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Immunochemical Analysis of the Distribution of the Desmosomal Protein Desmoglein I in Different Layers of Plantar Epidermis

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An antiserum raised against the bovine desmosomal protein desmoglein I (DG I), M_r approximately 160 kDa, was used in an immunochemical analysis of human plantar epidermis. Different layers of the tissue were prepared by means of horizontal freeze sectioning. Loosely attached surface layers were obtained by means of scraping of the skin surface with a scalpel. Tissue extracts were analysed by means of sodium dodecylsulphate polyacrylamide gel electrophoresis followed by immunoblotting. Significant amounts of a component with M_r approximately 160 kDa, reactive with the DG I-antiserum, were found in all layers except the loosely attached surface layers. In these layers the antiserum detected a component with M_r approximately 80 kDa, not found in other layers. This component may be a degradation product of DG I. Since DG I belongs to the group of transmembrane desmosomal proteins that is believed to constitute the link between the intracellular parts of desmosomes of opposing cells, it is concluded that desmosomes may play an important role in plantar stratum corneum cell cohesion, and that degradation of desmosomes may be an important step in desquamation in plantar epidermis.

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The stratum corneum serves as the physico-chemical barrier between the interior and exterior of the body. The pronounced mechanical resistance of the stratum corneum emanates from its building blocks, the corneocytes. With their tightly packed keratin fibres surrounded by an envelope of cross-linked proteins, these cells are highly adapted to their function (1). A question that is still without a definitive answer concerns the mechanisms by which the properties of individual corneocytes are transferred to the tissue as a functioning unit, i.e. mechanisms of intercellular cohesion in the stratum corneum. There are a number of structures and anatomical relationships that may be of importance. The folded nature of the cell surfaces (2), most pronounced in palmo-plantar stratum corneum, serves to increase the intercellular contact area. The lipid-rich intercellular substance (3, 4), and intercellular glycoproteins (5) may contribute to cell cohesion.

The extent to which desmosomes contribute to cell cohesion in the stratum corneum remains unclear. In viable epithelia the cohesive function of desmosomes is well established (6). During cornification, some desmosomes are probably degraded (7, 8, 9), leading to an apparent decrease in the number of desmosome-related structures in the intercellular space. This decrease is found mainly in non-palmoplantar skin (7). In palmo-plantar stratum corneum, as compared with viable epidermal layers, the intercellular space appears to contain a relatively unchanged number of structures resembling desmosomal plates in the electron microscope (10, 11). Correspondingly, a smaller fraction of the intercellular space of this tissue is occupied by lipid-rich material, compared with stratum corneum at other body sites. At the skin surface, where corneocytes dissociate and are shed, remaining desmosomes may be expected to have lost most of their cohesive capacity. The question then arises where this loss of desmosome cohesive function occurs, whether in the transition between granular and cornified layers, during the migration of the cells from the deepest layers of the stratum corneum towards the skin surface, or in direct association with desquamation.

Questions concerning mechanisms of cell cohesion in the stratum corneum are intimately related to the elucidation of the mechanisms of desquamation. A well-regulated desquamation is a prerequisite for an epidermal steady state. The shedding of cells at the skin surface may be expected to be a complicated process involving the interaction of several different mechanisms. The metabolism of intercellular lipids is most likely important (4), as is illustrated by such findings as in X-linked ichthyosis (12, 13).

This study attempted to elucidate the functional

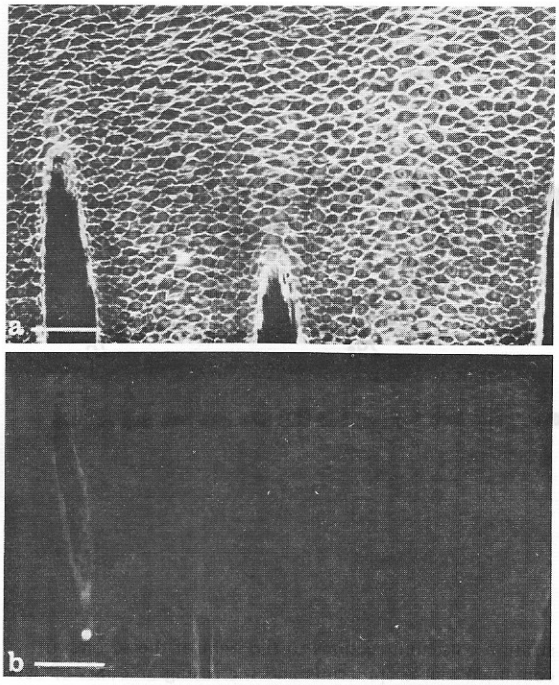

Fig. 1. Characterization of anti-DGI by immunofluorescence microscopy. 10-µm freeze-cut sections of bovine snout epithelium, fixed in acetone, were stained with rabbit serum diluted 1 : 50, followed by fluorescein isothiocyanate-conjugated swine anti-rabbit immunoglobulins (Dakopatts, Copenhagen, Denmark) diluted 1 : 20. *a*, Anti-DGI; *b*, preimmune serum. Bars = 100 µm.

state of desmosomes in plantar stratum corneum by means of immunochemical analysis of the distribution of the desmosomal protein desmoglein I (DGI) in different layers of plantar epidermis.

MATERIALS AND METHODS

Rabbit antiserum to DGI (anti-DGI)

DGI was purified by preparative sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) of bovine desmosomes prepared *ad modum* Gorbsky et al. (14). Excised gel slices containing DGI were homogenized in complete Freund's adjuvant, and injected subcutaneously into a rabbit. Booster doses were given after 4 and 8 weeks. The rabbit was bled before the first immunization and after 10 weeks. In Western blot analysis (15) of bovine desmosomes the antiserum reacted with DGI, but not with other desmosomal proteins or cytokeratins. In immunofluorescence microscopy it stained the cell periphery in bovine muzzle epidermis, but not intracellular structures (Fig. 1). A further description of anti-DGI is given elsewhere (A. Lundström & T. Egelrud, submitted for publication).

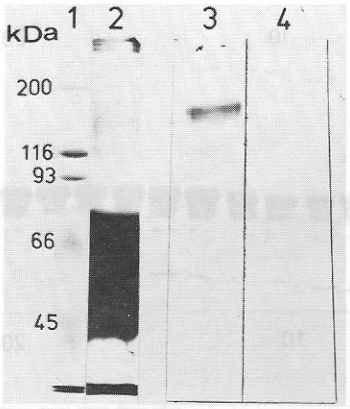

Fig. 2. Characterization of anti-DGI by immunoblotting. *1*, Molecular weight markers; *2–4*, identical extracts of cohesive stratum corneum, Coomassie blue stained SDS–PAGE (*2*), immunoblots with anti-DGI (*3*) and preimmune serum (*4*).

Whole plantar epidermis

Punch biopsies (4 mm) were taken from the central, weight-bearing parts of the heels of 8 male corpses, 20–60 years of age, that had been submitted to the Department of Forensic Medicine for autopsy. All had apparently normal skin and had not been bedridden. Biopsies were taken within 24 hours of death.

Plantar stratum corneum

Partially desquamated cells were collected by firmly scraping the central, weight-bearing parts of the heels of healthy male volunteers with a scalpel. Cohesive plantar stratum corneum was obtained with a skin transplantation knife as thin slices (approximately 0.25–0.35 mm) from the same areas. In experiments performed in order to obtain a quantitative estimate of the 'functionally desquamated' versus the cohesive layers of the tissue, unscraped slices of known thickness were soaked in phosphate-buffered saline for 5 min at room temperature. The surface that had faced outwards was then firmly scraped with a scalpel and the scrapings collected. The alkali-soluble protein in the scrapings and the remaining tissue slice was quantified according to Lowry et al. (16) as described earlier (17).

Serial sectioning

The 8 punch biopsies were analyzed after horizontal serial sectioning of the entire epidermis. The biopsies were frozen on dry ice, care being taken to obtain horizontal upper surfaces. Serial 30 µm sections, parallel to the skin surface, were prepared in a cryostat, the biopsies being mounted with the skin surface towards the knife. Every third section was mounted for phase contrast microscopy. The remaining sections were extracted separately for immunoelectrophoretic analysis.

SDS–PAGE and immunoblotting

The tissues were extracted in a buffer containing 0.1 M Tris-HCl, pH 9, 9 M urea, 2% SDS and 1% (v/v) β-mercaptoethanol. Plantar stratum corneum (20 mg/ml) or 30 µm freeze-

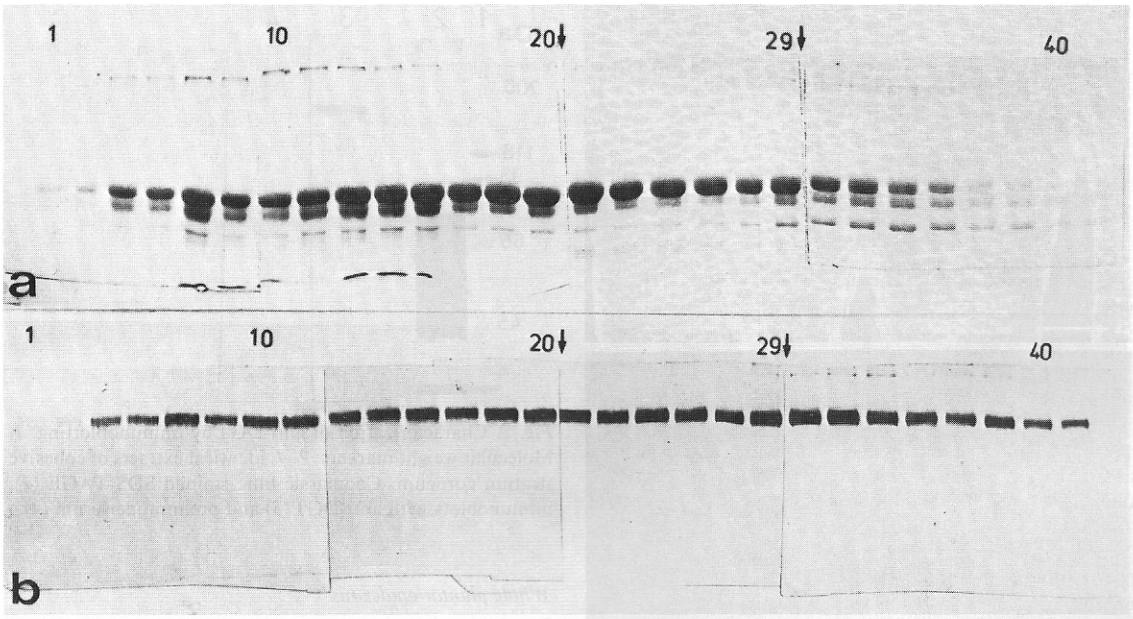

Fig. 3. Analysis of serial sections of plantar epidermis with SDS–PAGE and immunoblotting with anti-DG I. *a*, Coomassie Blue-stained SDS–PAGE gels; *b*, immunoblots with anti-DG I of gels run in the same way as those shown in *a*. *Numbers:* section numbers (skin surface to the left). *Arrows:* the sections to the left of the first arrow contained only stratum corneum, sections to the right of the second arrow contained viable epidermis and increasing amounts of dermis. Sections between the arrows contained decreasing amounts of stratum corneum and increasing amounts of viable epidermis. The first few sections from close to the skin surface contained only small fragments of tissue.

cut sections (50 µl/section) were incubated in this buffer for 15 h at 37°C. The extracts were prepared for SDS–PAGE by the addition of 0.67 volumes of a modified sample buffer (sample buffer *ad modum* Laemmli (18) with 2.5-fold higher concentrations of solutes), heated over a boiling water bath for 2 min, and centrifuged at 5000 *g* for 5 min. Analytical SDS–PAGE in 7.5% gels was performed *a.m.* Laemmli (18) with the Bio-Rad Minigel equipment (Bio-Rad, Richmond, Calif.) at 200 V. In all experiments two identical gels were run; one was stained with Coomassie Blue and dried between sheets of cellophane, the other gel was used for immunoblotting. The handling of gels and immunoblots was carried out in a standardized manner. Electrophoretic transfer of proteins separated by SDS–PAGE to nitrocellulose membranes (15) was carried out in a Semi-Dry Electroblotter A (Ancos, Ölstykke, Denmark), following the instructions supplied by the manufacturer. Immunostaining was performed with Tween 20 as blocking agent (19). Anti-DG I was used in dilution 1:1000. Alkaline phosphatase-conjugated goat anti-rabbit immunoglobulins (Bio-Rad) in dilution 1:3000 was used as second antibody. Bound conjugates were detected with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Bio-Rad) as substrates (20). A Shimadzu dual wavelength flying-spot scanner CS-9000 (Shimadzu, Kyoto, Japan) was used at 560 nm for densitometric scanning of dried SDS–PAGE gels (single beam, transmitted light) and immunoblots (reflected light, zig-zag mode).

RESULTS

Brief description of tissue

Epidermis from under the heels of non-bedridden individuals is subject to pronounced pressure as well as tensive and frictional forces. This leads to a hyperplastic response of the tissue, resulting in an elongation of rete ridges and the production of a stratum corneum which can achieve a thickness of 0.5–1 mm. While the major part of the stratum corneum is highly resistant to shearing forces, there is a thin layer of surface cells that may be described as 'functionally desquamated'. These surface cells can be removed by, e.g. moderate scraping with a scalpel. This is facilitated if the tissue has been briefly soaked in saline. In three pieces of plantar stratum corneum 0.22 mm, 0.30 mm and 0.35 mm thick, 7%, 5%, and 4%, respectively of the total protein was found in the cells loosely attached to the surface that had faced outwards in vivo. This would imply that the thickness of the functionally desquamated layers is around 15 µm.

Although not as pronounced as the dermo-epidermal junction, the transition zone between viable and

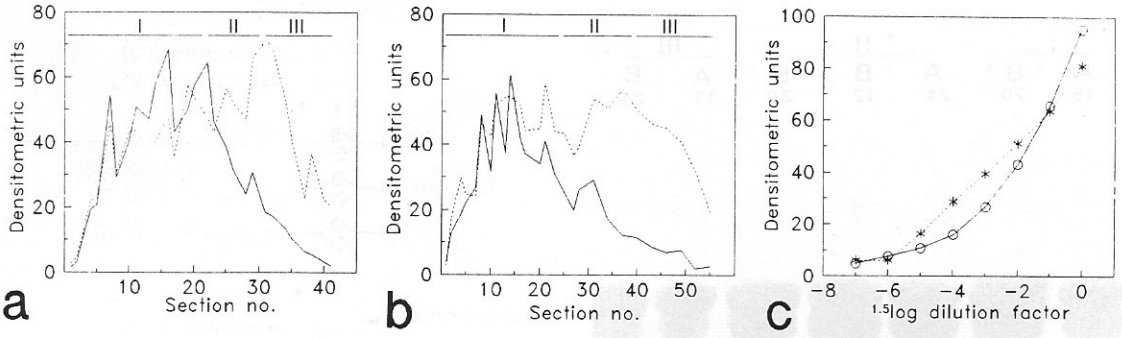

Fig. 4. Densitometric analysis of SDS–PAGE gels and immunoblots with anti-DGI. (*a*) Results from experiment presented in Fig. 3. (*b*) Results from a similar experiment with a biopsy from another individual. *I*, Sections containing only stratum corneum; *II*, sections containing decreasing amounts of stratum corneum and increasing amounts of viable epidermis; *III*, sections containing viable epidermis and dermis. ——, Total density obtained by scanning the distance between 70 and 40 kDa of lanes of Coomassie Blue-stained gels with transmitted light at 560 nm. – – –, Density of the peak at approx. 160 kDa obtained by scanning lanes of immunoblots with anti-DGI with reflected light at 560 nm. (*c*) Effect of varying the amount of material applied on the recorded values of densitometric analyses of an SDS–PAGE gel and the corresponding immunoblot with anti-DGI. Extracts of horizontal sections from the transition zone between cornified and viable layers of a biopsy of plantar epidermis were pooled and serially diluted with a factor of 1.5 with sample buffer. The diluted samples were subjected to SDS–PAGE and immunoblotting with anti-DGI as described in the text. The densitometric analysis was performed as described for (*a*) and (*b*). O——O, Coomassie Blue-stained lanes; *——* immunoblot.

cornified layers is undulating. The skin surface has also a wavy appearance. This means that a horizontal section of the epidermis at a given level will contain a mixture of cells that have migrated different distances from the basal layers and thus are in various stages of differentiation (the cells in the stratum corneum also undergo changes during their migration towards the skin surface (21). It also means that the transition between viable and cornified epidermal layers does not take place within one section. Instead there will be a gradual increase in the amount of viable cells in consecutive sections from the transition zone when the tissue is horizontally sectioned from the skin surface towards the dermis.

Distribution of DGI in plantar epidermis

The rabbit antiserum anti-DGI (but not the preimmune serum) detected a component in extracts of plantar stratum corneum with a molecular weight of around 160 kDa (Fig. 2). The electrophoretic mobility of this component could not be distinguished from the electrophoretic mobility of a similar component in viable plantar epidermis reacting with anti-DGI (see Fig. 3) or from DGI of bovine desmosomes used for immunization (not shown).

In the experiments presented in Figs. 3, 4 *a*, *b*, serial 30 µm horizontal sections of plantar epidermis, from the skin surface towards the dermis, were analyzed with SDS–PAGE and immunoblotting with anti-DGI, followed by densitometric scanning of Coomassie Blue-stained gels and immunoblots. Fig. 4 *c* shows the scanning densities as functions of relative concentrations of total protein (SDS–PAGE gels) and DGI (immunoblots). Every third section was examined by light microscopy. All sections were treated in a standardized manner before electrophoresis and the same amounts of extract were applied to the gels for all sections (i.e. the densitometric value for a lane containing the extract from a given section may be taken as a semi-quantitative estimate of the amount of total protein, mainly cytokeratins, or DGI in that section). From the results the following statements can be made: 1) The sections from the stratum corneum and the upper layers of viable epidermis contained more cytokeratins than the sections from deeper epidermal layers; 2) A DGI-like component was found in all sections; 3) In relation to the cytokeratin content the amount of DGI was highest in deeper epidermal layers; 4) There appeared to be no great differences in the amounts of DGI between sections from lower layers of the stratum corneum and sections from the uppermost parts of viable epidermis; and 5) If there was indeed a decrease in DGI concentration in relation to cytokeratin content in sections from superficial parts of the stratum corneum as compared with

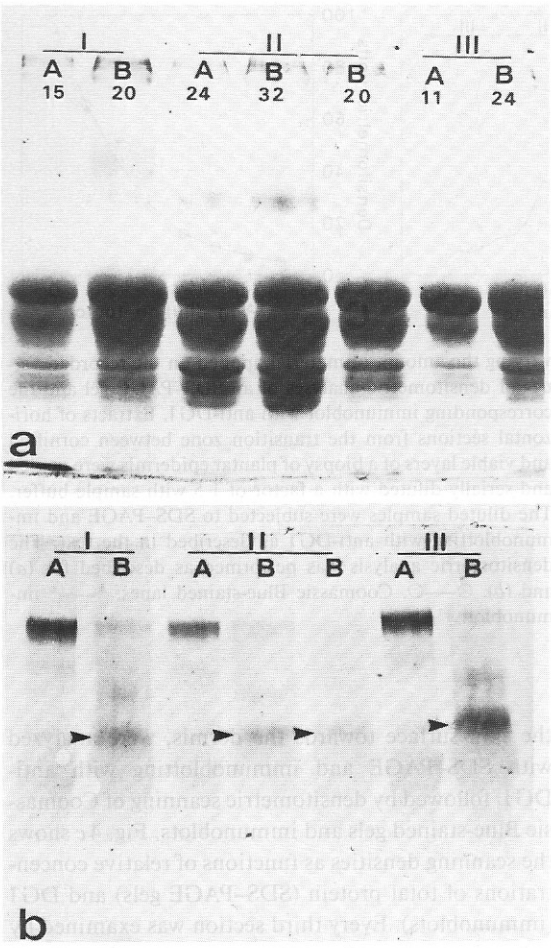

Fig. 5. Analysis of cohesive and "functionally desquamated" plantar stratum corneum with SDS–PAGE and immunoblotting with anti-DGI. The tissues were obtained and prepared as described in the text. (*a*) Coomassie Blue-stained gel; (*b*) immunoblot. Roman numerals refer to different individuals. (*A*) Cohesive tissue; (*B*) tissue obtained by means of scraping the skin surface with a scalpel. Arabic numerals in a depict densitometric units obtained as described in the text to Fig. 4 for each lane. Arrowheads in (*b*) indicate the putative degradation product of DGI with molecular weight approx. 80 kDa found only in extracts of loosely attached surface cells.

deeper cornified layers, these changes were too small to be detected in the present experiments.

As mentioned above, a strict separation in the stratum corneum between the superficial, 'functionally desquamated' cell layers and layers with cohesive cells cannot be achieved by horizontal sectioning. Loosely attached surface cells can, however, be collected by firmly scraping the skin surface with a scalpel. Fig. 5 shows the results of an experiment in which

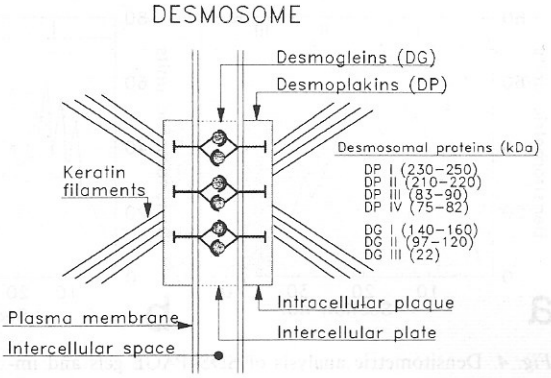

Fig. 6. Schematic presentation of current concepts of desmosomal structure.

surface cells—collected by scraping—and cohesive layers of the stratum corneum—obtained by means of cutting with a transplantation knife—from the central parts of the heels of three different individuals, were analysed by SDS–PAGE and immunoblotting with anti-DGI. The protein concentration of the extracts was adjusted so that most of the extracts of surface cells would contain more total protein than extracts of cohesive tissue (Fig. 5 *a*). In spite of this, a DGI-like component could be detected in all extracts of cohesive tissue, whereas this component was undetectable or barely detectable in extracts of surface cells (Fig. 5 *b*). Instead, these extracts contained a component reactive with anti-DGI, not found in cohesive tissue, with a molecular weight of around 80 kDa.

DISCUSSION

A schematic summary of current concepts concerning desmosomal structure is outlined in Fig. 6. DGI is a transmembrane glycoprotein whose cytoplasmic parts are anchored in the intracellular desmosomal plaque. Its extracellular parts are major constituents of the intercellular desmosomal plate (22, 23, 24). This structure is the result of the interaction of the desmosomal proteins of opposing cells and thus the part of the desmosome that mechanically links together two nearby cells. In electron micrographs numerous structures resembling desmosomal plates are found throughout palmo-plantar stratum corneum (10, 11). Theoretically, these structures could be made up of the extracellular parts of partially degraded desmogleins. Thus they could represent desmosomal remnants (25) with no remaining cohesive capacity. If, on the other hand, significant amounts of intact desmog-

leins can be found in the stratum corneum, it would strongly support the idea of an important role for desmosomes in stratum corneum cell cohesion.

A major conclusion of this work is that there were significant amounts of DGI in all layers of plantar epidermis except for the most superficial, 'functionally desquamated' layers of the stratum corneum, where possible degradation products of DGI were found. In relation to cytokeratin content, the concentration of DGI was greatest in the deeper layers of viable epidermis. This was mainly due to a low concentration of cytokeratins in these layers. On the basis of the present data it was not possible to decide whether or not there was a decrease in total tissue concentration of DGI during the transition between living and cornified layers. Nor was it possible to determine whether there was a continuous degradation of DGI within the stratum corneum or whether the apparent degradation that could be demonstrated in the loosely attached surface layers had occurred abruptly during the process of cell shedding. A more detailed analysis is needed to answer these questions. A major problem in this type of analysis is caused by the non-horizontal arrangement of the different tissue layers.

The present results are compatible with the thesis that desmosomes play a significant role in the cohesion of cells in plantar stratum corneum. They also suggest that a degradation of proteins of importance for the adhesive function of desmosomes, may be an important step in desquamation. This agrees well with our recent reports that trypsin treatment of deeper layers of plantar stratum corneum leads to an apparent degradation of the intercellular plates of desmosomes and cell dissociation (26), and that a unipolar shedding of cells from plantar stratum corneum in vitro is dependent on endogenous proteolysis (17).

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REFERENCES

1. Odland GF. Structure of the skin. In: Goldsmith LA, ed. Biochemistry and Physiology of the Skin. New York, Oxford: Oxford University Press, 1983; 1: 3–63.
2. Hashimoto K, Kanzaki T. Surface ultrastructure of human skin. Acta Derm Venereol (Stock) 1975; 55: 413–430.
3. Elias PM. Epidermal lipids, barrier function, and desquamation. J Invest Dermatol 1983; 80 (Suppl.): 44s–49s.
4. Williams ML, Elias PM. The extracellular matrix of stratum corneum: role of lipids in normal and pathological function. CRC Critical Reviews in Therapeutic Drug Carrier Systems 1987; 3: 95–122.
5. Brysk MM, Rajaraman S, Penn P, Barlow E. Glycoproteins modulate adhesion in terminally differentiated keratinocytes. Cell Tissue Res 1988; 253: 657–663.
6. Staehlin LA. Structure and function of intercellular junctions. Int Rev Cytol 1974; 39: 191–283.
7. Allen TD, Potten CS. Desmosomal form, fate, and function in mammalian epidermis. J Ultrastruct Res 1975; 51: 94–104.
8. King IA, Tabiowo A, Fryer PR. Evidence that major 78–44 kD concanavalin A-binding glycopolypeptides in pig epidermis arise from the degradation of desmosomal glycoproteins during terminal differentiation. J Cell Biol 1987; 105: 3053–3063.
9. King IA, Wood MJ, Fryer PR. Desmoglein II-derived glycopeptides in human epidermis. J Invest Dermatol 1989; 92: 22–26.
10. Selby CC. An electron microscope study of thin sections of human skin. II. Superficial cell layers of footpad epidermis. J Invest Dermatol 1957; 29: 131–149.
11. Brody I. Electron microscopy of normal human plantar epidermis. I. Strata lucidum and corneum. Z Mikrosk Anat Forsch 1972; 86: 305–331.
12. Shapiro LJ, Weiss R, Webster D, France JT. X-linked ichthyosis due to steroid-sulphatase deficiency. Lancet 1978; 1: 70–72.
13. Epstein EH Jr, Williams ML, Elias PM. Steroid sulfatase, X-linked ichthyosis, and stratum corneum cell cohesion. Arch Dermatol 1981; 117: 761–763.
14. Gorbsky G, Cohen SM, Shida H, Giudice GJ, Steinberg MS. Isolation of the non-glycosylated proteins of desmosomes and immunolocalization of a third plaque protein: Desmoplakin III. Proc Natl Acad Sci USA 1985; 82: 810–814.
15. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; 76: 4350–4354.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265–275.
17. Lundström A, Egelrud T. Cell shedding from human plantar skin in vitro: Evidence of its dependence on endogenous proteolysis. J Invest Dermatol 1988; 91: 340–343.
18. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680–685.
19. DeBlas AL, Cherwinski HM. Detection of antigens on nitrocellulose paper immunoblots with monoclonal antibodies. Anal Biochem 1983; 133: 214–219.
20. Blake MS, Johnston KH, Russel-Jones GI, Gotschlich EC. A rapid method for detection of alkaline phospha-

tase-conjugated anti-antibody on Western blots. Anal Biochem 1984; 136: 175–179.
21. Egelrud T, Lundström A. Stepwise modifications of keratin polypeptides during keratinization in palmar–plantar epidermis. Acta Derm Venereol (Stockh) 1989; 96: 105–110.
22. Skerrow CJ. Desmosomal proteins. In: Bereiter-Hahn J, Matoltsy AG, Sylvia Richards K, eds. Biology of the Integument. Berlin: Springer Verlag, 1986; 2: 762–787.
23. Schmelz M, Duden R, Cowin P, Franke WW. A constitutive transmembrane glycoprotein of Mr 165000 (desmoglein) in epidermal and non-epidermal desmosomes. II. Immunolocalization and microinjection studies. Eur J Cell Biol 1986; 42: 184–199.
24. Steinberg MS, Shida H, Giudice GJ, Shida M, Patel NH, Blaschuk OW. On the molecular organization, diversity and functions of desmosomal proteins. In: Junctional complexes of epithelial cells. Ciba Foundation Symposium. Chichester: Wiley, 1986; 3–25.
25. Elias PM. Epidermal lipids, membranes, and keratinization. Int J Dermatol 1981; 20: 1–19.
26. Egelrud T, Hofer P-Å, Lundström A. Proteolytic degradation of desmosomes in plantar stratum corneum leads to cell dissociation in vitro. Acta Derm Venereol (Stockh) 1988; 68: 93–97.