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$\alpha_6\beta_4$ integrin heterodimer is a component of hemidesmosomes

(cornea/immunoelectron microscopy/development)

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ABSTRACT **Antisera that recognize the α_6 and β_4 subunits of integrins were found by immunoelectron microscopy to localize to hemidesmosomes in the basal cells of mouse corneal epithelium. Immunoprecipitation experiments using extracts of metabolically labeled corneal epithelial cells indicate that the primary α_6-subunit-containing integrin heterodimer present is $\alpha_6\beta_4$ and not $\alpha_6\beta_1$. Here we extend previous studies to report that by immunofluorescence microscopy the α_6 integrin subunit colocalizes with bullous pemphigoid antigen and type VII collagen in newly forming hemidesmosomes in the developing 17-day fetal rabbit eye. Neither the composition of the anchoring filaments, which span the region between the hemidesmosomal plaque and the lamina densa of basement membrane where the globular domain of type VII collagen is located, nor the extracellular ligand of $\alpha_6\beta_4$ is known. Once anchoring filament proteins are identified, it will be of interest to determine whether any bind to $\alpha_6\beta_4$.**

Hemidesmosomes are cell–substrate adhesion junctions present along the basal cell–basement membrane junction of stratified squamous epithelia (1, 2). By comparison to desmosomes, biochemical characterization of the basic components of the hemidesmosome has been slow, due in part to difficulty in obtaining hemidesmosome-enriched cellular fractions. The only characterized component of the hemidesmosome is the bullous pemphigoid antigen (BPA), a 230-kDa protein present in the cytoplasmic plaque of the hemidesmosome (3–5). BPA is found within the electron-dense cytoplasmic plaque region of the hemidesmosome. The sequence of cDNA clones encoding the BPA molecule, although not entirely complete, shows no integral membrane segment (J. Stanley, personal communication). Several other monoclonal antibodies specific to hemidesmosomes have been reported, and their antigens await characterization (6, 7). Clearly, other molecules are present in both the cytoplasmic and the extracellular aspect of the hemidesmosome.

The molecule(s) that traverses the cell membrane in hemidesmosomes has yet to be identified; candidates include members of the integrin superfamily of extracellular matrix receptors (8–12). The integrins are integral membrane glycoproteins that function as cell–cell and cell–substrate adhesion molecules. As a result of our studies to localize and characterize integrin subunits present in corneal epithelium, we noted that antibodies to both the α_6 and β_4 integrin subunits bound to the basal cell–basement membrane zone in stationary epithelia. This prompted us to determine whether the heterodimer is associated with hemidesmosomes. The present study reports that α_6 and β_4 integrins colocalize with BPA and type VII collagen in hemidesmosomes of developing epithelia, that both α_6 and β_4 antibodies bind to hemidesmosomes as shown by immunoelectron microscopy, and that both $\alpha_6\beta_4$ and $\alpha_6\beta_1$ heterodimers can be identified in epithelia by immunoprecipitation.

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MATERIALS AND METHODS

Tissue Preparation. For adult corneal epithelium, New Zealand White (NZW) rabbit corneas and mouse (BALB/c) eyes were excised and frozen in Tissue Tek II OCT compound (Lab-Tek) for immunohistochemistry. For studies of the developing ocular surface epithelium, fetal eyes were obtained from NZW rabbits at 17 days of gestation and frozen in Tissue Tek II OCT compound for immunohistochemistry. To immunolocalize $\alpha_6\beta_4$ at the electron microscopic level, sheets of mouse corneal epithelium were isolated after incubation of corneas in 1 M NaCl in distilled water for 2 hr at room temperature. Epithelium was separated from the underlying basement membrane and stroma by gentle teasing with jewelers' forceps. These full-thickness epithelial sheets were either frozen in OCT compound for immunohistochemistry or processed for immunoelectron microscopy.

Antisera. G_0H_3, a rat monoclonal antibody that recognizes the α_6 integrin molecule from several species (13–16), was a gift from Arnoud Sonnenberg (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, The Netherlands). The polyclonal β_4 antiserum, a gift of Martin E. Hemler (Dana–Farber Cancer Institute and Harvard Medical School, Boston, MA), was prepared in rabbits against denatured human β_4 antigen, purified as described (17). The polyclonal β_1 antiserum 363 (18) was a gift of Richard O. Hynes (Howard Hughes Medical Institute and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA). The mouse monoclonal antibody to the carboxyl-terminal globular domain of human type VII collagen (7, 19, 20) was obtained from Robert E. Burgeson (Shriner's Hospital for Crippled Children, Portland, OR). Human antiserum to BPA was obtained through John Stanley (National Institutes of Health, Bethesda, MD).

Immunohistochemistry. Immunohistochemical localization procedures were as described (21). Negative control tissue sections (primary antibody omitted) were run routinely with each antibody-binding study. The secondary antibody was either tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-rat IgG, fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (both from Boehringer Mannheim), FITC-conjugated rabbit anti-mouse IgG (Calbiochem), or dichlorotriazinylaminofluorescein-conjugated donkey anti-rabbit IgG (The Jackson Laboratory). For double-labeling studies, a mixture of either α_6 and BPA or α_6 and type VII collagen antibodies was applied, followed by a mixture of TRITC anti-rat IgG and FITC anti-human or FITC anti-mouse IgG.

Abbreviation: BPA, bullous pemphigoid antigen.

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Immunoelectron Microscopy. Pre-embedding localization of α_6 and β_4 antibodies was done on epithelial sheets obtained from mouse corneas that had been incubated in 1 M NaCl. After washing in 20 mM Tris-buffered saline (pH 7.4), sheets were placed in 20 mM Tris buffer (pH 7.4) with 0.8% (wt/vol) bovine serum albumin, 0.1% (wt/vol) gelatin, and 5% (vol/vol) normal goat serum for 30 min at room temperature. Then sheets were washed for 5 min in the same buffer without goat serum (wash buffer) and incubated with α_6 or β_4 antibodies for 1–3 hr at room temperature. After primary antibody, the tissue was washed in large volumes of wash buffer, incubated in either goat anti-rat or goat anti-rabbit IgG conjugated to 5-nm gold (Biocell Laboratories), and then fixed and processed for electron microscopy (22).

Metabolic Labeling and Immunoprecipitation. Mice were killed by injection with sodium pentobarbital and the eyes were removed. Corneas were dissected and placed in methionine-free minimum essential medium (MEM) (Select-Amine kit, GIBCO) containing [^{35}S]methionine/[^{35}S]cysteine (Tran^{35}S-label, ICN) at 250 μCi/ml (1 μCi = 37 kBq) for 18 hr at 35°C. Medium was supplemented as described (22). After labeling, corneal epithelial sheets were then obtained from intact mouse corneas by incubation in Ca^{2+}- and Mg^{2+}-free MEM supplemented with 25 mM EDTA at 35°C for 60 min (23). Immunofluorescence microscopy with α_6 and β_4 antisera has shown that this technique removes both molecules along with the epithelium (data not shown). Epithelia were then extracted and immunoprecipitation was performed as described (9), with minor exceptions. Extraction buffer was buffer A [50 mM Tris, pH 8.0/0.15 mM NaCl/0.5 mM $CaCl_2$/0.5% (vol/vol) Nonidet P-40] supplemented with the following protease inhibitors: aprotinin (0.1 μM), leupeptin (1 μM), pepstatin (1 μM), and phenylmethylsulfonyl fluoride (0.2 mM). A 1:4 slurry of either protein A-Sepharose or anti-rat IgG-agarose (Sigma) was used to precipitate the primary antisera. Precipitated samples were run in 20-cm SDS/6% (wt/vol) polyacrylamide gels and processed for autoradiography.

RESULTS

α_6 and β_4 Colocalize in the Corneal Epithelium. The normal differentiated adult corneal epithelium is a non-keratinizing, stratified squamous epithelium of five to seven cell layers (Fig. 1*A*). The basal cells have hemidesmosomes at their basal aspect along their basement membrane (21). Both α_6 and β_4 localize to this area in mouse cornea by immunofluorescence microscopy (Fig. 1 *B* and *C*). This localization is similar in rabbit (Fig. 1*D*) and rat (data not shown). In some instances, α_6 is found to extend from the basal membrane up the lateral membranes (Fig. 1 *B* and *D*, arrows). Immunofluorescence microscopy with a β_1 antiserum (data not shown) reveals abundant β_1 around the apical and lateral membrane of basal cells, but little is present on their basal aspect. These data suggest that the α_6 between cells is present as an $\alpha_6\beta_1$ heterodimer and that most of the α_6 present is interacting with β_4 to form $\alpha_6\beta_4$ heterodimers.

α_6 in Corneal Epithelium Exists Primarily as an $\alpha_6\beta_4$ Heterodimer. Immunoprecipitation experiments were performed on metabolically labeled corneal epithelial extracts derived from 18-hr [^{35}S]methionine labeling in organ culture. We have used this approach extensively in the study of corneal wound healing; under the defined conditions, corneas are viable for at least 36 hr (24–26). Care is taken to inhibit endogenous protease activity and to extract tissues using conditions that allow heterodimers to remain intact. The β_4 antiserum immunoprecipitates 205- and 135-kDa proteins corresponding to β_4 and α_6, respectively (Fig. 2, lane 1). Immunoprecipitation with the α_6 antiserum (lane 2) yields the 135-kDa α_6 molecule and a 95-kDa protein that corresponds to β_1. For unknown reasons, we sometimes lost β_4 in immunoprecipitations using the α_6 antiserum. As a comparison, antiserum directed against β_1 (lane 3) brings down several proteins including precursor to β_1, mature β_1, and several other comigrating α bands including α_3 (shown by precipitation with α_3-specific antiserum; data not shown). Although α_6 appears absent from lane 3 (β_1 antiserum), small amounts were detected after longer exposure of the autoradiograms (not shown). We have confirmed the above identities of the individual integrin subunits by precipitation of extracts after SDS treatment. Boiling of samples in SDS followed by addition of Triton X-100 denatures $\alpha\beta$ heterodimers and allows precipitation of the individual subunits. The immunoprecipitation results indicate that the α_6 found in corneal epithelium forms heterodimers primarily with β_4 but also can be identified associated with β_1. In addition, $\alpha_6\beta_4$ heterodimers in these tissue extracts were less stable than $\alpha_6\beta_1$. There was no evidence that β_4 formed heterodimers with α chains other than α_6.

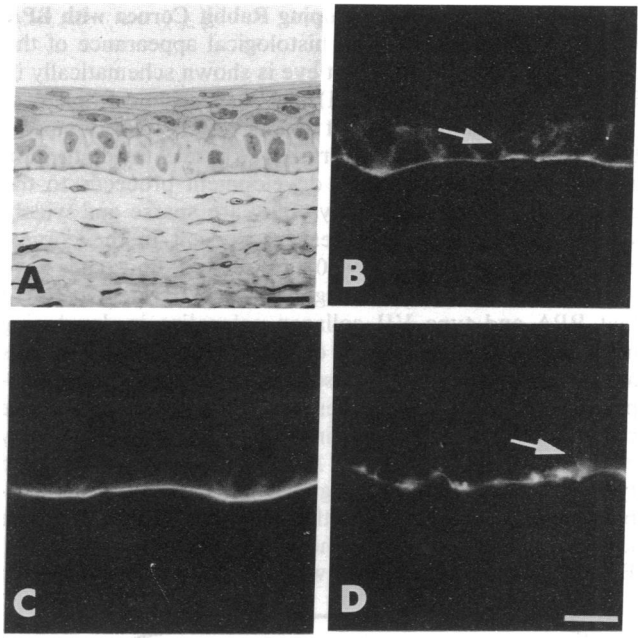

FIG. 1. Subunits α_6 and β_4 colocalize by immunofluorescence microscopy to the basal-cell basal membrane in corneal epithelium. (*A*) Light micrograph of a section of rabbit cornea showing the five to seven cell layers of the epithelium. (Bar = 20 μm.) (*B–D*) Immunofluorescence micrographs of sections of mouse (*B* and *C*) or rabbit (*D*) cornea. *B* and *D* show binding of α_6, and *C* shows binding of β_4. Arrows in *B* and *D* show binding of α_6 extending up lateral membranes of basal cells. (Bar = 10 μm.)

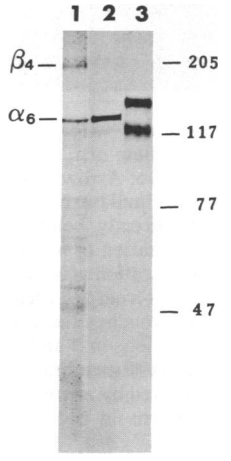

FIG. 2. Subunit α_6 is present as an $\alpha_6\beta_4$ heterodimer. Cell extracts of corneal epithelial sheets, labeled with [^{35}S]methionine, were extracted and integrins were precipitated with antiserum to α_6 (lane 1), β_4 (lane 2), or β_1 (lane 3). Since extracts were isolated without SDS, most heterodimers were preserved. In lane 3, the uppermost major band contains multiple α subunits, the lower major band is mature β_1, and the minor band below mature β_1 is the β_1 precursor. Markers at right are in kilodaltons.

α_6 Colocalizes in the Developing Rabbit Cornea with BPA and Type VII Collagen. The histological appearance of the developing 17-day fetal rabbit eye is shown schematically in Fig. 3*A*. We have shown (21) that a spectrum of hemidesmosome formation is present at this stage of development. Hemidesmosomes begin to form at the base of the epithelium at the lid margin, and their development proceeds to the conjunctiva, where at 17 days they are sparse and widely spaced. No hemidesmosomes are present in the corneal epithelium at this stage. By 20 days, hemidesmosomes have developed in the cornea. Using this system, we demonstrated that BPA and type VII collagen colocalize in developing hemidesmosomes. Thus, the developing rabbit eye provides a system for studying the assembly and histochemical composition of hemidesmosomes. Fig. 3 *B* and *C* show the colocalization of α_6 and BPA in the same section of the 17-day conjunctiva, and Fig. 3 *D* and *E* show colocalization of α_6 and type VII collagen in the region where the lid closely approaches the cornea. Note the exact colocalization of the known hemidesmosome-adhesion structure components, BPA and type VII collagen, with the integrin α_6 subunit. In addition, it is of interest in Fig. 3 *D* and *E* that the lid is seen to possess hemidesmosomal components whereas the corneal epithelium lacks these proteins at this stage of development. These data indicate the presence of α_6 in hemidesmosomes in the developing rabbit eye. Because of the species of origin (rabbit) of the β_4 antiserum, we could not confirm that α_6 was present in heterodimers with β_4 in the developing rabbit eye. The α_6 subunit does not appear to be present as $\alpha_6\beta_1$ heterodimers, since we have observed β_1 around the basal cell membranes in stationary and migrating epithelia of adult rat corneas, not correlating with the presence or absence of hemidesmosomes (data not shown).

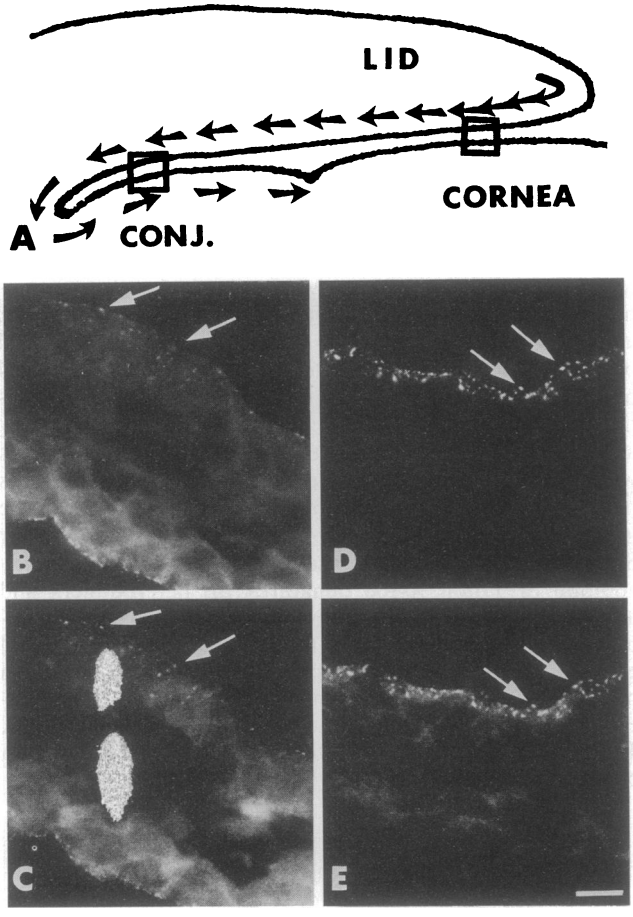

FIG. 3. Subunit α_6 colocalizes with BPA and with type VII collagen in the developing ocular surface epithelium of 17-day fetal rabbit. (*A*) Schematic of the developing rabbit eye. Arrows indicate direction of hemidesmosome formation over time and boxes indicate region from which sections in micrographs directly below were taken. CONJ., conjunctiva. (*B* and *C*) Colocalization of α_6 (*B*) and BPA (*C*) on the same section. (*D* and *E*) Colocalization of α_6 (*D*) and type VII collagen (*E*). The section in *D* and *E* is from the region of the developing rabbit eye in which the lid approaches the cornea, which at this stage lacks hemidesmosomes. Sections in *B* and *C* and in *D* and *E* are tangential to the plane of the basal cell membrane; thus a band of hemidesmosomes is visible. As indicated by arrows, there is absolute correlation between binding patterns in both sets of micrographs. (Bar = 10 μm.)

α_6 and β_4 Are Found in Hemidesmosomes by Immunoelectron Microscopy of NaCl-Released Epithelial Sheets. Electron microscopy of adult cornea shows abundant hemidesmosomes along the basal surface of basal epithelial cells (Fig. 4*A*). These hemidesmosomes have a distinct ultrastructural appearance. Extracellularly, thin (anchoring) filaments arise from the plaque and pass through the lamina lucida to the lamina densa of the basement membrane. Parallel and close to the cell membrane, a thin, electron-dense band is observed; anchoring filaments appear to emanate from this band. Treatment of mouse corneas with NaCl releases the epithelium as a sheet and preserves a portion of the hemidesmosomes visible by electron microscopy (Fig. 4*B*). Other treatments frequently used to isolate epithelial sheets, including digestion of corneas with dispase or incubation with EDTA (23), cause a complete disruption of hemidesmosomes. The α_6 and β_4 antigens are retained as part of NaCl-released epithelial sheets (Fig. 4 *C* and *D*). With anti-rat or anti-rabbit IgG conjugated to 5-nm gold as secondary antibody, α_6 (Fig. 4*E*) and β_4 (Fig. 4*F*) are localized to hemidesmosomes. The α_6 antibody localizes primarily to the extracellular region of the hemidesmosome. With the β_4 antibody, cytoplasmic binding of β_4 is observed. The polyclonal β_4 antibody recognizes epitopes on both the very large, 1000-amino acid cytoplasmic domain and the smaller extracellular domain, whereas the monoclonal α_6 antibody recognizes an epitope in the extracellular domain of α_6 (17). These data, combined with the lack of correlation between β_1 immunofluorescence and the presence of hemidesmosomes, and the abundance of $\alpha_6\beta_4$ heterodimers observed by immunoprecipitation, indicate that integrin $\alpha_6\beta_4$ heterodimers are components of hemidesmosomes.

DISCUSSION

The major finding of our study is the immunoelectron microscopic localization of both α_6 and β_4 to hemidesmosomal plaques of NaCl-released mouse corneal epithelial sheets. Further, we show the presence of $\alpha_6\beta_4$ heterodimers by immunoprecipitation of epithelial tissue and the colocalization of α_6, BPA, and type VII collagen in the developing rabbit eye. The 17-day fetal rabbit eye provides an excellent model for following the assembly of hemidesmosomes by stratified squamous epithelia (21). The hemidesmosomes start forming at the lid margin and spread back along the conjunctival epithelium and, at 20 days of fetal development, finally form in the cornea. Thus, one can observe a gradient of hemidesmosome formation along a continuous epithelium. Since the conjunctiva of the 17-day fetal rabbit has widely spaced hemidesmosomes, it is possible to colocalize hemidesmosomal components by immunofluorescence microscopy. We have previously shown (21) that type VII collagen and BPA colocalize in developing rabbit conjunctiva to the same site. In this paper we show that α_6 is present at the same location as BPA and type VII collagen. The synchronous appearance of α_6, BPA, and type VII collagen in developing rabbit cornea, the immunoelectron microscopy data, and the immunoprecipitation of $\alpha_6\beta_4$ make a strong case

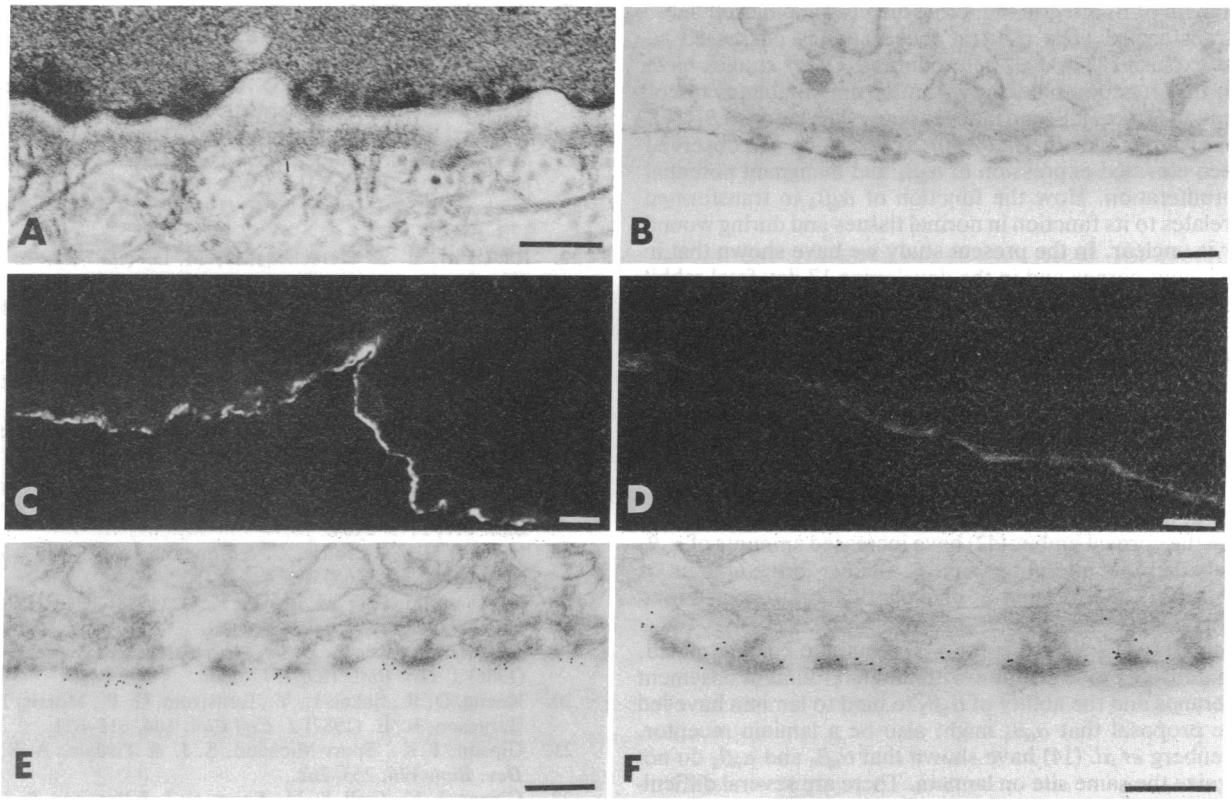

FIG. 4. Subunits α_6 and β_4 are found in hemidesmosomes by immunoelectron microscopy. (*A*) Electron micrograph of a normal rabbit cornea showing the hemidesmosomes along the basal aspect of the basal cells. (*B*) Electron micrograph of a NaCl-released epithelial sheet showing preservation of hemidesmosomes. This micrograph is also the secondary antibody control for the immunoelectron microscopy presented in *E* and *F*. (*C* and *D*) Immunofluorescence micrographs of NaCl-released sheets incubated with α_6 (*C*) or β_4 (*D*) antibody and then with fluorescent secondary antibody. Note preservation of binding of both α_6 and β_4 antisera after NaCl treatment. (*E*) Immunoelectron micrograph showing localization of α_6 to the extracellular region of hemidesmosomes. (*F*) Immunoelectron micrograph showing β_4 localization to both the intra- and the extracellular aspect of hemidesmosomes. (*A*, *B*, *E*, and *F*, bars = 0.2 μm; *C*, bar = 20 μm; *D*, bar = 10 μm.)

for a significant role for $\alpha_6\beta_4$ integrins in the formation and maintenance of hemidesmosomes.

Integrin α chains have three or four metal-binding domains responsible for the Ca^{2+} and/or Mg^{2+} requirement some integrins have for receptor–ligand interaction. One frequently used method of isolating corneal epithelial sheets is incubation of intact corneas with EDTA (23), which disrupts hemidesmosomes and releases the epithelium as an intact sheet. Additionally, Ca^{2+} is required for hemidesmosome reassembly (27). Disruption of integrin $\alpha_6\beta_4$ by EDTA might be the mechanism by which EDTA acts to disrupt hemidesmosomes.

The identification of $\alpha_6\beta_4$ in hemidesmosomes brings to three the number of known components in the hemidesmosome plus associated adhesion structures as determined by immunoelectron microscopy: BPA intracellularly (3, 4), type VII collagen extracellularly in anchoring fibrils (19), and the $\alpha_6\beta_4$ integrin heterodimer spanning the membrane. In addition to these proteins, Klatte *et al.* (6) used immunoelectron microscopy to identify monoclonal antibodies that recognize several as yet uncharacterized molecules found in hemidesmosomes; some of these could be identical to α_6 or β_4. The hemidesmosomes of the corneal epithelium appear analogous both morphologically and in their known components to those of all other stratified squamous epithelia, including the skin.

α_6 is unusual among the integrin α chains due to its ability to associate with more than one β chain. In many cell types, including platelets, fibroblasts, and muscle cells, α_6 forms heterodimers with β_1 (15, 16). In epithelial cells and in a variety of tumor cells, α_6 can be found associated with β_4 as well as with β_1 (28, 29). Other α chains that associate with more than one β chain include α_v, with β_1, β_3, or β_5 (30), and α_4, with β_1 or β_p, a novel β chain not yet fully characterized (31). Although $\alpha_6\beta_1$ has been shown recently to bind to the E8 fragment of laminin (14, 16), the ligand(s) for $\alpha_6\beta_4$ is not known. In its role as a laminin receptor, $\alpha_6\beta_1$ promotes neurite outgrowth and adhesion of a variety of different cell types (32, 33). α_6 can also be modified by phosphorylation. Murine macrophages treated with phorbol 12-myristate 13-acetate (PMA) develop the ability to adhere to laminin in an $\alpha_6\beta_1$-dependent fashion (34). Surprisingly, no change in either the synthesis or the cell-surface expression of $\alpha_6\beta_1$ was found, but an increase in phosphorylation of α_6, but not β_1, was observed. In addition, $\alpha_6\beta_1$ in the laminin-adherent, PMA-treated macrophages was found "anchored" to the cytoskeleton—i.e., in a Triton X-100-insoluble fraction—whereas in PMA-treated macrophages that adhered to plastic or fibronectin, $\alpha_6\beta_1$ was not anchored. These data indicate that the function of α_6 can be regulated by phosphorylation, possibly leading to an enhanced ability to associate with the cytoskeleton.

β_4, which has been completely characterized at the molecular level (35, 36), is distinct among integrin β chains. The β_4 cytoplasmic domain is over 1000 amino acids long; the cytoplasmic domains of the other integrin β chains are approximately 50 amino acids long (10). A portion of the large cytoplasmic domain has sequence homology to the type III repeat of fibronectin (35). Similar fibronectin type III repeats have also been observed in the intracellular muscle-like proteins twitchin and titan (37).

The large cytoplasmic domain of β_4 makes it a good candidate for a mediator of signal transduction across the plasma membrane. In fact, Falcioni *et al.* (29) have reported insulin-induced serine and tyrosine phosphorylation of the cytoplas-

mic domain of β_4 integrin in murine lung carcinoma cell lines. The transformed cells used in those studies possessed α_6 almost exclusively in $\alpha_6\beta_4$ heterodimers. Other studies have shown that function-blocking α_6 antiserum stimulates tumor cell growth *in vitro* (38) and induces phosphorylation of β_4 (39). In these studies and others (28, 40), a correlation was observed between elevated expression of $\alpha_6\beta_4$ and malignant potential and proliferation. How the function of $\alpha_6\beta_4$ in transformed cells relates to its function in normal tissues and during wound repair is unclear. In the present study we have shown that in normal adult cornea and in the developing 17-day fetal rabbit cornea, $\alpha_6\beta_4$ expression is limited to the basal aspect of the basal epithelial cells in regions where hemidesmosomes are located. In the epidermis, others have reported localization of $\alpha_6\beta_4$ to the basal cell–basement membrane zone by immunofluorescence microscopy (41). In addition to being the only cells to possess $\alpha_6\beta_4$, the basal cells are the only cells in these epithelial tissues that can divide; the suprabasal and apical cells are differentiated and normally are unlikely to divide. It will be of interest to determine whether the presumptive stem cells in the corneal limbus (42) have increased amounts of $\alpha_6\beta_4$ and whether any alterations in $\alpha_6\beta_4$—either in the amount of α_6 or β_4 or in their extent of phosphorylation—occur during wound healing.

The extracellular ligands for $\alpha_6\beta_4$ remain to be determined. The extensive colocalization with laminin found in basement membranes and the ability of $\alpha_6\beta_1$ to bind to laminin have led to the proposal that $\alpha_6\beta_4$ might also be a laminin receptor. Sonnenberg *et al.* (14) have shown that $\alpha_6\beta_4$ and $\alpha_6\beta_1$ do not recognize the same site on laminin. There are several difficulties in determining the ligand(s) interacting with $\alpha_6\beta_4$. One is the susceptibility of β_4 to proteolysis (28, 29, 35, 36). In this paper, we have shown that this problem can be overcome by liberal use of protease inhibitors during tissue extraction. A second problem is the "contamination" of $\alpha_6\beta_4$ preparations with $\alpha_6\beta_1$ heterodimers. A third problem, at least for studies of adhesion to laminin fragments, is the purification of laminin fragments. Other possible candidates for $\alpha_6\beta_4$ extracellular ligands include collagen IV, a recently reported anchoring filament protein (43), and heparan sulfate proteoglycan.

It is interesting to speculate that BPA found at the cytoplasmic aspect of the hemidesmosome could associate with the large integrin β_4 cytoplasmic domain. If so, BPA could play a role analogous to that of talin or α-actinin in the focal contact. BPA could organize intermediate filaments at one binding site and interact with integrin $\alpha_6\beta_4$ at another site in much the same way that talin binds to cytoskeletal elements as well as to the integrin β_1 cytoplasmic domain. The molecular analysis of BPA, when completed, will give us clues to its function in hemidesmosomes. If β_4 occurs only in hemidesmosomal plaques, its unique cytoplasmic domain could be a key element in hemidesmosome assembly.

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