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 $\alpha_3\beta_1$ by CHO fibroblasts results in the acquisition of collagen and $\alpha_2\beta_1$ 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 desmcollins (Collins et al., 1991; Wheeler et al., 1991), contribute to epidermal cell-cell adhesion. Finally, integrins $\alpha_2\beta_1$, $\alpha_3\beta_1$, 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 $\alpha_2\alpha_6$ (Carter et al., 1991). As mediators of basal cell-BMZ adhesion, $\alpha_2\beta_1$, $\alpha_6\beta_4$, 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 $\alpha_3\beta_1/\alpha_2\beta_1$ 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 $\alpha_3\beta_1$ and $\alpha_2\beta_1$ 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 PIB5, an anti-$\alpha_3\beta_1$ mAb, detaches keratinocytes (KC) in culture from epiligrin (Carter et al., 1991). We now report that PIB5 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 $\alpha_3\beta_1$ and $\alpha_2\beta_1$ may interact to mediate ICA in epidermis.

1. Abbreviations used in this paper: BMZ, basement membrane zone; ECM, extracellular matrix; FA, focal adhesion; ICA, intercellular adhesion; KC, keratinocyte.
**Materials and Methods**

**Cells and Cell Cultures**

The FEPE1L-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⁺, stratify in organotypic cultures, form FAs, express the same surface 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) by 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 (10³ cells) with α2 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 genitin (G418, GIBCO/BRL, Gaithersburg, MD). After two weeks of culture, clones expressing both the highest 1% level of α2β1 were isolated by sorting with FACStar (Becton-Dickinson) using the 12Fl anti-α2 specific monoclonal antibody (Takada and Hemler, 1989). Stable α2 overexpressers were maintained in medium containing 100 µg/ml G418.

**Antibodies**

Antibodies to the integrin receptors α2β1 (PIB5, PIF2, P4E7), α3β1 (PIH5, PH6, P4B4), α2β3 (PG49, P4C2), α3β1 (P1D6, P1F8), and β1 (P4C10) have been previously described (Wayner and Carter, 1987; Wayner et al., 1988; Carter et al., 1990a). P1B5 is an anti-β1 based on precloning experiments and comparison of peptide fragments generated by protease digestion of P1F1 antigen and bona fide β1, A1A5 (anti-β1), G0H3 (anti-α3), ECD2-C, and HECD-1 (anti-E-cadherins) were gifts from Drs. Martin E. Hemler (Dana-Farber Cancer Institute, Boston, MA), Arnoud Sonnenberg (Central Laboratory of the Netherlands, Amsterdam, Holland), and Masatoshi Takeichi (Kyoto University, Kyoto, Japan), respectively. OC-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 mouse mAbs followed by affinity-purified FITC-conjugated rabbit anti-mouse IgG. Antibodies were diluted in 1% BSA/PBS and coverslips were washed in PBS after each incubation. Using this approach, all anti-αβ1 (PIH5, P4B4, PH6) and anti-αβ3 (PIB5, PIF2) mAbs tested stained intercellular contacts. Where indicated (Fig. 2 F) KC were grown in the presence of PIB5 overnight, washed, fixed, and incubated with FITC-conjugated rabbit anti-mouse. This prolonged incubation of PIB5 with cells resulted in complete digestion of the PIB5, as determined by SDS-PAGE. Residual pIbin was inactivated by the addition of iodoacetamide (final concentration 0.01 M) and the F(ab') was dialyzed into PBS. The dialysis was run over a protein G-sepharose column to deplete the preparation of Fe fragments. Less than 10 ng intact PIB5 per 20 µg F(ab') was detected by SDS-PAGE analysis.

**ICA Assay**

Cells were trypsinized and plated in 48-well plates and 10 cm dishes. Upon reaching confluence, cells in 48-well plates were used as the adhesion substrate. Cells were labeled for 90 min with 200 µCi of Na²³/CrO₄ (specific activity 5 mCi/ml, 3Cr, New England Nuclear, Boston, MA)/ml of KGM, harvested, rinsed twice in PBS, and resuspended in KGM. Approximately 10³ cells were added (in 300 µL) to each well of a 48-well plate. Test mAbs (either 100 µL of hybridoma supernatant or purified antibody diluted in 100 µL of SP2) were added at time zero (1.4 final dilution). Adhesion assays were performed at 22°C for 3 h, unless otherwise indicated. At the conclusion of this time, wells were rinsed twice with PBS and cells were solubilized in 0.5% SDS/0.25 M NaOH, and counted in a gamma counter.

For the aggregation assay shown in Fig. 2 H, cells were treated as for the suspension phase of the ICA but plated on plastic in the absence of an adherent monolayer or an adhesive ligand.

**Preparation of αβ3 Beads**

Confluent 15 cm dishes of FEPE1L-8 cells were labeled for 16 h with 50 µCi/ml of trans-35S-label (ICN Biochemicals Inc., Irvine, CA) in KGM. Cells were solubilized in 1% Triton X-100, 0.5% BSA, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% NaN₃ (lysis buffer), centrifuged for 30 min at 15000 g, and postnuclear supernatants were incubated for 16 h with PIH5 or PH6 (anti-αβ1), isotype-matched anti-α3, anti-α4 (PG49), anti-α3 mAbs, or SP2 bound to protein A-Sepharose. αβ3-beads were prepared by immobilizing anti-integrin-associated receptors were washed four times with 1% Empigen BB, 50 mM Tris-HCl (pH 7.5), 400 mM NaCl (wash buffer), and resuspended in KGM for use in adhesion assays. All steps before the adhesion assay were performed at 4°C. Aliquots were removed for gel analysis and scintillation counting. ICA assays with 35S-labeled cells were performed in parallel as a control for these experiments. After washing wells, beads were counted visually and then solubilized and counted by scintillation spectroscopy. Of note, more counts were associated with αβ3β1-beads than with αβ1β1-beads. This could reflect greater synthesis of αβ3β1 vs αβ1β1, or more efficient coating of αβ3β1 vs αβ1β1 onto beads. In either case, this explains the apparent discrepancy between 35S-counts and αβ3β1-beads associated with FEPE1L-8 cells in the presence of SP2 (Fig. 4 a and b). Adhesion in these experiments was specific by the following criteria: (a) adhesion of receptor-coated beads to FEPE1L-8 cells was inhibitable by inclusion of mAb to that receptor; (b) little to no adhesion was seen when receptor was purified from preclreated lysates; and (c) no adhesion was seen with SP2-bound beads. Similar experimental results were obtained on at least three occasions.

**Immobilization of Integrins for Use as an Adhesion Substrate**

48-well polystyrene plates were serially incubated at 32°C with (α) 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 FEPE1L-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, contaminating 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 (PIB5, PH6, P1D6, 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/0.2% 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 washed to high density in thirty 15 cm plates (~10⁶ cells), rinsed, harvested by scraping into PBS in the presence of protease inhibitors, and dounce-homogenized in 0.34 M su-
crose, 50 mM borate buffer, 2 mM EDTA without detergent to solubilize cytoplasmic components (Wayner and Carter, 1987). All steps were performed at 4°C in the presence of 1 mM NEM, 1 mM PMSF. Lyastes were centrifuged at 100,000 g for 1 h and the pellet (containing the plasma membranes) was solubilized in 300 ml of cell lysis buffer. Lyastes were run over mAb-columns at a rate of 1 ml/min. Columns were rinsed with 10 vol of PBS, 5 vol of 1% boctylglucoside, PBS and eluted with 30 mls of boctylglucoside/PBS containing 50 mM triethylamine (pH 11). Fractions (1.5 ml) were collected into 150 µl of 1 M Tris, pH 7.4. Fifty µl aliquots of each fraction were separated by 8% SDS-PAGE, and stained with silver nitrate in order to evaluate purity of the integrin preparations as well as to quantitate receptor yields. The receptors eluted in fractions 5-9. Approximately 100 µg of receptor was purified from thirty confluent 15 cm plates.

The purity of eluted receptor preparations was extensively evaluated. The following criteria excluded antibody contamination. First, no staining of immunoglobal subunits was seen when column fractions were analyzed by SDS-PAGE and silver staining of gels. Second, eluates were adsorbed onto cowasphere beads (Duke Scientific Corp., Palo Alto, CA) and allowed to incubate with surfaces coated with specific integrin ligands, anti-integrin mAbs, or rabbit anti-mouse IgG, and subsequently washed. Binding to integrin ligands or anti-integrin mAbs was observed but no binding of covaspheres to secondary mAbs was observed, as might be expected if mAb were present in the eluates. Finally, no contaminating IgG was found in αβ1 or αβ2 preparations using dot-blot immunoassays (see Results and Table 1). In these immunoassays, 1-10 µl aliquots of receptors were spotted onto nitrocellulose along with aliquots of serial dilutions of control proteins. After drying, nitrocellulose strips were blocked with BSA, and incubated for 1 h each in primary antibody (anti-collagen 1 and 4 [1:500] Chemicon International, Inc., Temicula, CA), anti-fibronectin [R790], anti-laminin [R5929], rabbit anti-mouse serum [Zymed Labs, Inc., South San Francisco, CA] or anti-epiligrin (PIE1, Carter et al., 1991) followed by HRP-coupled secondary with extensive washing between steps. Bound antibody was then detected with the Amersham Enhanced Chemiluminescence System (Amersham Corp., Arlington Heights, IL). All primaries used except PIE1 were rabbit polyclonal antisera. Using the same assay, no contamination of receptor preparations by other integrins was detected. The potential contamination of column eluates by other integrins and CD44 determined by ELISA (as described below) was < 7%.

ELISA Receptor-Receptor Adhesion Assay

These assays were similar in principle to the assays depicted in Fig. 1 with three differences. (a) Antibodies were not used to plate-immobilize integrins; (b) one of the integrins was allowed to interact in solution rather than being immobilized on Sepharose beads; and (c) binding of the soluble integrin was measured by using antibodies to that integrin rather than isocitopic labeling of integrins. Polyserynec 96-well plates were incubated overnight with purified matrix proteins or affinity-purified adhesion receptors, washed with PBS, and blocked with 1% BSA/PBS. Plates coated in this way were used as "adhesion substrates" for soluble integrins. Soluble receptors were added, allowed to interact with "adhesion surfaces" for 3 h (± inhibitory mAbs or soluble protein ligands), and then washed with PBS to remove unbound receptor. Binding of receptors to plastic and to each other was quantitated by sequentially incubating wells with appropriate anti-integrin mAbs, peroxidase-conjugated rabbit anti-mouse IgG, and 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), a colorimetric reagent. "Cross-detection" of one integrin by antibodies to another was < 7% for all receptor/mAb combinations.

The percent specific adhesion of soluble integrin X to immobilized integrin Y was then calculated in the following manner: % specific adhesion (soluble integrin X immobilized integrin Y) = OD measured (integrin X with anti-integrin X) minus OD cross-detection (integrin Y by anti-integrin X) divided by OD 100% adhesion (integrin X on plastic detected with anti-integrin X).

Preliminary experiments were performed to determine optimal working concentrations of each of the receptors. These experiments showed that a "plateau concentration" existed for each integrin beyond which increasing integrin concentrations did not result in higher adhesion. This suggested that the interaction was saturable. The mAbs used to inhibit receptor-receptor interactions did not interfere with detection of bound integrins in this assay.

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 cells were fixed in MeOH after washing, stained with 1% crystal violet, solubilized with 1% deoxycholate, and quantitated by reading the absorbance at 595 nm using an ELISA plate reader.

Results

Integrins αβ1 and αβ2 Localize to Interacellular Contacts

In cell culture, αβ1 localizes to FAs in areas of low cell density and to intercellular contacts in touching cells (Fig. 2 F and Carter et al., 1990a,b, 1991). Interaction with epiligrin induced αβ1 localization in FAs (Carter et al., 1991). Intercellular localization could be detected with all anti-αβ1 mAbs (PIB5, PIF2, P2E6, P4E7). Similar dual distributions of αβ1 was also observed in cryostat sections of normal human palm epidermis (Fig. 2, B-D). αβ1 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. αβ1 was detected on the lateral and apical surfaces of cells in culture (Fig. 2 E) or palm skin (Fig. 2 A). αβ2 localization in FAs only occurred with the addition of an exogenous collagen substrate (Carter, 1990a). These results indicate that the subcellular localization of αβ1 and αβ2 may be dependent on the microenvironment of the cell in deep versus shallow rete ridges. Furthermore these results suggest that the intercellular localization of αβ1 and αβ2 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-αβ1 mAbs failed to induce cell aggregation. The results summarized in Fig. 2 suggested that integrins αβ1 and αβ2 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 αβ1 and αβ2, 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, 1988). 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
Table I. Adhesion of α3β1- or α4β1-beads to immobilized α3β1

<table>
<thead>
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<th>Source of immobilized integrins:</th>
<th>α3β1-beads</th>
<th>α4β1-beads</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>P1B5-activated</td>
<td>Control</td>
</tr>
<tr>
<td>Immobilized Integrin Exp.</td>
<td>Immobilizing mAb</td>
<td>E1L8</td>
</tr>
<tr>
<td>Exp. I</td>
<td>(RooM)</td>
<td>615 ± 69</td>
</tr>
<tr>
<td>α3β1</td>
<td>(P1B5)</td>
<td>1733 ± 45</td>
</tr>
<tr>
<td>Exp. II</td>
<td>(RooM)</td>
<td>583 ± 39</td>
</tr>
<tr>
<td>α3β1</td>
<td>(P1B5)</td>
<td>399 ± 17</td>
</tr>
<tr>
<td>α3β1</td>
<td>(P1B5)</td>
<td>805 ± 117</td>
</tr>
<tr>
<td>α3β1</td>
<td>(P1B5)</td>
<td>886 ± 120</td>
</tr>
<tr>
<td>Exp. III</td>
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</tr>
<tr>
<td>α3β1</td>
<td>(+ soluble antiβ1)</td>
<td>7213 ± 1507</td>
</tr>
<tr>
<td>α3β1</td>
<td>(+ soluble antiβ1)</td>
<td>1607 ± 174</td>
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Binding of α3β1-coated beads to plate-immobilized α3β1. The experimental design used in these experiments is outlined in Fig. 1. α3β1 or α4β1 (4 in Fig. 1) was purified onto Sepharose beads (6 in Fig. 1) from [35S]methionine-labeled FEPE1L-8 and HT1080 cells, respectively, and incubated with integrins immobilized via anti-integrin mAbs on adhesion plates from control or P1B5-treated, unlabeled FEPE1L-8 cell lysates. FEPE1L-8 cells were treated with intact P1B5 in experiments I and II, and with P1B5 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-β, (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 P1B5-activated versus control FEPE1L-8 lysates are depicted in the third column of each panel. Results of three independent experiments are shown.

This study. Similar results were obtained with P1B5 in conditioned culture supernatant, affinity-purified P1B5 or P1B5 F(ab') (10 µg/ml final concentration). This suggests that serum or high Ca in hybridoma supernatants did not stimulate ICA. P1B5 F(ab') was a potent stimulator of ICA (Fig. 3), although it contained no detectable intact P1B5. This data excludes the possibility that P1B5 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 P1H5 or P4C10) did not induce ICA or cell aggregation. Second, P1B5 also induced aggregation of suspension cells. Third, we observed P1B5-induced ICA when cells were plated on anti-α2β1 or anti-α5β1, and thus, not susceptible to P1B5-induced detachment. Finally, P1B5 did not stimulate the adhesion of FEPE1L-8 cells to substrate ligands such as COL, FN, LM, KC ECM. These data suggested that P1B5 can trigger ICA in addition to its effects on cell-substratum adhesion.

Integrins α3β1 and α4β1 Participate in P1B5-induced ICA

It was possible that P1B5 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 P1B5-induced ICA. Only mAbs known to inhibit epidermal adhesion were used for these experiments. Anti-E-cadherins (HECD-1, ECCD-2) (Takeichi, et al., 1987) were used to test the specificity of integrin interactions. Data obtained using this assay are presented in Table I.
Figure 2. Intercellular localization of integrins α2β1 and α3β1 in human palm and cultured keratinocytes and PIB5-induced cell aggregation. Localization of integrins α2β1 (2A, 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 α2β1 in basal cells of the deep rete ridges and the relative lack of basal α3β1 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 PIB5. PIH5, 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 PIB5-induced ICA although they could disrupt baseline epidermal adhesion. This suggested that ACAMs/cadherins do not mediate PIB5-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, α2β1, and α3β1 (P4C10, P4E7, and P4B4, respectively) significantly inhibited PIB5-induced ICA, as shown in Fig. 3. Two other anti-β, specific mAbs, PIFI and A1A5, also inhibited PIB5-induced ICA (data not shown). Antibodies recognizing other integrins expressed by epidermal cells, such as α6 (G0H3), α9β1 (PID6), 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-β1 specific mAbs. Anti-α2β1-coated beads incubated with lysates immunodepleted of α2β1 by serial preclearing did not bind appreciably to an FEPEIL-8 monolayer (data not shown). This
suggests either that little α2β1 is expressed on the apical surface of epidermal cells or that this apical α2β1 is inaccessible. Little binding was observed between α2β1-beads and FEPEIL-8 cells in the presence of PIB5 or SP2 (Fig. 4B). This served as both a specificity control and, with the PIB5 F(ab’) data, suggested that homophilic α2β1 interactions are not responsible for PIB5-induced ICA. Taken together, these results demonstrate the similarity between the interactions of two FEPEIL-8 cells or α2β1-beads and FEPEIL-8 cells. This suggests that α2β1 is one of the molecules mediating PIB5-induced ICA.

α3β1, Beads Bind to Immobilized α3β1

A further modification in the adhesion assay allowed us to measure inter-integrin interactions in a cell-free system. Results of these experiments are summarized in Table I. As depicted in Fig. 1, integrins were immobilized on two different solid phases, sepharose beads or plastic wells, via anti-integrin mAbs. FEPEIL-8 cells were used as the source of all integrins except α4β1, which was isolated from HT1080 cells. Three different anti-α3β1 specific mAbs (PIB5, P1F2, and P4E7) were used to plate-immobilize α3β1. Once again, isotype-matched mAbs were used for bead-immobilization of α3β1 and α2β1 (left and right, respectively). Epidermal cells used as the source of plate-immobilized integrins were pre-treated with intact PIB5 (Exp. I and II), PIB5 F(ab’) (Exp. III) or SP2 (labeled control). Treated cells were solubilized, and cell lysates were then incubated with wells precoated with anti-integrin mAbs (as indicated in second

Figure 3. PIB5 stimulates ICA. Bars represent the percent adhesion of 51Cr-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 (PI1 and A1A5) are not shown. Other mAbs used were P4G9 (anti-α4β1), P66 (anti-αβ1), G003 (anti-α6), P1G12 (anti-CD44), HECD-1 and ECCD-2 (anti-E-cadherins), and GC-4 (anti-ACAM). For results shown, all except PIB5 F(ab’), ECCD, HEC, and GC4 were used as hybridoma supernatant. Results using purified PIB5 at 10 μg/ml were similar to that shown for PIB5 F(ab’).
Figure 4. PIB5 stimulates the adhesion of $\alpha_2\beta_1$-coated beads to an FEPEIL-8 monolayer. (a) Binding of $\alpha_2\beta_1$ and $\alpha_3\beta_1$ 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 $[^{35}S]$cpm (with indicated standard deviations) is shown on the Y-axis. The left depicts binding of $\alpha_2\beta_1$-beads while the right depicts binding of $\alpha_3\beta_1$-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-$\beta_1$) and PIB5 + P4E7 (anti-$\alpha_3\beta_1$), respectively. In all experiments $<$10% of $\alpha_2\beta_1$ counts and $<$1% of $\alpha_3\beta_1$ counts were associated with FEPEIL-8 cells in the presence of SP2. Bead-associated cpm were higher for $\alpha_3\beta_1$ coated compared to $\alpha_2\beta_1$ coated beads. The ICA of $^{51}$Cr-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 $\alpha_2\beta_1$-beads (left column) or $\alpha_3\beta_1$-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.

This modification allowed us to use immobilized integrins as an adhesion substrate in place of confluent epidermal cells. As shown in Table I, little specific adhesion was observed between $\alpha_2\beta_1$-beads and $\alpha_3\beta_1$ purified from control-treated epidermal cells. Stimulation of basal adhesion (two to 14-fold) was observed in each experiment performed when $\alpha_2\beta_1$ was purified from PIB5-treated cells. This stimulated interaction could be inhibited by soluble anti-$\beta_1$ mAbs (Exp. III). PIB5 did not stimulate the interaction between $\alpha_2\beta_1$-beads and immobilized $\alpha_3\beta_1$ or $\alpha_3\beta_1$-beads and immobilized $\alpha_2\beta_1$, demonstrating its selectivity for $\alpha_2\beta_1$/$\alpha_3\beta_1$ interactions. The magnitude of this stimulation varied depending on the particular anti-$\alpha_3\beta_1$ antibody used to immobilize $\alpha_3\beta_1$ 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 $\alpha_2\beta_1$-beads. Once again, these results suggest that $\alpha_2\beta_1$ and $\alpha_3\beta_1$ are the mediators of PIB5-induced ICA.

Affinity-purified $\alpha_2\beta_1$ and $\alpha_3\beta_1$ Preferentially Interact

In order to obtain independent confirmation of an interaction between integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$, 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. $\alpha_3\beta_1$ and CD44 were also affinity-purified for use as controls on PID6 and P4G9 columns, respectively. The integrins purified from column from left).
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% β-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 α3 (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, α3 or α5 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/β-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 quantitate 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 α3β1 and immobilized α3β1. This interaction is inhibited to basal levels by the addition of soluble anti-α3β1 (P1B5) or anti-α2β1 (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 α3β1, but not to immobilized CD44 (see Fig. 6, middle) or immobilized α3β1 (data not shown). Again, this interaction was inhibited by anti-α3β1 (P1B5) or anti-β1 (P4C10) mAbs. Of note, the addition of soluble ligands for α3β1 and α5β1, 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 $\alpha_2\beta_1$ 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 $\alpha_2\beta_1$ to immobilized $\alpha_3\beta_1$ 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 $\alpha_2\beta_1$ to immobilized fibronectin was specifically inhibited, confirming that the purified $\alpha_3\beta_1$ was functional. Taken together, these results suggest that the observed $\alpha_2\beta_1/\alpha_3\beta_1$ 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 $\alpha_2\beta_1$ was acquired along with expression of $\alpha_3\beta_1$. For these experiments, CHO K1 cells, which express abundant $\alpha_2\beta_1$, but low levels of integrins $\alpha_3\beta_1$ 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 $\alpha_2\beta_1$ (mean fluorescent intensity 123 in $\alpha_2$-CHO compared to 4 in parental CHO). Expression of endogenous $\alpha_2$ and total $\beta_1$ integrins was not significantly increased. The binding of parental CHO cells to $\alpha_2$-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 $\alpha_2\beta_1$, 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 $\alpha_5\beta_1$. 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-
Only slight increases in staining of u2-CHO cells with 7E2, PB1, 131), PB1 (anti-hamster u), or irrelevant mouse IgG, as indicated. Cho

cho

Figure served. Mean fluorescent intensity of staining with 12F1 increased or mouse IgG was detected. In contrast, marked increases in stain-

ing of u:-CHO cells with 12FI, an anti-a specific mAb was ob-

served. Mean fluorescent intensity of staining with 12FI increased from 4 for CHO cells to 123 for u:-CHO. Integrin expression by CHO and α2-transfected CHO. CHO (dotted line) and α2-CHO (solid line) cells were stained with saturating quantities of 12F1 (anti-human α2), 7E2 (anti-hamster β1), PBI (anti-hamster α3), or irrelevant mouse IgG, as indicated. Only slight increases in staining of α2-CHO cells with 7E2, PBI, or mouse IgG was detected. In contrast, marked increases in staining of α2-CHO cells with 12F1, an anti-α2 specific mAb was observed. Mean fluorescent intensity of staining with 12F1 increased from 4 for CHO cells to 123 for α2-CHO. The Journal of Cell Biology, Volume 120, 1993 532

E-cadherins or ACAMs, participate in basal but not P1BS-

meditated ICA. Importantly, these same studies showed that other cell-cell adhesion receptors, such as E-cadherins or ACAMs, participate in basal but not PIB5-mediated 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 (LFA1-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 αβ binding sites (i.e., higher avidity) than αβ. Alternatively, the affinity of αβ for epiligrin may be higher than that for αβ. Both of these would result in cell-substrate adhesion being dominant to cell-cell adhesion. Thus, only in situations where the αβ-epiligrin interaction is prevented can the interaction with αβ occur. Finally, PIB5 may be inducing a conformational change in αβ, which increases its affinity for a cell-surface coreceptor while reducing its affinity for a matrix ligand.

Discussion

PIB5 Induces Epidermal ICA Mediated Through Integrins αβ and αβ

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 αβ and αβ 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

Purified αβ and αβ Can Interact in Vitro

We show that purified integrins αβ and αβ 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 αβ and αβ, just as it stimulated an interaction between intact cells. In contrast, a significant basal level of interaction was observed between affinity-purified αβ and αβ, and PIB5 did not stimulate this further (Fig. 6). This suggests that the PIB5-affinity column selectively purifies the “active” form of αβ, and/or activates all the αβ. Alternatively, differences in deter-

gents used or αβ immobilization techniques may have affected the conformation of αβ. Results using intact cells and mAb-immobilized integrins also suggested there was a directionality to the interaction (Fig. 4 and data not shown). While αβ-beads could interact with an epidermal monolayer, αβ-beads did not. This was surprising in view of the expression of both αβ and αβ by epidermal cells. No such directionality was observed using affinity-purified integrins αβ and αβ (Fig. 6). This could reflect differences in accessibility of αβ on a cell surface versus in a deter-

gent solution. The observation that anti-αβ beads (i.e., PIB5-beads or PIH6-beads) bound poorly to a monolayer of intact epidermal cells was consistent with this interpretation. In any case, a selective interaction between integrins αβ and αβ is observed using two different cell-free systems.

A number of alternate explanations for these observations were evaluated. First, antibody cross-linking of two mole-

ules of αβ are excluded by both the PIB5 F(ab’) data and data using purified integrins. It is unlikely that we are actu-

ally 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 αβ ligands (and thus, not susceptible to PIB5-detachment). Interactions between inte-

grins on one surface and mAb domains on the other could artifically give the appearance of integrin-integrin bind-

ing. This is unlikely because isotype-matched mAbs were used to immobilize test and control integrins. Furthermore,
Figure 8. \(\alpha_2\)-CHO acquire collagen and \(\alpha_3\beta_1\) binding. The adhesion of CHO or \(\alpha_2\)-CHO to various ligand proteins was measured. Shown here are the results obtained using FN, col, \(\alpha_3\beta_1\), and \(\alpha_2\beta_1\) as ligands. Adhesion was measured in the presence of SP2 or the indicated inhibitory mAb (PIH5, anti-\(\alpha_2\) or P4C2, anti-\(\alpha_3\beta_1\)). Results of one experiment, representative of four performed, is shown. Briefly, wells were coated overnight with the indicated ligands (5 \(\mu\)g/ml for collagen and FN, 1:4 dilution for \(\alpha_3\beta_1\) and \(\alpha_2\beta_1\)), rinsed, and then blocked for 1 h with 5 \(\mu\)g/ml BSA before use. Inhibitors (1:4 final dilution) were added at time zero. After 90 min at 37\(^\circ\)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 \(\alpha_2\)-CHO to collagen and \(\alpha_3\beta_1\) was observed. PIH5 completely inhibited \(\alpha_2\)-CHO binding to collagen and \(\alpha_3\beta_1\).

the selective interaction between affinity-purified \(\alpha_3\beta_1\) and \(\alpha_2\beta_1\) also makes this unlikely by providing independent evidence for an \(\alpha_3\beta_1/\alpha_2\beta_1\) interaction measured in the absence of immobilizing mAbs. Finally, it is still formally possible that some as yet unidentified molecule copurifies with either \(\alpha_3\beta_1\) or \(\alpha_2\beta_1\), isolated in various ways, and actually bridges these two integrins. If true, one must propose that the \(\alpha_3\beta_1/\alpha_2\beta_1\) interaction is stabilized by PIH5, and inhibited by anti-\(\beta_1\), anti-\(\alpha_3\), and anti-\(\alpha_2\) specific mAbs. Although hard to exclude absolutely, we think this is unlikely for a number of reasons. First, addition of soluble ligands for these integrins, 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 \(^{35}\)S-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 \( \alpha_2 \)-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 \( \alpha_2-X-\alpha_3 \) interaction. The simplest explanation compatible with all the data is that purified integrins \( \alpha_2 \beta_1 \) and \( \alpha_2 \tilde{\beta}_1 \) 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 (LFA1/ICAM1, VLA4/VCAM1) (Marlin 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 \( \alpha \)-CHO transfectants. CHO cells acquired the ability to adhere to collagen and \( \alpha_2 \tilde{\beta}_1 \), when they were transfected with \( \alpha \_ \) cDNAs. Both these interactions were selective and inhibitable. Like the interaction of affinity-purified integrins, \( \alpha_2 \)-CHO adhesion to purified \( \alpha_2 \tilde{\beta}_1 \) 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 \( \alpha_2 \)-CHO establish the premise that immobilized integrins can support cell adhesion.

**Potential Role of \( \alpha \_2 \tilde{\beta}_1 \) and \( \alpha_3 \tilde{\beta}_1 \), Interactions in Cell–Cell Adhesion In Vivo**

Based on the data presented above, we infer that \( \alpha_3 \tilde{\beta}_1 \) and \( \alpha_2 \tilde{\beta}_1 \), 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 \( \alpha_3 \tilde{\beta}_1 \) 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-\( \alpha_2 \tilde{\beta}_1 \) or anti-\( \alpha_3 \tilde{\beta}_1 \) was observed (data not shown). Thus, PIB5 treatment and commitment to differentiation both appear to induce ICA mediated by integrins \( \alpha_2 \beta_1/\alpha_3 \tilde{\beta}_1 \). (d) Although intercellular localization of \( \alpha_2 \tilde{\beta}_1 \) was detected by all anti-\( \alpha_2 \tilde{\beta}_1 \) mAbs tested, this was most striking when cells were incubated in the presence of PIB5 prior to fixation (Fig. 2F). Here, PIB5 is inducing changes in integrin distribution that occur during stratification.

The recent report of tyrosine phosphorylation occurring after PIB5-induced \( \alpha_2 \tilde{\beta}_1 \), cross-linking suggests that \( \alpha_2 \tilde{\beta}_1 \) can participate in signal transduction (Kornberg et al., 1991). Thus, a single agent, PIB5, can induce cell-substrate detachment, cell–cell adhesion, \( \alpha_2 \beta_1-\alpha_3 \tilde{\beta}_1 \) interactions in vitro, and biochemical changes consistent with signal transduction. It is tempting to speculate that these events are inter-related, 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.

The authors gratefully acknowledge the gifts of mAbs from numerous investigators. Y. Takada thanks Wilma Puzon for technical assistance. We thank Dr. Frank Symington for stimulating and helpful discussions.

This work was supported by National Institutes of Health grants K11 HL02216 (to B. E. Symington), R01-Ca49259 (to W. G. Carter), GM-47517 (to Y. Takada), and an American Cancer Society grant CD-453F to W. G. Carter.

Received for publication 28 April 1992 and in revised form 28 August 1992.

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