Expression of Distinct Desmocollin Isoforms in Human Epidermis

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Previous evidence suggested the presence of two distinct desmocollin isoforms in human epidermis. These isoforms have now been distinguished at the protein level using monoclonal and polyclonal antibodies against N-terminal fragments of desmosomal glycoprotein (DG) IV/V isolated from plantar callus and antibodies against a fusion protein containing the extracellular domain of DGII/III.

Immune blotting of glycoprotein fractions from whole epidermis, plantar callus, psoriatic scales and cultured keratinocytes showed that intact DGIV/V and its proteolytic fragments consistently migrated faster than DGII/III during SDS-PAGE. The apparent Mr difference between the two isoforms was in the range 2–5 kD. DGIV/V was the predominant species in epidermal tissue but was much less prominent in cultured cells by immune-blotting and immune precipitation. This is consistent with the differentiation-related expression of desmocollins revealed by immunofluorescence. DGIV/V was strongly expressed in the upper spinous/granular layer of the epidermis whereas DGII/III was more prominent in the basal layers of the tissue. The DGIV/V monoclonal (LH50) recognized an N-terminal, Ca^{++}-sensitive epitope, because its staining of unfixed epidermal tissue was markedly influenced by Ca^{++} levels. Ca^{++} inhibition was observed at concentrations as low as 50 μM, suggesting its possible physiologic significance. Ca^{++} inhibition of LH50 binding was also observed in an enzyme-linked immunosorbent assay system using denatured glycoproteins although higher concentrations were required. It remains to be seen whether direct effects of Ca^{++} on desmocollin conformation are involved in the regulation of keratinization by extracellular Ca^{++}. *J Invest Dermatol 100:373–379, 1993*

The desmosome is a cell-surface organelle involved in the maintenance of cell-cell adhesion within most, if not all, epithelial tissues. It is now becoming increasingly clear that desmosome structure can vary both within individual tissues and between different types of epithelia. Thus there are significant differences in the molecular composition of the desmosomal plaque in simple and stratified epithelia [1–3]. However, there is less information about the intercellular glycoproteins that might be expected to play a more direct role in the adhesive process.

Most biochemical studies of desmosomal glycoproteins have utilized subcellular fractions from bovine muzzle epidermis [4–8] and revealed the presence of two major glycoprotein components — the desmogleins and the desmocollins. A number of immunologic observations hinted at possible species and tissue heterogeneity for these glycoproteins particularly for the desmocollins. Thus polyclonal antibodies against bovine desmocollin precipitated glycoproteins of varying Mr from stratifying and non-stratifying epithelial cells [8]. Giudice *et al* [9] found that similar antibodies reacted with desmosomal glycoproteins in bovine epidermis, cornea, and esophagus although there were differences in apparent Mr. However, only one of four monoclonals recognized desmocollins in all three stratified epithelia. Cowin *et al* [10] reported that monoclonals against bovine desmocollin did not stain desmosomes in simple epithelial tissues, Parrish *et al* [11] described polyclonal anti-desmocollin antibodies that recognized desmosomes in meninges but only stained suprabasal desmosomes in epidermis.

cDNA clones encoding bovine desmocollin have recently been isolated by several groups and independent sequence analysis has shown homology with the cadherin family of cell-adhesion molecules [12–14]. The desmocollin gene transcript is subject to alternative splicing resulting in two messages, one of which contains a premature stop codon, and this accounts for the presence of two desmocollin polypeptides (apparent Mr about 115 and 100 kD) on sodium dodecylsulfate (SDS) gels of desmosomes from tissue and in cultured cell extracts. Immune precipitation of MDCK cells has suggested the presence of a third possible variant probably also differing in its cytoplasmic domain [15].

In a previous study we raised polyclonal antibodies against major 46/48-kD glycopeptides from plantar callus [16] and showed that these fragments arose from the degradation of a desmocollin-like glycoprotein during terminal differentiation [17]. Direct amino acid sequencing revealed considerable homology with bovine desmocollin and suggested that these 46/48-kD fragments comprised the most N-terminal portion of intact human desmocollin [18]. However these amino acid sequences were not found in the human

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Abbreviations:

BSA: bovine serum albumin
Con A: concanavalin A
DG: desmosomal glycoprotein
EDTA: ethylenediaminetetraacetic acid
ELISA: enzyme-linked immunosorbent assay
IgG: immunoglobulin G
PBS: phosphate-buffered saline, 8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4/13.7 mM NaCl/2.7 mM KCl, pH 7.4
SDS-PAGE: sodium dodecylsulfate–polyacrylamide gel electrophoresis

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desmocollin clone isolated from a human keratinocyte cDNA library [19]. On the basis of these observations we suggested that human epidermis contained two similar, but genetically distinct, desmocollin isoforms and presented preliminary evidence that these were differentially expressed in the epidermis [18]. The human clones that were originally isolated represented one desmocollin gene that produced DGII and DGIII polypeptides by alternative splicing and this gene has now been localized to chromosome 9 [20]. The other desmocollin isoform was represented by the related, but distinct, polypeptides DGIV and DGV, which were degraded to 46- and 48-kD glycopeptides during terminal differentiation. cDNA clones encoding human DGIV/V have now been isolated (I.A. King, unpublished data) and show similarity to the desmocollin clones originally isolated from bovine epidermis. A second bovine desmocollin equivalent to human DGII/III has recently been described [21].

We have now examined the expression of these distinct desmocollin isoforms in human epidermis using polyclonal and monoclonal antibodies generated against the 46/48-kD fragments of DGIV/V and a polyclonal antibody against the extracellular region of DGII/III. With these reagents we can clearly distinguish between DGII/III and DGIV/V polypeptide chains in both epidermal tissue and epidermal cells.

MATERIALS AND METHODS

Isolation of Concanavalin A (Con A)-Binding Glycoproteins Glycoproteins were isolated from pooled human plantar callus and psoriatic scales as described previously [16] except that sodium deoxycholate was omitted from the extraction and dialysis buffers. Omission of detergent had no significant effect on the overall yield or protein composition of the isolated material. Dialyzed extracts were subjected to one cycle of freeze thawing (−70°C) to precipitate keratins, which were removed by centrifugation, before application to the Con A column. Human leg skin was obtained from amputation specimens and was separated into epidermal and dermal fractions after brief (20 to 30 seconds) immersion in water at 55 to 60°C. Con A-binding glycoproteins were isolated from leg epidermis and from cultured cells as for plantar callus except that 1% sodium deoxycholate was included (to ensure recovery of immunoreactive membrane-bound components) and the freeze-thawing step was omitted.

Antibodies Polyclonal antisera against the 46/48-kD band from plantar callus were raised in rabbits as described previously [16].

The monoclonal antibody LH50 directed against human DGIV/V was generated essentially according to Kohler and Milstein [22] from mice immunized with total Con A-binding glycoproteins from plantar callus. Mice received intra-peritoneal injections of 50 μg of protein and were boosted intravenously 4 d before fusion. In a second fusion mice were immunized with electrophoretically purified 46/48-kD glycopeptides from plantar callus and this generated three additional monoclonals with similar immunochemical properties to LH50. Splenocytes from positive mice were fused with SP2/AGO/14 cells using 50% polyethylene glycol. Fusion products were plated out [23] in 96-well plates in azaserine-hypoxanthine selection medium (Sigma) and after 2 weeks were screened by an enzyme-linked immunosorbent assay (ELISA) assay using microtitre plates coated with callus glycoproteins [24]. Positive wells were expanded, checked for reaction with callus glycoproteins by immunoblotting, and cloned by limiting dilution in the presence of splenocyte feeder cells. Clones were rescreened, expanded, and cloned again and hybridoma supernatant was removed for further analysis. LH50 was identified as an immunoglobulin G (IgG) using the Amersham isotyping kit.

Polyclonal antibodies against human DGII/III were raised in rabbits using a 2084-bp fragment of the recombinant extracellular region expressed as a TrpE fusion protein in the pATH 2 expression vector [25].

SDS-Polyacrylamide Gel Electrophoresis (PAGE) and Immunoblotting Electrophoresis was usually performed on 8% polyacrylamide [26] and proteins were transferred to nitrocellulose [27] and stained with amido black as before [17]. Immunostaining was as described previously [17] except that horseradish peroxidase-conjugated second antibody and 4-chloro-1-napthol substrate (BioRad) were substituted for ^{125}I-labeled protein A. Gels containing ^{35}S-labeled proteins were stained with coomassie blue, destained, and processed by fluorography.

Immunofluorescence Pieces of fresh human skin were mounted and frozen as described previously [28]. Cryostat sections (5 μ) were air-dried and incubated with the appropriate undiluted hybridoma supernatant. In some experiments the supernatant was adjusted to 1-5 mM ethylenediaminetetraacetic acid (EDTA). Alternatively it was dialyzed twice against at least 100 vol of phosphate-buffered saline (PBS) and was used with or without the addition of up to 5 mM $CaCl_2$. After 30 min, sections were washed, incubated with fluorescein-conjugated rabbit anti-mouse IgG (1:40, Dakopatts), washed, and mounted as before [28]. Human keratinocytes grown on washed cover slip (4- to 10-d) cultures were treated briefly with 0.1% EDTA to remove 3T3s, fixed in methanol at 4°C, and permeabilized with 0.25% Triton X-100 in PBS before incubation with polyclonal antibodies followed by fluorescein-conjugated swine anti-rabbit IgG. Superficial stratum corneum cells were scraped from the inner forearm with a scalpel blade, transferred to a glass slide in PBS, air dried, and fixed with methanol before staining.

Cell Culture and Metabolic Label Human keratinocyte cultures were initiated and propagated from juvenile foreskin in regular culture medium as described previously [29] and were usually used after one or two passages. A431 cells (derived from an epidermoid carcinoma) were grown in Dulbecco's minimum essential medium supplemented with antibiotics and 10% fetal calf serum. Confluent cells were rinsed with PBS and stored at −70° before glycoprotein isolation. For metabolic labeling confluent 3-cm dishes were incubated with 0.5 ml of methionine-free Eagle's minimum essential medium containing 5% dialyzed fetal calf serum, antibiotics, and 25 to 50 μCi/ml of L-[^{35}S] methionine (cell labeling grade approximately 1000 Ci/mmole, ICN Radiochemicals) for 18 h.

Immune Precipitation [^{35}S]methionine-labeled cells were washed with PBS, scraped off the dish in a small volume of 1% SDS, and heated at 100°C for 5 min. Samples were diluted with 4 vol of a stock solution to give the following final concentrations: 1% bovine serum albumin, 1% NP40, 0.5% sodium deoxycholate, 0.2% SDS, 0.15 M NaCl, 1.25 mM phenylmethylsulfonyl fluoride, 50 mM Tris-HCl, pH 7.5. After clarification by centrifugation, 1-ml samples were incubated sequentially (1 h at 4°C) with 25 μl of protein A-agarose beads coated with 1) pre-immune rabbit antibodies, 2) DGII/III fusion protein antibodies, and 3) polyclonal DGIV/V antibodies. The antibodies were coupled to the protein A beads using 20 mM dimethylpimelimidate at pH 9.0 [30]. The beads were washed three times using the above immune-precipitation solution, once with 1% NP40, 0.5 M NaCl, 1 mM EDTA, 1.25 mM phenylmethylsulfonyl fluoride, 50 mM Tris HCl, pH 7.5, and were eluted by boiling in SDS sample buffer [17].

RESULTS

Epidermal Tissue DGII/III and DGIV/V were readily distinguished by immune blotting of glycoprotein fractions isolated from various human epidermal sources.

In whole human epidermis the polyclonal callus antibodies and LH50 both recognized major 115- and 100-kD bands (DGIV and V) as well as proteolytic degradation products at about 80 kD and 54 to 46 kD (Fig 1, tracks 2 and 3). The DGIV band consistently appeared as a doublet at low gel loadings or when very dilute antibody was used. The DGV band was usually more strongly stained than DGIV, suggesting it may be the more prominent species in intact epidermis. The DGII/III fusion protein antibody recognized a major band that migrated with an Mr about 5 kD greater than DGIV and was tentatively identified as DGII (Fig 1, track 4). A discrete DGIII band was never observed in these blots. It is probably

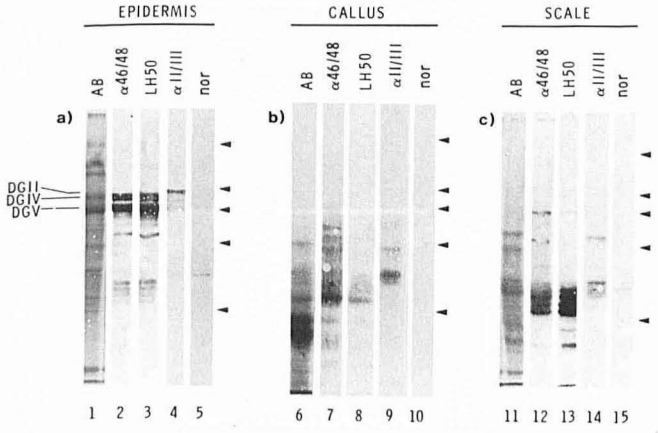

Figure 1. Immune blotting of epidermal glycoproteins. Con A–binding glycoproteins (50 to 100 μg/track) isolated from *(a)* whole human leg epidermis, *(b)* plantar callus, and *(c)* psoriatic scale were separated by SDS-PAGE and transferred to nitrocellulose. Replicate strips were stained with amido black (AB; tracks 1, 6, 11) or probed with antiserum against 46/48-kD glycopeptides (α 46/48; tracks 2, 7, 12; 1:250), the DGIV/V monoclonal (LH50, tracks 3, 8, 13; 1:25), antiserum against the DGII/III fusion protein (α II/III; tracks 4, 9, 14; 1:250), or normal rabbit serum (nor; tracks 5, 10, 15; 1:250). *Arrowheads*, position of Mr standards (200,000, 116,000, 93,000, 68,000 and 43,000) in descending order.

obscured by the much larger amounts of DGIV/V that are present in the tissue and that migrate in the same region of the gel.

In plantar callus the polyclonal and monoclonal DGIV/V antibodies recognized major proteolytic degradation products in the Mr range 80 to 46 kD (Fig 1, tracks 7 and 8). The DGII/III antibody also detected glycopeptide fragments in callus (Fig 1, track 9) but these consistently migrated in a higher Mr range (85 to 64 kD) than those identified by the DGIV/V antibodies.

In psoriatic scale small amounts of intact DGV were detected as well as the proteolytic fragments of DGIV/V generated during terminal differentiation (Fig 1, tracks 12 and 13). No intact DGII or DGIII bands were observed with the fusion protein antibody (Fig 1, track 14). However, the degradation products of DGII/III migrated significantly slower than the equivalent DGIV/V glycopeptide fragments.

DGII/III and DGIV/V were also distinguished by indirect immunofluorescence of unfixed human skin sections (Fig 2). The DGII/III antibody produced stronger staining in the basal layer of the epidermis and this became weaker towards the outer layers of the tissue (Fig 2*c*). The polyclonal DGIV/V antibody stained throughout the living layers of the epidermis but this was much stronger in the upper half of the tissue (Fig 2*a*). Staining with LH50 (and the other DGIV/V monoclonals) was restricted to the upper living layers of the epidermis (Fig 2*b*) and appeared to start about one-third to one-half way through the spinous layer. The differences between the polyclonal and monoclonal DGIV/V antibodies probably reflects the low level of cross-reactivity of the former reagent with other epithelial cadherins (see Figs 7 and 8).

The staining with LH50 was markedly influenced by Ca^{++} concentrations indicating that it recognized a Ca^{++}-sensitive epitope in the extracellular domain of DGIV/V. Removal of Ca^{++} from the hybridoma supernatant by chelation with EDTA (Fig 3*b*) or by dialysis (Fig 3*c*) produced considerable enhancement of cell-surface staining. This enhanced staining was reduced when Ca^{++} was added back to the dialyzed hybridoma supernatant (Fig 3*d*). Similar effects of Ca^{++} were observed with the other DGIV/V monoclonals (results not shown). This effect appeared to be specific because Ca^{++} did not affect the staining obtained with non-desmocollin antibodies produced during the same fusion as the DGIV/V monoclonals. Similar Ca^{++} effects were observed in unfixed sections of human breast, leg, and foreskin, indicating that this was not a site-specific phenomenon.

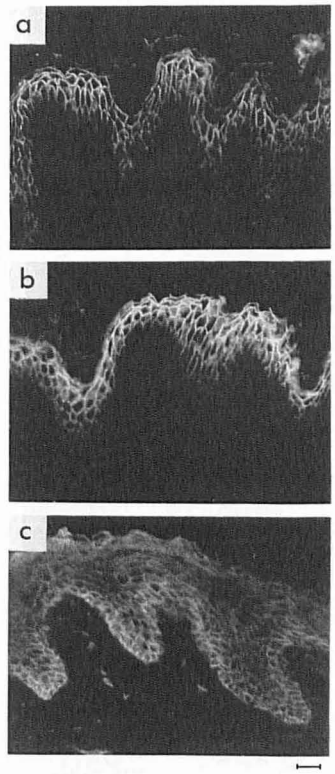

Figure 2. Indirect immunofluorescence of human foreskin. Unfixed cryostat sections were stained using *(a)* anti-46/48 kD glycopeptide serum diluted 1:50, *(b)* LH50 diluted 1:5, and *(c)* the DGII/III fusion protein antiserum diluted 1:50. *Bar*, 20 μm.

To obtain an estimate of the Ca^{++} levels required to inhibit LH50 staining, serial foreskin sections were stained using dialyzed hybridoma supernatant supplemented with increasing concentrations of $CaCl_2$ (Fig 4). Addition of 5 μM Ca^{++} had no effect on LH50 staining. Some inhibition of staining was observed at 50 μM excess Ca^{++} and this was quite marked at 500 μM. Addition of 5 mM Ca^{++} almost completely abolished LH50 staining.

Inhibition of LH50 binding to DGIV/V by Ca^{++} was also demonstrated in an ELISA system using Con A–binding glycoproteins isolated from human leg epidermis and adsorbed onto microtiter plates (Fig 5). In these assays Ca^{++} concentrations greater than 1 mM produced significant inhibition. Again this appeared to be specific because Ca^{++} had no effect on the binding of a non-desmocollin antibody (Fig 5*b*). The Ca^{++} level required for inhibition in this system was at least an order of magnitude greater than that effective in indirect immunofluorescence. This discrepancy probably reflects binding of LH50 to urea-denatured glycoproteins in the ELISA, whereas the immunofluorescent assay involved binding to native glycoproteins. Ca^{++} had no effect on the binding of LH50 to SDS-denatured DGIV/V blotted onto nitrocellulose after SDS-PAGE (I. King, unpublished). It would therefore appear that Ca^{++} changes the native conformation of DGIV/V, resulting in reduced accessibility of the LH50 epitope.

Although fragments of DGII/III and DGIV/V were detected in the horny layer of epidermis by immune blotting (Fig 1), none of the desmocollin antibodies produced clear cell-surface staining in the stratum corneum of human skin sections (Figs 2–4). However, DGIV/V staining was clearly seen on superficial stratum corneum cells after scraping from the skin surface. Figure 6 shows methanol-fixed stratum corneum cells stained with polyclonal DGIV/V antibodies and with LH50. Both antibodies produced distinctive punctate staining over the surface of the cells. The staining appeared to be concentrated along the edges of the polyhedral stratum corneum cell envelope. No such staining was observed with the DGII/III antibodies.

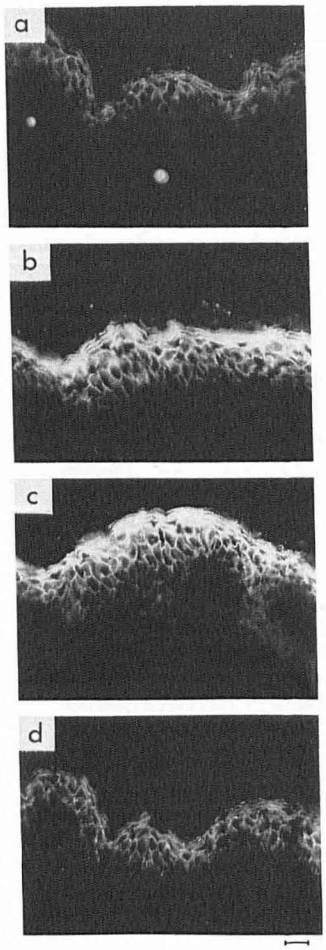

Figure 3. Effect of Ca^{++} depletion on LH50 staining in human breast skin. Unfixed cryostat sections were stained using undiluted LH50 hybridoma supernatant after *(a)* no treatment, *(b)* addition of 2 mM EDTA, *(c)* dialysis against PBS, and *(d)* dialysis against PBS and addition of 2 mM $CaCl_2$. Photomicrographs were taken using the automatic exposure meter on the internal camera of the microscope. *Bar*, 20 μm.

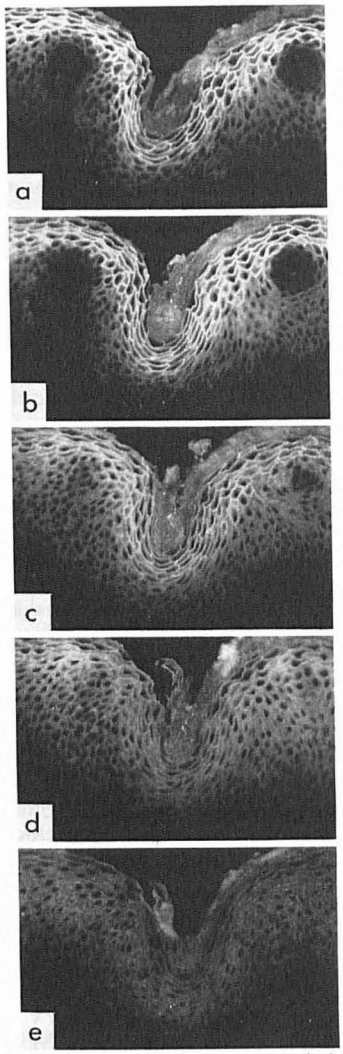

Figure 4. Ca^{++} sensitivity of LH50 staining in human foreskin. Serial sections of human foreskin were stained using extensively dialysed LH50 (diluted 1 : 5) supplemented with *(a)* no $CaCl_2$, *(b)* 5 μM $CaCl_2$, *(c)* 50 μM $CaCl_2$, *(d)* 500 μM $CaCl_2$, and *(e)* 5000 μM $CaCl_2$. All photomicrographs were taken with the same exposure. *Bar*, 20 μM.

Cultured Epidermal Cells DGII/III and DGIV/V were both expressed in cultured human keratinocytes and the two species could be distinguished by both immune blotting and immune precipitation.

Figure 7 shows immune blotting of con A–binding glycoproteins isolated from pooled keratinocyte cultures. In contrast with whole epidermal tissue (Fig 1), distinct DGII and DGIII bands were readily detected in cultured cells using the anti-fusion protein antibody (Fig 7, track 4). These bands consistently migrated more slowly than the DGIV and DGV bands (Fig 7, tracks 2 and 3), consistent with an Mr difference of about 2–3 kD. Differences were also noted in the degradation products of the two desmocollin isoforms. Qualitative comparison with immune blots of whole epidermal tissue (Fig 1) suggested that DGII/III was more prominent and DGIV/V was less prominent in cultured keratinocytes. DGIV was usually more strongly stained than DGV in cultured cells in contrast with epidermal tissue where DGV appeared to predominate. A band at about 120 kD recognized by the polyclonal DGIV/V antibodies (Fig 7, track 2) was probably a cross-reacting component common to stratifying and non-stratifying cells in culture because 1) it was never detected in glycoprotein preparations from epidermal tissue, 2) it was not recognized by the DGIV/V monoclonal LH50 (Fig 7, track 3), and 3) it was the major immunoreactive component in non-stratifying A431 cells (immune blot not shown, see immune precipitations below).

Desmocollin expression in cultured human keratinocytes was also examined by immune-precipitation of [^{35}S]methionine-labeled cells and was compared with the non-stratifying A431 cell line. DGII and III were readily precipitated from cultured keratinocytes by the fusion protein antibody (Fig 8, track 2). These components were also detected in A431 cells but only after prolonged exposure of the fluorogram (results not shown). LH50 was found to be ineffective in immune precipitation probably because its affinity was too low. However the polyclonal callus antibodies precipitated two bands (DGIV and V) that migrated with an Mr 2–3 kD lower than DGII and III (Fig 8, track 3). These components were not detected in A431 cells even after prolonged exposure of the fluorogram. The 120-kD component co-precipitated by the DGIV/V polyclonal migrated more slowly than DGII and was the major band immune precipitated from A431 cells (Fig 8, track 6). It is probably the same cross-reacting component observed on immune blots (Fig 7) and may be another epithelial member of the cadherin family such as E-cadherin.

LH50 never stained cultured keratinocytes in indirect immunofluorescence although a variety of fixation/permeabilization protocols were tested. Nor was staining seen with the other monoclonal antibodies generated against the N-terminal fragments of

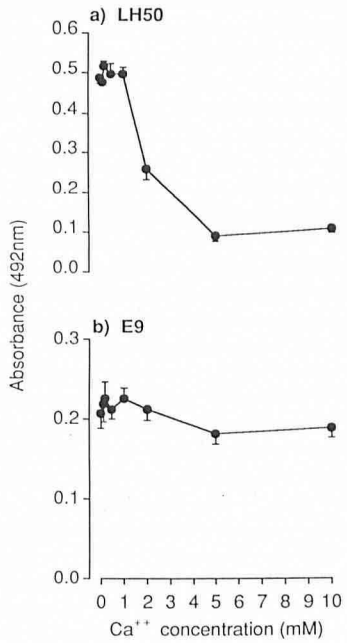

Figure 5. Effect of Ca^{++} on LH50 binding to epidermal glycoproteins in ELISA. Wells were coated with Con A–binding glycoprotein from human leg epidermis and incubated with *(a)* dialysed LH50 and *(b)* dialysed E9 (a non-desmocollin, epidermal glycoprotein antibody). Antibodies were diluted 1:10 and supplemented with increasing concentrations of $CaCl_2$. LH50 binding was determined using horseradish peroxidase-conjugated rabbit anti-mouse IgG [24].

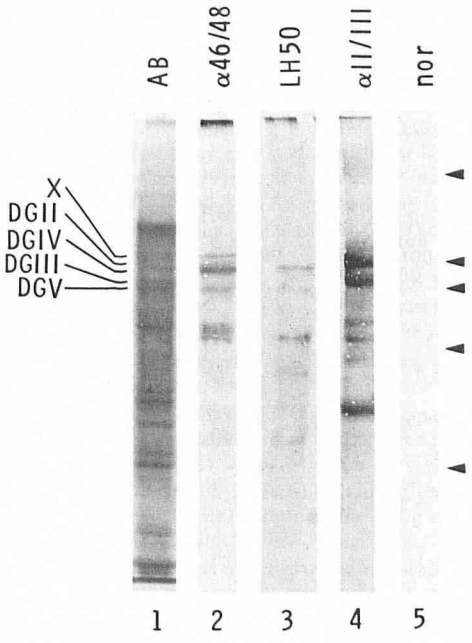

Figure 7. Immune blotting of human keratinocyte glycoproteins. Con A–binding glycoproteins from cultured cells (about 100 μg/track) were separated by SDS-PAGE and transferred to nitrocellulose. Replicate strips were 1) stained with amido black (AB) or probed with 2) anti–46/48-kD glycopeptide serum (α 46/48; 1:250), 3) the DGIV/V monoclonal (LH50; 1:25), 4) the DGII/III fusion protein antiserum (α II/III; 1:250), and 5) normal rabbit serum (nor; 1:250). Mr standards are indicated by *arrowheads* as in Fig 1. X, 120-kD component recognized by polyclonal, but not monoclonal, DGIV/V antibodies.

DGIV/V suggesting that these epitopes are not exposed under cell culture conditions. Keratinocyte cultures were therefore stained with the polyclonal DGII/III and DGIV/V antibodies to determine whether the different distributions seen in epidermal tissue were reflected in cultured cells (Fig 9). However, similar staining patterns were observed with both antibodies. They both stained the interface between small cells at the margin of keratinocyte colonies as well as the surface of larger flattened cells overlying the colonies.

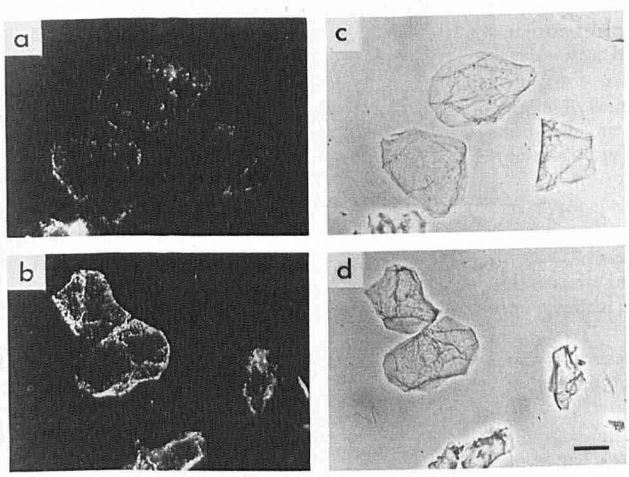

Figure 6. Indirect immunofluorescence of human stratum corneum cells scraped from the inner forearm. Cells were air-dried onto a glass slide, fixed with methanol (4°C, 15 min), and stained using (*a* and *c*) undiluted LH50 and (*b* and *d*) the anti–46/48-kD glycopeptide serum diluted 1:50. *a* and *b* show immune fluorescent staining whereas *c* and *d* show the corresponding phase images. *Bar*, 20 μm.

DISCUSSION

The results presented in this article are consistent with our previous evidence for the heterogeneity of desmocollins in human epidermis [18]. We have now been able to distinguish the two desmocollin isoforms in epidermal tissue and cultured cells at the protein level. DGII/III and its proteolytic fragments consistently migrated with a higher apparent Mr than the DGIV/V polypeptides. This size difference appears to be greater than that predicted for bovine type I and II desmocollins where the Mrs deduced from cDNA sequencing differ by less than 0.5 kD [21]. Preliminary analysis of human cDNA clones confirms that the human DGIV/V polypeptides are probably slightly shorter than DGII/III (I.A. King, unpublished). It remains to be seen whether there are also differences in the degree and nature of post-translational modifications such as glycosylation and phosphorylation.

The two desmocollin isoforms are clearly expressed at different stages of the keratinization process. DGII/III staining was stronger in the basal layers and became attenuated towards the outer layers of the tissue whereas DGIV/V staining was much stronger in the upper spinous and granular layers of the epidermis. Some staining of the lower epidermal layers was observed with the polyclonal DGIV/V antibodies but this probably reflects a low level of cross-reactivity because it was not observed with the DGIV/V monoclonals. The possibility that the LH50 epitope was masked in the lower layers of the tissue must be considered given the failure of this antibody to stain cultured cells. However this is considered unlikely because recent *in situ* hybridizations have shown that expression of the DGIV/V messages is also restricted to the upper spinous/granular layers of the epidermis (J.A. Arnemann *et al*, submitted). Moreover, Holton *et al* [31] showed indirect immunofluorescence of human skin with polyclonal antibodies against a synthetic N-terminal peptide of bovine type I desmocollin, which is highly homologous to the N-terminus of the callus glycopeptides [18]. This antibody also produced strong cell-surface staining in the upper layers of human epidermis.

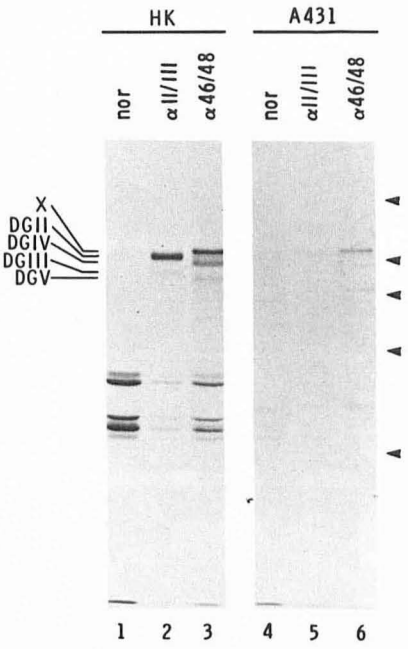

Figure 8. Immune precipitation of ^{35}S-methionine–labeled human keratinocytes (HK) and A431 cells. Metabolically labeled cells were extracted as described in *Materials and Methods* and extracts were incubated sequentially with antibodies covalently coupled to Protein A–agarose beads. 1 and 4, normal rabbit antibodies; 2 and 5, DGII/III fusion protein antibodies; 3 and 6, antibodies against 46/48-kD glycopeptides from DGIV/V. X, 120-kD component co-precipitated from HK by the DGIV/V antibodies that is also present in A431 cells.

The relative abundance of the two desmocollin isoforms in epidermal tissue and cultured keratinocytes is also consistent with their differential expression during keratinization. DGII/III was much more prominent in cultured keratinocyte cultures that do not stratify to the same extent as epidermal tissue. It will clearly be of interest to examine the relative abundance of DGII/III and DGIV/V isoforms under culture conditions that promote extensive stratification such as growth at an air-liquid interface [32]. We were not able to demonstrate differential distribution of these isoforms in cultured keratinocyte colonies by immunofluorescent staining. The DGIV/V polyclonals clearly stained the surface of proliferative cells at the margin of expanding colonies. It may be that DGIV/V expression is switched on earlier in cultured cells than in tissue but this seems unlikely given its relatively low abundance. Alternatively this may reflect the cross-reactivity of the polyclonal DGIV/V antibodies with the 120-kD component that was prominent in stratifying and non-stratifying epithelial cells in culture but was not present in epidermal tissue. In this respect the failure of LH50 and the other DGIV/V monoclonals to stain cultured keratinocytes was particularly disappointing. Anti-peptide antibodies generated against the deduced DGIV/V sequence may be necessary to answer this point.

It is now well established that Ca^{++} plays an important role in the regulation of epidermal differentiation. The rapid assembly of desmosomes is an early morphologic event following Ca^{++} switching of keratinocyte monolayers [33] and this is followed by stratification and the expression of various differentiation markers [34–36]. It has been suggested that the effect of Ca^{++} on differentiation is mediated by activation of protein kinase C via breakdown of phosphatidylinositol [37]. However, the recent finding that the desmosomal glycoproteins belong to the cadherin gene family suggests that Ca^{++} might also have a direct effect on these components. Putative Ca^{++} binding domains have been identified in deduced desmoglein [38–40] and desmocollin sequences [12–14,19,21] and Ca^{++}-protected fragments of the desmocollins have been demonstrated [41]. Moreover it now appears that there is a Ca^{++} gradient maintained across the epidermis with the highest levels found in the extracellular spaces of the upper spinous and granular layers [42]. It was therefore of considerable interest that the LH50 epitope was identified as a Ca^{++}-sensitive region in the N-terminus of DGIV/V. Ca^{++} inhibition of LH50 binding was most marked in native DGIV/V as demonstrated in the immunofluorescence assays. The Ca^{++} effect was observed at concentrations as low as 50 μM, indicating that it may be of physiologic significance for epidermal differentiation. It is not clear whether DGII/III is equally responsive to Ca^{++} because monoclonals against Ca^{++}-sensitive domains of this isoform are not yet available.

The heterogeneity of the desmocollins raises important questions about the role of the different isoforms in desmosome-mediated cell adhesion during differentiation. Precisely how desmocollins function in the adhesive process is unclear although analogy with the "classical" cadherins [43] suggests that some form of homotypic association may be involved. Although DGII/III was more prominent in the basal layers of the epidermis it appeared to persist throughout the living layers by immunofluorescence. However, it is not clear whether these polypeptides persist in these upper layers as intact adhesive glycoproteins. Proteolytic degradation products of DGII/III were detected in the horny layers of callus and psoriatic scales by immune blotting although we were unable to stain the surface of superficial stratum corneum cells. Presumably desmocollins are inactive as adhesion molecules after partial degradation because this would disrupt linkage between the extracellular adhesive domain and the cytoskeleton. It may be important to determine at which stage of keratinization DGII/III is degraded to assess its continuing contribution to desmosome-mediated adhesion. DGIV/V, which is expressed later in keratinization, is also subject to proteolytic degradation and this probably occurs during transition to the stratum corneum. Intact DGIV and DGV were never detected in normal stratum corneum or plantar callus [16] although intact DGV was present in some samples of psoriatic scale (Fig 1). This probably reflects the absence of a discrete granular layer and the incomplete keratinization characteristic of this disorder. Furthermore, proteolytic fragments of DGIV/V were not detected in desmosomes isolated from the spinous layers of bovine epidermis (I.A. King, unpublished). It is therefore likely that DGIV/V plays a more prominent role than DGII/III in desmosome-mediated cell adhesion in the upper living layers of the epidermis. Precisely how DGIV/V contributes to the strength and stability of these adhesive junctions and the overall protective function of the epidermis remains to be established.

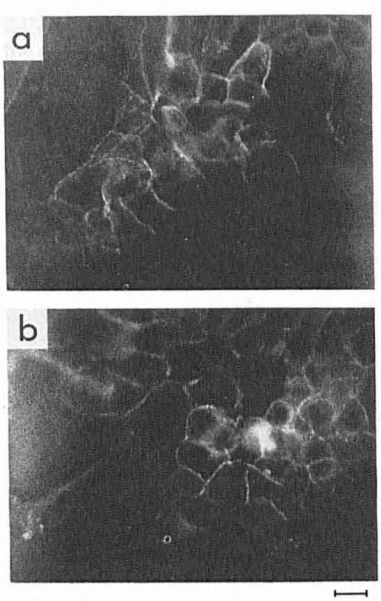

Figure 9. Indirect immunofluorescence of cultured human keratinocytes. Cells on cover slips were fixed and permeabilized before staining with *(a)* DGII/III fusion protein antiserum and *(b)* anti–46/48-kD glycopeptide serum both at 1:50 dilution. *Bar,* 20 μm.

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