Integrin α6/β4 Complex Is Located in Hemidesmosomes, Suggesting a Major Role in Epidermal Cell–Basement Membrane Adhesion

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Abstract. The α6/β4 complex is a member of the integrin family of adhesion receptors. It is found on a variety of epithelial cell types, but is most strongly expressed on stratified squamous epithelia. Fluorescent antibody staining of human epidermis suggests that the β4 subunit is strongly localized to the basal region showing a similar distribution to that of the 230-kD bullous pemphigoid antigen. The α6 subunit is also strongly localized to the basal region but in addition is present over the entire surfaces of basal cells and some cells in the immediate suprabasal region. By contrast staining for β1, α2, and α3 subunits was very weak basally, but strong on all other surfaces of basal epidermal cells. These results suggest that different integrin complexes play differing roles in cell–cell and cell–matrix adhesion in the epidermis.

Immunoelectron microscopy showed that the α6/β4 complex at the basal epidermal surface is strongly localized to hemidesmosomes. This result provides the first well-characterized monoclonal antibody markers for hemidesmosomes and suggests that the α6/β4 complex plays a major role in epidermal cell–basement membrane adhesion. We suggest that the cytoplasmic domains of these transmembrane glycoproteins may contribute to the structure of hemidesmosomal plaques. Immunoultrastructural localization of the BP antigen suggests that it may be involved in bridging between hemidesmosomal plaques and keratin intermediate filaments of the cytoskeleton.

The α6/β1 and α6/β4 integrins are cell surface glycoprotein complexes each containing a presumably identical α subunit and unique β subunits (Sonnenberg et al., 1987, 1988a; Hemler et al., 1988, 1989; Kajiji et al., 1989). Both complexes are members of the superfamilv of integrins which mediate cell–cell and cell–matrix interactions (Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Buck and Horwitz, 1987). In addition, the α6/β1 complex is part of the very late activation antigen subfamily of integrins, the members of which share a common β1 subunit but have different α subunits (Hemler et al., 1987a; Hemler, 1990). The integrin α6/β1 was first identified on platelets (Sonnenberg et al., 1987; Hemler et al., 1988) and since then has been found on a variety of other cell types, including epithelial (tumor) cells (Hemler et al., 1989; Sonnenberg et al., 1990a), macrophages (Shaw et al., 1990), and lymphocytes (Shimizu et al., 1990). Inhibition studies using α6-specific antibodies have shown that the α6/β1 complex functions as a specific receptor for laminin (Sonnenberg et al., 1988b). The binding site for α6/β1 has been located on the long arm of laminin in the elastase-derived fragment E8 (Aumailley et al., 1990; Sonnenberg et al., 1990b; Hall et al., 1990). On macrophages and lymphocytes, the α6/β1 integrin appears to mediate cell adhesion to laminin in an activation-dependent manner (Shaw et al., 1990; Shimizu et al., 1990). Cytoskeletal association and phosphorylation of the α6 subunit have been suggested as possible molecular events in this activation (Shaw et al., 1990). The role of the α6/β1 complex as an adhesion receptor is further indicated by the observation that invasion of transformed cells through reconstituted basement membranes could be strongly inhibited by an α6-specific monoclonal antibody (Dedhar and Saulnier, 1990). That the α6/β1 complex is also important in tissue morphogenesis is shown in a recent report, in which inhibition of kidney development by antibodies against α6 was described (Sorokin et al., 1990). Complexes of α6/β4 have been found on carcinoma cell lines of diverse type (Sonnenberg et al., 1988a, 1990a; Hem-
Some evidence exists that on colon carcinoma cell lines the α6/β4 protein acts as a laminin receptor (Lotz et al., 1990). However, although α6/β4 has been shown to be present on mammary tumor cells, its function as a laminin receptor on these cells has not been demonstrated (Sonnenberg et al., 1990b). Expression of α6/β4 in mouse (Falcioni et al., 1986) and human tumor cells (Kimmel and Carey, 1986) has been associated with metastasis.

Recently, the cDNA for β4 has been cloned and sequenced (Suzuki and Naitoh, 1990; Hogervorst et al., 1990). Comparison of the primary structure of this β subunit with other β subunits indicated some common structural features such as homologous cysteine-rich domains and a putative transmembrane segment, but also a unique feature: the presence of an exceptionally long cytoplasmic domain of ~1,000 amino acids.

In a former study, we reported on the distribution of α6 and β4 subunits in adult and neonatal mice (Sonnenberg et al., 1990a). We used immunoperoxidase reactions to demonstrate that nearly all epithelial tissues express both α6 and β4 subunits. Expression of the α6 subunit was seen mostly on the basal surface of epithelial cells, but basolateral distributions were also observed. By contrast, the expression of the β4 subunit always appears to be confined to the basal surface. Expression of α6/β4, however, is not restricted to epithelial cells, since staining for α6 and β4 has also been demonstrated in peripheral nerves (Kennel et al., 1986; Falcioni et al., 1988), but by immunoperoxidase staining skin was found to be strongly positive (Sonnenberg et al., 1990). There is also evidence for expression of α6 and β4 in placental membranes by trypsin treatment (Aplin et al., 1984). Screening of the resulting hybridomas was carried out initially by immunofluorescence using tissue sections. Epitopes were selected that were located exclusively at the basal surface of the epithelium. Bullous pemphigoid serum that had been shown by Western blotting to recognize the 230kd bullous pemphigoid antigen (Stanley et al., 1981) was kindly provided by Dr. F. Wojnarowskij (Department of Dermatology, Slade Hospital, Oxford, UK). 5 nm gold-conjugated goat anti-rat IgG was purchased from Janssen Pharmaceutica (Beerse, Belgium). 9 nm gold-conjugated protein A was kindly provided by Dr. G. Griffith (European Molecular Biological Laboratory, Heidelberg, Germany).

**Immunoelectron Microscopy**

An interesting discrepancy between previous studies of the distribution of α6/β4 complex is that the complex was not detected in skin by two-site monoclonal antibody assay (Kennel et al., 1986; Falcioni et al., 1988), but by immunoperoxidase staining skin was found to be strongly positive (Sonnenberg et al., 1990a). This suggests that α6/β4 may be present in an insoluble form perhaps as part of a junctional structure associated with the cytoskeleton. In this study we show by immunoelectron microscopy that the α6/β4 complex in basal epidermal cells is localized in hemidesmosomes, suggesting that it plays an important role in epidermal cell adhesion to the basement membrane.
Immunogold Labeling of Epidermal Cell Sheets. Human breast skin excised at mastectomy was rinsed in cold HBSS and dissected to obtain the epidermis with a small amount of underlying dermis. The epidermis was then detached as a sheet by collagenase treatment as follows. Strips of dissected skin (1 cm × 1 mm) were incubated in collagenase (Clostridiopeptidase A, EC 3.4.24.3; Sigma C-2139; Sigma Chemical Co.) to 160 U/ml in HBSS at 37°C for 30 to 60 min. After digestion the tissue fragments were transferred to HBSS containing 10% FBS at room temperature and the epidermis peeled away from the underlying stroma with fine forceps. In some experiments chromatographically purified collagenase (Sigma C-0773; Type VII; Sigma Chemical Co.) was used with similar results. Epidermal sheets were incubated in PBS (pH 7.4) containing 1% BSA (PBS/BSA) for 30 min at room temperature. This was followed by incubation for 1 h at room temperature in monoclonal antibody at dilutions of 0-1:200 with PBS plus 5% FBS (PBS/FBS). After three 5-min washes with PBS/BSA epidermal sheets were incubated with either anti-mouse IgG or anti-rat IgG as appropriate diluted 1:50 with PBS/FBS. Samples were then washed three times for 10 min in PBS and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4°C for a minimum of 2 h. Washing with cacodylate buffer was followed by post fixation in 1% osmium tetroxide, rinsing in distilled water, dehydrated through graded alcohols and dried acetone, and embedding in Spurr resin. Ultrathin sections were viewed and photographed in a Philips EM201 either unstained or after staining with uranyl acetate and lead citrate.

Labeling and Immunoprecipitation of Integrins

Cells were surface labeled with 125I using lactoperoxidase (Sonnenberg et al., 1988a) and lysed with a buffer containing 1% NP-40, 100 mM NaCl, 4 mM EDTA, and 25 mM Tris-HCl, pH 7.5. The lysates were clarified by centrifugation at 15,000 rpm and precleared by incubation with protein A-Sepharose (Pharmacia, Uppsala, Sweden). After incubation with antibodies against integrin subunits, immunocomplexes were collected with protein A-Sepharose. Affinity-purified rabbit anti-rat IgG was included in immunoprecipitations involving the rat monoclonal antibodies BIES, GoH3, and 439-9B. Immunoprecipitated material was analyzed by SDS-PAGE.

Results

Antibody Characterization

To characterize antibodies against integrin α6, β1, and β4 subunits, immunoprecipitations were performed from detergent extracts of 125I surface-labeled HBL-100 cells. Fig. 1, lane I shows that antibody A-1A5 immunoprecipitated the β1 subunit with associated α subunits, consistent with previous data that this antibody binds to the β1 subunit (Hemler et al., 1983). Antibody GoH3 to integrin α6 immunoprecipitated the α6 subunit together with β1 and β4 subunits (Fig. 1, lane 2); the relative intensities suggest that this cell line expresses α6/β4 strongly and α6/β1 weakly (Sonnenberg et al., 1990a). Fig. 1, lane 3 shows that antibody 439-9B to the β4 subunit (Falcioni et al., 1988) immunoprecipitates α6/β4, and lane 4 shows that antibody 5B5 also precipitates α6/β4. The 5B5 epitope is absent from the surface of platelets (which contain α6β1 but not α6β4, data not shown), indicating that 5B5 recognizes a β4-associated epitope.

Immunofluorescence and Immunoelectron Microscopic Analysis of Integrins in Human Skin

Immunofluorescent staining of cryostat sections of normal human breast skin with antibodies against β4 antigen showed an apparently continuous line of reactivity along the basal surface of the basal epidermal cells (Fig. 2 a). A similar appearance of continuous linear basal reactivity was obtained by staining with bullous pemphigoid serum (Fig. 2 b) and with anti-laminin antibody (not shown). This indicates that all three of these antigens are strongly localized to the basal layer.
Figure 2. Fluorescent staining of cryostat sections of human breast skin with (a) anti-β4 (5B5), (b) BP serum, (c) anti-α6 (GoH3), and (d) anti-β1 (A-1A5). Anti-β4 gives strong staining of the basal region of the basal epidermal cells (a), which at the light microscope level appears similar in distribution to the staining given by BP serum (b). Anti-α6 also gives strong staining of the basal region, but, in addition, gives weaker but positive staining of the lateral and apical surfaces of the basal cells and some suprabasal cells (arrows in c). It is shown below that all three of these antigens are associated with hemidesmosomes. That the staining for these antigens appears continuous in the basal regions in these fluorescence micrographs probably reflects the extremely high density of hemidesmosomes. In contrast with the other three antigens, β1 is located principally on the apical and lateral surfaces of basal cells, sometimes with an apparently punctate distribution (d). Sparse punctate staining of the basal surfaces of basal cells can sometimes be seen (arrow in d). Bar, 20 μm.

epidermal–basement membrane region. Staining with antibody to the α6 integrin subunit also gave intense staining of the basal epidermal surface but, in addition, outlined the entire surfaces of basal cells as well as cells in the immediate suprabasal region (Fig. 2 c). Antibody to the β1 subunit gave strong staining of the cell–cell contacts of basal keratinocytes and weaker positive staining of cells in the immediate suprabasal layers (Fig. 2 d). Staining of the cell–matrix contacts with this antibody was weakly positive and quite distinct from the strong continuous staining obtained for β4, α6, and bullous pemphigoid antigen. The α2 and α3 subunits were found to have similar distribution to β1, that is prominent in cell–cell contacts but scarcely found in contact with the basement membrane (not shown).

Ultrastructural localization of the integrin α3, α6, and β1 subunits was possible by using the enhanced immunoperoxidase reaction (see Materials and Methods), whereas localization of β4 could also be demonstrated by immunogold labeling of fixed tissue sections. The ultrastructural immunoperoxidase staining of human skin confirmed the localization of the α6 and β4 along the basal surfaces of basal epidermal cells (Fig. 3, a and b). The α3 and β1 subunits were detected on surfaces of basal cells that were in contact with other cells, but were scarcely found on those in contact with the basement membrane (Fig. 3, c and d). Thus, the β1- and β4-containing integrins on basal cells appeared to be quite distinct.

The basal epithelial cells are attached to the basement membrane by numerous hemidesmosomes. The ultrastructural appearance of these hemidesmosomes is shown in a
Figure 3. Ultrathin sections of human skin incubated with mAbs (a) anti-α6 (GoH3); (b) anti-β4 (439-9B); (c) anti-α3 (143); (d) anti-β1 (A-1A5), and peroxidase conjugate. The electron micrographs show the basal cells of the epidermis lying on the basement membrane (BM). In a and b the peroxidase reaction product (arrows) is seen on the surface of the cells in contact with the basement membrane. In c and d reaction (arrows) is seen on the lateral cell surface. The surface in contact with the basement membrane is unstained. Hemidesmosomes (arrowheads) are clearly visible. N, nucleus; D, desmosomes; M, melanin. Bars, 1,000 nm.

Figure 4. (a) Thin section of mouse ear skin fixed with 2.5% glutaraldehyde and 1% OsO₄ and embedded in a mixture of LX112 and Araldite. An area of a basal cell and the underlying connective tissue is shown with hemidesmosomes consisting of an electron dense plaque (arrows) underneath the plasma membrane; a basement membrane (BM) is shown immediately underlying the hemidesmosome and the connective tissue of the dermis with collagen fibrils (C). (b) Ultrathin cryosection of mouse ear skin incubated with anti-β4 (346-11A) and gold-conjugated second antibody. All hemidesmosomes are labeled (arrows). Bars: (a) 200 nm; (b) 100 nm.
Figure 5. Protein A-gold staining of ultrathin cryostat sections of human breast skin with (a) anti-α6 (GoH3), (b) anti-β4 (5B5), and (c) BP serum. The skin sample was not fixed before freezing, sectioning and staining because the epitopes recognized by the monoclonal antibodies are sensitive to paraformaldehyde treatment. We always experience some nonspecific background staining with this technique as indicated in the control (d), which was incubated with complete hybridoma culture medium, followed by anti-mouse Ig and protein A-gold. (a) Concentration of labeling for α6 at the extracellular regions of hemidesmosomes in the region of the lamina lucida (arrows). (b) Partly tangential section showing strong association of β4 staining with hemidesmosomes (arrows). (c) Staining with BP serum with the inner plaque region of hemidesmosomes (arrows). The staining is not intense but is comparable both in intensity and location with that found in previous studies (see text). Photographs are at different magnifications; Bars, 200 nm.

Localization of α6 and β4 as well as bullous pemphigoid antigen in the basal region of keratinocytes was also carried out on ultrathin frozen sections of human breast skin. The α6 subunit was strongly localized to hemidesmosomes by this technique. The majority of gold particles were located at the external face of the hemidesmosomal plasma membrane in the lamina lucida region of the basement membrane (Fig. 5 a). β4 subunit also showed hemidesmosomal localization and was especially intensely labeled in sections where the hemidesmosomes were cut obliquely (Fig. 5 b), presumably because this allowed easier access to the labeling reagents. Bullous pemphigoid antigen was strongly localized to the basal cytoplasm of the basal keratinocytes (Fig. 5 c). Rather than being directly associated with hemidesmosomal plaques that are close to the plasma membrane, the labeling appeared to be between the plaques and the so-called inner plaques where the intermediate filaments insert (see Tanaka
et al., 1990). No consistent labeling of the basal surface for \( \beta_1 \) integrin was obtained by this technique.

In thin sections of human tongue embedded in LR White resin, the morphology of the basement membrane and the hemidesmosomes proved to be best preserved (Fig. 6). Although the degree of labeling in this experiment was not as high as in the ones shown in Figs. 4 b and 5 b, the localization of the \( \beta_4 \) subunit in hemidesmosomes was clearly visible.

To analyze further the localization of integrins on the basal epidermal surface, collagenase-separated human breast epithelium was labeled with subunit-specific antibodies. This technique allows better access of antibodies and gold-labeled reagents to the basal cell surface and, because the cells are not permeabilized before labeling, provides unequivocal evidence for the extracellular localization of antibody binding sites. Fig. 7, a and b shows that \( \alpha_6 \)- and \( \beta_4 \)-specific antibodies reacted with the exposed extracellular faces of hemidesmosomes. In a sample of 141 hemidesmosomes for anti-\( \beta_4 \) and 113 hemidesmosomes for anti-\( \alpha_6 \), all showed labeling. Areas of membrane between hemidesmosomes were largely but not entirely devoid of label. Counts of gold particles associated with the exposed basal surface showed that with \( \beta_4 \)-specific antibodies 89.7 % of gold particles were located on hemidesmosome and with \( \alpha_6 \)-specific antibodies 87.3 % of gold particles were associated with hemidesmosomes. The \( \beta_1 \)-specific antibody gave positive but much less intense labeling of the exposed basal surface, but showed no obvious association with hemidesmosomes (Fig. 7 c). Controls in which the first antibodies were omitted showed complete absence of attached gold particles (Fig. 7 d). No labeling for cytoplasmic antigens such as bullous pemphigoid and cytokeratin was obtained by this technique. These results confirm that the \( \alpha_6 \) and \( \beta_4 \) integrin subunits are associated with hemidesmosomes on the surface of basal keratinocytes. Further, the binding sites for the A-1A5 and 5B5 antibodies are confirmed to be extracellular. The labeling of the basal surface for \( \beta_1 \) subunit is weak but positive.

**Expression of Integrins in Primary Keratinocyte Cultures**

To support the immunohistochemical and immunoelectron microscopical observations, the expression of integrins on keratinocytes was examined by immunoprecipitation. Primary cultures of keratinocytes were \( ^{125} \)I surface labeled and lysates of these cells were treated with monoclonal antibodies against integrin \( \alpha \) and \( \beta \) subunits. As shown in Fig. 8, keratinocytes express the \( \alpha_2 \) and \( \alpha_3 \) subunits in association with \( \beta_1 \), whereas the \( \alpha_6 \) subunit was found primarily associated with \( \beta_4 \). Furthermore, primary keratinocytes expressed \( \alpha_5 \beta_1 \) at low levels, but \( \alpha_1 \beta_1 \) or \( \alpha_4 \beta_1 \) were not detectable.

**Discussion**

Hemidesmosomes are cell–matrix junctions that have been characterized and defined by their ultrastructural appearance (Weiss and Ferris, 1954; Kelly, 1966; Shienvold and Kelly, 1976; Ellison and Garrod, 1984). Cytoplasmically they consist of dense plaques that are associated with the inner face of the basal plasma membrane. Bundles of intermediate filaments run through the peripheral cytoplasm to associate with the inner aspect of the dense plaque. It has been suggested that these filaments are continuous, and that rather than terminating at the edge of the plaque they loop back into the cytoplasm. Filaments of different ultrastructure extend between the plaque and the tonofilaments (Kelly, 1966). Extracellularly, hemidesmosomes are associated with the lamina lucida of the basement membrane where anchoring filaments which traverse the basement membrane can sometimes be detected (Ellison and Garrod, 1984).

In this study we have investigated the localization of the \( \alpha_6/\beta_4 \) complex in epidermis. We have shown that this complex is localized in hemidesmosomes in the basal epidermal cells. This localization suggests that the \( \alpha_6/\beta_4 \) complex plays an important role in binding epidermal cells to the basement membrane. Presumably the basement membrane contains a component that acts as a ligand for the \( \alpha_6/\beta_4 \) complex though this ligand has not yet been identified. Our results provide the first well characterized monoclonal antibody markers for hemidesmosomes.

\( \alpha_6 \) and \( \beta_4 \) are transmembrane glycoproteins, the cytoplasmic domain of \( \beta_4 \) being unusually large and markedly different from that of any of the other known integrins (Suzuki and Naitoh, 1990; Hogervorst et al., 1990). The membrane–proximal cytoplasmic structure of the hemidesmosomes is a dense plaque and it seems likely that cytoplasmic domains of hemidesmosomal glycoproteins contribute to the structure of this plaque. The unusual cytoplasmic domain of \( \beta_4 \) may be specialized for participation in plaque formation. It is interesting to note that one of the major desmosomal glycoproteins, dgl or desmoglein, whose cytoplasmic domain is
located in the desmosomal plaque (Miller et al., 1987; Steinberg et al., 1987), also has a large and unusual cytoplasmic domain (Parrish et al., 1990; Koch et al., 1990), while being related to cadherins in its extracellular domain (Koch et al., 1990). However, there is no apparent homology between the cytoplasmic amino acid sequences of β4 and dgl.

α6 and β4 subunits are expressed in nearly all epithelial cells as well as peripheral nerves and certain subsets of endothelial cells (Sonnenberg et al., 1990a; Kennel, S. J., V. Godfrey, L. Y. Ch'ang, T. K. Lankford, L. J. Foote, and A. Makkinje, manuscript in preparation). Not all of these possess hemidesmosomes so the α6/β4 complex must also reside in nonplaque locations. Different cytoplasmic interactions in various locations may be mediated by alternative structures of the β4 cytoplasmic domain generated by cell type–specific alternative mRNA splicing (Tamura et al., 1990). These authors have also proposed that the unique structure of β4 suggests its involvement in unique cytoskeletal interactions.

We suggest that interaction between the cytoskeleton and transmembrane glycoproteins is indirect. A hemidesmosome-associated protein that has been extensively studied in recent years is the bullous pemphigoid (BP)1 antigen (230 kD)

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1. Abbreviation used in this paper: BP, bullous pemphigoid.
Figure 8. Analysis of integrins on human keratinocytes. Lysates of 125I-labeled keratinocytes were immunoprecipitated with mAbs anti-\(\alpha\)2 (10G11), anti-\(\alpha\)3 (J\(\alpha\)43), anti-\(\alpha\)4 (B5-G10), anti-\(\alpha\)5 (BIE5), anti-\(\alpha\)6 (GoH3), anti-\(\beta\)1 (A-1A5) and anti-\(\beta\)4 (439-9B). Samples were analyzed on SDS-polyacrylamide (5%) gels under nonreducing (left) and reducing (right) conditions.

(Stanley et al., 1981, 1988). This molecule is recognized by autoantibodies present in sera from patients with the bullous pemphigoid disease and has been localized intracellularly, in association with the hemidesmosomal plaque (Westgate et al., 1985). Our present immunolocalization studies, together with those of Westgate et al. (1985) and Tanaka et al. (1990) lead us to suggest that the BP antigen may occupy a location in hemidesmosomes similar to that which we have proposed for desmoplakins in desmosomes, that is between the membrane-associated plaque and the intermediate filaments (Miller et al., 1987; Garrod et al., 1990). An association between BP antigen and keratin filaments has been suggested previously (Jones et al., 1986) and is further supported by the recent finding that the carboxyl-terminal domain of BP antigen and that of two desmosomal plaque proteins, desmoplakins I and II, are very similar (Green et al., 1990). It has been suggested that the desmoplakins I and II are involved in linking the keratin filaments to the cell surface and that they may interact with the keratin network via these homologous domains. However, O'Keefe et al. (1989) were unable to demonstrate interaction between isolated desmoplakins and keratin filaments, while other desmosomal components have been shown to exhibit keratin binding activity. These are desmocollin (Tsukita and Tsukita, 1985), B6P (Kapprell et al., 1988), and a 140-kD protein related to lamin B (Cartaud et al., 1990). It may be that the component that binds hemidesmosomes to intermediate filaments has yet to be identified. Another candidate is the 180-kD antigen recognized by some bullous pemphigoid autoantibodies (Diaz et al., 1990), which has been localized to hemidesmosomal plaques (Klatte et al., 1989).

It has been reported that formation of new hemidesmosomes during epidermal attachment to the basement membrane is dependent upon extracellular Ca\(^{2+}\) (Trinkaus-Randall and Gipson, 1984). It is possible that this may be interpreted in relation to the proposed involvement of the \(\alpha\)6/\(\beta\)4 complex in hemidesmosomal adhesion since integrin-mediated adhesion is well known to be divalent cation dependent (Ruoslahti and Pierschbacher, 1987). This is analogous to the finding that Ca\(^{2+}\)-dependent adhesion of desmosomes may be ascribed to the relationship between desmosomal glycoproteins and cadherins (Holton et al., 1990; Koch et al., 1990; Collins et al., 1991).

A protein of 125-kD that is recognized by a monoclonal antibody, prepared against a protein preparation containing hemidesmosomal components, has recently been described by Klatte et al. (1989). This protein has been localized at the lamina lucida side of the hemidesmosomes. Because of its localization and size, the 125-kD protein might be similar to the \(\alpha\)6 integrin subunit.

In skin, the \(\alpha\)2/\(\beta\)1 and \(\alpha\)3/\(\beta\)1 integrins were strongly expressed at the lateral borders of basal epidermal cells, but only weakly at the basal side. The presence of these two integrins at cell–cell borders was noted previously (Peltonen et al., 1989; Larjava et al., 1990; Carter et al., 1990), and it was proposed that the observed distribution of the \(\beta\)1 integrins in skin indicates a role of these molecules in cell–cell interactions in epidermis (Peltonen et al., 1989). Consistent with this, antibodies against \(\beta\)1 were found to dissociate colonies of cultured keratinocytes (Larjava et al., 1990). Our finding that \(\alpha\)6 shows weak cell–cell border localization in addition to strong basal location suggests, that it, too, may have a role in cell–cell interaction. Basolateral distribution of \(\alpha\)6 has been observed previously in other epithelia (Sonnenberg et al., 1990a; Sorokin et al., 1990).

Location of the \(\alpha\)6/\(\beta\)4 integrin complex to hemidesmosomes emphasizes the marked difference in biochemical composition between hemidesmosomes and desmosomes which has been demonstrated by several groups (Jones et al., 1986; Miller et al., 1987; Schwarz et al., 1990). Since loss of adhe-
In addition to bullous pemphigoid, loss or abnormality of desmosomes has been reported in certain diseases including various types of carcinoma (Schenk, 1979; White and Gohari, 1984) and junctional epidermolysis bullosa (Tidman and Eady, 1986). Our results provide a basis for the investigation of the involvement of hemidesmosomal adhesion in these processes.

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