

Desmosomal adhesion inhibits invasive behavior

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Edited by Elaine V. Fuchs, University of Chicago, Chicago, IL, and approved April 17, 1998 (received for review October 27, 1997)

ABSTRACT Recent studies of human disease and transgenic animal experiments have clearly demonstrated the importance of desmosomes in normal tissue architecture. Furthermore, desmosomal components are down-regulated in certain types of carcinomas, suggesting a possible role for desmosomes in suppression of invasion and metastasis. However, there is no functional evidence to support such a hypothesis. To obtain such evidence, we needed to generate desmosomal adhesion in an invasive cell line. We show that expression of multiple desmosomal components (the desmosomal cadherins, desmocollin and desmoglein, and the armadillo protein, plakoglobin) in nonadhesive L929 fibroblasts generates adhesion in aggregation assays. This adhesion is specifically blocked by short peptides corresponding to the putative cell adhesion recognition sites of desmocollin and desmoglein. This result provides an experimental demonstration of the functional importance of the cell adhesion recognition sites of desmocollin and desmoglein and indicates that both desmosomal cadherins are specifically involved in this adhesion. Moreover, whereas parental L929 cells are strongly invasive into collagen gels, we show that invasion is substantially inhibited in cells transfected with desmosomal components. Invasion is restored by treating the transfected cells with anti-adhesion peptides, indicating that desmosomal adhesion specifically blocks invasion in culture. Our results support the suggestion that desmosomes have a role in suppression of tumor spreading.

The intercellular junctions known as desmosomes are multi-molecular membrane domains that provide intercellular adhesion and membrane anchors for the intermediate filament cytoskeleton. Much evidence now attests to their importance in tissue architecture. Loss of keratinocyte adhesion caused by autoantibodies to desmogleins (Dsgs) (1) or by mutations of plakophilin 1 (2) demonstrates a role for desmosomes in the structure and morphogenesis of the epidermis. Furthermore, dominant negative and null mutations of the pemphigus vulgaris antigen, Dsg3, cause loss of epidermal cell adhesion, and plakoglobin (PG) null mutations cause epidermal defects and embryonic lethal defects in cardiac muscle (3–6). Moreover, we and others have shown that desmosome expression or staining for desmosomal components is abnormal in certain types of human carcinomas (7–11). Thorough immunohistochemical studies of pathological material have shown that loss of staining for desmosomal components correlates with invasion or metastasis in transitional cell carcinoma (7) and squamous cell carcinoma of head and neck (8–10). It is therefore conceivable that desmosomes have an invasion and metastasis suppressor function.

It has proved difficult to demonstrate desmosomal adhesion by transfection of nonadhesive cells. Previous studies in which desmocollin (Dsc), Dsg, or both, in conjunction with PG, have

been expressed in nonadhesive L929 cells have failed to demonstrate an adhesive function for these components (12–14). Such results appear to suggest that these components, either alone or in combination, are insufficient to generate adhesion at least in the context of a fibroblastic host cell. Recently, however, binding studies have provided direct evidence for heterophilic interaction between Dsc and Dsg (15), and adhesion has been obtained by transfection of fibroblasts with human Dsg1, Dsc2, and PG (16).

In this study, we carried out multiple transfections of nonadhesive L929 cells with a combination of desmosomal components that has not previously been used. These experiments generated cell lines that exhibited substantial intercellular adhesion. In a collagen invasion assay, these cells showed suppression of invasion that was reversed by the addition of specific peptides, indicating that desmosomal adhesion inhibits invasion. This evidence suggests a functional role for desmosomal adhesion in regulating cell behavior.

MATERIALS AND METHODS

Construction of Plasmids. Eukaryotic expression vectors, driven by the β -actin promoter, with cDNA encoding neomycin resistance (pBATneo) or full-length mouse E-cadherin (pBATEM2) were kindly donated by M. Takeichi (Kyoto University, Japan). Vectors pBATDsc1a and pBATDsc1b encoding full-length bovine Dsc1a and Dsc1b, respectively, have been described previously (12). Vectors pBATPG and pBATDsg1 were constructed by replacing the E-cadherin sequence in pBATEM2 with cDNA encoding full-length human PG and full-length bovine Dsg1, respectively (kindly provided by P. Cowin; New York University Medical Center). Vector pHyg encoding hygromycin resistance has been described (17), and vector pBabepuro encoding puromycin resistance was kindly donated by H. Land (Imperial Cancer Research Fund, London).

Transfection. Subconfluent L929 cells were transfected in 9-cm Petri dishes by using the calcium phosphate method with three different successive selections. Initially, L929 cells were transfected as described (12) with pBATneo (10 μ g/ml), pBATDsc1a (50 μ g/ml), and pBAT Dsc1b (50 μ g/ml) and were selected with 800 μ g/ml G-418 sulfate. In all instances, positive cell lines were identified by flow cytometry and Western blotting. A cell line BE11, expressing both Dsc1a and Dsc1b, was then further transfected with pHyg (10 μ g/ml), pBATPG (50 μ g/ml), and pBATDsg1 (50 μ g/ml) and selected with 500 μ g/ml hygromycin. A positive cell line (11D6) expressing all transfected components was then retransfected with pBATDsc1a (50 μ g/ml), pBATDsc1b (50 μ g/ml), and pBabepuro (10 μ g/ml) and then selected with puromycin (6 μ g/ml). A positive cell line (AB5) was further characterized

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This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: Dsg, desmoglein; PG, plakoglobin; Dsc, desmocollin; CAR, cell adhesion recognition.

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because of its extensive ability to aggregate in suspension (see below).

Western Blotting. Cell lysates were separated by SDS/PAGE and transferred onto a nitrocellulose membrane. Blots were blocked with 5% skim milk and probed with the following antibodies: (i) 52-3D (diluted 1:500), a mouse anti-Dsc monoclonal antibody that reacts with the cytoplasmic domains of Dsc1a and Dsc1b (12); (ii) rabbit polyclonal antibodies RO851 and RO852 (diluted 1:2000) raised against Dsc1a- and Dsc1b-specific peptides, respectively, from the alternatively spliced C-terminal domains and characterized by Western blotting on bovine nasal epidermis; (iii) 3a, a guinea pig anti-Dsg (diluted 1:1000) kindly provided by P. Cowin; or (iv) a rabbit anti-PG (diluted 1:500) kindly provided by R. Kemler (Max Planck Institute for Immunobiology, Freiberg, Germany). Primary antibodies were detected by using the appropriate peroxidase-conjugated secondary antibody and an ECL detection system (Amersham).

Immunofluorescence Microscopy. L929 cell clones were grown to confluence on Costar polycarbonate filters, which were washed with PBS and cut into a number of segments. For desmosomal cadherin staining, cells were briefly washed with PBS/1% BSA, permeabilized with 0.1% Triton X-100 in PBS/1% BSA for 20 min, washed, and then blocked with PBS/1% BSA containing 5% normal goat serum on ice for 10 min. The cells were incubated with either antibody 3a against Dsg (diluted 1:100) or HM6 (12) against Dsc (diluted 1:100) for 2 h on ice. Cells were extensively washed with PBS/1% BSA and fixed with 2% paraformaldehyde for 10 min. After the filters were washed with PBS/1% BSA, they were incubated in 0.02 M glycine before incubation in the appropriate Cy3-conjugated secondary antibody (Jackson ImmunoResearch). Double immunofluorescence was performed as described above by using the same antibody concentrations and by using Cy3- and fluorescein isothiocyanate-conjugated secondary antibodies to distinguish between primary antibodies. Filters were washed several times, mounted in Gelvatol, and examined under a Zeiss Axioplan microscope. For PG staining, cells were fixed with 50% methanol/50% acetone, washed repeatedly with PBS, and further permeabilized with 0.1% Triton X-100. After the filters were blocked as described above, they were incubated with monoclonal antibody 11E4 (14) (diluted 1:10) for 1 h, washed extensively, and then incubated with mouse Cy3-conjugated secondary antibody for 1 h. Cells were then washed and mounted as described above.

Flow Cytometry. L929 cell clones were cultured for 24 h in bacteriological dishes. Cells were harvested by trituration, washed in Hepes-buffered saline (HEBS)/CaCl₂ [137 mM NaCl/5 mM KCl/5.5 mM glucose/0.8 mM Na₂HPO₄/21 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid/2 mM CaCl₂ (pH 7.05)] and a single-cell suspension obtained by passage through a 70- μ m sieve. Cells (10⁶) were fixed for 15 min in 400 μ l of HEBS/CaCl₂ containing 2% formaldehyde and then washed with HEBS/CaCl₂. Fixed cells were incubated for 45 min in either 200 μ l of antibody HM6 (diluted 1:100) or antibody 3a (diluted 1:40). Cells were repeatedly washed and then incubated in 200 μ l of the appropriate fluorescein isothiocyanate-conjugated secondary antibody (1:200; Jackson ImmunoResearch). Cells were further washed and resuspended in 400 μ l of HEBS/CaCl₂ for analysis on a FACScan flow cytometer (Becton Dickinson).

Aggregation Assays. Single-cell suspensions of L929 cell clones were obtained as described above. Cells were washed and suspended at about 5×10^5 /0.5 ml of HEBS/CaCl₂ with 10 μ g/ml DNase I (Sigma). In some cases, cells were resuspended in 0.5 ml of HEBS/CaCl₂ containing peptides (see below). Cells were placed in the wells of 24-well tissue culture dishes (preblocked with 1.5% BSA), rotated at 75 rpm for 2 h at 37°C, and viewed by phase-contrast microscopy. In some experiments, the ability of two different transfected clones to

interact was assessed by labeling one with the fluorescent dye 5 (and 6)-carboxy-2',7'-dichlorofluorescein diacetate succinimidyl ester (10 μ M; Molecular Probes). Reciprocal labeling was carried out to control for the possible effect of the label.

Peptides. Peptides were synthesized on an Applied Biosystems 431A peptide synthesizer with FASTMoc chemistry. The lyophilized peptides were desalted on a PD-10 column (Pharmacia), and the concentration of the peptide was estimated by using the BCA protein assay kit (Pierce) with BSA as a standard. The four peptides synthesized were: IYAYAT-TADG, Dsc1 peptide containing cell adhesion recognition (CAR) site; ATYIDYTAGA, scrambled Dsc1 peptide; IY-CRALNSLG, Dsg1 peptide containing CAR site; and TMQN-LNDRSLASYL, 13-amino acid sequence from the 1A rod domain of human keratin-10.

Invasion Assays. Collagen gels were prepared by mixing type I collagen (extracted from rat tail tendon) in 0.1% acetic acid (8 ml of 1.5 mg/ml), 10 \times MEM (1 ml), and 0.125 M NaOH/0.26 M NaHCO₃ (1 ml). The collagen mixture (0.5 ml) was rapidly aliquoted into the wells of a 24-well tissue culture dish and allowed to set at 37°C. Cells (2×10^5) in 0.5 ml of MEM/10% fetal calf serum were layered gently on the surface of the collagen gel. To examine the effect of peptides on invasion, L929 cell clones were resuspended in MEM/10% fetal calf serum containing either Dsc peptide (525 μ g/ml) or Dsg peptide (550 μ g/ml), or both, before layering on the gel. At 24-h intervals, the number of cells that had invaded per cm² was determined by using a Zeiss Axiovert 10 microscope. The total number of cells was also counted at intervals to ensure that there was no difference in cell proliferation.

RESULTS

Transfection of L929 Cells with Desmosomal Components.

Cells were transfected with Dsc1a and Dsc1b by using neomycin resistance, and a positive cell line (BE11) expressing both Dsc1a and Dsc1b was used for further experiments. This cell line was not adhesive in aggregation assays (data not shown) and therefore was further transfected with Dsg1 and PG by using hygromycin resistance. Several cell lines expressing all four components were identified. One of these, 11D6, showed readily detectable levels of Dsg1 and PG, although its Dsc expression was very low (Fig. 1*a*). It also showed slight aggregation (Fig. 3*A* and *B*). To enhance Dsc expression, we decided to retransfect 11D6 cells with Dsc1a and Dsc1b by using puromycin selection. By this means, we obtained a number of cell lines that expressed readily detectable amounts of both the a and b forms of Dsc1, as well as Dsg1 and PG.

Several transfected cell lines showed aggregation, but most were unstable, becoming nonadhesive before they could be fully characterized. Two cell lines were more stable, and one of these, AB5, was selected for further study. AB5 showed significant expression of Dsc1a/Dsc1b but somewhat reduced expression of Dsg1 and PG compared with the parent line, 11D6 (Fig. 1*a*). Both Dsc1a/Dsc1b and Dsg1 were clearly detectable at the cell surface by flow cytometry (Fig. 1*b*). The AB5 cell line showed substantial adhesion in aggregation assays (Fig. 3*C*), the aggregates produced being larger, although less regular and compact, than those produced by a transfected L929 cell line, R16, expressing E-cadherin (Fig. 3*D*; ref. 12). Quantitation showed that the degree of aggregation exhibited by cell line AB5 was comparable to that obtained by expression of E-cadherin (Fig. 4). The adhesion produced by AB5 cells was also predominantly calcium-dependent because aggregates formed in calcium-free medium were substantially smaller (Fig. 3*E*).

Inhibition of Aggregation with CAR Peptides: Demonstration of Desmosomal Cadherin-Specific Adhesion. E-cadherin has a CAR site centered around the tripeptide HAV (18). In the desmosomal cadherins Dsc1 and Dsg1, the corresponding

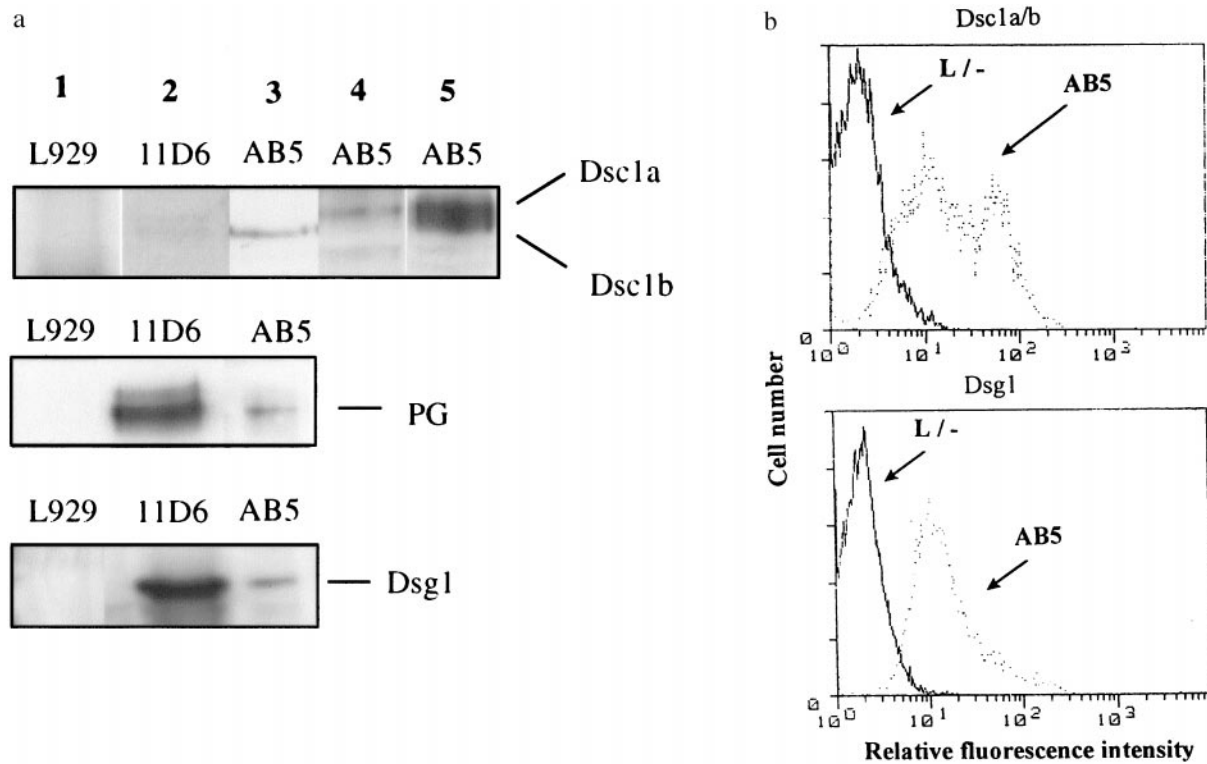


FIG. 1. (a) Analysis of transfected L929 cell clones by immunoblot. (Top) Cell lines L929 (lane 1) and 11D6 (lane 2) probed with a pan-Dsc-specific antibody 52-3D and cell line AB5 probed with Dsc1b-specific antibody RO851 (lane 3), Dsc1a-specific antibody RO852 (lane 4), and 52-3D (lane 5). (Middle) Cell lines L929 (lane 1), 11D6 (lane 2), and AB5 (lane 3) probed with rabbit anti-PG. (Bottom) Cell lines L929 (lane 1), 11D6 (lane 2) and AB5 (lane 3) probed with a Dsg antibody 3a. Cell line 11D6 expresses low levels of Dsc1a and Dsc1b and high levels of Dsg1 and PG, whereas cell line AB5 expresses high levels of Dsc1a and Dsc1b and lower levels of Dsg1 and PG. (b) Analysis of transfected L929 cell clones by flow cytometry. AB5 and untransfected L929 cells were subjected to FACS analysis by using antibody HM6 against Dsca/Dscb (Upper) and antibody 3a against Dsg1 (Lower).

tripeptide sequences are YAT and RAL, respectively (19). These are regarded as putative CAR sites. To determine the functional role of these putative CAR sites and to show that the

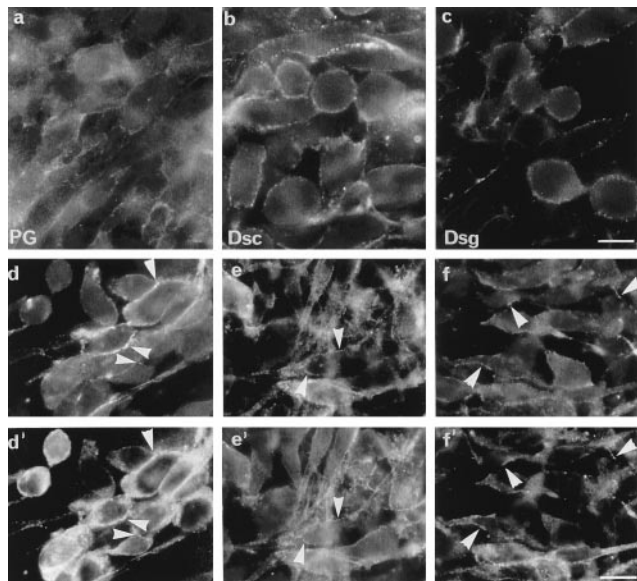


FIG. 2. Immunofluorescent staining of AB5 cells by using antibodies 11E4 (a), HM6 (b), and 3a (c) against PG, Dsca/Dscb, and Dsg1, respectively. (Bar = 18 μ m.) Double immunofluorescence of AB5 cells was performed to examine colocalization of PG (d) and Dsg1 (d'), PG (e) and Dsc (e'), and Dsc (f) and Dsg1 (f') by using the antibodies described above. Arrowheads indicate areas of colocalization. (Bar = 12 μ m.)

adhesion generated in AB5 cells specifically involved desmosomal cadherins, 10-mer peptides centered around the Dsc1 and Dsg1 CAR sites were synthesized and added to aggregation assays (see *Materials and Methods* for complete sequences). Each peptide gave substantial inhibition of aggregation (Fig. 3 H and I). The Dsc1 and Dsg1 peptides were used at a concentration of 0.48 mM (525 μ g/ml) and 0.43 mM (550 μ g/ml), respectively. These were the lowest effective concentrations to result in maximum inhibition of invasion of AB5 cells into collagen gels, as quantitatively determined by dose-response analysis. A number of nonspecific peptides including a scrambled Dsc1 peptide had no effect on the ability of AB5 cells to aggregate in suspension (data not shown). Furthermore, neither of the desmosomal peptides inhibited aggregation of an L929 cell line transfected with the E-cadherin cDNA (12), nor did a 10-mer peptide corresponding to the CAR site of E-cadherin inhibit the aggregation of AB5 cells (not shown).

In support of the specificity of AB5 cell adhesion we showed that AB5 does not adhere to the parental cell line (Fig. 3 F and G). Furthermore, we isolated a revertant of AB5 that had lost expression of Dsg1 and PG (results not shown) and was unable to aggregate (Fig. 4). In addition, inhibition of AB5 adhesion (ca. 45%) was obtained with polyclonal antibody 3a to Dsg (Fig. 4). No function-blocking antibodies to Dsc1 are currently available. Our polyclonal anti-Dsc1 antibody that reacts with the extracellular domain of the molecule (20) had no effect on adhesion.

Taken together these results indicate that the putative CAR sites contribute functionally to desmosomal adhesion, that the adhesion generated in AB5 cells is specifically mediated by desmosomal cadherins, and that it involves a contribution from both Dsc1 and Dsg1.

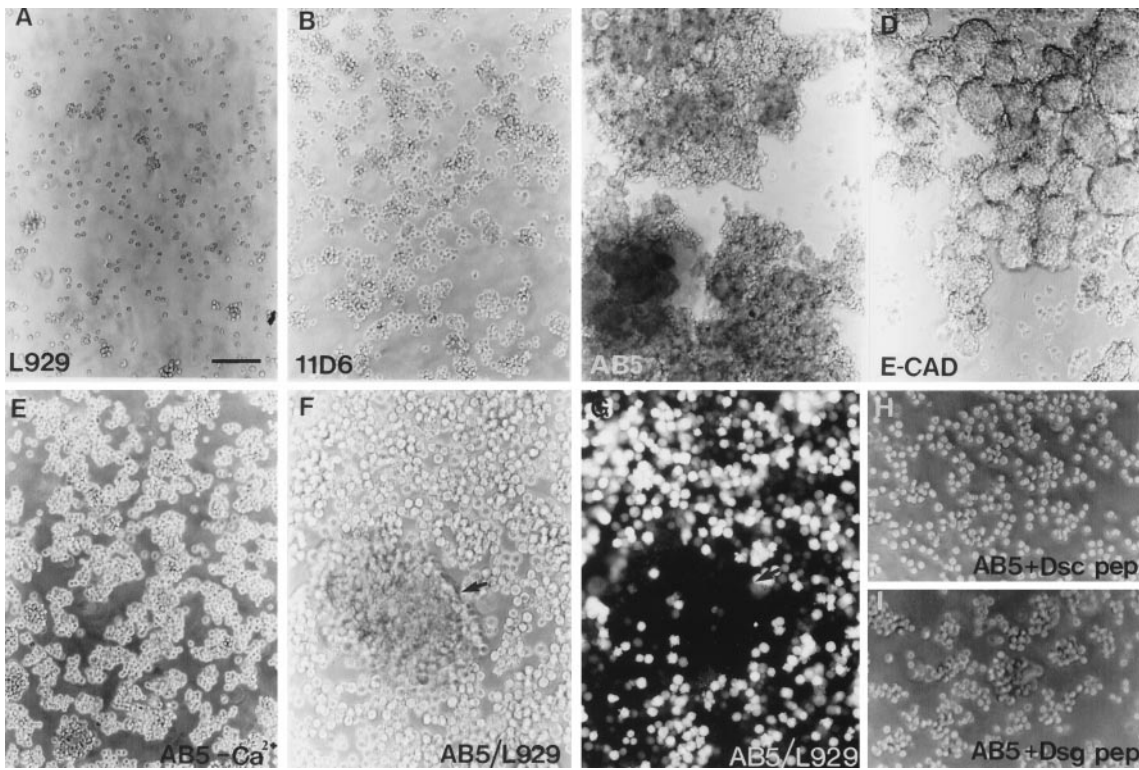


FIG. 3. Aggregation assays for cellular adhesion. (A) L929 cells. (B) 11D6 cells. (C) AB5 cells. (D) R16 cells. (E) AB5 cells in the absence of calcium. (F) Phase contrast of untransfected L cells (fluorescently labeled) and AB5 cells (unlabeled) after mixed aggregation. The aggregate (arrow) consists almost entirely of unlabeled AB5 cells, whereas when AB5 cells were fluorescently labeled and L929 cells were unlabeled the aggregates consisted almost entirely of labeled cells (results not shown). (G) Immunofluorescence of the same field of view. (H) AB5 cells in the presence of Dsc1 CAR peptide. (I) AB5 cells in the presence of Dsg1 CAR peptide. In B, cells were aggregated for 16 h; in all other cases cells were aggregated for 1 h. (Bar = 80 μm in A, B, C, D, E, H, and I; bar = 40 μm in F and G.)

Previous work has suggested that desmosomal adhesion is initiated in epithelial cells by prior cellular interaction via E-cadherin (21, 22) and also that expression of E-cadherin in non-desmosome-bearing cells can elicit up-regulation of desmosomal components (23). Our results might be explained if expression of the desmosomal components in L929 cells caused up-regulation of classical cadherin expression. L929 cells express α - and β -catenin; therefore, up-regulation of cadherin expression could result in aggregation independent of desmosomal component expression. To test this possibility, we carried out immunofluorescence and immunoblotting studies with a number of cadherin antibodies, including ECCD-2 (24) against E-cadherin and two pan-cadherin antibodies (25), all of which reacted with nondesmosomal cadherins in controls. Cadherin expression was not detected in AB5 cells (results not shown). Furthermore, no up-regulation of β -catenin expression could be detected in AB5 cells by immunoblotting (results not shown). By contrast, β -catenin expression is strongly up-regulated in R16 cells that express E-cadherin (12). We conclude that there is no detectable up-regulation of cadherin expression in AB5 cells and that there is no prior requirement for adhesion mediated by E-cadherin or other classical cadherins for desmosomal adhesion to develop between these transfected cells.

Transfected Cells Do Not Form Desmosomes. By using fluorescent staining with antibodies specific for Dsc, Dsg, and PG, we showed that all three components were present at the regions of contact between adhering AB5 cells and were not extractable by Triton X-100 (Fig. 2*a-c*) and that there was some colocalization of components (Fig. 2*d-f'*). The staining for Dsc and Dsg was distinctly punctate, whereas that for PG was mostly uniformly distributed at the plasma membrane. Electron microscopy showed long lengths of close membrane apposition between AB5 cells but no membrane densities

resembling desmosomes or indications of intermediate filament attachment (not shown). We conclude that expression of these four components generates adhesion but does not result in full desmosome assembly.

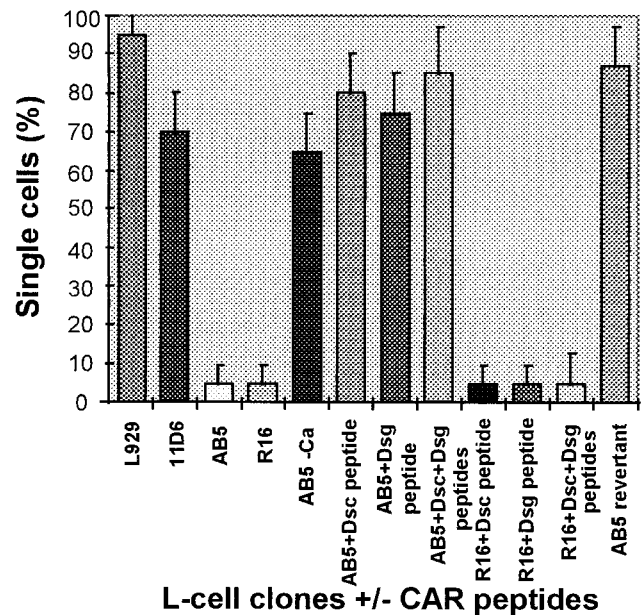


FIG. 4. Quantitation of aggregation assays. Aggregation of L929 cell clones in the presence or absence of CAR peptides were quantitated after 1 h with the exception of 11D6P2 (11D6 cells transfected with puromycin vector alone), which was allowed to aggregate for 16 h. Quantitation was by counting single cells and expressing these counts as a percentage of total cells. Error bars indicate the SEM.

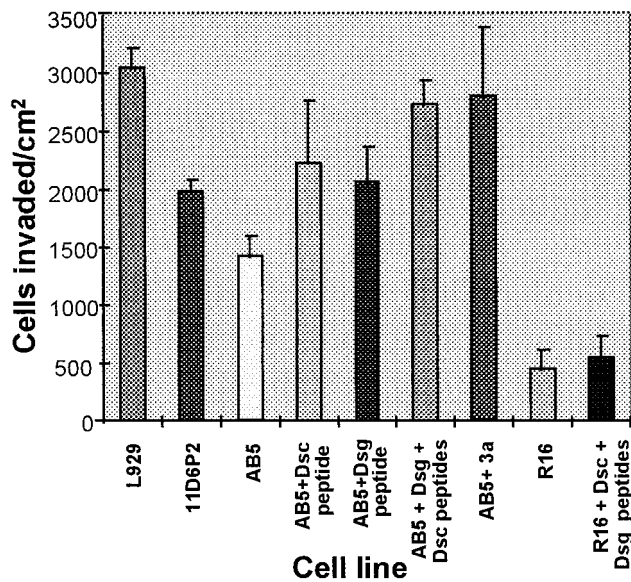


FIG. 5. Invasion is suppressed by desmosomal adhesion and suppression is reversed by anti-adhesion peptides. L929 clones were plated onto collagen gels, and the number of cells that invaded the gels were counted after 48 h. Figures are expressed as mean values of a number of experiments + SEM. For further details, see the text.

Desmosomal Adhesion Inhibits Invasion. Untransfected L929 cells showed substantial invasion into collagen gels (26) (Fig. 5). Compared with these, the weakly aggregating 11D6P2 cells (11D6 cells subjected to control transfection with the puromycin vector) showed about 35% inhibition of invasion, whereas AB5 cell invasion was inhibited by more than 53% (Fig. 5), both results being statistically significantly different from control values ($P < 0.001$ in both cases, ANOVA). Consistent with previous studies (26), E-cadherin expressing cells (R16) also showed strong inhibition of invasion (Fig. 5). Addition of Dsc1- or Dsg1-specific adhesion peptide to AB5 cells before plating on collagen gels substantially restored their invasiveness. Substantial restoration of invasiveness toward control levels was also achieved with both peptides together (Fig. 5), but the results were not statistically significantly different from those obtained with either of the peptides alone ($P > 0.05$ in both cases). Our dose-response analysis showed that no greater blocking of inhibition of invasion occurred even with a peptide concentration of double that used in the assay shown. As control experiments, a Dsc1-scrambled peptide and a 13-amino acid keratin-10 peptide were used, and neither had any effect on cell invasion (not shown). Furthermore, anti-Dsg antibody 3a also restored invasiveness (Fig. 5), whereas anti-Dsc1 antibody JCMC (20) had no effect (not shown). We conclude that desmosomal adhesion specifically inhibits cellular invasion into collagen gels.

DISCUSSION

This study shows that multiple transfection of a nonadhesive L929 cell line with full-length Dsc1a and Dsc1b, Dsg1, and PG cDNAs can generate strong cell aggregation and that the desmosomal adhesion thus generated is able to inhibit cellular invasion into collagen gels. Furthermore, our results demonstrate the functional importance of the CAR sites of Dsg and Dsc (RAL and YAT, respectively) in adhesion mediated by these glycoproteins.

To generate adhesion, we used a combination of conditions that had not been previously used in transfection experiments. First, we used cDNAs for desmosomal cadherins that are codistributed in tissues. Thus, we used the Dsc and Dsg

isoforms, Dsc1 and Dsg1, that are normally expressed together in differentiated cells of stratified epithelia. However, Marcozzi *et al.* (16) recently demonstrated that the combination of Dsc2a, Dsg1 and PG can generate aggregation comparable to that which we obtained, whereas Chitaev and Troyanovsky (15) demonstrated heterophilic interaction between Dsg2 and Dsc1a, suggesting that desmosomal adhesion is not isoform specific. Such results are consistent with the long-standing observations that desmosomes form readily between cells of different tissues and species (27, 28). Second, we used both a and b forms of Dsc1. Third, we used three different selection systems to obtain stable expression of the proteins in the same cells.

Our experiments resulted in several cell lines exhibiting cell-cell adhesion, many of which were unstable. This observation is consistent with those of Marcozzi *et al.* (16). We also obtained a number of other cell lines with the same four desmosomal components, but they showed either weak or no aggregation. Unlike AB5, which showed reduced expression of Dsg1 and PG after retransfection with Dscs, the other multiple expressors retained strong expression of Dsg1 and PG (not shown). These results are consistent with the dominant negative effect of Dsg on desmosomal adhesion reported by others (29) and may be related to the observations that Dsgs bind 6 times more PG than Dscs (14).

Only the "a" form of Dsc binds PG and no function for the "b" form has yet been discovered. When retransfecting the 11D6 cell line, we transfected some cells with either the a or the b form as well as both a and b together. Neither of the single transfections yielded aggregating cell lines. Our results thus suggest that the b form is required for aggregation to take place. However, this is contrary to recent observations of Marcozzi *et al.* (16). The lack of a strongly aggregating cell line with only Dsc1a and Dsg could be attributed to the dominant negative effect of Dsg.

The inhibition of AB5 cell aggregation by the Dsc1 and Dsg1 CAR peptides clearly demonstrates that the adhesion we have generated is a specific event involving the desmosomal glycoproteins. This result also provides direct evidence that these sites are functionally involved in desmosomal adhesion. Crystallographic studies of conventional cadherins indicate that the HAV CAR site is located on the face of the molecule involved in adhesion dimer formation (30). Unlike the conventional cadherins, neither of the desmosomal cadherins alone can form strong homophilic interactions (12, 14). It remains to be determined therefore how their CAR sites are involved in binding interactions when both molecules are present in the same cells.

Our observations that either of the CAR peptides and an antibody specific for Dsg were able, individually, to produce substantial inhibition of aggregation and invasion is consistent with a role for both Dsc and Dsg in these events and with reports of heterophilic interaction between Dsc and Dsg (15, 16). Interaction between the components is further indicated by our observations that Dsc, Dsg, and PG colocalize in detergent-insoluble puncta at the cell membrane. Although punctate staining for desmosomal glycoproteins was observed at the boundaries between transfected cells, our extensive ultrastructural studies did not reveal the presence of electron-dense structures resembling desmosomal plaques at the cell membrane. We speculate that additional desmosomal components such as desmoplakin and plakophilin may be required to obtain assembly of complete desmosomes. Some support for this suggestion comes from the recent description of human plakophilin 1 mutations that result in the ectodermal dysplasia/skin fragility syndrome (2). The affected individual lacks detectable plakophilin 1 in the epidermis. Immunofluorescent staining of the affected epidermis also reveals abnormal diffuse distribution of desmoplakin and abnormal expression and aggregation of keratin filaments. Few desmosomes are

detectable by electron microscopy and most of those present are small with barely detectable plaques. This could be because the absence of plakophilin results in detachment of desmoplakin (and keratin filaments) from the desmosomes resulting in loss of plaque structure. Thus, the absence of plakophilin and/or desmoplakin from our transfected cells may account for the absence of ultrastructurally detectable desmosomes.

Our ultrastructural observations also failed to reveal any association of intermediate filaments with the cell periphery in AB5 cells. The insolubility of the puncta may therefore be consistent with the rapid entry of one component, Dsg, into the insoluble fraction after synthesis rather than to cytoskeletal attachment (31). Lack of cytoskeletal attachment may be expected because AB5 cells do not express desmoplakin, which is known to mediate desmosome-intermediate filament attachment (32, 33). By contrast, α - and β -catenin mediate attachment of E-cadherin to the actin cytoskeleton, and this may be significant in relation to the difference in appearance of aggregates formed by E-cadherin and desmosome-transfected L929 cells. Cytoskeletal attachment may enable the E-cadherin-expressing cells to form small tight aggregates while desmosomal expressers remain large and loose. Thus, aggregation that appears quantitatively equally effective can give rise to aggregates with differing morphology. Cytoskeletal attachment may also account for the greater effectiveness of E-cadherin in suppressing collagen invasion.

We have used a simple collagen invasion assay to provide functional evidence for an involvement of desmosomal adhesion in suppression of invasion. This assay has been widely used to investigate the invasive properties of cells. Of particular relevance here is its use to demonstrate the invasion suppressor property of E-cadherin (26). The results we have obtained indicate that specific, peptide-inhibitable desmosomal adhesion inhibits invasion of L929 cells into collagen gels. As well as demonstrating the specificity of the phenomenon, the ability of the CAR peptides and an anti-Dsg1 antibody to restore invasive behavior to the transfected cells indicates that changes in neither cell motility nor cell proliferation can account for the observations.

The human PG gene localizes to chromosome 17q21, is subjected to loss of heterozygosity in breast and ovarian cancer (34), and may be down-regulated in early metastasis of human solid tumors (35). Recently, loss of heterozygosity of chromosome 18q12.1, on which the desmosomal glycoprotein cluster is located, has been reported in human squamous cell carcinoma lines (36). Previous studies have shown that desmosome expression and staining for desmosomal components are abnormal in certain types of human carcinomas and, furthermore, that loss of staining for desmosomal components correlates with invasiveness (7–11). Our results provide functional evidence consistent with the hypothesis that desmosomes have a tumor invasion and metastasis suppressor function.

We thank Professor Martin Humphries, Dr. Charles Streuli, and Dr. Carolyn Byrne for helpful discussion. This work was supported by the Biotechnology and Biological Sciences Research Council and the Wellcome Trust.

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