Interaction of Integrins $\alpha_3\beta_1$ and $\alpha_2\beta_1$: Potential Role in Keratinocyte Intercellular Adhesion

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Abstract. The colocalization of integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ at intercellular contact sites of keratinocytes in culture and in epidermis suggests that these integrins may mediate intercellular adhesion (ICA). P1B5, an anti-$\alpha_3\beta_1$ mAb previously reported to inhibit keratinocyte adhesion to epiligrin, was also found to induce ICA. Evidence that P1B5-induced ICA was mediated by $\alpha_2\beta_1$ and $\alpha_3\beta_1$ was obtained using both ICA assays and assays with purified, mAb-immobilized integrins. Selective binding of $\alpha_2\beta_1$-coated beads to epidermal cells or plate-bound $\alpha_3\beta_1$ was observed. This binding was inhibited by mAbs to integrin $\alpha_2$, or $\beta_1$ subunits and could be stimulated by P1B5. We also demonstrate a selective and inhibitable interaction between affinity-purified integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$. Finally, we show that expression of $\alpha_2\beta_1$ by CHO fibroblasts results in the acquisition of collagen and $\alpha_3\beta_1$ binding. Binding to both of these ligands is inhibited by P1H5, an anti-$\alpha_2\beta_1$ specific mAb. Results of these in vitro experiments suggest that integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ can interact and may do so to mediate ICA in vivo. Thus, $\alpha_3\beta_1$ mediates keratinocyte adhesion to epiligrin and plays a second role in ICA via $\alpha_2\beta_1$.

A variety of adhesion receptors maintain the integrity and polarity of the stratified epidermis of the skin (Fuchs, 1990). Some of these mediate cell-substrate adhesion while others contribute to intercellular adhesion (ICA). For example, hemidesmosomes anchor basal cells to the basement membrane zone (BMZ) (Staehelin, 1974). A family of Ca$^{2+}$-dependent, protease-sensitive receptors, including cadherins, L-CAM, uvomorulin, and ACAM, mediate homophilic interactions at the adherens junctions (Volk and Geiger, 1986; reviewed in Takeichi, 1991). Cadherins and the structurally related desmosomal components, known as desmogleins and desmocollins (Collins et al., 1991; Wheeler et al., 1991), contribute to epidermal cell-cell adhesion. Finally, integrins $\alpha_2\beta_1$, $\alpha_4\beta_1$, and $\alpha_6\beta_4$ mediate cell-substrate and cell-cell contacts (Hynes, 1987; Carter et al., 1990a,b). Among these adhesion molecules, the expression of hemidesmosomes and integrins is restricted to proliferative basal and suprabasal cell layers in normal skin (Carter et al., 1990a,b; Adams and Watt, 1991; Hertle et al., 1991). The four layers of the skin arise from differentiation and stratification of epidermal stem cells, a subset of the basal cell population (Lavker and Sun, 1983; Potten and Morris, 1988; Fuchs, 1990). An identified trigger of epidermal cell differentiation in vitro is detachment from the substratum (Adams and Watt, 1990; Fuchs, 1990). By analogy, basal cell-BMZ detachment seems likely to be a physiologic trigger of epidermal cell differentiation. The epidermal BMZ contains laminin, collagen type IV, proteoglycans, and epiligrin (Fuchs, 1990; Carter et al., 1990a,b, 1991). Epiligrin induces formation of both focal adhesions (FAs) via $\alpha_2\beta_1$, and hemidesmosome-like structures via $\alpha_3\beta_1$ (Carter et al., 1991). As mediators of basal cell-BMZ adhesion, $\alpha_2\beta_1$, $\alpha_3\beta_1$, and epiligrin may be involved in regulating epidermal cell division and differentiation. Immunofluorescence studies of cultured KC demonstrated that epidermal stratification is accompanied by reduced $\alpha_2\beta_1/\alpha_3\beta_1$ expression at FAs and increased $\alpha_2\beta_1/\alpha_3\beta_1$ expression at intercellular contact sites (Carter et al., 1990; Larjava et al., 1990). Not only are these data consistent with basal to lateral relocation of these integrins but they also suggest that integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ may play a role in ICA.

We were interested in examining whether integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ do play a role in ICA. We previously reported that PIBS, an anti-$\alpha_2\beta_1$ mAb, detaches keratinocytes (KC) in culture from epiligrin (Carter et al., 1991). We now report that PIBS triggers ICA between epidermal cells in culture. We also describe interactions occurring between integrins $\alpha_3\beta_1$ and $\alpha_2\beta_1$ in epidermal cells and in cell free systems. Given the restricted expression of these integrins in skin and their relocation during epidermal stratification, our data suggest that integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ may interact to mediate ICA in epidermis.
**Materials and Methods**

**Cells and Cell Cultures**

The FEPPEIL-8 human cell line was generated by KC transfection with human papilloma virus 16 (Kaur and McDougall, 1988). HPV-transformed KC cell lines have previously been shown to differentiate in response to Ca2+; they are also useful in organotypic cultures, form FAs, express the same integrin profile as human foreskin KC, and produce little endogenous matrix (Carter et al., 1990a; Kaur and Carter, 1992, in press). Normal KC were prepared as described by Boyce and Ham (1985) in a sequential digestion of tissue with dispase (Grade II; Boehringer-Mannheim, Indianapolis, IN) to separate dermis from epidermis, followed by trypsin digestion of the epidermis to release cells. KC and KC cell lines were grown in KC growth medium containing bovine pituitary extract (KGM; Clonetics, San Diego, CA).

**CHO Cell Culture, Transfection, and Analysis**

CHO K1 cells were maintained in DME supplemented with 10% FBS, nonessential amino acids, penicillin (100 U/ml) and streptomycin (100 μg/ml). Electroporation was used to cotransfect CHO K1 cells (10^7 cells) with pEG cDNA (Takada and Hemler, 1989) in PBJ-1 vector (10 μg) and pCDneo plasmid (1 μg). PBJ-1 is an SR-alpha promoter-based vector (Takabe et al., 1988), kindly provided by Dr. Mark Davis (Stanford University, Stanford, California). 3 d after transfection, cells were transferred to medium containing 700 μg/ml genetin (481, Gibco/BRL, Gaithersburg, MD). After two weeks of culture, clones expressing the highest 1% level of α5β1 were isolated by sorting with FACStar (Becton-Dickinson) using the 12F1 anti-α2 specific monoclonal antibody (Takada and Hemler, 1989). Stable α2 overexpressers were maintained in medium containing 100 μg/ml 481.

**Antibodies**

Antibodies to the integrin receptors α5β1 (P1B5, PIF2, P4E7), α6β1 (P1H5, P1H6, P4B4), α4β1 (P4G9, P4C2), α5β1 (P1D6, P1F8), and β1 (P4C10) have been previously described (Wayner and Carter, 1987; Wayner et al., 1988; Carter et al., 1990a). P1F1 is an anti-β1 based on precluring experiments and comparison of peptide fragments generated by protease digestion of P1F1 antigen and bone fide β1 (A5A5 anti-β1), G0H3 (anti-α5), ECCD-2, and HECDO-1 (anti-E-cadherins) were gifts from Drs. Martin E. Hemler (Dana-Farber Cancer Institute, Boston, MA), Arnaud Sonenberg (Central Laboratory of the Netherlands, Amsterdam, Holland), and Masatoshi Takeichi (Kyoto University, Kyoto, Japan), respectively. GC-4 (anti-ACAM) was purchased from the Sigma Chemical Co. (St. Louis, MO).

**Immunofluorescence and Immunocytochemistry**

Immunofluorescence was performed as previously described (Carter et al., 1990b). Briefly, KC grown on acid-washed coverslips in KGM were permeabilized with 1% Triton X-100/PBS and sequentially incubated with affinity-purified rabbit anti-α5 and goat anti-rabbit IgG. Antibodies were diluted in 1% BSA/PBS and coverslips were washed in PBS after each incubation. Using this approach, all integrin receptors were visualized and then solubilized and counted by scintillation spectrometry. Of note, more counts were associated with α5β1-beads than with α4β1-beads. This could reflect greater synthesis of α5β1 vs α4β1, or more efficient coating of α5β1 vs α4β1 onto beads. In either case, this explains the apparent discrepancy between radio-labeling of α5β1 and α4β1 associations with FEPPEIL-8 cells in the presence of SP2 (Fig. 4 a and h). Adhesion in these experiments was specific by the following criteria: (a) adhesion of receptor-coated beads to FEPPEIL-8 cells was inhibitable by inclusion of mAb to that receptor; (b) little to no adhesion was seen when receptor was purified from preclurred lysates; and (c) no adhesion was seen with SP2-bound beads. Similar experimental results were obtained on at least three occasions.

**Immunofluorescence as an Adhesion Substrate**

48-well polystyrene plates were serially incubated at 22°C with (a) 10 μg/ml affinity-purified rabbit anti-mouse IgG (Zymed Labs, Inc., San Francisco, CA) in PBS for 2 h, (b) 0.5% BSA in PBS for 1 h, and (c) anti-integrin mAb for 2 h. Radiolabeled or cold FEPPEIL-8 lysates were then added to wells, incubated on ice for 2 h and washed four times before use as an adhesion substrate. Specificity of receptor immobilization was determined by solubilizing antibody-bound proteins in sample buffer followed by SDS-PAGE analysis. Of note, containing bands were common to all immobilized integrins. Integrins on Sepharose beads, prepared as described above, were allowed to interact with polystyrene plates coated with integrins, as depicted in Fig. 1.

**Affinity Purification of Integrins**

Antibodies (P1B5, P1H6, P4D6, and P3H9) were purified from conditioned culture medium by affinity-chromatography on protein A-agarose. Purified mAbs were coupled to Affigel A according to the manufacturer's instructions (BioRad Laboratories, Richmond, CA), stored in PBS/0.02% NaN3 and equilibrated with cell lysis buffer (1% Triton X-100, 25 mM Tris pH 7.5, 0.5 M NaCl before use). Cells were allowed to high density in thirty 15 cm plates (~10^9 cells), rinsed, harvested by scraping into PBS in the presence of protease inhibitors, and dounce-homogenized in 0.34 M su-
results in the absence of P1B5. Although interassay variability in basal cell-cell adhesion which was above background. We measured the effects of anti-integrin mAbs on the ICA density and to intercellular contacts in touching cells (Fig. 2 F and Carter et al., 1990a,b, 1991). Interaction with epiligrin induced α3β1 localization in FAs (Carter et al., 1991). Intercellular localization could be detected with all anti-α2β1 mAbs (P1B5, P1F2, P2E6, P4E7). Similar dual distributions of α3β1 was also observed in cryostat sections of normal human palm epidermis (Fig. 2, B–D). α3β1 localized to the basal surface of basal cells in deep rete ridges (DR, Fig. 2, B and C) and to the lateral surfaces of basal cells in shallow rete ridges (SR, Fig. 2, B and D). The areas of interest are shown at higher magnification in the inserts. α3β1 was detected on the lateral and apical surfaces of cells in culture (Fig. 2 E) or palm skin (Fig. 2 A). α2β1 localization in FAs only occurred with the addition of an exogenous collagen substrate (Carter, 1990a). These results indicate that the subcellular localization of α3β1 and α2β1 may be dependent on the microenvironment of the cell in deep versus shallow rete ridges. Furthermore these results suggest that the intercellular localization of α3β1 and α2β1 in culture and tissue may reflect a physiological ICA process.

PIB5 Induces Aggregation of Epidermal Cells in Suspension

We found that PIB5 consistently and specifically induced aggregation of epidermal cells in suspension (Fig. 2 H). Other anti-integrins were capable of detaching cells from substrate ligands but did not induce cell aggregation (Fig. 2 G and not shown). Further, other anti-α2β1 mAbs failed to induce cell aggregation. The results summarized in Fig. 2 suggested that integrins α2β1 and α3β1 might interact with each other given an appropriate stimulus, such as that delivered by PIB5. Next we examined whether there was evidence for a functional interaction between α2β1 and α3β1 that might mediate cell–cell adhesion.

PIB5 Stimulates ICA

We measured the effects of anti-integrin mAbs on the ICA of the HPV-immortalized human keratinocyte (KC) line, FEPE1L-8 (Kaur and McDougall, 1998). FEPE1L-8 cells are similar to primary KC in their surface adhesion receptor profile and in their ability to form FAs on keratinocyte matrix (unpublished observations and Kaur and Carter, 1992, in press). ICA was found to consist of two components: basal ICA, that was not inhibited by anti-integrins, and PIB5 induced cell–cell adhesion which was above background. We shall subsequently refer to the PIB5-stimulated cell-cell adhesion as “PIB5-induced ICA.” Basal ICA was defined as the adhesion of ⁵¹Cr-labeled FEPE1L-8 cells in suspension to an unlabeled, confluent monolayer of FEPE1L-8 cells in the absence of PIB5. Although interassay variability in basal ICA was observed, PIB5 stimulated ICA by at least 100% in over 50 adhesion assays performed during the course of

CHO Cell-Substrate Adhesion Assays

Cell-substrate adhesion assays using the CHO cells were essentially as described previously (Symington et al., 1989). The only modification was that
Table I. Adhesion of αβ1- or αβ4-bands to immobilized αβ1-

| Source of immobilized integrins: | αβ1-bands | αβ4-bands |
|----------------------------------|-----------|-----------|
|                                  | P.B5-activated | Control | P.B5/Control |
|                                  | E1L8      | E1L8     | E1L8       |

| Immobilized Integrin | (Immobilizing mAb) | P.B5  | Control | P.B5/Control |
|----------------------|---------------------|-------|---------|--------------|
| Exp. I               | αβ1 (P.B5)         | 1733  | 854     | 2.1          |
|                      | αβ1 (Ro64)         | 615   | 700     | 0.9          |
| Exp. II              | αβ1 (P.D3)         | 399   | 380     | 1.0          |
|                      | αβ1 (P.E14)        | 805   | 199     | 4.0          |
|                      | αβ1 (P.B6)         | 886   | 332     | 2.8          |
| Exp. III             | αβ1 (P.D3)         | 399   | 380     | 1.0          |
|                      | αβ1 (P.E14)        | 805   | 199     | 4.0          |
|                      | αβ1 (P.B6)         | 886   | 332     | 2.8          |

This table shows the adhesion of αβ1- or αβ4-bands to immobilized αβ1-bands. The experimental design used in these experiments is outlined in Fig. 1. αβ1, or αβ4, (4 in Fig. 1) was purified onto Sepharose beads (6 in Fig. 1) from 35S-labeled FEPE1L-8 and HT1080 cells, respectively, and incubated with integrins immobilized via anti-integrin mAbs on adhesion plates from control or PIBS-treated, unlabeled FEPE1L-8 cell lysates. FEPE1L-8 cells were treated with intact PIBS in experiments I and II, and with PIBS F(ab') in experiment III. The immobilized integrin (2 in Fig. 1) and immobilizing mAb (1 in Fig. 1) are listed in the two columns at the left. Soluble anti-β1 (P4C10) (3 in Fig. 1) was added at time zero in Exp. III. After a 3 h incubation, wells were washed twice, and bound, bead-associated counts were determined by scintillation spectroscopy after solubilization in SDS/NaOH. Bound counts (+/- standard error for quadruplicate samples) are depicted in the first two columns and the ratio of counts bound by receptor purified from PIBS-activated versus control. FEPE1L-8 lysates are depicted in the third column of each panel. Results of three independent experiments are shown.

Figure 1. Schematic representation of adhesion assay using immobilized, mAb-captured integrins. This assay was designed to measure inter-integrin interactions in a cell-free system. Integrins αβ1, or αβ4 (2) were immobilized on plastic plates via anti-integrin antibodies (I). These integrins were derived from detergent solubilized, unlabeled FEPE1L-8 cells pretreated with either SP2 or PIBS. 35Smethionine-labeled epidermal cells and HT1080 cells were used as the source of bead-immobilized integrins αβ1 and αβ4, respectively (4). Sepharose beads (6) were coated with anti-αβ1 mAb PIBS5 or the isotype-matched anti-αβ4 mAb P4G9 (5).

In some experiments, soluble anti-β1 mAb (P4C10, 3) was added at time zero to test the specificity of integrin interactions. Data obtained using this assay are presented in Table I.

This study. Similar results were obtained with PIBS in conditioned culture supernatant, affinity-purified PIBS or PIBS F(ab') (10 μg/ml final concentration). This suggests that serum or high Ca in hybridoma supernatants did not stimulate ICA. PIBS F(ab') was a potent stimulator of ICA (Fig. 3), although it contained no detectable intact PIBS. This data excludes the possibility that PIBS was simply bridging cells via its two antigen-binding sites. No differences were observed in ICA whether 51Cr-labeled cells were prepared for the assay by brief trypsinization, exposure to 2 mM EDTA, or scraping, suggesting that the adhesion components involved were not sensitive to the detachment protocol.

It could be argued that we were not measuring ICA but simply the increased adhesion of labeled cells in suspension to substratum. This was excluded in a number of ways. First, several mAbs capable of detaching adherent cells (such as PIBS5 or P4C10) did not induce ICA or cell aggregation. Second, PIBS also induced aggregation of suspension cells. Third, we observed PIBS-induced ICA when cells were plated on anti-αβ1 or anti-αβ4, and thus, not susceptible to PIBS-induced detachment. Finally, PIBS did not stimulate the adhesion of FEPE1L-8 cells to substrate ligands such as COL, FN, LM, KC ECM. These data suggested that PIBS can trigger ICA in addition to its effects on cell-substratum adhesion.

Integrins αβ1, and αβ4, Participate in PIBS-induced ICA

It was possible that PIBS was stimulating ICA mediated through cadherins, ACAMs, or other known epidermal intercellular adhesion molecules. We therefore tested the effect of mAbs to these molecules on PIBS-induced ICA. Only mAbs known to inhibit epidermal adhesion were used for these experiments. Anti-E-cadherins (HECD-1, ECCD-2) (Takeichi.
Intercellular localization of integrins α2β1 and α3β1 in human palm and cultured keratinocytes and P1B5-induced cell aggregation. Localization of integrins α2β1 (A, P4B4) and α3β1 (B–D, P1B5) in cryostat sections of adult human palm skin. Shallow and deep rete ridges are labeled SR and DR, respectively. C and D represent enlargements of the deep and shallow rete ridges, shown in B, respectively. Boxed regions of C and D are further magnified in the insets. Note the relative lack of apical α3β1 in basal cells of the deep rete ridges and the relative lack of basal α2β1 in basal cells of shallow rete ridges (arrows).

Panels E and F show the fluorescent localization of α2β1 (E, using P1H5) and α3β1 (F, using P1B5) in intercellular contact sites of cultured keratinocytes. H shows the marked cell aggregation induced by P1B5. P1H5, an anti-α2β1 capable of detaching cells did not induce cell aggregation (G). Equal cell numbers were plated in G and H.

1991), and anti-ACAM (GC-4) (Volk and Geiger, 1986) did not inhibit P1B5-induced ICA although they could disrupt baseline epidermal adhesion. This suggested that ACAMs/cadherins do not mediate PIBS-induced ICA. The trypsin and calcium insensitivity of PIB5-induced ICA were also inconsistent with ICA mediated by ACAM/cadherins (data not shown) (Takeichi, 1991). Only mAbs against integrin β1, α3β1, and α2β1 (P4C10, P4E7, and P4B4, respectively) significantly inhibited PIB5-induced ICA, as shown in Fig. 3. Two other anti-β specific mAbs, PIF1 and A1A5, also inhibited PIB5-induced ICA (data not shown). Antibodies recognizing other integrins expressed by epidermal cells, such as α6, α5β1, or αvβ3, did not inhibit PIB5-induced ICA. These data suggested that PIB5 was inducing ICA via integrins α2β1 and α3β1.

α2β1 Beads Bind to Confluent Epidermal Cells in a Specific and PIB5-inducible Manner

We reasoned that the receptors mediating PIB5-induced ICA might also interact with each other in a PIB5-inducible manner in cell free conditions. Initially we measured the adhesion of purified, radiolabeled integrins to confluent monolayers of FEPEIL-8 cells. The integrins were bound to protein A-sepharose beads via anti-integrin mAbs to increase valency. As shown in Fig. 4, PIB5 stimulated the binding of α2β1-beads to FEPEIL-8 cells. PIB5-induced adhesion could be inhibited by anti-α2β1 or anti-β specific mAbs. Anti-α2β1-coated beads incubated with lysates immunodepleted of α3β1 by serial preclearing did not bind appreciably to an FEPEIL-8 monolayer (data not shown). This
Figure 3. PIB5 stimulates ICA. Bars represent the percent adhesion of $^{51}$Cr-labeled FEPEIL-8 cells in suspension to a confluent monolayer of FEPEIL-8 cells. At time zero, hybridoma supernatants (1:4 final dilution), purified mAbs, or F(ab') (both at 10 μg/ml final concentration) were added to sample wells, as indicated. Shown are the results at 3 h, although PIB5 stimulation was seen at all time points tested. Basal adhesion was equivalent in the presence of SP2 or no additives. Samples were performed in triplicate and associated standard deviations are indicated. Significant reductions in PIB5-induced adhesion were only observed with anti-α2β1 (P4B4), anti-α3β1 (P4E7), or anti-β1 (P4C10). Similar results obtained with two other anti-β1 mAbs (PIF1 and A1A5) are not shown. Other mAbs used were P4G9 (anti-α4β1), P1D6 (anti-α6β1), GOH3 (anti-α6), PIG12 (anti-CD44), HEC1 and ECCD-2 (anti-E-cadherins), and GC4 (anti-ACAM). For results shown, all except PIB5 F(ab'), ECCD, HEC1, and GC4 were used as hybridoma supernatant. Results using purified PIB5 at 10 μg/ml were similar to that shown for PIB5 F(ab').
PIB5 stimulates the adhesion of α2β1-coated beads to an FEPEIL-8 monolayer. (a) Binding of α2β1 and α3β1 to FEPEIL-8. Radiolabeled integrins were immobilized via anti-integrin mAbs onto protein A-sepharose beads and the beads were then incubated with confluent FEPEIL-8 cell monolayers in the presence of the indicated mAbs (final dilution 1:4). After 3 h, wells were washed twice, solubilized with SDS-NaOH, and bound cpm were measured by scintillation spectroscopy. FEPEIL-8-associated [35S]cpm (with indicated standard deviations) is shown on the Y-axis. The left depicts binding of α2β1 beads while the right depicts binding of α3β1 beads to FEPEIL-8 cells. Solid bars represent adhesion in the presence of SP2 (white) or PIB5 (black). Horizontal-striped and cross-hatched bars represent adhesion in the presence of soluble PIB5 + PIF1 (anti-β1) and PIB5 + P4E7 (anti-α3β1), respectively. In all experiments ~10% of α2β1 counts and <1% of α3β1 counts were associated with FEPEIL-8 cells in the presence of SP2. Bead-associated cpm were higher for α3β1 coated compared to α2β1 coated beads. The ICA of 51Cr-labeled FEPEIL-8 cells in suspension to confluent FEPEIL-8 monolayers was measured in each case as a positive control for the experiment. Similar results were obtained in four independent experiments. (b) Photomicrograph depicts binding of α2β1 beads (left column) or α3β1 beads (right column) to an intact FEPEIL-8 monolayer in the presence of the soluble mAbs indicated at the left. The bar represents 100 microns.

As shown in Table I, little specific adhesion was observed between α2β1-beads and α3β1 purified from control-treated epidermal cells. Stimulation of basal adhesion (two to 14-fold) was observed in each experiment performed when α2β1 was purified from PIB5-treated cells. This stimulated interaction could be inhibited by soluble anti-β1 mAbs (Exp. III). PIB5 did not stimulate the interaction between α3β1-beads and immobilized α3β1, or α2β1-beads and immobilized α2β1, demonstrating its selectivity for α2β1/α2β1 interactions. The magnitude of this stimulation varied depending on the particular anti-α2β1 antibody used to immobilize α2β1 on plastic, from twofold when PIB5 (IgG1) was used to 14-fold when PIF2 (IgG1) was used. This may reflect differences in mAb affinities or epitope densities. Finally, because mAbs were used to immobilize integrins in these experiments, it was formally possible that we were actually measuring interactions between integrins on one surface and mAb domains on the other surface. This could involve interactions with constant or variable regions of IgG. Use of isotype-matched reagents for immobilization of test and control integrins minimized this possibility and results using affinity-purified integrins, described below, exclude this. Most importantly, the integrin-integrin adhesion measured in this cell-free system was very similar to the adhesion observed between two cells or between adherent cells and α2β1-beads. Once again, these results suggest that α2β1 and α3β1 are the mediators of PIB5-induced ICA.

Affinity-purified α2β1 and α3β1 Preferentially Interact

In order to obtain independent confirmation of an interaction between integrins α2β1 and α3β1, we affinity-purified these integrins on PIH6 and PIB5 mAb-columns, respectively. This allowed us to measure integrin-integrin interactions without immobilizing integrins via antibodies. α2β1 and CD44 were also affinity-purified for use as controls on PID6 and P4G9 columns, respectively. The integrins purified from
Figure 5. Affinity-purified α3β1 and α2β1 contain no detectable collagen, epiligrin, fibronectin, laminin, or immunoglobulin. (A) Lysates from 35S-labeled cells were passed over affinity-columns of P1B5 and P1H6, and eluted with PBS/1% b-octylglucoside/50 mM triethylamine. Aliquots of α3β1 (left) and α2β1 (right), as indicated, were separated by 7% SDS-PAGE. Arrowheads mark positions of α (top) and β (bottom) subunits. The faint 110 kD bands represent β1 precursors. Migration positions of molecular weight markers are indicated at left. Lower Mr background bands were visualized with all column purified adhesion receptors. Silver staining of gels revealed no detectable antibody contamination of α3 or α2. (B) Aliquots of purified receptor were spotted onto strips of nitrocellulose, allowed to dry, and blocked with BSA before incubation with detecting antibodies. The left represents strips reacted with anti-integrin mAbs to evaluate potential contamination of receptor preparations by other integrins. One and 10 μl aliquots of α3β1, α2β1, and CD44 or 1 μg of FN were spotted onto nitrocellulose. Detection was with P3H9 (anti-CD44), P1B5 (anti-α3) or P1H6 (anti-α2). No contamination of α2 by α1 (and vice versa) was detected. In the right panel, potential matrix protein or IgG contamination of receptor preparations was evaluated. 10 μL aliquots of receptors were used, as indicated. For each detecting antibody used, serial 10-fold dilutions (1, 0.1, 0.01, and 0.001 μg [1 ng]), of appropriate positive control proteins were used. Concentrated KC culture supernatant was also spotted onto nitrocellulose. Although exposure lengths sufficient to allow detection of 1 ng of each of the test proteins led to increased background, no positive signals in α2, α1 or α3 preparations were detected. The CD44 preparation appeared to cross-react with all the antibodies.

Results from the ELISA-type adhesion assay are summarized in Fig. 6. Purified integrins or CD44 were coated on 96-well plates for subsequent use as an adhesion surface. Soluble, affinity-purified integrins (in PBS/b-octylglucoside, pH 7.5) were added to the plastic wells and after incubation unbound receptors were removed by washing with PBS. A three step enzyme-linked mAb detection was used to quantify both the efficiency of receptor binding to plastic and the interaction between two receptors. As shown in the left panel, there is an interaction between soluble α2β1 and immobilized α3β1. This interaction is inhibited to basal levels by the addition of soluble anti-α2β1 (P1B5) or anti-α3β1 (P1H6) specific antibodies, and reduced by EDTA (2 mM). None of the antibodies used as soluble inhibitors interfered with the detection of bound integrins. Soluble α3β1 bound to immobilized α2β1, but not to immobilized CD44 (see Fig. 6, middle) or immobilized α2β1 (data not shown). Again, this interaction was inhibited by anti-α2β1 (P1B5) or anti-β1 (P4C10) mAbs. Of note, the addition of soluble ligands for α2β1 and α3β1, such as collagen, FN, GRGDS,
Figure 6. Interaction of affinity-purified $\alpha_2\beta_1$ and $\alpha_3\beta_1$. The specific adhesion of soluble affinity-purified integrins to various ligands (matrix proteins or affinity-purified integrins) is shown here (+/- standard deviation). Protein ligands were allowed to passively coat wells of multiwell plates. A selective and inhibitable interaction between $\alpha_2\beta_1$ and $\alpha_3\beta_1$ was observed in six separate experiments. All affinity-purified integrins were functionally active in PBS/1% b-octylglucoside. Integrin aliquots used were shown to be free of contaminating mAbs as described in Methods. Inhibitory antibodies used (PIH6, anti-$\alpha_2\beta_1$; PIB5, anti-$\alpha_3\beta_1$; P4C10, anti-$\beta_1$) were added at time zero (1:4 final dilution). In the middle panel, the three columns depict binding of soluble $\alpha_3\beta_1$ to immobilized CD44 in the presence of no inhibitors, soluble anti-$\alpha_3\beta_1$, and soluble anti-$\beta_1$, from left to right.

LM, or concentrated KC culture supernatant (enriched in epiligrin), did not inhibit receptor–receptor interactions. Furthermore, treatment of receptor preparations with polyclonal anti-Col 1 or 4, anti-FN, anti-LM, monoclonal anti-epiligrin, or rabbit anti-mouse IgG, to remove putative contaminants, did not inhibit subsequent receptor–receptor interactions as might be expected if one of these proteins was bridging integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$.

Although some binding of soluble $\alpha_3\beta_1$ to immobilized $\alpha_2\beta_1$ was observed, standard errors were large and the interaction was not inhibited by anti-$\beta_1$ mAbs, suggesting that it was not specific (Fig. 6, right). In contrast, the binding of soluble $\alpha_3\beta_1$ to immobilized fibronectin was specifically inhibited, confirming that the purified $\alpha_3\beta_1$ was functional. Taken together, these results suggest that the observed $\alpha_3\beta_1/\alpha_3\beta_1$ interaction is specific.

$\alpha_2$ Transfectants Selectively Bind to Affinity-purified $\alpha_3\beta_1$

Having shown that integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ may interact to mediate epidermal ICA, and could interact selectively in cell-free systems, we set out to determine whether binding to $\alpha_3\beta_1$ was acquired along with expression of $\alpha_2\beta_1$. For these experiments, CHO K1 cells, which express abundant $\alpha_3\beta_1$, but low levels of integrins $\alpha_2\beta_2$ and $\alpha_5\beta_1$, were transfected with a human $\alpha_2$ cDNA (Takada and Hemler, 1989). As shown in Fig. 7, $\alpha_2$-CHO transfectants express significantly increased levels of $\alpha_3\beta_1$ (mean fluorescent intensity 123 in $\alpha_2$-CHO compared to 4 in parental CHO). Expression of endogenous $\alpha_3$ and total $\beta_1$ integrins was not significantly increased. The binding of parental CHO cells or $\alpha_2$–CHO to a variety of ligands, including collagen (a previously described ligand for $\alpha_2\beta_1$), and purified $\alpha_3\beta_1$, was then measured. Consistent with their high level expression of $\alpha_3\beta_1$, parental CHO cells bound well to FN only (Fig. 8). In contrast, the $\alpha_2$–CHO acquired the ability to bind to collagen and to purified $\alpha_3\beta_1$, but not to purified $\alpha_3\beta_1$. Binding of $\alpha_2$–CHO to both collagen and $\alpha_3\beta_1$ was drastically reduced by PIH5, an anti-$\alpha_2$ antibody. Binding of $\alpha_2$–CHO to $\alpha_3\beta_1$ was also completely inhibited by the combination of P4C10 and P4E7 (an anti-$\beta_1$ and anti-$\alpha_3$, respec-
PIB5 Induces Epidermal ICA Mediated Through Integrins \( \alpha_2\beta_1 \) and \( \alpha_3\beta_1 \)

PIB5 induced epidermal cell–cell adhesion. We show that this is a true increase in ICA, rather than an artifact of mAb-induced cross-bridging of cells. Furthermore, we found no evidence that PIB5 was inducing adhesion to substrate proteins such as FN, LM, Col, or to tissue culture plastic. Cell adhesion studies using mAbs implicated integrins \( \alpha_3\beta_1 \) and \( \alpha_6\beta_1 \) in PIB5-induced ICA. Importantly, these same studies showed that other cell–cell adhesion receptors, such as E-cadherins or ACAMs, participate in basal but not PIB5-induced ICA. These results suggested that PIB5 is inducing a heterophilic integrin interaction. This distinguishes the effects of PIB5 from those of other mAbs reported to induce cell–cell adhesion via induction of homophilic interactions (CD39, VLA-4) (Kansas et al., 1991; Bednarczyk and McIntyre, 1990) or interactions between receptors of two distinct classes (LFA-1-ICAM) (Keizer et al., 1988).

How might the same antibody (PIB5) have disparate effects on cell-substrate versus cell–cell adhesion? Several explanations are possible. Epiligrin, because of its large size and tendency to aggregate, may have more \( \alpha_3\beta_1 \) binding sites (i.e., higher avidity) than \( \alpha_6\beta_1 \). Alternatively, the affinity of \( \alpha_3\beta_1 \) for epiligrin may be higher than that for \( \alpha_6\beta_1 \). Both of these would result in cell-substrate adhesion being dominant to cell–cell adhesion. Thus, only in situations where the \( \alpha_3\beta_1 \)-epiligrin interaction is prevented can the interaction with \( \alpha_6\beta_1 \) occur. Finally, PIB5 may be inducing a conformational change in \( \alpha_3\beta_1 \) which increases its affinity for a cell-surface coreceptor while reducing its affinity for a matrix ligand.

Purified \( \alpha_3\beta_1 \) and \( \alpha_6\beta_1 \) Can Interact in Vitro

We show that purified integrins \( \alpha_3\beta_1 \) and \( \alpha_6\beta_1 \) can interact, using both affinity-purified integrins and mAb-immobilized integrins. Several features distinguished the interaction of affinity-purified integrins from the interaction of mAb-immobilized integrins or intact cells. PIB5 induced an interaction between mAb-immobilized \( \alpha_3\beta_1 \) and \( \alpha_6\beta_1 \), just as it stimulated an interaction between intact cells. In contrast, a significant basal level of interaction was observed between affinity-purified \( \alpha_3\beta_1 \) and \( \alpha_6\beta_1 \), and PIB5 did not stimulate this further (Fig. 6). This suggests that the PIB5-affinity column selectively purifies the “active” form of \( \alpha_3\beta_1 \) and/or activates all the \( \alpha_6\beta_1 \). Alternatively, differences in detergents used or \( \alpha_6\beta_1 \) immobilization techniques may have affected the conformation of \( \alpha_3\beta_1 \). Results using intact cells and mAb-immobilized integrins also suggested there was a directionality to the interaction (Fig. 4 and data not shown). While \( \alpha_6\beta_1 \)-beads could interact with an epidermal monolayer, \( \alpha_6\beta_1 \)-beads did not. This was surprising in view of the expression of both \( \alpha_6\beta_1 \) and \( \alpha_3\beta_1 \) by epidermal cells. No such directionality was observed using affinity-purified integrins \( \alpha_3\beta_1 \) and \( \alpha_6\beta_1 \) (Fig. 6). This could reflect differences in accessibility of \( \alpha_3\beta_1 \) on a cell surface versus in a detergent solution. The observation that anti-\( \alpha_3\beta_1 \) beads (i.e., PIB5-beads or PH56-beads) bound poorly to a monolayer of intact epidermal cells was consistent with this interpretation. In any case, a selective interaction between integrins \( \alpha_3\beta_1 \) and \( \alpha_6\beta_1 \) is observed using two different cell-free systems.

A number of alternate explanations for these observations were evaluated. First, antibody cross-linking of two molecules of \( \alpha_3\beta_1 \) are excluded by both the PIB5 F(ab') data and data using purified integrins. It is unlikely that we are actually measuring cell-substrate adhesion made possible by PIB5 detachment of adherent monolayer cells since PIB5 selectively stimulated interactions between suspension cells (Fig. 2 H) and between mAb-immobilized integrins (Table I). Furthermore, PIB5 stimulated ICA even when cells were plated on proteins that are not \( \alpha_6\beta_1 \) ligands (and thus, not susceptible to PIB5-detachment). Interactions between integrins on one surface and mAb domains on the other could artifactually give the appearance of integrin–integrin binding. This is unlikely because isotype-matched mAbs were used to immobilize test and control integrins. Furthermore,
Figure 8. α2-CHO acquire collagen and α3β1 binding. The adhesion of CHO or α2-CHO to various ligand proteins was measured. Shown here are the results obtained using FN, col, α5β1, and α3β1 as ligands. Adhesion was measured in the presence of SP2 or the indicated inhibitory mAb (P1H5, anti-α2 or P4C2, anti-α3β1). Results of one experiment, representative of four performed, is shown. Briefly, wells were coated overnight with the indicated ligands (5 μg/ml for collagen and FN, 1:4 dilution for α5β1 and α3β1), rinsed, and then blocked for 1 h with 5 μg/ml BSA before use. Inhibitors (1:4 final dilution) were added at time zero. After 90 min at 37°C, wells were washed twice, then adherent cells were fixed, stained, and solubilized in deoxycholate. Binding was quantitated by reading absorbance at OD 595 on an ELISA plate-reader. Percent adhesion is indicated in each panel. Adhesion (and spreading) of both cell lines to FN was maximal and defined as 100%. Binding to BSA was <5% for both cell lines. Although the cells appeared less well-spread, significant adhesion of α2-CHO to collagen and α3β1 was observed. P1H5 completely inhibited α2-CHO binding to collagen and α3β1.

the selective interaction between affinity-purified α3β1 and α2β1 also makes this unlikely by providing independent evidence for an α3β1/α2β1 interaction measured in the absence of immobilizing mAbs. Finally, it is still formally possible that some as yet unidentified molecule copurifies with either α3β1 or α2β1; isolated in various ways, and actually bridges these two integrins. If true, one must propose that the α3β1−X-α2β1 interaction is stabilized by PIB5, and inhibited by anti-β1, anti-α3, and anti-α2 specific mAbs. Although hard to exclude absolutely, we think this is unlikely for a number of reasons. First, addition of soluble ligands for these integrin, such as keratinocyte matrix, collagen, fibronectin, laminin, or GRGDS, did not interfere with cell-cell or integrin-integrin interactions, as would be expected if one of these molecules were X. Background bands visible by 35S-labeling, Coomassie blue or silver staining of gels were com-
mon to all receptors purified. Depletion of putative contaminants from purified receptor preparations using polyclonal mAbs did not reduce receptor–receptor interactions. Finally, if PIB5 were stabilizing an interaction mediated through X, it should stimulate the interaction of purified receptors or \( \alpha_2-\text{CHO} \) with \( \alpha_2 \beta_1 \). Yet PIB5 did not stimulate these interactions. This is not compatible with the hypothesis that PIB5 is stabilizing the \( \alpha_2-X-\alpha_2 \) interaction. The simplest explanation compatible with all the data is that purified integrins \( \alpha_2 \beta_2 \) and \( \alpha_2 \beta_1 \) can interact in vitro and may do so with appropriate stimulation in intact cells. While integrins have previously been shown to interact with members of the immunoglobulin family of receptors (LFA/ICAM1, VLA4/VCAMI) (Martin and Springer, 1987; Elices et al., 1990; Springer, 1990), and to bind to a variety of matrix and plasma proteins (Wayner and Carter, 1987), this is the first demonstration of an inter-integrin interaction.

**Cells Can Interact with Purified Integrins**

Perhaps the strongest evidence for a role of \( \alpha_2/\alpha_1 \) interactions in intercellular adhesion is provided by experiments using the \( \alpha_2-\text{CHO} \) transfectants. CHO cells acquired the ability to adhere to collagen and \( \alpha_2 \beta_1 \) when they were transfected with \( \alpha_2 \) cDNAs. Both these interactions were selective and inhibitable. Like the interaction of affinity-purified integrins, \( \alpha_2-\text{CHO} \) adhesion to purified \( \alpha_3 \beta_1 \) was not stimulated by PIB5 treatment. It is thus unlikely that PIB5 is necessary for stabilizing \( \alpha_2/\alpha_1 \) interactions mediated by a bridging molecule. In any event, adhesion data using \( \alpha_2-\text{CHO} \) establish the premise that immobilized integrins can support cell adhesion.

**Potential Role of \( \alpha_2 \beta_1 \) and \( \alpha_3 \beta_1 \) Interactions in Cell–Cell Adhesion In Vivo**

Based on the data presented above, we infer that \( \alpha_2 \beta_2 \) and \( \alpha_2 \beta_1 \), which can interact with each other in cell free systems, mediate PIB5-induced ICA. Furthermore, we propose that similar events may occur during epidermal stratification. We base this on the following observations. (a) Tissue staining of human palm epidermis suggests that the cell–substrate or cell–cell distribution of integrin \( \alpha_2 \beta_2 \) is dependent on the location of the basal cell in deep or shallow rete ridges. This correlates with increased cell proliferation in deep rete ridges compared to shallow rete ridges (Lavkar and Sun, 1983; our unpublished results). (b) Not only can PIB5 detach epidermal cells from epiligrin, a component of the epidermal BMZ (Carter et al., 1991), but work in progress shows that immobilized epiligrin can antagonize the effects of PIB5 on ICA. This suggests that PIB5 is accelerating or accentuating an ongoing physiologic process. Because basal cell–BMZ detachment is a known trigger for epidermal differentiation (Adams and Watt, 1990; Fuchs, 1990), PIB5 appears to be mimicking the physiologic trigger of epidermal differentiation. Furthermore, it appears that cell–cell interactions predominate when cell-basement membrane attachment is disrupted. (c) When epidermal cells are allowed to differentiate and stratify by 10 d of culture in high calcium, high basal ICA inhibitable by anti-\( \alpha_2 \) or anti-\( \alpha_2 \) was observed (data not shown). Thus, PIB5 treatment and commitment to differentiation both appear to induce ICA mediated by integrins \( \alpha_2 \beta_2/\alpha_3 \beta_1 \). (d) Although intercellular localization of \( \alpha_2 \beta_1 \) was detected by all anti-\( \alpha_2 \beta_1 \) mAbs tested, this was most striking when cells were incubated in the presence of PIB5 prior to fixation (Fig. 2 F). Here, PIB5 is inducing changes in integrin distribution that occur during stratification. The recent report of tyrosine phosphorylation occurring after PIB5-induced \( \alpha_2 \beta_1 \) cross-linking suggests that \( \alpha_3 \beta_1 \) can participate in signal transduction (Kornberg et al., 1991). Thus, a single agent, PIB5, can induce cell–substrate detachment, cell–cell adhesion, \( \alpha_2 \beta_2/\alpha_3 \beta_1 \) interactions in vitro, and biochemical changes consistent with signal transduction. It is tempting to speculate that these events are interrelated, and that PIB5 is actually mimicking the physiologic trigger of epidermal basal cell division that gives rise to the differentiating daughter cell which will move up the epidermis, resulting in epidermal stratification. Our data suggest that while cadherins and ACAMs mediate the basal ICA, integrins are involved in the stimulated ICA. Thus, the dynamic nature of integrin-mediated interactions may be utilized for establishing transient intercellular contacts as cells leave the basement membrane. This may explain the apparent redundancy in epidermal cell–cell adhesion structures and is certainly compatible with the data presented. Experiments designed to address these questions are underway.

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**References**

Adams, J. C., and F. M. Watt. 1990. Changes in keratinocyte adhesion during terminal differentiation: reduction in fibronectin binding precedes \( \alpha_3 \) integrin loss from the cell surface. *Cell*. 63:425–435.

Barrandon, Y., and H. Green. 1987. Cell migration is essential for sustained growth of keratinocyte colonies: The roles of TGF-\( \alpha \) and EGFR. *Cell*. 50:1131–1137.

Bednarecky, J. L., and B. W. McIntyre, 1990. A mAb to VLA-4 alpha chain induces homotypic lymphocyte aggregation. *J. Immunol.* 144:777–784.

Boyce, S. T., and R. G. Ham. 1985. Cultivation, frozen storage, and clonal growth of normal human epidermal keratinocytes in serum free medium. *J. Tissue Culture Methods*. 9:83–93.

Carter, W. G., P. Kaur, S. Gil, F. J. Gahr, and E. A. Wayner. 1990a. Distinct functions for integrins \( \alpha_2 \beta_1 \) in focal adhesion and \( \alpha_2 \beta_1 \) bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. *J. Cell Biol*. 111:3141–3154.

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

Carter, W. G., M. C. Ryan, and P. J. Gahr. 1991. Epiligrin, a new cell adhesion ligand for integrin \( \alpha_3 \beta_1 \) in epithelial basement membranes. *Cell*. 65:599–610.

Collins, J. E., P. K. Logan, T. P. Kenny, J. MacGervie, J. L. Holton, and D. R. Garrod. 1991. Cloning and sequence analysis of desmosomal glycoproteins 2 and 3 (desmocollins): cadherin-like desmosomal adhesion molecules with heterogeneous cytoplasmic domains. *J Cell Biol*. 133:381–391.

Elices, M. J., L. Osborn, Y. Takada, C. Crouse, S. Luhowski, M. E. Hemier, and R. R. Lobb. 1990. VCAM-1 on activated endothelium interacts with the leucocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell*. 60:577–584.

Fuchs, E. 1990. Epidermal differentiation: the bare essentials. *J. Cell Biol*. 111:2807–2814.

Hertle, M. D., J. C. Adams, and F. M. Watt. 1991. Integrin expression during terminal differentiation. *J. Cell Biol*. 113:381–391.

Hynes, R. O. 1987. Integrins: a family of cell surface adhesion receptors. *Science*. 235:1106–1117.
and biochemistry of human CD39. *J. Immunol.* 146:2235–2244.

Kaur, P., and J. K. McDougall. 1988. Characterization of primary human keratinocytes transformed by human papillomavirus type 18. *J. Virol.* 62:1917–1924.

Kaur, P., and W. G. Carter. 1992. Integrin expression and differentiation in transformed epidermal cells is regulated by fibroblasts. *J. Cell Sci.* 103:755–763.

Keizer, G. D., W. Visser, M. Vliem, and C. G. Figdor. 1988. A mAb (NKI-L16) directed against a unique epitope on the human LFA-1 antigen induces homotypic cell–cell interactions. *J. Immunol.* 140:1393–1400.

Kornberg, L. J., S. H. Earp, C. E. Turner, C. Prockop, and R. L. Juliano. 1991. Signal transduction by integrins: increased protein tyrosine phosphorylation caused by clustering of β1 integrins. *Proc. Natl. Acad. Sci. USA.* 88:8392–8396.

Larjava, H., J. Peltonen, S. K. Akiyama, S. S. Yamada, H. M. Gralnick, J. Uitto, and K. M. Yamada. 1990. Novel function for β1 integrins in keratinocyte cell–cell interactions. *J. Cell Biol.* 110:803–815.

Lavker, R. M., and T.-T. Sun. 1983. Epidermal stem cells. *J. Invest. Derm.* 81(Suppl. 1):121s–127s.

Marlin, S. D., and T. A. Springer. 1987. Purified ICAM-1 is a ligand for lymphocyte function associated antigen 1. *Cell.* 51:813–819.

Potten, C. S., and R. J. Morris. 1988. Epithelial stem cells in vivo. *J. Cell Sci.* (Suppl.): 10:45–62.

Rheinwald, J. G., and H. Green. 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell.* 6:317–344.

Springer, T. A. 1990. Adhesion receptors of the immune system. *Nature (Lond.)* 346:425–434.

Stachelin, L. A. 1974. Structure and function of intercellular junctions. *Int. Rev. Cytol.* 39:191–238.

Symington, B. E., F. W. Symington, and L. R. Rohrschneider. 1989. Phorbol ester induces increased expression, altered glycosylation, and reduced adhesion of K562 erythroleukemia fibroblast receptors. *J. Biol. Chem.* 264:13258–13266.

Takabe, Y., M. Seiki, J.-I. Fujisawa, P. Hoy, K. Yokota, K.-I. Arai, M. Yoshida, and N. Arai. 1988. SR-alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell. Biol.* 8:466–472.

Takeichi, M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science (Wash. DC).* 251:1451–1455.

Volk, T., and B. Geiger. 1986. A-CAM: a 135-kD receptor of intracellular adherens junctions. *J. Cell Biol.* 103:1441–1464.

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.

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.

Wheeler, G. N., A. E. Parker, C. L. Thomas, P. Attiliotti, D. Poynter, J. Arnnenna, A. J. Rutman, S. C. Pidsley, F. M. Watt, D. A. Rees, R. S. Buxton, and A. I. Magee. 1991. Desmosomal glycoprotein DGI, a component of intercellular desmosome junctions, is related to the cadherin family of cell adhesion molecules. *Proc. Natl. Acad. Sci. USA.* 88:4796–4800.