Expression of β_1, β_3, β_4, and β_5 Integrins by Human Epidermal Keratinocytes and Non-Differentiating Keratinocytes

Josephine C. Adams and Fiona M. Watt

Keratinocyte Laboratory, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London, England WC2A 3PX

Abstract. We have compared the adhesive properties and integrin expression profiles of cultured human epidermal keratinocytes and a strain of nondifferentiating keratinocytes (ndk). Both cell types adhered to fibronectin, laminin, and collagen types I and IV, but ndk adhered more rapidly and at lower coating concentrations of the proteins. Antibody blocking experiments showed that adhesion of both cell types to fibronectin was mediated by the $\alpha_5\beta_1$ integrin and to laminin by $\alpha_3\beta_1$ in synergy with $\alpha_2\beta_1$. Keratinocytes adhered to collagen with $\alpha_2\beta_1$, but an antibody to α_2 did not inhibit adhesion of ndk to collagen. Both cell types adhered to vitronectin by α_v-containing integrins. Immunoprecipitation of surface-iodinated and metabolically labeled cells showed that in addition to $\alpha_2\beta_1$, $\alpha_3\beta_1$, and $\alpha_5\beta_1$, both keratinocytes and ndk expressed $\alpha_6\beta_4$ and $\alpha_v\beta_5$. ndk expressed all these integrins at higher levels than normal keratinocytes. ndk, but not normal keratinocytes, expressed $\alpha_v\beta_1$ and $\alpha_v\beta_3$; they also expressed $\alpha_1\beta_1$, an integrin that was not consistently detected on normal keratinocytes. Immunofluorescence experiments showed that in stratified cultures of normal keratinocytes integrin expression was confined to cells in the basal layer; terminally differentiating cells were unstained. In contrast, all cells in the ndk population were integrin positive. Our observations showed that the adhesive properties of ndk differ from normal keratinocytes and reflect differences in the type of integrins expressed, the level of expression and the distribution of integrins on the cell surface. ndk thus have a number of characteristics that distinguish them from normal basal keratinocytes.

THE integrins constitute a large family of cell surface molecules involved in cell–cell and cell–matrix interactions. Integrins are heterodimers, consisting of non-covalently associated α and β subunits, both of which are transmembrane glycoproteins (Hynes, 1987). The integrins are presently classified on the basis that one β subunit can associate with several different α subunits: the β_1 and β_3 subgroups are widely expressed and are principally involved in cell–matrix interactions, whereas β_2 integrins are expressed on leukocytes and are involved in cell–cell interactions (reviewed by Hemler, 1990). However, it is now apparent that one α subunit can also associate with different β subunits, examples being the $\alpha_6\beta_1$ and $\alpha_6\beta_4$ integrins (Sonnenberg et al., 1988*a*,*b*; Kajiji et al., 1989) and the $\alpha_v\beta_1$, $\alpha_v\beta_3$, or $\alpha_v\beta_5$ integrins (Bodary and McLean, 1990; Vogel et al., 1990; Cheresh et al., 1989; Ramaswamy et al., 1990). Heterodimer composition is important in determining ligand specificity (Cheresh et al., 1989; Bodary and McLean, 1990; Sonnenberg et al., 1990*a*; Vogel et al., 1990), but there is also evidence that the same integrin can have different functions when expressed on different cell types (Elices and Hemler, 1989; Languino et al., 1989).

One tissue in which integrins are believed to play an important role in determining the spatial organization of the cells is the epidermis. Immunofluorescence studies have shown that integrins are expressed by the basal layer of proliferating keratinocytes attached to the basement membrane, but are largely absent from the suprabasal layers of terminally differentiating cells (Fradet et al., 1984; Sonnenberg et al., 1986; Wayner et al., 1988; De Strooper et al., 1989; Peltonen et al., 1989). Keratinocytes can be grown in culture as stratified sheets that have the same basic organization as the epidermis (Rheinwald and Green, 1975; Watt, 1988). We have shown that when cells are induced to undergo terminal differentiation in culture they lose adhesiveness for extracellular matrix proteins and no longer express β_1 integrins. However, the reduction in adhesiveness precedes loss of integrins by several hours. In the case of $\alpha_5\beta_1$, there is direct evidence for a reduction of fibronectin-binding ability prior to loss from the cell surface. These changes may help to ensure that cells which are committed to terminal differentiation are selectively expelled from the basal layer (Adams and Watt, 1990).

We recently isolated a strain of nondifferentiating keratinocytes (ndk)[1] (Adams and Watt, 1988). These cells

Dr. Adams' present address is Vascular Research Division, Department of Pathology, Brigham and Women's Hospital, Longwood Medical Research Center, 221 Longwood Ave., Boston, MA 02115.

1. *Abbreviations used in this paper*: ECM, extracellular matrix; ndk, nondifferentiating keratinocytes.

were identified as keratinocytes on the basis that they express the keratin profile characteristic of normal keratinocytes in culture. ndk are not immortalized or tumorigenic and yet lack the capacity for terminal differentiation. The cells do not stratify and do not require feeder cell support. Before confluence they move as individual cells and have prominent ruffled membranes; their motile phenotype appears to be a result, at least in part, of autocrine production of scatter factor (Adams et al., 1991). Normal keratinocytes are induced to undergo terminal differentiation when placed in suspension (Green, 1977), a process that can be inhibited by fibronectin or antibodies to β_1 integrins (Adams and Watt, 1989). When ndk are placed in suspension they undergo growth arrest but do not terminally differentiate (Adams and Watt, 1988).

The properties of ndk suggest that they have altered cell–cell and cell–matrix adhesive properties. The aim of the present report was to compare the adhesive properties and profile of integrin expression of ndk with those of normal keratinocytes to obtain further insights into the ndk phenotype.

Materials and Methods

Materials

Human plasma fibronectin was obtained from Calbiochem (Cambridge, UK) or Blood Products (Elstree, Herts, UK). Human placental type IV collagen and mouse EHS laminin were obtained from Sigma Chemical Co. (Poole, UK). Bovine type I collagen (Vitrogen 100) was obtained from Collaborative Research Inc. (Lexington, MA). Human vitronectin was purchased from Telios Pharmaceuticals Inc. (San Diego, CA). GRGDSP and GRGESP peptides were obtained from Novabiochem (Nottingham, UK). ^{125}[I]iodine (sp act 100 mCi/ml), α^{32}P[dATP] (sp act 3,000 Ci/mmol) ^{125}I-Protein A (sp act 100 μCi/ml), ^{35}[S]methionine, and ^{35}[S]cysteine (both sp act 10 mCi/ml) were from Amersham International (Amersham, UK).

The mAbs to integrin subunits used in this study are shown in Table I and were generous gifts from the investigators indicated below. Mouse mAbs: TS2/7 and B-5G10 from M. Hemler (Dana-Farber Cancer Research Institute, Boston, MA), 12F1 from V. Woods (University of California, San Diego, CA), J143 from A. Albino (Sloan-Kettering Memorial Institute, New York, NY), P1B5 and P1E6 from E. Wayner (Oncogen, Seattle, WA), 5E8 from R. Bankert (Roswell Park Memorial Institute, Buffalo, NY), 13C2 and 23C6 from M. Horton (ICRF, London), and Y2/51 from D. Mason (John Radcliffe Hospital, Oxford). Rat monoclonals: BIE5 and BIIG2 from C. Damsky (University of California, San Francisco, CA), Mab 16 and Mab 13 from K. Yamada (NCI, Bethesda, MD), GoH3 from A. Sonnenberg (University of Amsterdam, The Netherlands), and 439-9B from S. Kennel (Oak Ridge National Laboratory, TN). Where more than one monoclonal to the same integrin subunit was used for immunofluorescence or immunoprecipitation the results obtained with each were the same.

Rabbit antiserum to the fibronectin receptor, principally reactive with the β_1 subunit, was obtained from E. Ruoslahti (Cancer Research Foundation, La Jolla, CA). Rabbit antiserum to a synthetic peptide corresponding to the 39 carboxy-terminal amino acids of the human β_1 subunit was obtained from R. Hynes (MIT, Boston, MA). An antiserum to the same peptide, prepared according to the method of Marcantonio and Hynes (1988) was obtained from S. Goodman (Max-Planck Rheumatology Unit, Erlangen, Germany). Rabbit antiserum to a synthetic peptide corresponding to amino acids 757-776 of the human β_5 integrin subunit was obtained from M. Hemler (Ramaswamy and Hemler, 1990). A mouse monoclonal raised against human involucrin was prepared by S. Young (ICRF, London; Young et al., submitted for publication).

A cDNA probe for the human β_5 integrin subunit (2M; Ramaswamy and Hemler, 1990) was kindly provided by M. Hemler.

Cell Culture

Normal human epidermal keratinocytes (strain a, passages 5–14) were isolated and cultured according to the method of Rheinwald and Green (1975) on a feeder layer of J2 3T3 cells, in medium composed of three parts DME and one part Ham's F12, supplemented with 10% FCS (Imperial Laboratories, Andover, Hants, UK), 1.8×10^{-4} M adenine, 5 μg/ml insulin (Sigma Chemical Co.), 0.5 μg/ml hydrocortisone (Calbiochem, Cambridge Bioscience, Cambridge, England), 10^{-10} M cholera toxin (ICN Biomedicals, High Wycombe, Berks, UK), and 10 ng/ml EGF (a gift from Dr. George-Nascimento, Chiron Corporation, Emeryville, CA) (FAD + FCS + HICE; Allen-Hoffmann and Rheinwald, 1984). ndk (passages 5–12), a strain of keratinocytes which are unable to undergo terminal differentiation, were cultured in the same medium as the normal keratinocytes, but without a feeder cell layer (Adams and Watt, 1988). Both cell types were grown at 37°C in a humidified 5% CO_2 atmosphere. Culture medium was changed every 2–3 d.

Table I. Antibodies to Integrins Used in This Study

Specificity	Name	Species	Reference
α_1	TS2/7	mouse	Hemler et al., 1984
α_2	12F1	mouse	Pischel et al., 1987
	P1E6*	mouse	Carter et al., 1990
	5E8*	mouse	Zylstra et al., 1986
α_3	J143	mouse	Kantor et al., 1987
	P1B5*	mouse	Wayner and Carter, 1987
α_4	B-5G10*	mouse	Hemler et al., 1987
α_5	BIIG2*	rat	Werb et al., 1989
	BIE5*	rat	Hall et al., 1990
	16*	rat	Akiyama et al., 1989
α_6	GoH3*	rat	Sonnenberg et al., 1986
α_v	13C2*	mouse	Horton et al., 1985
$\alpha_v\beta_3$ complex	23C6	mouse	Davies et al., 1989
β_1	13*	rat	Akiyama et al., 1989
	DH12	mouse	De Strooper et al., 1988
	363‡	rabbit	Marcantonio & Hynes, 1988
	Rx‡	rabbit	(from S. Goodman)
β_3	Y2/51	mouse	von dem Borne et al., 1989
placental vitronectin receptor	αVNR‡*	rabbit	Suzuki et al., 1986
β_4	439-9B	rat	Kennel et al., 1989

* Antibodies known to perturb cell adhesion. ‡ Polyclonal antibodies; 363 and Rx were raised against a 39-mer peptide corresponding to the human β_1 subunit carboxy-terminus. See text for anti-β5 antiserum and anti-fibronectin receptor antiserum.

Preparation of Sections through Keratinocyte Cultures

Confluent stratified cultures of keratinocytes were detached from the culture dish as an intact sheet by incubation with 2.5 mg/ml dispase (Grade II; Boehringer Mannheim GmbH, Mannheim, Germany) for 30–60 min in serum-free DME at 37°C (Green et al., 1979). The sheet was rinsed in PBS, draped over Whatman No. 1 filter paper and frozen in isopentane in a bath of dry ice and methanol. 6-μm sections were cut at right angles to the surface of the filter paper.

Indirect Immunofluorescent Staining

Keratinocytes and J2 feeder cells, or ndk, were plated onto glass coverslips and grown for 2–3 d. The cells were rinsed in PBS and fixed in 3.7% formaldehyde for 10 min at room temperature. For involucrin staining, fixed cells were permeabilized in methanol for 5 min on ice. The cells were incubated with primary antibody for 45 min, washed extensively in PBS, incubated with appropriate FITC-conjugated second antibody (ICN Biomedicals, High Wycombe, Bucks, UK) for 45 min, washed again in PBS, mounted in Gelvatol (Monsanto Co., St. Louis, MO), and examined under epifluorescence using a Zeiss Axiophot microscope (Zeiss, Oberkochen, Germany).

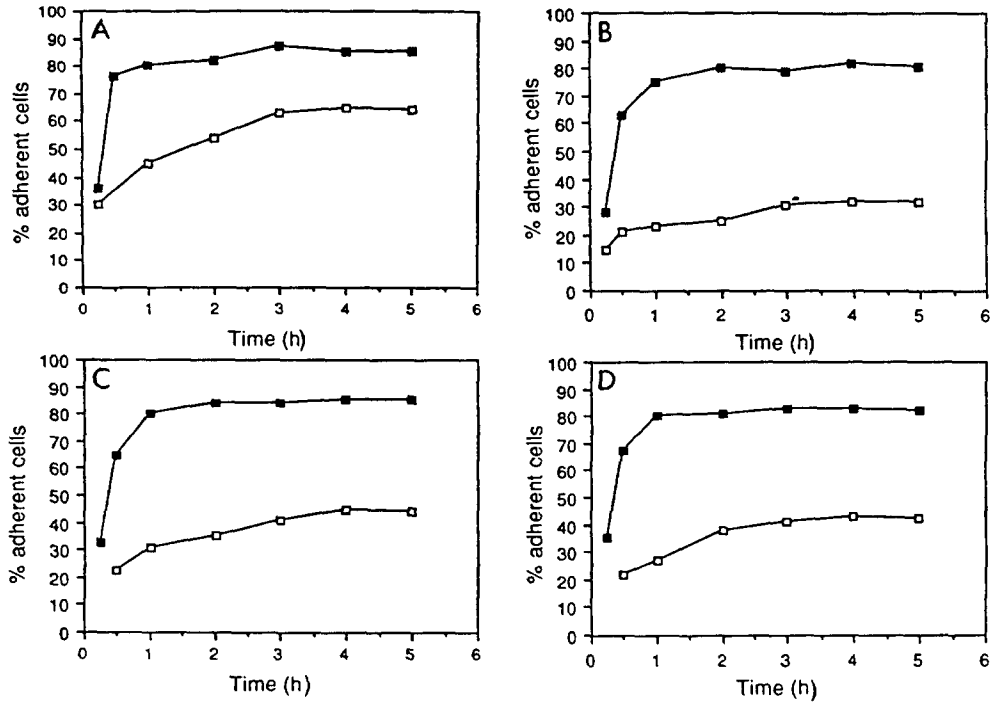


Figure 1. Adhesion of keratinocytes and ndk to ECM glycoproteins: time dependence. 10^4 keratinocytes (□) or ndk (■) were allowed to attach to wells coated with 100 μg/ml of fibronectin (*A*), laminin (*B*), collagen type I (*C*), or collagen type IV (*D*) for the indicated lengths of time at 37°C. Points shown are mean values from three separate experiments.

Cell Adhesion Assays

ECM glycoproteins, diluted in PBS, were left to adsorb to bacteriological plastic microtitre wells (Flow 76-208-05) overnight at 4°C. The plates were rinsed three times in PBS and uncoated plastic blocked by incubation for 1 h with PBS containing 0.5 mg/ml heat denatured BSA. The plates were rinsed again, and 10^4 keratinocytes or ndk cells, prepared by trypsin/EDTA treatment, were added to each well in 100 μl of serum-free FAD medium. Cells were allowed to adhere at 37°C for varying periods of time and then the nonadherent cells were removed by gently washing the plates in PBS containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$. Adherent cells were fixed, stained with methylene blue, and photographed. Adhesion was quantified by counting cells from the photographs. Adhesion-blocking antibodies, when used, were added to the wells before adding the cells.

Radioactive Labeling of Integrins

Cell surface iodination was carried out on trypsinized keratinocytes or ndk, using the lactoperoxidase-glucose oxidase method (Hynes, 1973). Typically, 1 mCi of ^{125}[I]iodine was used to label 10^7 cells. After labeling, the cells were washed three times in PBS containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ and lysed for 15 min on ice in 1% NP-40 in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 2 mM PMSF, and 0.01% leupeptin. For metabolic labeling of integrins, keratinocytes or ndk were incubated overnight (18–20 h) in FAD + HICE containing 10% dialyzed FCS, and 50 μCi/ml each of ^{35}[S]methionine and ^{35}[S]cysteine. The cells were then rinsed in PBS containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$, scraped from the dish, and lysed as indicated above.

Immunoprecipitation and Gel Electrophoresis

The lysates prepared from keratinocytes and ndk were clarified by centrifugation at 14,000 rpm for 5 min. Aliquots were equalized on the basis of TCA-precipitable radioactivity; typically 2×10^6 to 4×10^6 cpm of iodinated material or 1×10^6 to 2×10^6 cpm of ^{35}S-labeled material were used per precipitation. In the metabolic labeling experiments, this corresponded to the lysates from ~5×10^5 ndk or 9×10^5 keratinocytes per immunoprecipitation. Lysates were incubated with primary antibodies, under conditions of antibody excess, for 2 1/2 h on ice. If required, rabbit anti-rat IgG serum or rabbit anti-mouse IgG serum (ICN Biomedicals) was added for the last hour. 50 μl of a 1:1 (vol/vol) suspension of protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) was then added and the mixture tumbled for 45 min at 4°C. Beads and immune complexes were washed as described (Adams and Watt, 1988), resuspended in SDS-PAGE sample buffer, boiled for 5 min, and resolved on 7.5% polyacrylamide gels according to the method of Laemmli (Laemmli, 1970). Generally, no reducing agent was added, but in some experiments 5% (vol/vol) of 2-mercaptoethanol was added to the sample buffer.

Gels were stained with Coomassie blue and destained; those containing iodinated samples were dried down immediately and exposed to Kodak XAR5 x-ray film at room temperature, using an intensifying screen. Those containing metabolically labeled samples were treated with 'Amplify' (Amersham, UK) before drying and then exposed to XAR5 x-ray film at −70°C.

Preparation of mRNA and Northern Analysis

Poly A^+ RNA was prepared from cultured keratinocytes, using the "Quick-prep" mRNA Purification Kit (Pharmacia Fine Chemicals), according to the manufacturer's instructions. Dami cell mRNA was a gift from Dr. Sheryl Greenberg (Department of Haematology, Brigham and Women's Hospital) (Greenberg et al., 1988). 1-μg samples were electrophoresed on a 1% agarose gel containing formaldehyde, using standard procedures (Sambrook et al., 1989). After electrophoresis, the gel was incubated in 0.05 M NaOH, 0.15 M NaCl for 30 min, and then in 0.1 M Tris-HCl, pH 7.5 for a further 30 min.

RNA was transferred from the gel to a nylon membrane (Zetaprobe, Bio-Rad Laboratories) over 1 h, using a Posiblot vacuum transfer apparatus (Stratagene) and then U.V. cross-linked to the membrane.

25 ng of β_5 cDNA probe was radiolabeled by random priming, using a DNA multiprime labelling kit (Amersham International). Blots were prehybridized for 4 h at 42°C in 6 × SSC, 5 × Denhardt's solution, 50% formamide, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA (Sigma Chemical Co.) and hybridized overnight at 42°C in the same buffer containing 2×10^6 cpm/ml of β_5 probe. Blots were washed three times for 15 min each at room temperature in 2 × SSC, 0.1% SDS, and then washed twice in the same solution at 60°C, for 20 min each wash. Blots were autoradiographed at −70°C using an intensifying screen and Kodak XAR5 x-ray film.

Results

Adhesive Properties of Keratinocytes and ndk

The adhesion of ndk and cultured epidermal keratinocytes to ECM glycoproteins was compared in short term, serum-free cell adhesion assays. The proteins chosen were those to which keratinocytes are exposed in vivo (Martin et al., 1988)

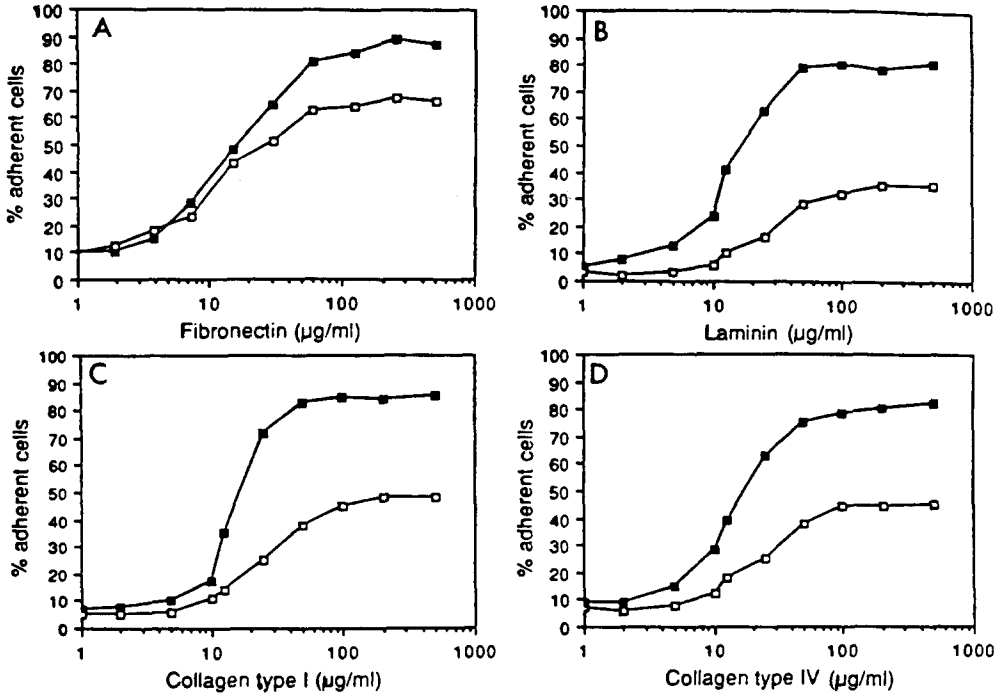


Figure 2. Adhesion of keratinocytes and ndk to ECM glycoproteins: concentration dependence. 10^4 keratinocytes (□) or ndk (■) were allowed to attach to wells coated with the indicated concentrations of fibronectin (*A*), laminin (*B*), collagen type I (*C*), or collagen type IV (*D*) for 3 h at 37°C. Points shown are mean values from three separate experiments.

or in vitro when cultured on 3T3 feeder cells (Alitalo et al., 1982).

In time course experiments, irrespective of the substratum used, maximal adhesion of ndk cells was observed after 1 h of incubation, whereas maximal adhesion of keratinocytes occurred after three to four hours (Fig. 1, *A–D*; Adams and Watt, 1990). Under our assay conditions keratinocytes that have initiated terminal differentiation (usually 20–30% of the population) do not adhere (Adams and Watt, 1990). Between 75 and 90% of ndk adhered to each matrix glycoprotein, whereas the percentage of adherent basal keratinocytes varied depending on the matrix used: the highest numbers of keratinocytes adhered to fibronectin (60 to 65%); intermediate numbers to collagen types I and IV (40 to 45%) and the lowest numbers to laminin (33%), as reported previously (Clarke et al., 1985; Adams and Watt, 1990).

Both cell types adhered at 37°C but not at 4°C, and adhesion was not prevented by pretreatment of cells with cycloheximide. These results indicate that adhesion required metabolic energy or membrane fluidity and did not require new protein synthesis (data not shown).

In experiments in which cells were allowed to attach for 3 h to surfaces coated with increasing concentrations of each matrix glycoprotein, adhesion of both keratinocytes and ndk was found to be saturable. To achieve maximum adhesion of ndk to laminin, collagen type I or type IV, coating concentrations of about 50 μg/ml were required, whereas keratinocytes required coating concentrations of about 100 μg/ml. However, a fibronectin coating concentration of 100 μg/ml was needed to cause maximum adhesion of both cell types (Fig. 2, *A–D*; Adams and Watt, 1990).

Effects of Anti-integrin Antibodies on the Adhesion of Keratinocytes and ndk

To test for the involvement of integrins in cell adhesion to ECM glycoproteins, cell adhesion assays were carried out in the presence of function-blocking mAbs directed against integrin subunits (Table I). Adhesion of both cell types to fibronectin, collagen type IV and laminin was completely inhibited by an anti-β_1 mAb. An anti-α_5 mAb inhibited adhesion of both cell types to fibronectin but not to collagen type IV or laminin; inclusion of an anti-α_3 antibody caused no further inhibition. An anti-α_2 mAb inhibited keratinocyte adhesion to collagen type IV, and did not affect adhesion to fibronectin or laminin. The anti-α_3 mAb used decreased keratinocyte and ndk adhesion to laminin by ~30% and did not affect cell adhesion to fibronectin or collagen type IV. Adhesion of both cell types to laminin was further inhibited when antibodies to both α_2 and α_3 were present. The anti-α_6 antibody did not affect the adhesion of either cell type to fibronectin, laminin, or collagen (Fig. 3, *A* and *B*).

We found that both cell types could adhere to vitronectin, although the percentage of adherent ndk was greater (80% compared to 38%). The anti-α_v mAb inhibited adhesion of both cell types to vitronectin but not to fibronectin. An anti-$\alpha_v\beta_3$ monoclonal, used at the same dilution, had little effect on ndk or keratinocyte adhesion. The anti-β_1 mAb did not affect keratinocyte adhesion to vitronectin but did partially inhibit ndk adhesion. Thus, both cell types used α_v-containing integrin(s) in adhesion to vitronectin but not to fibronectin; in ndk β_1-containing integrin(s) also appeared to be involved (Fig. 3 *C*).

Identification of Integrins Expressed on the Surface of Keratinocytes and ndk

The adhesion assay results demonstrated that both keratinocytes and ndk used β_1 integrins to adhere to fibronectin, laminin and collagen types I and IV, and α_v-containing integrin(s) to adhere to vitronectin. ndk adhered and spread more quickly on all matrix glycoproteins than normal keratinocytes; keratinocytes differed in adhesiveness to the different glycoproteins whereas ndk did not. To examine the

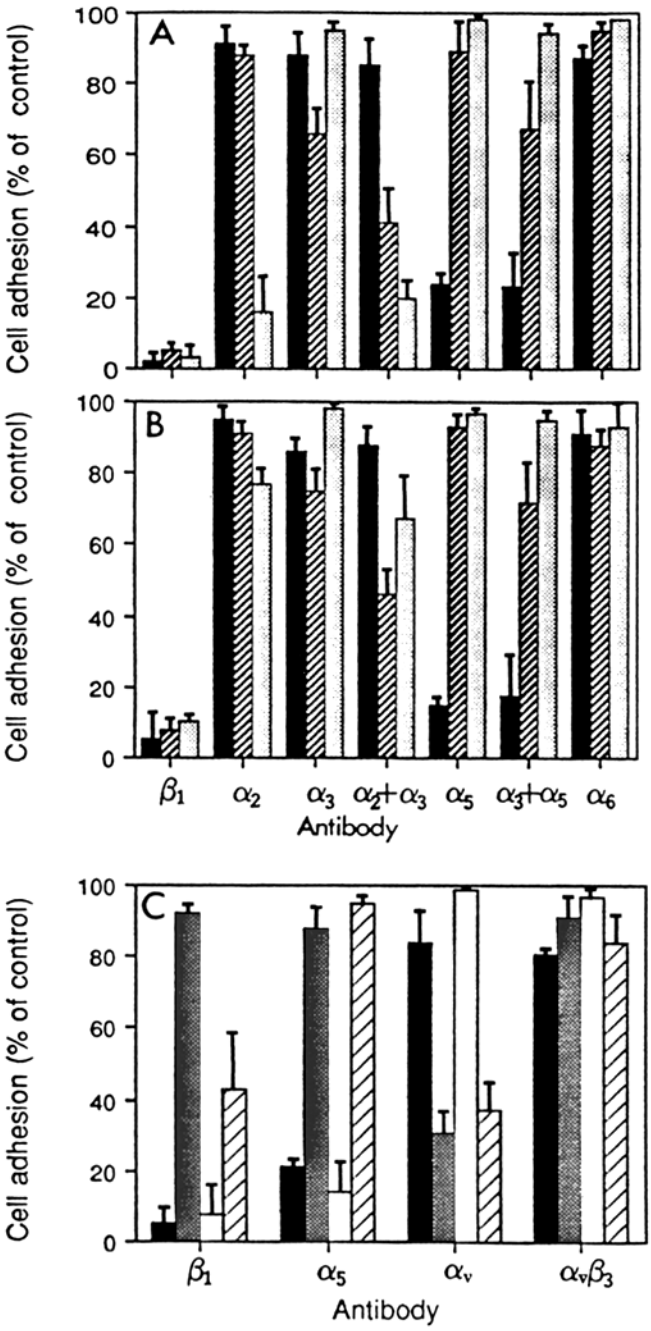


Figure 3. Inhibition of cell adhesion with integrin subunit-specific monoclonal antibodies. (*A*) Keratinocyte adhesion to fibronectin (■), laminin (▨) or collagen type IV (▤), all used at coating concentrations of 20 μg/ml. The antibody concentrations chosen were those that were maximally effective. β_1, 100 μg/ml Mab13 IgG; α_2, 1:100 dilution of 5E8 ascites; α_3, 1:2 dilution of PlB5 conditioned medium; α_2 + α_3, 5E8 ascites used at 1:100 dilution plus a 1:2 dilution of PlB5 conditioned medium (final concentrations); α_5, 1:4 dilution of BIIG2 conditioned medium; α_3 + α_5, 1:4 dilutions of PlB5 and BIIG2 conditioned media (final concentrations); α_6, 1:2 dilution of GoH3 conditioned medium. (*B*) ndk adhesion; conditions the same as in *A*, except that coating concentrations of 15 μg/ml were used. (*C*) Keratinocyte (■, ▨) or ndk (□, ▨) adhesion to fibronectin (■, □ coating concentration 20 μg/ml) or vitronectin (▨, ▨ coating concentration 15 μg/ml). β_1, 100 μg/ml Mab 13 IgG. α_5, 1:4 dilution of BIIG2 conditioned medium; α_v, 13C2; $\alpha_v\beta_3$, 23C6 (both used at 1:50 dilution of ascites). Values are means from duplicate experiments, bars indicate SEM.

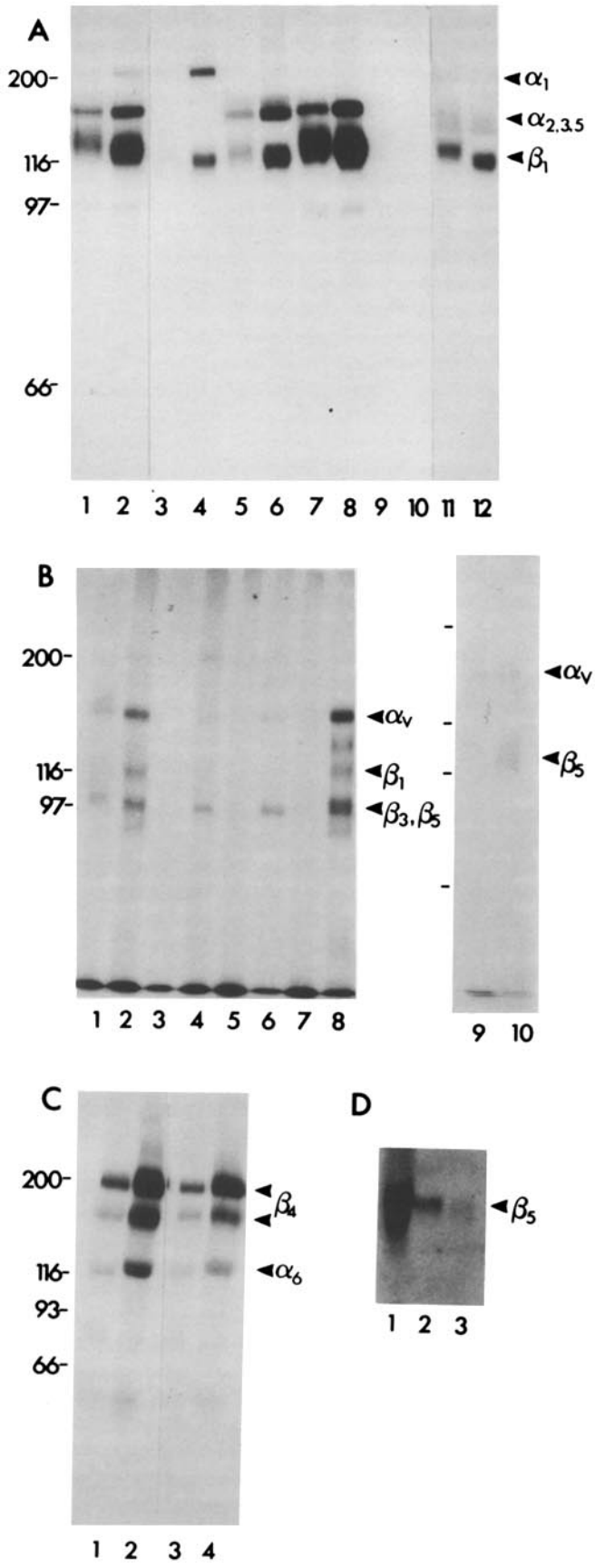
biochemical basis for these differences in adhesive properties, keratinocytes and ndk were surface iodinated, lysed, and aliquots equalized on the basis of TCA-precipitable radioactivity were immunoprecipitated using a panel of monoclonal and polyclonal antibodies to integrin subunits (see Table I).

An antiserum raised against a peptide corresponding to the carboxy-terminus of the β_1 chain, which co-precipitates all α subunits associated with the β_1 subunit, immunoprecipitated from both cell types cell surface proteins which migrated under nonreducing conditions with apparent molecular weights of 120 and 160 kD. An additional protein of 200 kD was precipitated from ndk cells (Fig. 4 *A*, lanes *1* and *2*). The 120-kD band corresponds to the β_1 subunit and the higher molecular weight bands to α subunits. The intensity of all three bands was greater in ndk, suggesting either that these integrins were more readily iodinated on ndk, or that ndk possessed higher levels of β_1 integrins on the cell surface. The results also suggested that ndk cells contained a different complement of α/β_1 heterodimers; therefore, we carried out immunoprecipitations using monoclonal antibodies specific for particular α subunits which are known to associate with the β_1 subunit (Hynes, 1987; Hemler, 1990).

We found that both keratinocytes and ndk cells expressed $\alpha_2\beta_1$, $\alpha_3\beta_1$, and $\alpha_5\beta_1$ and that all of these integrins were present at higher levels on ndk (Fig. 4 *A*, lanes *5–8*, *11*, and *12*). Neither cell type expressed $\alpha_4\beta_1$ (Fig. 4 *A*, lanes *9* and *10*). ndk expressed $\alpha_1\beta_1$, α_1 corresponding to the 200-kD band seen in the ndk anti-β_1 immunoprecipitation (Fig. 4 *A*, lane *2*). In some experiments, very low levels of $\alpha_1\beta_1$ could be detected in keratinocytes but this result was not consistently reproducible (Fig. 4 *A*, lanes *3* and *4*).

The apparent molecular weights of the α_1, α_2, α_3, and α_5 subunits were in the range expected from published studies on other cell types; mean values from four separate experiments are: α_1, 200 kD; α_2, 160 kD; α_3, 160 kD; α_5, 130 kD to 160 kD (c.f., Hemler, 1990). As reported for other epithelial cells (Werb et al., 1989), the α_5 band was very broad and iodinated poorly in comparison with the other α subunits. Interestingly, the β_1 subunit in the ndk integrin complexes consistently migrated slightly faster than the β_1 subunit in the keratinocyte integrin complexes, giving it an apparent molecular weight of 116 kD compared to 120 kD (compare Fig. 4 *A*, lanes *2*, *4*, *6*, *8*, and *12* with lanes *1*, *5*, *7*, and *11*). This difference was also observed when the immunoprecipitates were resolved under reducing conditions (not shown).

We used several antibodies directed against the α_v or β_3 subunits to investigate the expression of β_3 integrins on keratinocytes and ndk. A monoclonal to the α_v subunit precipitated proteins of apparent molecular weight 140 and 100 kD from keratinocytes; from ndk it precipitated bands of 140, 116, 90 kD and a doublet of 100 and 97 kD (Fig. 4 *B*, lanes *1* and *2*). Monoclonals directed against either an $\alpha_v\beta_3$ complex-dependent epitope or the β_3 subunit did not precipitate any proteins from keratinocytes but did precipitate the 140-kD protein and a single band at 97 kD from ndk (Fig. 4 *B*, lanes *3–6*). Affinity purified polyclonal IgG, raised against purified placental vitronectin receptor (Suzuki et al., 1986), very weakly precipitated the 140- and 100 kD proteins from keratinocytes and precipitated the same five proteins from ndk as the anti-α_v monoclonal, plus an addi-



tional protein of apparent molecular weight 130 kD (Fig. 4 *B*, lanes *7* and *8*). The 116-kD band in ndk extracts corresponded in size to the β_1 subunit and could be precipitated by the β_1 peptide antiserum from dissociated integrin complexes (data not shown). The 100-kD band immunoprecipitated by anti α_v in keratinocytes and ndk had the same apparent molecular weight as the β_5 subunit (Cheresh et al., 1989; Ramaswamy and Hemler, 1990). When precipitations were carried out using an antiserum raised against a synthetic peptide corresponding to the carboxy-terminus of the human β_5 subunit, an $\alpha_v\beta_5$ complex was clearly precipitated from ndk cells (Fig. 4 *B*, lane *10*). In these precipitates, it was noticeable that the β_5 subunit was precipitated as a doublet of rather diffuse bands. The same proteins were also precipitated from keratinocyte extracts (Fig. 4 *B*, lane *9*). Since the latter complex was only just detectable, we confirmed the presence of β_5 in keratinocytes by probing keratinocyte mRNA on a Northern blot with a human β_5 cDNA. A single 3.5-kb message was detected, as observed in Dami, a human megakaryocyte line (Fig. 4 *D*; Ramaswamy and Hemler, 1990).

In conclusion, keratinocytes express $\alpha_v\beta_5$ whereas ndk express $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$. The additional bands seen in the ndk precipitations (Fig. 4 *B*) may represent associated cell surface proteins, other integrin subunits, or proteolytic degradation products.

Monoclonals to the α_6 or β_4 subunits precipitated proteins of apparent molecular weight 200 and 180 kD, corresponding to the β_4 subunit (Kajiji et al., 1989; Hemler et al., 1989; Sonnenberg et al., 1990*b*), and 120 kD, corresponding to the α_6 subunit (Sonnenberg et al., 1987, 1988*a*), from both keratinocytes and ndk. All three proteins appeared more abundant in ndk; there was no difference in the mo-

Figure 4. Immunoprecipitation of surface-iodinated integrins and Northern blot of the β_5 subunit. (*A*) β_1 integrins. Aliquots of lysates prepared from surface-iodinated keratinocytes (lanes *1*, *3*, *5*, *7*, *9*, and *11*) or ndk (lanes *2*, *4*, *6*, *8*, *10*, and *12*), were equalized on the basis of TCA-precipitable radioactivity and immunoprecipitated with antibodies to the β_1 subunit (363 polyclonal, lanes *1* and *2*) and mAbs to the α_1 (TS2/7, lanes *3* and *4*), α_2 (12F1, lanes *5* and *6*), α_3 (J143, lanes *7* and *8*), α_4 (B-5G10, lanes *9* and *10*), or α_5 (BIE5, lanes *11* and *12*) subunits. All lanes are from the same gel. Lanes *11* and *12* are a longer exposure than lanes *1–10*. (*B*) α_v integrins. Lysates from keratinocytes (lanes *1*, *3*, *5*, *7*, and *9*) or ndk (lanes *2*, *4*, *6*, *8*, and *10*) were immunoprecipitated with mAbs to α_v (13C2, lanes *1* and *2*), an $\alpha_v\beta_3$ complex-dependent epitope (23C6, lanes *3* and *4*), β_3 (Y2/51, lanes *5* and *6*), an affinity purified polyclonal IgG raised against placental vitronectin receptor (αVNR, lanes *7* and *8*) or an antiserum to a peptide corresponding to amino acids 757–776 of the human β_5 subunit (lanes *9* and *10*). (*C*) β_4 integrins. Lysates of keratinocytes (lanes *1* and *3*) or ndk (lanes *2* and *4*) were immunoprecipitated with antibodies to α_6 (GoH3, lanes *1* and *2*) or β_4 (439-9B, lanes *3* and *4*) subunits. Both pairs of lanes are from the same gel. All samples were resolved on 7.5% polyacrylamide gels under nonreducing conditions. Molecular weight markers are as indicated in kD. Markers for *B*, lanes *9* and *10* are the same as for *A*. (*D*) Northern blot probed with a cDNA probe for β_5. 1 μg mRNA was loaded per track. Dami, a megakaryocyte cell line, served as a positive control (lane *1*); normal keratinocytes (lanes *2* and *3*). The blot was also probed for β actin to confirm that equal amounts of mRNA were loaded (not shown).

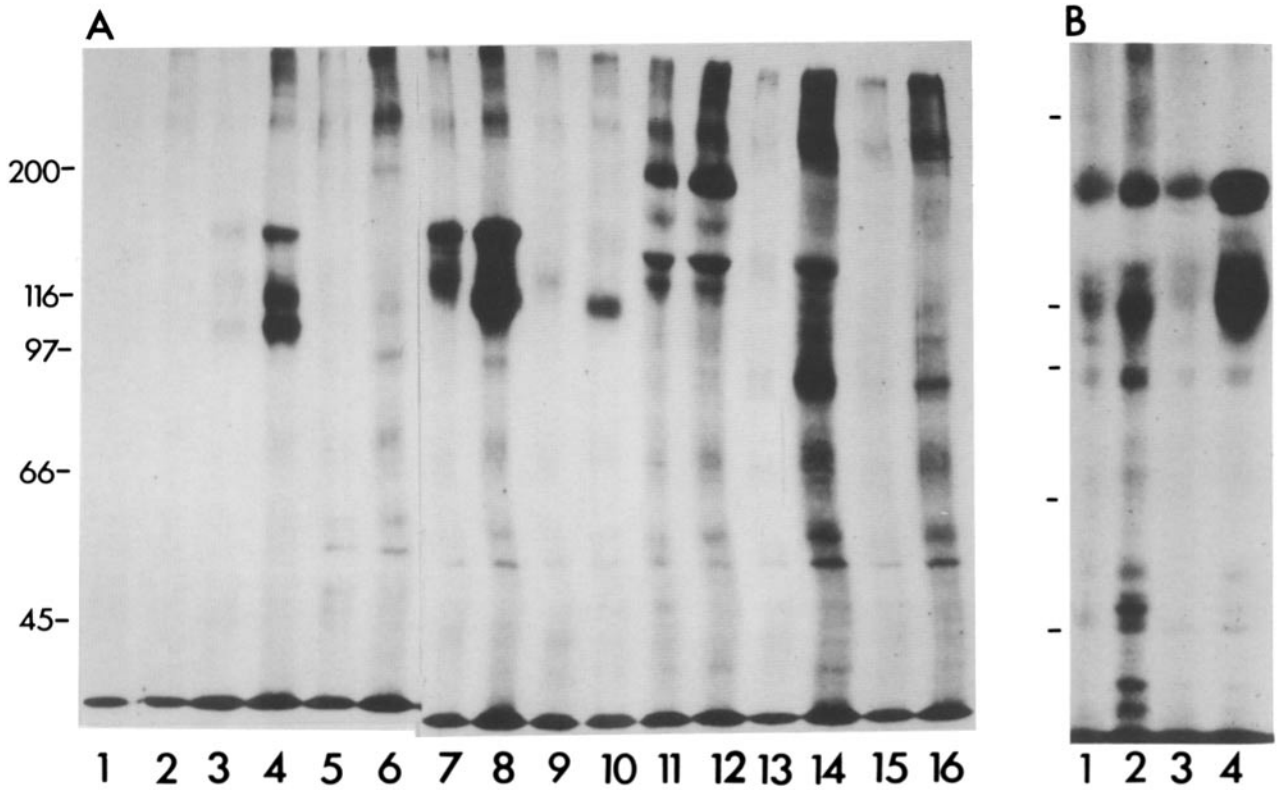


Figure 5. Immunoprecipitation of integrins labeled with ^{35}[S]methionine and ^{35}[S]cysteine. (*A*) Aliquots of cell lysates of keratinocytes (lanes *1*, *3*, *5*, *7*, *9*, *11*, *13*, and *15*) or ndk (lanes *2*, *4*, *6*, *8*, *10*, *12*, *14*, and *16*), equalized on the basis of TCA-precipitable counts, were immunoprecipitated with nonimmune mouse IgG (lanes *1* and *2*), antiserum Rx to the β_1 subunit (lanes *3* and *4*) and mAbs to α_1 (TS2/7, lanes *5* and *6*), α_3 (J143, lanes *7* and *8*), α_5 (BIIG2, lanes *9* and *10*), α_6 (GoH3, lanes *11* and *12*), α_v (13C2, lanes *13* and *14*), and β_3 (Y2/51, lanes *15* and *16*). All lanes are from the same exposure of the same gel. (*B*) Aliquots of cell lysates of keratinocytes (lanes *1* and *3*) or ndk (lanes *2* and *4*) immunoprecipitated with anti-α_2 (12F1; lanes *1* and *2*) or anti-α_3 (J143; lanes *3* and *4*). Nonreduced samples were resolved on a 7.5% polyacrylamide gel.

bilities of the subunits between the two cell types. There was no evidence for association of α_6 with β_1 or for the existence of additional α subunits associated with β_4 (Fig. 4 *C*, lanes *1–4*).

Biosynthesis of Integrins by Keratinocytes and ndk

The use of surface-iodinated cells does not permit quantitative comparisons between integrins, since different heterodimers may not be equally accessible to the iodinating agents. We therefore used metabolic labeling with ^{35}[S]methionine and ^{35}[S]cysteine to obtain more information on the relative levels of integrins synthesised by each cell type and to investigate further the observation that ndk apparently expressed higher levels of integrins than keratinocytes (Fig. 5).

In metabolic labeling experiments, additional proteins were present in the immunoprecipitations, some of which correspond to α or β subunit precursors. For example, precipitation with the anti-β_1 serum precipitated the β_1 precursor as well as the mature α and β subunits (Fig. 5 *A*, lanes *3* and *4*) and anti-α_6 precipitated an additional band of 140 kD (Fig. 5 *A*, lanes *11* and *12*). The experiments indicated that $\alpha_1\beta_1$ was synthesized by ndk but was not detectable in keratinocytes (Fig. 5 *A*, lanes *5* and *6*) and confirmed that neither cell type made $\alpha_4\beta_1$ (not shown). In keratinocytes, the β_1 and β_4 integrins were more abundant that the α_v-containing integrin. Of the keratinocyte β_1 integrins, $\alpha_2\beta_1$ and $\alpha_3\beta_1$ were labeled to approximately equal intensity and $\alpha_5\beta_1$ was labeled less intensely, suggesting that it was indeed less abundant than the other β_1 integrins. All integrins were present in greater amounts in ndk lysates than in keratinocyte lysates, but the relative abundance of the β_1 integrins was the same.

Localization of Integrins on Cultured Keratinocytes

Integrin distribution was examined in small stratified colonies of human epidermal keratinocytes which had been grown on J2 feeder cells and then fixed in formaldehyde, but not permeabilized. In the center of these colonies, the basal layer of keratinocytes is covered by layers of terminally differentiating involucrin-positive cells (Fig. 6 *a*; Watt, 1988). Thus in nonpermeabilized preparations, staining of the basal cells can only be observed at the margins of the colony, which consist of a single cell layer.

The anti-β_1 mAbs Mab13 and DH12 both gave intense staining of the cell–cell contact zones of the basal keratinocytes and did not stain the suprabasal cells (Fig. 6 *b*; Nicholson and Watt, 1991). Different mAbs to both α_2 (12F1, 5E8, and P1E6) and α_3 (J143 and P1B5) produced similar, basal cell-specific staining patterns (Fig. 6, *c* and *d* and results not shown). mAbs to α_5 (BIIG2 and Mab16) produced a different and somewhat varied staining pattern: a few basal cells were uniformly and diffusely stained, but in most colonies

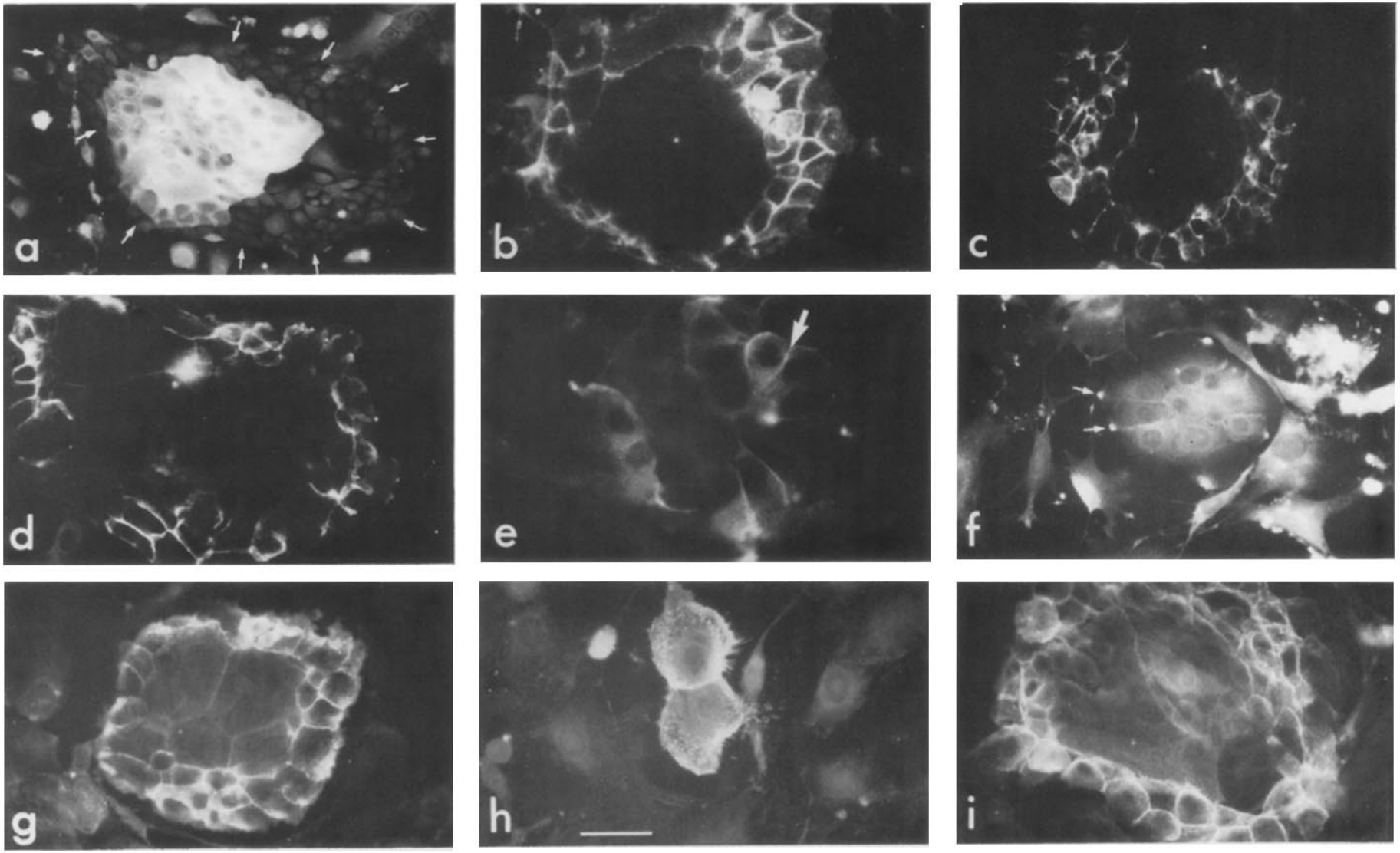


Figure 6. Localization of integrins in keratinocyte colonies by indirect immunofluorescence. Keratinocyte colonies were fixed in formaldehyde (*b–i*) or fixed and permeabilized in methanol (*a*). Cells were stained with a mAb to involucrin, to visualize terminally differentiating cells in the upper layers of the colonies (*a*), or mAbs to integrin subunits. *b*, Mab13, β_1; *c*, 5E8, α_2; *d*, PlB5, α_3; *e*, BIIG2, α_5; *f*, 13C2, α_v; *g* and *h*, 439-9B, β_4; *i*, GoH3, α_6. Arrows in *a* indicate the margins of the colony. Arrow in *e* indicates intense staining in area of cell–cell contact. Arrows in *f* indicate areas of intense staining at the free margins of the cells. This colony consists entirely of basal cells. Bars: (*a*, *b*, *f*, *g–i*) 50 μm; (*c*, *d*) 73 μm; (*e*) 36 μm.

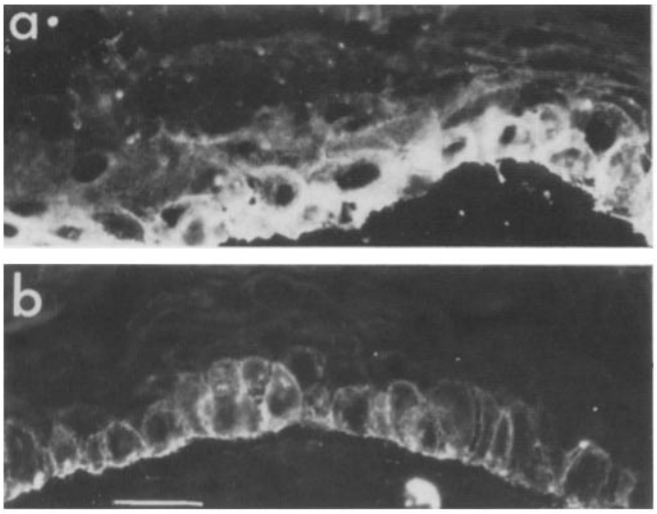


Figure 7. Localization of integrins in keratinocyte cultures by indirect immunofluorescence. Transverse frozen sections of confluent keratinocyte cultures were stained with monoclonal antibodies to (*a*) β_1 (DH12) or (*b*) α_6 (GoH3) integrin subunits. Bar, 50 μm.

spotty staining of varying intensity which delineated the cell–cell contact areas of basal cells was obtained (Fig. 6 *e*; and Nicholson and Watt, 1991). Intense, localized staining at the free edges of some cells was also observed (data not shown).

As expected from the immunoprecipitation results, no staining of keratinocyte colonies was obtained using the anti-β_3 mAb. The anti-α_v monoclonal produced faint spotty staining of cell–cell contact areas between basal cells and, in some cells, intense staining of areas at the free margins of the cells. The feeder cells were also strongly stained by this antibody (Fig. 6 *f*), but suprabasal cells were not stained.

Antibodies directed against α_6 or β_4 produced basal cell-specific staining patterns in which the majority of staining was localized to cell–cell contact areas. Additionally, staining was observed at the free edges of basal cells; this was somewhat heterogeneous and appeared to be associated with cell-surface microvilli and filopodia in contact with the substratum (Fig. 6, *g–i*). Filopodial staining was very marked in small groups of cells (Fig. 6 *h*).

The observation that staining for integrins was reduced or absent on keratinocytes undergoing terminal differentiation was confirmed by staining transverse sections of confluent keratinocyte cultures which had been released from the culture dish by treatment with dispase. The anti-β_1 monoclonal stained the entire margin of cells in the basal layer (Fig. 7 *a*). The monoclonal to the α_6 subunit (which in keratinocytes is associated exclusively with the β_4 subunit; see Figs. 4 and 5) also stained the entire margin of basal layer cells (Fig. 7 *b*). In some areas, the basal margin was stained more intensely than the lateral and apical margins; in the ventral cytoplasm brightly stained spots were sometimes observed, possibly corresponding to internalized hemidesmosome-like structures (not shown; Carter et al., 1990*b*).

Expression of Integrins on ndk

ndk were grown on glass coverslips and stained with anti-integrin mAbs after fixation in formaldehyde. All cells were stained by these antibodies; this is consistent with the fact that the cell population does not contain any terminally differentiated cells (Adams and Watt, 1988). However, the staining patterns obtained with antibodies to different integrin subunits were markedly different. Staining with anti-β_1 or anti-α_3 mAbs was localized to cell surface microvilli and to areas of membrane ruffling (Fig. 8, *a* and *c*; Nicholson and Watt, 1991). In contrast, the anti-α_1 and anti-α_2 monoclonals stained tiny spots within areas of membrane ruffling (Fig. 8 *b* and data not shown). The anti-α_5 mAbs gave intense spotty staining in localized regions of ruffled membrane and in areas at the trailing edge of the cells. Regions of apposed cell membranes also stained (Fig. 8 *d*; Nicholson and Watt, 1991). The staining obtained with anti-α_2 or anti-α_5 monoclonals was more heterogeneous than that observed with the anti-β_1 or anti-α_3 antibodies; thus, for example, not all areas of ruffled membrane were stained.

The anti-α_v mAb gave intense staining of patches of ruffled membrane. More spotty staining could be distinguished in some cells at the cell margin and underlying the spread portion of the cytoplasm (Fig. 8 *g*). In contrast, the anti-β_3 mAb only stained membrane ruffles (Fig. 8 *h*). This suggests that $\alpha_v\beta_3$ has a different subcellular distribution to $\alpha_v\beta_1$ or $\alpha_v\beta_5$; thus the different α_v-containing integrins may have separate functions.

The anti-α_6 and β_4 mAbs produced intense staining of cell surface microvilli and filopodia in contact with the substratum (Fig. 8, *e* and *f*). These staining patterns were similar to those obtained with the anti-β_1 and anti-α_3 antibodies.

Discussion

In this paper we have investigated the adhesive properties and integrin expression profiles of cultured human epidermal keratinocytes and a non-differentiating strain of keratinocytes, termed ndk. The results of the adhesion-blocking experiments indicate that both cell types use $\alpha_5\beta_1$ to adhere to fibronectin and $\alpha_3\beta_1$ to adhere to laminin. In keratinocytes $\alpha_2\beta_1$ was involved in adhesion to collagen type IV and also functioned synergistically with $\alpha_3\beta_1$ in adhesion to laminin; in ndk $\alpha_2\beta_1$ appears to be only involved in adhesion to laminin. ndk also express $\alpha_1\beta_1$, which has been identified as a collagen or laminin receptor in various cell types (Hemler, 1990; Hall et al., 1990); this receptor may thus be the major ndk collagen receptor. Other investigators have also reported a role for $\alpha_5\beta_1$ in keratinocyte adhesion to fibronectin (Carter et al., 1990*a*) and for $\alpha_2\beta_1$ in adhesion to collagen types I and IV (Staquet et al., 1990) or collagen type I and laminin (Carter et al., 1990*a*,*b*). Evidence regarding the role of $\alpha_3\beta_1$ is conflicting: adhesion blocking experiments have indicated that it is involved in keratinocyte adhesion to fibronectin and collagen type IV (Staquet et al., 1990), or to fibronectin and laminin (Carter et al., 1990*a*,*b*). However, $\alpha_5\beta_1$ is the major keratinocyte integrin which binds to fibronectin–Sepharose (Adams and Watt, 1990). $\alpha_3\beta_1$ and $\alpha_2\beta_1$ may also function in cell–cell adhesion (Kaufmann et al., 1989; Larjava et al., 1990; Carter et al., 1990*a*). It seems possible that β_1 integrins in keratinocytes function in initial adhesive contacts to extracellular matrix glycoproteins and then relocate to cell–cell contact areas (Carter et al., 1990*a*).

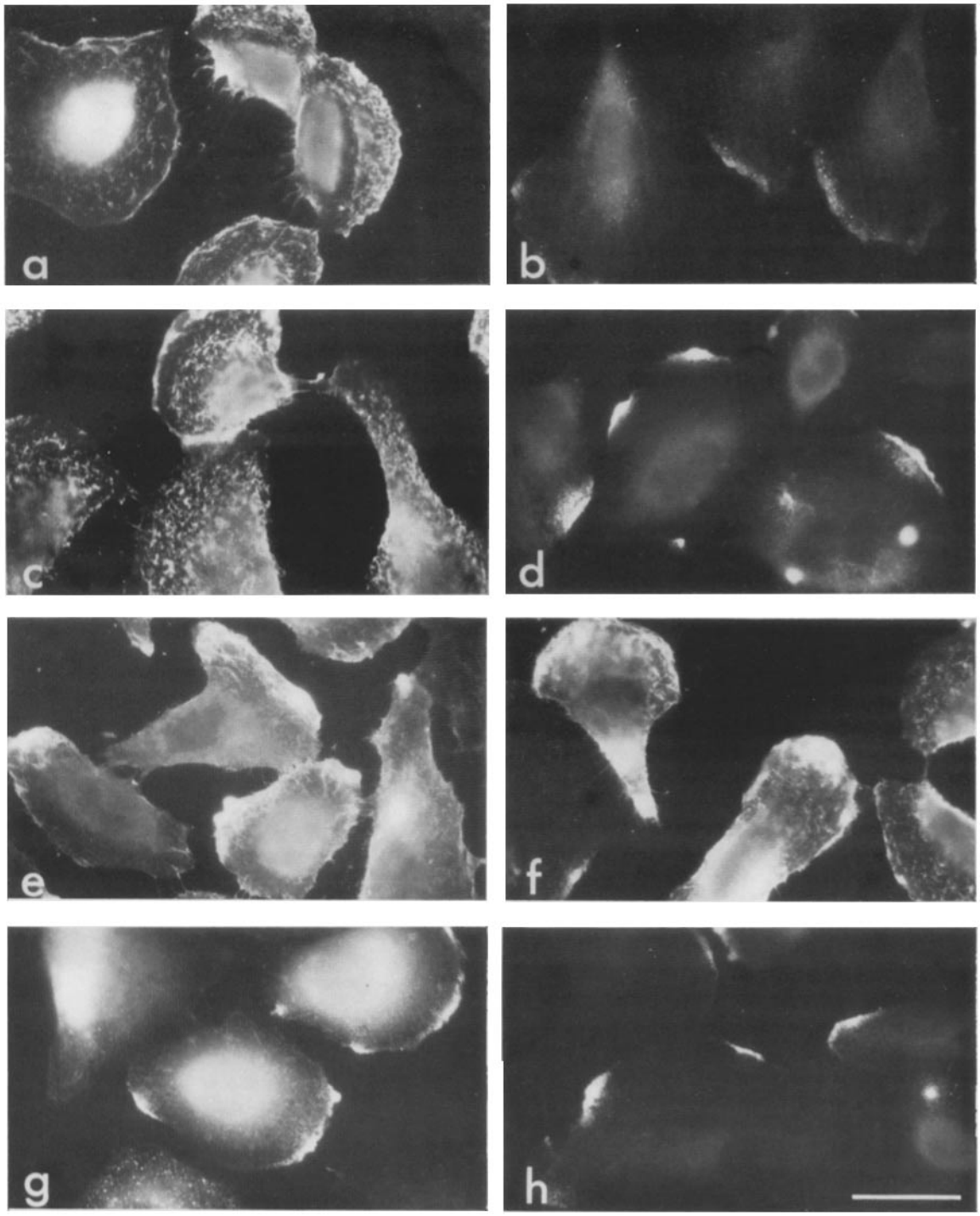


Figure 8. Localization of integrins in ndk cultures by indirect immunofluorescence. ndk were fixed in formaldehyde and stained with mAbs to integrin subunits. *a*, Mabl3, β_1; *b*, PlE6, α_2; *c*, J143, α_3; *d*, Mabl6, α_5; *e*, GoH3, α_6; *f*, 439-9B, β_4; *g*, 13C2, α_v; *h*, Y2/51, β_3. Bar, 30 μm.

Adhesion to vitronectin was mediated by α_v-containing integrins. Consistent with a previous report, β_1-containing integrins were not involved in keratinocyte adhesion to vitronectin (Larjava et al., 1990). In keratinocytes, α_v was associated with the β_5 subunit; this is in agreement with the finding that these cells do not express β_3 integrins (Figs. *4* and *5*; Staquet et al., 1990; Marchisio et al., 1991) and the detection of β_5 in other epithelial cells (Cheresh et al., 1989; Ramaswamy and Hemler, 1990). $\alpha_v\beta_5$ has been reported to bind fibronectin as well as vitronectin (Cheresh et al., 1989). However, the keratinocyte α_v integrin only mediated adhesion to vitronectin (see Fig. 3) and did not bind to fibronectin–Sepharose (Adams and Watt, 1990).

In ndk, α_v- and β_1-containing integrins appeared to be involved in adhesion to vitronectin, and indeed ndk were found to express $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$. Both $\alpha_v\beta_1$ and $\alpha_v\beta_5$ function

as fibronectin receptors in certain cell types (Vogel et al., 1990; Cheresh et al., 1989), however, in ndk, α_v containing integrins only appeared to mediate adhesion to vitronectin.

The $\alpha_6\beta_4$ integrin did not appear to be involved in adhesion of either cell type to fibronectin, laminin, or collagen, since an α_6 antibody, which inhibits the adhesion of $\alpha_6\beta_1$-positive cells to laminin (Sonnenberg et al., 1988*b*, 1990*a*), did not prevent adhesion to any of these matrix glycoproteins. However, in assays in which keratinocytes are allowed to adhere for 12 h, $\alpha_6\beta_4$ does appear to play some role in cell–matrix adhesion (De Luca et al., 1990; Carter et al., 1990*b*). By this time, proteins secreted by the keratinocytes themselves form part of the extracellular matrix (Carter et al., 1990*b*). Recently, $\alpha_6\beta_4$ has been localized to hemidesmosomes in vivo (Stepp et al., 1990), suggesting that its ligand may be a component of anchoring filaments or an as yet uncharacterized basement membrane component. It has also been proposed that this integrin may be involved in the regulation of cell proliferation (Sacchi et al., 1989).

Immunoprecipitation experiments revealed both quantitative and qualitative differences between the integrins expressed by ndk and keratinocytes and provide possible explanations for the greater adhesiveness of ndk to extracellular matrix proteins. The levels of all integrins were greater in ndk than keratinocytes, whether assessed by surface iodination or metabolic labeling, and this might account for the increased adhesiveness. However, levels of β_1 integrins on the cell surface do not always correlate with the ligand binding activity, as in the case of $\alpha_5\beta_1$ in keratinocytes (Adams and Watt, 1990), of $\alpha_4\beta_1$, $\alpha_5\beta_1$ and $\alpha_6\beta_1$ in lymphocytes (Shimizu et al., 1990) and of $\alpha_6\beta_1$ in neurons (Neugebauer and Reichardt, 1991). Posttranslational modifications of integrin subunits, by phosphorylation or glycosylation, have been correlated with alterations in cell adhesiveness (see Adams and Watt, 1990 for discussion). The immunoprecipitations indicate that the β_1 subunit may undergo different posttranslational modifications in ndk and keratinocytes, since the β_1 precursors comigrate (see Fig. 5, lanes *3* and *4*), yet the mature β_1 in keratinocytes has a higher apparent molecular weight than that of ndk. The keratinocyte β_1 is also larger than the β_1 subunit of dermal fibroblasts (Larjava et al., 1989) or MG63 osteosarcoma cells (Adams, unpublished observations). Furthermore, in both cell types, the breadth of the β_1 subunit band in the iodinated immunoprecipitations (Fig. 4) varied depending on which α subunit was being precipitated. This may indicate that the β_1 subunit undergoes different posttranslational modifications depending on the α subunit with which it is associated. Finally, ndk express integrins which normal keratinocytes do not: $\alpha_v\beta_1$, which binds vitronectin and, in some cases, fibronectin (Bodary et al., 1990; Vogel et al., 1990), and $\alpha_v\beta_3$, which can bind vitronectin, fibrinogen, von Willebrand factor, osteopontin, and thrombospondin (Hemler, 1990).

Indirect immunofluorescent staining of the keratinocyte colonies indicated that the localization of all integrins was restricted to basal cells. This is in agreement with the distribution patterns of integrins in the intact epidermis (see references cited in Introduction), with immunoprecipitations from surface-iodinated keratinocytes (Adams and Watt, 1990) and with mRNA levels for the α_5 and β_1 integrin subunits (Nicholson and Watt, 1991). As previously reported, antibodies against the β_1, α_2, and α_3 subunits stained cell–cell contact areas (Larjava et al., 1989; Carter et al., 1990*a*). Thus, suprabasal, terminally differentiating cells must use other mechanisms to adhere to each other (Watt, 1984).

α_v and α_5 had a more patchy and variable distribution on keratinocytes than α_2 and α_3, and were sometimes located at the free margins of the cells. Antibodies to α_6 and β_4 also stained free cell margins and filopodia, in addition to cell–cell contact areas. The basal monolayer of keratinocytes at the periphery of a stratified colony migrates outwards as the colony enlarges (Barrandon and Green, 1987); thus the distribution of these integrins may reflect a role in cell migration (Bretscher, 1989). The staining patterns we observed were of unpermeabilized cells; β_1 integrins have been found in the focal contacts of permeabilized keratinocytes in short term cultures (Carter et al., 1990*a*; Guo et al., 1990) and $\alpha_6\beta_4$ has been localized to hemidesmosome-like structures called stable anchoring contacts (Carter et al., 1990*b*).

In ndk cultures, no terminally differentiating cells are present and all cells stained with every anti-integrin antibody. The distribution of the integrins did not relate in a simple way to the distribution of the extracellular matrix proteins identified as their ligands in adhesion assays: fibronectin and collagen type IV are found in a network over and around the cells, whereas laminin is localized in small patches on ruffled membranes (Adams, Nicholson, and Watt, unpublished observations). We have recently reported that when cell–cell adhesion of ndk is induced by the polyanionic detergent, suramin, the β_1 integrins redistribute to regions of cell–cell contact (Adams et al., 1991).

Finally, our experiments have yielded more information about the phenotype of ndk cells. The presence of integrins on all ndk is consistent with a basal keratinocyte phenotype (and see Adams and Watt, 1988). However, other characteristics, such as synthesis of the $\alpha_v\beta_1$ and $\alpha_v\beta_3$ integrins, are not shared with normal basal keratinocytes. ndk expressed $\alpha_1\beta_1$, an integrin that is expressed at moderately high levels until 15 wk of gestation in human fetal epidermis (Hertle et al., 1991) and this would suggest that ndk resemble fetal keratinocytes more closely than adult keratinocytes. Nevertheless, β_3 is never detected in developing epidermis (Hertle et al., 1991), raising two other possibilities: that ndk are a minor epidermal population, perhaps found in adnexal structures, or that for some reason β_3 expression has been induced in culture. Our experiments do show that the adhesive properties of ndk differ from normal basal keratinocytes and may be attributed to differences in the type of integrins expressed, the level of expression and the distribution of the receptors on the cell surface.

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References

Adams, J. C., R. A. Furlong, and F. M. Watt. 1991. Production of scatter factor by ndk, a strain of epithelial cells, and inhibition of scatter factor activity by suramin. *J. Cell Sci.* 98:385–394.

Adams, J. C., and F. M. Watt. 1988. An unusual strain of human keratinocytes which do not stratify or undergo terminal differentiation in culture. *J. Cell Biol.* 107:1927–1938.

Adams, J. C., and F. M. Watt. 1989. Fibronectin inhibits the terminal differentiation of human keratinocytes. *Nature (Lond.).* 340:307–309.

Adams, J. C., and F. M. Watt. 1990. Changes in keratinocyte-extracellular matrix interactions during terminal differentiation: reduction in fibronectin binding precedes loss of the $\alpha_5\beta_1$ integrin from the cell surface. *Cell.* 63:425–435.

Akiyama, S. K., S. S. Yamada, W.-T. Chen, and K. M. Yamada. 1989. Analysis of fibronectin receptor function with monoclonal antibodies: roles in cell adhesion, migration, matrix assembly and cytoskeletal organisation. *J. Cell Biol.* 109:863–875.

Alitalo, K., E. Kuismanen, R. Myllyla, U. Kiistala, S. Askoseljavaara, and A. Vaheri. 1982. Extracellular matrix proteins of human epidermal keratinocytes and feeder 3T3 cells. *J. Cell Biol.* 94:497–505.

Allen-Hoffmann, B. L., and J. G. Rheinwald. 1984. Polycyclic aromatic hydrocarbon mutagenesis of human epidermal keratinocytes in culture. *Proc. Natl. Acad. Sci. USA.* 81:7802–7806.

Barrandon, Y., and H. Green. 1987. Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor α and epidermal growth factor. *Cell.* 50:1131–1137.

Bodary, S. C., and J. W. McLean. 1990. The integrin β_1 subunit associates with the vitronectin receptor α_v subunit to form a novel vitronectin receptor in a human embryonic kidney cell line. *J. Biol. Chem.* 265:5938–5941.

von dem Borne, A. E. G. Kr., P. W. Modderman, L. G. Admiraal, and H. K. Nieuwenhuis. 1989. *In* Leucocyte Typing IV. W. Knapp, B. Dörken, W. R. Gilks, E. P. Rieber, R. E. Schmidt, H. Stein, and A. E. G. Kr. von dem Borne, editors. Oxford University Press. 951–966.

Bretscher, M. S. 1989. Endocytosis and recycling of the fibronectin receptor in CHO cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1341–1348.

Carter, W. G., E. A. Wayner, T. S. Bouchard, and P. Kaur. 1990*a*. The role of integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ in cell-cell and cell-substrate adhesion of human epidermal cells. *J. Cell Biol.* 110:1387–1404.

Carter, W. G., P. Kaur, S. G. Gil, P. J. Gahr, and E. A. Wayner. 1990*b*. Distinct functions for integrins $\alpha_3\beta_1$ in focal adhesions and $\alpha_6\beta_4$/bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. *J. Cell Biol.* 111:3141–3154.

Cheresh, D. A., J. W. Smith, H. M. Cooper, and V. Quaranta. 1989. A novel vitronectin receptor integrin ($\alpha_v\beta_x$) is responsible for distinct adhesive properties of carcinoma cells. *Cell.* 57:59–69.

Clarke, R. A. F., J. M. Folkvord, and R. L. Wertz. 1985. Fibronectin, as well as other extracellular matrix proteins, mediate human keratinocyte adherence. *J. Invest. Dermatol.* 85:378–383.

Davies, J., J. Warwick, N. Totty, R. Philp, M. Helfrich, and M. Horton. 1989. The osteoclast functional antigen, implicated in the regulation of bone resorption, is biochemically related to the vitronectin receptor. *J. Cell Biol.* 109:1817–1826.

De Luca, M., R. N. Tamura, S. Kajiji, S. Bondanza, P. Rossino, R. Cancedda, P. C. Marchisio, and V. Quaranta. 1990. Polarized integrin mediates human keratinocyte adhesion to basal lamina. *Proc. Natl. Acad. Sci. USA.* 87:6888–6892.

De Strooper, B., M. Saison, M. Jaspers, M. Spaepen, F. Van Leuven, H. Van Den Berghe, and J. J. Cassiman. 1988. Monoclonal DH12 reacts with cell surface and a precursor form of the β subunit of the human fibronectin receptor. *Cell Biol. Int. Rep.* 12:9–16.

De Strooper, B., B. Van Der Schueren, M. Jaspers, M. Saison, M. Spaepen, F. Van Leuven, H. Van Den Berghe, and J. J. Cassiman. 1989. Distribution of the β_1 subgroup of the integrins in human cells and tissues. *J. Histochem. Cytochem.* 37:299–307.

Elices, M. J., and M. E. Hemler. 1989. The human integrin VLA-2 is a collagen receptor on some cells and a collagen/laminin receptor on others. *Proc. Natl. Acad. Sci. USA.* 86:9906–9910.

Fradet, Y., C. Cordon-Cardo, T. Thomson, M. E. Daly, W. F. Whitmore, K. O. Lloyd, M. R. Melamed, and L. J. Old. 1984. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. USA.* 81:224–228.

Green, H. 1977. Terminal differentiation of cultured human epidermal cells. *Cell.* 11:405–416.

Green, H., O. Kehinde, and J. Thomas. 1979. Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. *Proc. Natl. Acad. Sci. USA.* 76:5665–5668.

Greenberg, S. M., D. S. Rosenthal, T. A. Greeley, R. Tantravahi, and R. I. Handin. 1988. Characterization of a new megakaryocytic cell line: the Dami cell. *Blood.* 72:1968–1977.

Guo, M., K.-I. Toda, and F. Grinnell. 1990. Activation of human keratinocyte migration on type I collagen and fibronectin. *J. Cell Sci.* 96:197–205.

Hall, D. E., L. F. Reichardt, E. Crowley, B. Holley, H. Moezzi, A. Sonnenberg, and C. H. Damsky. 1990. The $\alpha_1\beta_1$ and $\alpha_6\beta_1$ integrin heterodimers mediate cell attachment to distinct sites on laminin. *J. Cell Biol.* 110:2175–2184.

Hemler, M. E. 1990. VLA proteins in the integrin family: structures, functions and their role on leukocytes. *Ann. Rev. Immunol.* 8:363–400.

Hemler, M. E., C. Crouse, and A. Sonnenberg. 1989. Association of the VLA α^6 subunit with a novel protein. *J. Biol. Chem.* 264:6529–6535.

Hemler, M. E., F. Sanchez-Madrid, T. J. Flotte, A. M. Krensky, S. J. Burakoff, A. K. Bhan, T. A. Springer, and J. L. Strominger. 1984. Glycoproteins of 210,000 and 130,000 M. W. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. *J. Immunol.* 132:3011–3018.

Hemler, M. E., C. Huang, Y. Takada, L. Schwarz, J. L. Strominger, and M. L. Clabby. 1987. Characterisation of the cell surface heterodimer VLA-4 and related peptides. *J. Biol. Chem.* 262:11478–11485.

Hertle, M. D., J. C. Adams, and F. M. Watt. 1991. Integrin expression during human epidermal development in vivo and in vitro. *Development.* 112:193–206.

Horton, M. A., D. Lewis, K. McNulty, J. A. S. Pringle, and T. J. Chambers. 1985. Monoclonal antibodies to osteoclastomas (giant cell bone tumours): definition of osteoclast-specific antigens. *Cancer Res.* 45:5663–5669.

Hynes, R. O. 1973. Alterations of cell-surface proteins by viral transformation and by proteolysis. *Proc. Natl. Acad. Sci. USA.* 70:3170–3174.

Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549–554.

Kajiji, S., R. N. Tamura, and V. Quaranta. 1989. A novel integrin ($\alpha_E\beta_4$) from human epithelial cells suggests a fourth family of integrin adhesion receptors. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:673–680.

Kantor, R. R. S., M. J. Mattes, K. O. Lloyd, L. J. Old, and A. P. Albino. 1987. Biochemical analysis of two cell surface glycoprotein complexes, very common antigen 1 and very common antigen 2. *J. Biol. Chem.* 262:15158–15165.

Kaufmann, R., D. Frösch, C. Westphal, L. Weber, and C. E. Klein. 1989. Integrin VLA-3: ultrastructural localization at cell–cell contact sites of human cell cultures. *J. Cell Biol.* 109:1807–1815.

Kennel, S. J., L. J. Foote, R. Falcioni, A. Sonnenberg, C. D. Stringer, C. Crouse, and M. E. Hemler. 1989. Analysis of the tumor-associated antigen TSP-180. *J. Biol. Chem.* 264:15515–15521.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature (Lond.).* 227:680–685.

Languino, L. R., K. Gehlsen, E. Wayner, W. Carter, E. Engvall, and E. Ruoslahti. 1989. Endothelial cells use $\alpha_2\beta_1$ as a laminin receptor. *J. Cell Biol.* 109:2455–2462.

Larjava, H., J. Peltonen, S. K. Akiyama, S. S. Yamada, H. R. Galnick, J. Uitto, and K. M. Yamada. 1990. Novel function for β_1 integrins in keratinocyte cell-cell interactions. *J. Cell Biol.* 110:803–815.

Marcantonio, E. E., and R. O. Hynes. 1988. Antibodies to the conserved cytoplasmic domain of the integrin β_1 subunit react with proteins in vertebrates, invertebrates, and fungi. *J. Cell Biol.* 106:1765–1772.

Marchisio, P. C., S. Bondanza, O. Cremona, R. Cancedda, and M. De Luca. 1991. Polarized expression of integrin receptors ($\alpha_6\beta_4$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, and $\alpha_v\beta_5$) and their relationship with the cytoskeleton and basement membrane matrix in cultured human keratinocytes. *J. Cell Biol.* 112:761–773.

Martin, G. R., R. Timpl, and K. Kuhn. 1988. Basement membrane proteins—molecular structure and function. *Adv. Protein Chem.* 39:1–50.

Neugebauer, K. M., and L. F. Reichardt. 1991. Cell-surface regulation of β_1-integrin activity on developing retinal neurons. *Nature (Lond.).* 350:68–71.

Nicholson, L. J., and F. M. Watt. 1991. Decreased expression of fibronectin and the $\alpha_5\beta_1$ integrin during terminal differentiation of human keratinocytes. *J. Cell Sci.* 98:225–232.

Peltonen, J., H. Larjava, S. Jaakkola, H. Gralnick, S. K. Akiyama, S. S. Yamada, K. M. Yamada, and J. Uitto. 1989. Localisation of integrin receptors for fibronectin, collagen, and laminin in human skin. *J. Clin. Invest.* 84:1916–1923.

Pischel, K. D., M. E. Hemler, C. Huang, H. G. Bluestein, and V. L. Woods. 1987. Use of the monoclonal antibody 12F1 to characterise the differentiation antigen VLA2. *J. Immunol.* 138:226–233.

Ramaswamy, H., and M. E. Hemler. 1990. Cloning, primary structure and properties of a novel human integrin β subunit. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1561–1568.

Rheinwald, J. G., and H. Green. 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell.* 6:331–344.

Sacchi, A., G. Piaggio, M. G. Rizzo, R. Falcioni, and S. J. Kennel. 1989. Stimulation of tumour cell growth *in vitro* by a monoclonal antibody to a tumour specific protein (TSP-180) present on the surface of 3LL cells. *Clin. Exp. Metastasis.* 7:41–54.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Vol. I, Chapter 7.

Shimizu, Y., G. A. Van Seventer, K. J. Horgan, and S. Shaw. 1990. Regulated expression and binding of three VLA (β_1) integrin receptors on T cells. *Nature (Lond.).* 345:250–253.

Sonnenberg, A., H. Daams, M. A., van der Valk, J. Hilken, and J. Hilgers. 1986. Development of mouse mammary gland: identification of stages in differentiation of luminal and myoepithelial cells using monoclonal antibodies and polyvalent antiserum against keratin. *J. Histochem. Cytochem.* 34: 1037–1046.

Sonnenberg, A., F. Hogervorst, A. Osterop, and F. E. M. Veltman. 1988*a*. Identification and characterization of a novel antigen complex on mouse mammary tumor cells using a monoclonal antibody against platelet glycoprotein Ic. *J. Biol. Chem.* 263:14030–14038.

Sonnenberg, A., P. W. Modderman, and F. Hogervorst. 1988*b*. Laminin receptor on platelets is the integrin VLA-6. *Nature (Lond.).* 336:487–489.

Sonnenberg, A., H. Janssen, F. Hogervorst, J. Calafat, and J. Hilgers. 1987.

A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. *J. Biol. Chem.* 262:10376–10383.

Sonnenberg, A., C. J. T. Linders, P. W. Modderman, C. H. Damsky, M. Aumailley, and R. Timpl. 1990*a*. Integrin recognition of different cell binding fragments of laminin (P1, E3, E8) and evidence that $\alpha_6\beta_1$ but not $\alpha_6\beta_4$ functions as the major receptor for fragment E8. *J. Cell Biol.* 110:2145–2155.

Sonnenberg, A., C. J. T. Linders, J. H. Daams, and S. J. Kennel. 1990*b*. The $\alpha_6\beta_1$ (VLA-6) and $\alpha_6\beta_4$ protein complexes: tissue distribution and biochemical properties. *J. Cell Sci.* 96:207–217.

Staquet, M. J., B. Levarlet, C. Dezutter-Dambuyant, D. Schmitt, and J. Thivolet. 1990. Identification of specific human epithelial cell integrin receptors as VLA proteins. *Exp. Cell Res.* 187:277–283.

Stepp, M. A., S. Spurr-Michaud, A. Tisdale, J. Elwell, and I. K. Gipson. 1990. $\alpha_6\beta_4$ integrin is a component of hemidesmosomes. *Proc. Natl. Acad. Sci. USA.* 87:8970–8974.

Suzuki, S., W. S. Argraves, R. Pytela, H. Arai, T. Krusius, M. D. Pierschbacher, and E. Ruoslahti. 1986. cDNA and amino acid sequence of the cell adhesion protein receptor recognizing vitronectin reveal a transmembrane domain and homologies with other adhesion protein receptors. *Proc. Natl. Acad. Sci. USA.* 86:8614–8616.

Vogel, B. E., G. Tarone, F. G. Giancotti, J. Gailit, and E. Ruoslahti. 1990. A novel fibronectin receptor with an unexpected subunit composition ($\alpha_v\beta_1$). *J. Biol. Chem.* 265:5934–5937.

Watt, F. M. 1984. Selective migration of terminally differentiating cells from the basal layer of cultured human epidermis. *J. Cell Biol.* 98:16–21.

Watt, F. M. 1988. Proliferation and terminal differentiation of human epidermal keratinocytes in culture. *Biochem. Soc. Trans.* 16:666–668.

Wayner, E. A., and W. G. Carter. 1987. Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique α and common β subunits. *J. Cell Biol.* 105:1873–1884.

Wayner, E. A., W. G. Carter, R. S. Piotrowicz, and T. J. Kunicki. 1988. The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J. Cell Biol.* 107:1881–1891.

Werb, Z., P. M. Tremble, O. Behrendtsen, E. Crowley, and C. Damsky. 1989. Signal transduction though the fibronectin receptor induces collagenase and stromolysin gene expression. *J. Cell Biol.* 109:877–889.

Zylstra, S., F. A. Chen, S. K. Ghosh, E. A. Repasky, U. Rao, H. Takita, and R. B. Bankert. 1986. Membrane-associated glycoprotein (gp 160) identified on human lung tumours by a monoclonal antibody. *Cancer Res.* 46:6446–6451.