Cells cultured from human dermal papillae also stained for interstitial collagens, type IV collagen and laminin. However, similar results were obtained when cultured dermal fibroblasts were stained with the same antibodies. The expression of basement membrane proteins in human dermal papillae resembles that seen in follicles from other mammalian species and suggests that this is relevant to dermal papilla function. Cultured dermal papilla cells express a similar pattern of interstitial collagens and basement membrane proteins to those seen in tissue sections but this finding is not specific to dermal papilla cells. *J Invest Dermatol 96:93–97, 1991*

The dermal papilla is a specialized structure situated at the base of the hair follicle. Together with the dermal sheath that surrounds the follicle, the dermal papilla is derived from a condensation of mesenchymal cells, which appears early in the course of hair follicle morphogenesis directly beneath the epithelial germ [1]. Recombinant experiments on the adult rat vibrissa follicle have shown that the dermal papilla is responsible for inducing differentiation of the hair bulb matrix [2,3]. This parallels embryologic events, where development of the epithelial component is dependent upon inductive stimuli from the mesenchyme [4,5].

Once established during embryonic development, the cellular population of the dermal papilla is thought to remain fairly constant throughout successive hair cycles in adult life [6,7]. However, there are major changes in the vascular supply, cellular morphology, and volume of the dermal papilla extracellular matrix during the hair growth cycle [8,9]. At the onset of anagen, the developing epithelial matrix grows down to invest the papilla. Mesenchymal cells of the dermal papilla display ultrastructural evidence of synthetic activity and become separated by an extensive extracellular matrix that is maintained throughout anagen. The volume of the extracellular matrix diminishes during catagen to become almost indiscernible as telogen is reached.

Although the signals responsible for mediating dermal papilla function are not well understood, interactions with extracellular matrix are thought to have an important influence on cell behavior and development [10]. Interstitial collagens and basement membrane proteins form two major groups of extracellular matrix molecules and in this paper we report on their expression and distribution in the dermal papilla and dermal sheath of human hair follicles, and describe the changes that occur during the hair growth cycle. We have also stained cells cultured from human dermal papillae using the same panel of antibodies in order to assess whether cultured cells retain the same pattern of matrix production as that seen in the parent tissue.

MATERIALS AND METHODS

Materials The following antibodies were used: goat anti-type I collagen, goat anti-type III collagen (Sera-Lab Ltd); monoclonal mouse anti-type IV collagen (ICN Biomedicals Ltd); rabbit anti-laminin (a gift from Unilever Research). Bullous pemphigoid serum was obtained from two patients attending the Department of Dermatology in Sheffield. Biotinylated second antibodies and avidin-biotin-peroxidase complex were purchased from Vector Laboratories and FITC-conjugated second antibodies from Serotec Ltd. The primary antibodies were diluted 1 : 100 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin. Bullous pemphigoid serum was used at a dilution of 1 : 20. Cell culture consumables were purchased from Flow Laboratories. Other reagents were

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Abbreviations:
BPA: bullous pemphigoid antigen
FITC: fluoroscein isothiocyanate
PBS: phosphate-buffered saline

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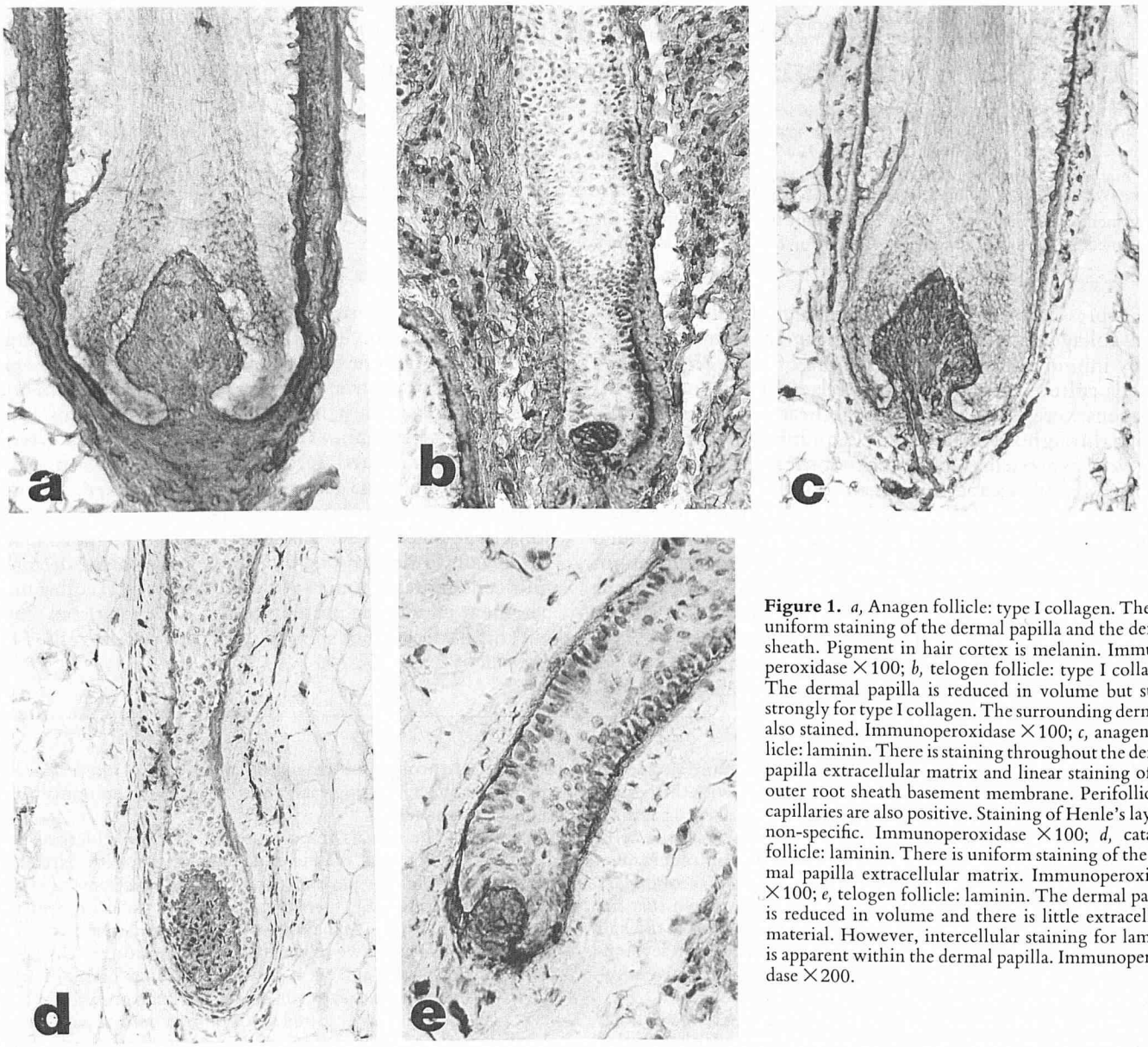

Figure 1. *a,* Anagen follicle: type I collagen. There is uniform staining of the dermal papilla and the dermal sheath. Pigment in hair cortex is melanin. Immunoperoxidase ×100; *b,* telogen follicle: type I collagen. The dermal papilla is reduced in volume but stains strongly for type I collagen. The surrounding dermis is also stained. Immunoperoxidase ×100; *c,* anagen follicle: laminin. There is staining throughout the dermal papilla extracellular matrix and linear staining of the outer root sheath basement membrane. Perifollicular capillaries are also positive. Staining of Henle's layer is non-specific. Immunoperoxidase ×100; *d,* catagen follicle: laminin. There is uniform staining of the dermal papilla extracellular matrix. Immunoperoxidase ×100; *e,* telogen follicle: laminin. The dermal papilla is reduced in volume and there is little extracellular material. However, intercellular staining for laminin is apparent within the dermal papilla. Immunoperoxidase ×200.

from Sigma and 8-well tissue culture slides were from ICN Biomedicals.

Tissue Sections Normal scalp tissue was obtained during the routine excision of various benign lesions (e.g., cysts, nevi), snap frozen, and stored at −70°C until required. Six-μm cryostat sections were mounted on poly-l-lysine coated glass slides, air dried, and fixed in acetone. Over 20 biopsies were studied.

Cell Culture Dermal papilla cell cultures were initiated using an established technique [11]. Briefly, dermal papillae were isolated by microdissection of anagen hair follicles. The papilla explants were placed in 25-cm^2 flasks in Medium 199 supplemented with antibiotics and 20% fetal bovine serum and cultured at 37°C in a humidified atmosphere containing 5% CO_2. Attachment of papilla explants and initiation of cell outgrowths usually occurred by day 5. The cells were subcultured after 2–4 weeks and thereafter at 1–2-week intervals. Third or fourth passage cells from 8 cell lines were used in the immunochemical studies. Cells were harvested as a single cell suspension using Trypsin-EDTA, counted in a hemocytometer chamber, and then seeded into 8-well tissue culture chamber slides at a density of 5000 cells/well. After 48 h in culture, the cells were washed with PBS, fixed in ice-cold methanol, and air dried. In some experiments, immunostaining was performed on two-week primary cultures initiated directly on 8-well slides. For comparative purposes, six dermal fibroblast cell lines, established from explants of interfollicular papillary dermis of the same biopsy specimens, were also studied.

Immunochemistry Tissue sections were washed in PBS and then incubated sequentially with blocking serum, primary antibody, biotinylated second antibody, and avidin-biotin-peroxidase complex. All incubations were for 30 min with three 5-min washes in PBS containing 1% bovine serum albumin between each stage. Antibody binding was visualized with diamino-benzidine and the sections were then counterstained with hematoxylin. FITC-conjugated second antibodies were used for studying the distribution of bullous pemphigoid antigen (BPA) and for the cell culture studies and specimens examined using a Leitz Orthoplan fluorescence mi-

croscope. Controls for both tissue sections and cell cultures included replacing the primary antibody with non-immune serum or irrelevant antibodies and, for type I and type IV collagen, the use of antigen-absorbed antibodies.

RESULTS

Tissue Sections

Type I Collagen and Type III Collagen: The distribution of staining for type I and type III collagen was identical. Both antibodies stained interfollicular dermis and throughout the dermal sheath surrounding the hair follicle. Dermal papillae of anagen and catagen follicles displayed uniform dense staining of the extracellular matrix (Fig 1*a*). The residual extracellular matrix in dermal papillae of telogen follicles also stained for types I and III collagen (Fig 1*b*).

Type IV Collagen and Laminin: The staining patterns with antibodies to type IV collagen and laminin were similar. Both antibodies stained the outer root sheath basement membrane throughout the hair cycle. In dermal papillae of anagen follicles (Fig 1*c*), staining was evident throughout the extracellular matrix and was not confined to vascular structures within the papilla or the papilla-epithelial interface, although staining was accentuated in these sites in some follicles. This pattern was maintained during catagen (Fig 1*d*). In telogen follicles, the volume of the dermal papilla extracellular matrix was reduced but intercellular staining for laminin and type IV collagen was still apparent within the dermal papilla (Fig 1*e*). Staining of the basement membrane at the papilla-epithelial interface could also be discerned in telogen follicles.

Bullous Pemphigoid Antigen: There was linear staining for BPA of the outer root sheath basement membrane in continuity with staining of the epidermal basement membrane. In anagen follicles, staining extended to the lower tip of the hair bulb and sometimes around the neck of the dermal papilla but neither the basement membrane around the body of the dermal papilla nor the dermal papilla extracellular matrix stained for BPA (Fig 2*a*). However, in catagen and telogen follicles, staining for BPA became continuous around hair follicle epithelium with clear linear staining at the papilla-epithelial interface (Fig 2*b*).

Cell Cultures Cultured dermal papilla cells stained strongly with antibodies to type I and type III collagen (Fig 3*a,b*). Both antibodies also recognized fibrillar extracellular material. The staining pattern in two-week primary cultures was identical to that seen in passaged cells. Cytoplasmic staining for type IV collagen was seen in all eight dermal papilla cell lines (Fig 3*c*). Staining was most pronounced in cells spreading at the margins of cell aggregates. Fibrillar extracellular material was sometimes seen particularly in the region of cell aggregates. Six dermal papilla cell lines displayed cytoplasmic staining for laminin. This was concentrated in the perinuclear region and no extracellular staining was seen (Fig 3*d*). Six dermal fibroblast lines were also studied. All showed positive staining for type I collagen, type III collagen, type IV collagen, and laminin that was indistinguishable from that seen in dermal papilla cell cultures.

All controls were negative.

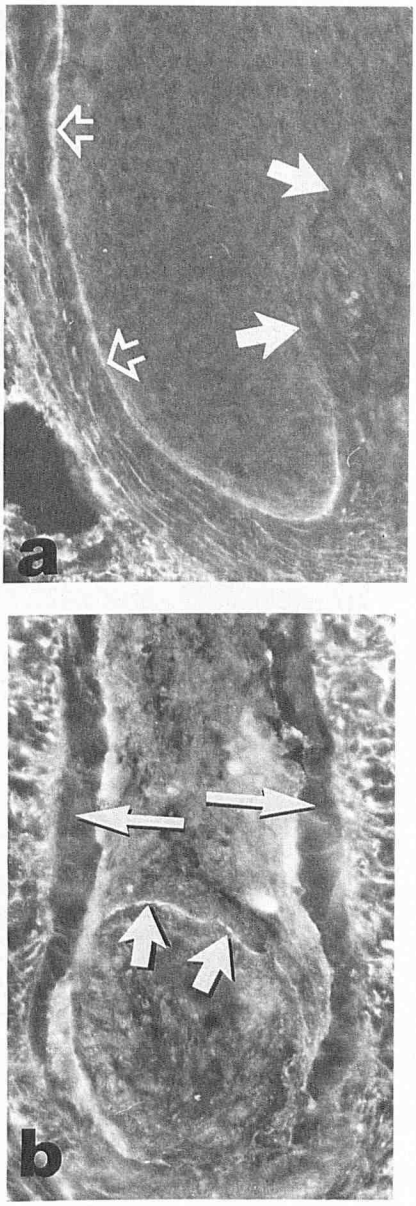

Figure 2. *a,* Anagen follicle: bullous pemphigoid antigen. There is linear staining of the outer root sheath basement membrane *(open arrows)* extending to the lower tip of the hair bulb and around the stalk of the dermal papilla. There is no staining of the epithelial basement membrane around the body of the dermal papilla *(closed arrows).* Immunofluorescence ×250; *b,* late catagen follicle: bullous pemphigoid antigen. Linear staining is seen at the interface between follicular epithelium and the dermal papilla *(short arrows).* There is a well-developed glassy membrane *(long arrows),* which is not stained. Immunofluorescence ×250.

DISCUSSION

Previous immunohistochemical studies have shown that the dermal papillae of rat pelage follicles contain type IV collagen, laminin, and other basement membrane components such as chondroitin-6-sulphate [12–14]. The papilla matrix in human follicles is also rich in type IV collagen and laminin, the distribution of which is not confined to sites such as the epithelial basement membrane and vascular structures. The source of this material is uncertain and it is possible that it derives from endothelia within the dermal papilla or from epithelial cells of the hair bulb matrix. However, interrupted linear electron-dense structures resembling basement membrane have been observed around the mesenchymal cell population in the dermal papilla [15], suggesting that these cells are capable of synthesizing basement membrane molecules.

Unlike type IV collagen and laminin, staining for BPA was limited to the hair follicle epithelial basement membrane. Moreover, the distribution of BPA staining in the hair follicle was not uniform and altered during the hair growth cycle. The absence of staining for BPA around developing hair bulbs has been reported in mouse and rat skin [13,16]. In adult human follicles, this phenomenon is restricted to the papilla-epithelial interface during anagen. It is possi-

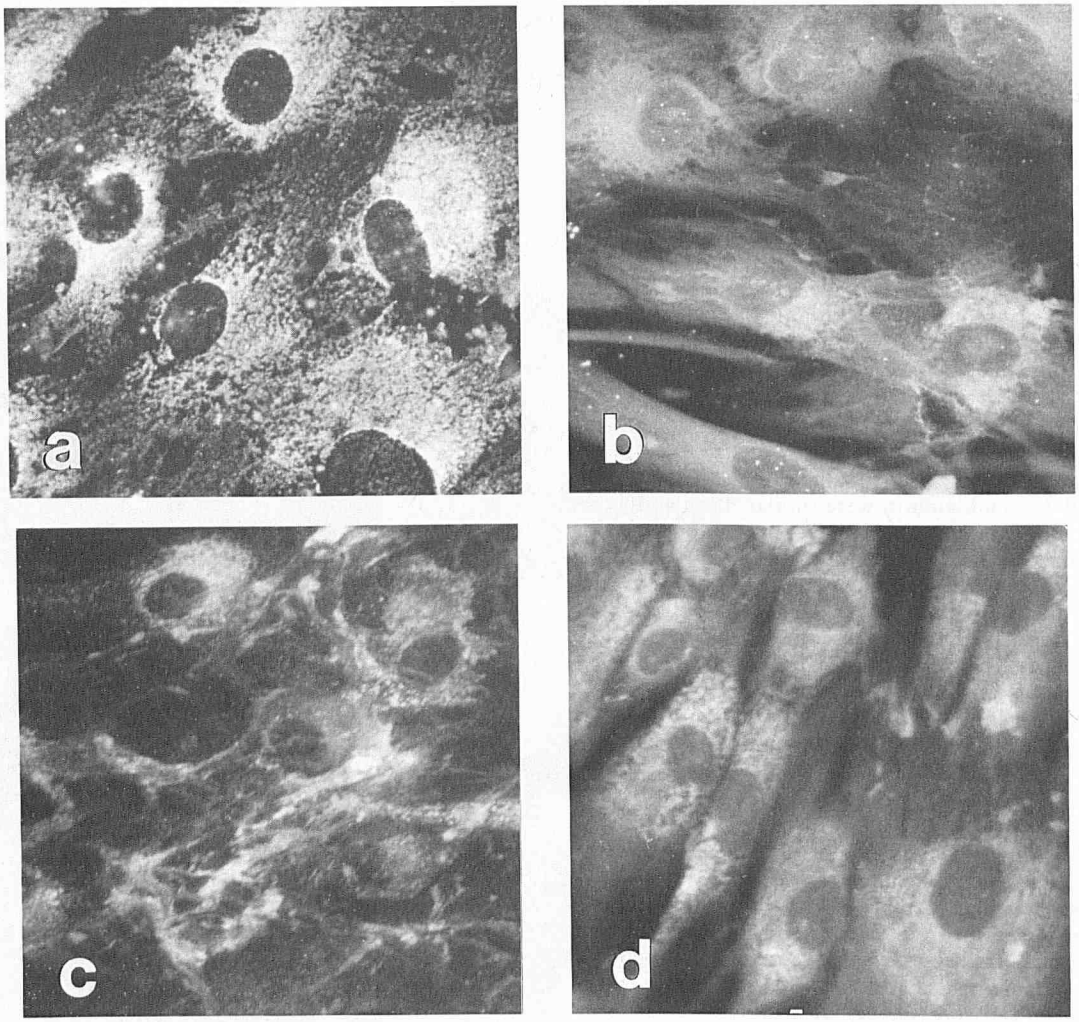

Figure 3. Immunofluroescence of cultured dermal papilla cells for *a*, type I collagen; *b*, type III collagen; *c*, type IV collagen, *d*, laminin. Magnification ×500.

ble that the absence of BPA staining around anagen dermal papillae is due to masking or degradation of the antigen. It seems more likely, however, that basal cells of the hair matrix epithelium do not express BPA. Bullous pemphigoid antigen is known to be synthesized by cultured epidermal keratinocytes [17,18], and is found in association with hemidesmosomes [19]. In the rat, the hair matrix epithelial basement membrane surrounding the dermal papilla does not contain hemidesmosomes [20]. There is little published information on this topic in human follicles. We have not observed hemidesmosomes around the dermal papilla in human scalp follicles by electronmicroscopy (unpublished observations), although they have been reported in this site in beard follicles [15]. Basement membranes have a variety of biologic functions, including structural organization and compartmentalization, the filtering of macromolecules, and the modulation of cell metabolism and differentiation [21]. The prominent expression of type IV collagen and laminin within the dermal papilla, particularly when compared with the absence of such expression in the interfollicular dermis, argues in favor of a significant role in dermal papilla function. However, the continued expression of basement membrane material in catagen papillae suggests that these molecules are not directly involved in regulating the growth of epithelial cells in the hair bulb matrix. In contrast, there is a clear change in immunoreactivity for BPA during the hair cycle. Whether this indicates a direct functional role for BPA in ordering epithelial differentiation in the hair bulb or is secondary to the changes in differentiative status of hair bulb epithelium during the hair growth cycle has yet to be determined.

Whereas there are striking similarities in the distribution and expression of basement membrane components in the dermal papillae of human scalp and rat pelage follicles, the same is not true of interstitial collagens. We found that human dermal papillae were strongly immunoreactive for type I and type III collagen throughout the hair growth cycle. Collagen fibers can be seen within the human dermal papilla at electronmicroscopy (although they are more sparse than in the interfollicular dermis and are not arranged into bundles) [15]. In contrast, Couchman reported that the papillae of rat pelage follicles do not stain for type I collagen and are only weakly positive for type III collagen [14]. Neither the function of collagen fibers in the dermal papilla nor the reason for this interspecies difference is known. However, it may be relevant that whereas the anatomy of human hair follicles is similar to that of rodent pelage follicles, their growth behavior is not identical, most notably in the very much longer anagen phase in scalp and beard skin.

Cultured dermal papilla cells show distinctive morphologic and behavioral properties [22,23]. The cells are more spread out than dermal fibroblasts and tend to arrange into aggregates, particularly when grown on collagen gel. Early passage cells cultured from rat whisker papillae also retain the ability to induce hair growth when reimplanted into the intact animal [24]. Rat papilla cells express type IV collagen and laminin but not type I and little type III collagen in

early primary culture, thus resembling the in vivo phenotype [14]. Human papilla cells also stain for type IV collagen and laminin and, unlike rat cells, this is unaffected by in vitro age in that the pattern of staining for these basement membrane molecules was maintained through several passages. Furthermore, human papilla cells express interstitial collagens from the outset of the primary culture. This observation is consistent with the difference in tissue staining patterns between human and rat dermal papillae. However, the specificity of our findings is questioned by the fact that identical staining patterns for interstitial collagens and basement membrane proteins were obtained when we studied interfollicular dermal fibroblasts. Whereas these cells are not generally regarded as being responsible for synthesizing basement membrane proteins in vivo, it has been shown that cultured dermal fibroblasts express type IV collagen and laminin genes [25] and release laminin into culture supernatants [26]. A further complication is introduced by the work of Katsuoka and colleagues [27]. Using biochemical techniques, they found that cultured dermal papilla cells produced a greater proportion of type III collagen than dermal fibroblasts, but they were unable to detect synthesis of type IV collagen by either cell type. These discrepancies may be due to differences in methodology or antibody specificities. However, it is clear that we need to characterize human dermal papilla cells more fully if we are to rely on culture techniques for analyzing dermal papilla function.

In conclusion, the similarity in the expression of basement membrane proteins in hair follicles from various mammalian species, including man, suggests an important role in dermal papilla function. The reason for the species differences in the expression of interstitial collagens is less clear, but may relate to variations in follicular growth characteristics. Work designed to characterize other extracellular matrix components in hair follicle mesenchyme and in cultured dermal papilla cells is currently in progress.

REFERENCES

1. Pinkus H: Embryology of Hair. In: Montagna W, Ellis RA (eds.). The Biology of Hair Growth. Academic Press, New York, 1958, pp 1–32
2. Oliver RF: The experimental induction of whisker growth in the hooded rat by implantation of dermal papillae. J Embryol Exp Morphol 18:43–51, 1967
3. Oliver RF: The induction of hair follicle formation in the adult hooded rat by vibrissa dermal papillae. J Embryol Exp Morphol 23:219–236, 1970
4. Kollar EJ: The induction of hair follicles by embryonic dermal papillae. J Invest Dermatol 55:374–378, 1970
5. Sengel P: The Morphogenesis of Skin. Cambridge University Press, Cambridge, 1976
6. Wessells NK, Roessner KD: Non-proliferation in dermal condensations of mouse vibrissae and pelage hairs. Dev Biol 12:419–433, 1965
7. Pierard GE, de la Brassinne M: Modulation of dermal cell activity during hair growth in the rat. J Cutan Pathol 2:35–41, 1975
8. Young RD: Morphological and ultrastructural aspects of the dermal papilla during the growth cycle of the vibrissal follicle in the rat. J Anat 131:355–365, 1980
9. Montagna W, Chase HB, Malone JD, Melaragno HP: Cyclic changes in the polysaccharides of the papilla of the hair follicle. Q J Microscop Sci 93:241–245, 1952
10. Buck CA, Horwitz AF: Cell surface receptors for extracellular matrix molecules. Annu Rev Cell Biol 3:179–205, 1987
11. Messenger AG: The culture of dermal papilla cells from human hair follicles. Br J Dermatol 110:685–689, 1984
12. Couchman JR, Gibson WT: Expression of basement membrane components through morphological changes in the hair growth cycle. Dev Biol 108:290–298, 1985
13. Westgate GE, Shaw DA, Harrap GJ, Couchman JR: Immunohistochemical localization of basement membrane components during hair follicle morphogenesis. J Invest Dermatol 82:259–264, 1984
14. Couchman JR: Rat hair follicle dermal papillae have an extracellular matrix containing basement membrane components. J Invest Dermatol 87:762–767, 1986
15. Hashimoto K, Shibazaki S: Ultrastructural study on differentiation and function of hair. In: Kobori T, Montagna W (eds.). Biology and Disease of Hair. University Park Press, Baltimore, pp 23–57
16. Bard S, Micouin C, Thivolet J, Sengel P: Heterogeneous distribution of bullous pemphigoid antigen during hair development in the mouse. Arch Anat Microscop Exp 70:141–148, 1981
17. Woodley D, Didierjean L, Regnier M, Saurat JH, Prunieras M: Bullous pemphigoid antigen synthesized *in vitro* by human epidermal cells. J Invest Dermatol 75:148–151, 1980
18. Stanley JR, Hawley-Nelson P, Yuspa SH, Shevach EM, Katz SI: Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. Cell 24:897–903, 1981
19. Mutasim DF, Takahashi Y, Labib RS, Anhalt GJ, Patel HP, Diaz LA: A pool of bullous pemphigoid antigen(s) is intracellular and associated with the basal cell cytoskeleton-hemidesmosome complex. J Invest Dermatol 84:47–53, 1985
20. Hardy MH, Van Exan RJ, Sonstegard KS, Sweeny PR: Basal lamina changes during tissue interactions in hair follicles—an *in vitro* study of normal dermal papillae and Vitamin A-induced glandular morphogenesis. J Invest Dermatol 80:27–34, 1983
21. Timpl R, Dziadek M: Structure, development and molecular pathology of basement membranes. Int Rev Exp Pathol 29:1–112, 1986
22. Jahoda CAB, Oliver RF: Vibrissa dermal papilla aggregative behaviour *in vivo* and *in vitro*. J Embryol Exp Morphol 79:211–224, 1984
23. Messenger AG, Senior HJ, Bleehen SS: The *in vitro* properties of dermal papilla cell lines established from human hair follicles. Br J Dermatol 114:425–430, 1986
24. Jahoda CAB, Horne KA, Oliver RF: The induction of hair growth by implantation of cultured dermal papilla cells. Nature 311:560–562, 1984
25. Olsen DR, Chu M, Uitto J: Expression of basement membrane zone genes coding for type IV procollagen and laminin by human skin fibroblasts *in vitro:* elevated $\alpha 1$ (IV) collagen mRNA levels in lipoid proteinosis. J Invest Dermatol 90:734–738, 1988
26. Woodley DT, Stanley JR, Reese MJ, O'Keefe EJ: Human dermal fibroblasts synthesize laminin. J Invest Dermatol 90:679–683, 1988
27. Katsuoka K, Mauch C, Schell H, Hornstein OP, Krieg T: Collagen-type synthesis in human-hair papilla cells in culture. Arch Dermatol Res 280:140–144, 1988