5-11-1992

The integrin alpha 6 beta 4 is a laminin receptor

Edward C. Lee
New England Deaconess Hospital

Margaret M. Lotz
New England Deaconess Hospital

Glenn D. Steele Jr.
New England Deaconess Hospital

See next page for additional authors

Follow this and additional works at: https://escholarship.umassmed.edu/cancerbiology_pp

Part of the Cancer Biology Commons, and the Neoplasms Commons

Repository Citation
https://escholarship.umassmed.edu/cancerbiology_pp/123

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in Cancer Biology Publications and Presentations by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
The integrin alpha 6 beta 4 is a laminin receptor

Authors
Edward C. Lee, Margaret M. Lotz, Glenn D. Steele Jr., and Arthur M. Mercurio
Abstract. In this study, the putative laminin receptor function of the \( \alpha 6 \beta 4 \) integrin was assessed. For this purpose, we used a human cell line, referred to as clone A, that was derived from a highly invasive, colon adenocarcinoma. This cell line, which expresses the \( \alpha 6 \beta 4 \) integrin, adheres to the ES and not to the PI fragment of laminin. The adhesion of clone A cells to laminin is extremely rapid with half-maximal adhesion observed at 5 min after plating. Adhesion to laminin is blocked by GoH3, an \( \alpha 6 \) specific antibody (60% inhibition), as well as by A9, a \( \beta 4 \) specific antibody (30% inhibition). Most importantly, we demonstrate that \( \alpha 6 \beta 4 \) binds specifically to laminin-Sepharose columns in the presence of either Mg\(^{2+}\) or Mn\(^{2+}\) and it is eluted from these columns with EDTA but not with NaCl. The \( \alpha 6 \beta 4 \) integrin does not bind to collagen-Sepharose, but the \( \alpha 2 \beta 1 \) integrin does bind. Clone A cells do not express \( \alpha 6 \beta 1 \) as evidenced by the following observations: (a) no \( \beta 1 \) integrin is detected in \( \beta 1 \) immunoblots of GoH3 immunoprecipitates; and (b) no \( \alpha 6 \beta 1 \) integrin is seen in GoH3 immunoprecipitates of clone A extracts that had been immunodepleted of all \( \beta 4 \) containing integrin using the A9 antibody. These data establish that laminin is a ligand for the \( \alpha 6 \beta 4 \) integrin and that this integrin can function as a laminin receptor independently of \( \alpha 6 \beta 1 \).

The function of the \( \alpha 6 \) integrin subunit has been complicated by the finding that this subunit can also associate with a different \( \beta \) subunit, namely \( \beta 4 \) (Hemler et al., 1989; Kajitii et al., 1989). The \( \beta 4 \) subunit is expressed primarily on epithelial cells and their oncogenically transformed derivatives, although it also found in endothelial and some neuronal cells (reviewed in Quaranta and Jones, 1991). An obvious question, based on the behavior of \( \alpha 6 \beta 1 \), is whether \( \alpha 6 \beta 4 \) can function as a laminin receptor, and, if so, whether this function differs from the regulated behavior of \( \alpha 6 \beta 1 \). This question has caused considerable controversy in the recent literature. Although we reported that \( \alpha 6 \beta 4 \) can function as a laminin receptor on colon carcinoma cells based on the ability of an \( \alpha 6 \) specific antibody to block adhesion (Lotz et al., 1990), this function for \( \alpha 6 \beta 4 \) has not been widely accepted. Several papers have concluded, for example, that the ligand for \( \alpha 6 \beta 4 \) must be distinct from that of \( \alpha 6 \beta 1 \) (Sonnenberg et al., 1990) and have emphasized that \( \alpha 6 \beta 4 \) has not been shown to bind laminin affinity columns (Deluca et al., 1990; Quaranta and Jones, 1991). One tacit assumption in many of these studies has been that the \( \alpha 6 \)-dependent adhesion of cells that express \( \alpha 6 \beta 4 \) is mediated by low levels of \( \alpha 6 \beta 1 \) and not \( \alpha 6 \beta 4 \). Moreover, recent studies on \( \alpha 6 \beta 4 \) have not addressed this laminin receptor function directly, but have focused on its function in stratified epithelial cells such as keratinocytes (Carter et al., 1990; Quaranta and Jones, 1991; Sonnenberg et al., 1991; Stepp et al., 1990). These groups have postulated that \( \alpha 6 \beta 4 \) may play a critical role in the assembly and maintenance of hemidesmosomes. This integrin is localized along the basal surface of keratinocytes suggesting its probable function in cell to basement membrane inter-

© The Rockefeller University Press, 0021-9525/92/05/671/8 $2.00
The Journal of Cell Biology, Volume 117, Number 3, May 1992 671-678
action. However, the speculation in these studies is that keratinocyte α6β4 binds to a basement membrane component other than laminin, although a laminin receptor function has not been excluded. The current opinion of a laminin receptor function for α6β4 is exemplified by a statement contained in a recent commentary on this subject (Quaranta and Jones, 1991): “the scanty published information neither conclusively supports nor formally disproves laminin as a ligand for α6β4.”

The ambiguities associated with a laminin receptor function for the α6β4 integrin prompted us to examine this issue in more detail. For this purpose, we used a human cell line, referred to as clone A, that was derived from a highly invasive colon adenocarcinoma. This cell line, which expresses α6 in association with β4 and not β1, adheres extremely rapidly to the E8 fragment of laminin. The data obtained establish that laminin is a ligand for the α6β4 integrin and that this integrin can function as a laminin receptor.

Materials and Methods

Cells

The clone A cell line obtained from Dr. D. Dexter (Du Pont, Wilmington, DE) was derived from a poorly differentiated human colon adenocarcinoma (Dexter et al., 1979). The in vitro morphology and growth characteristics of this cell line have been described previously (Dexter et al., 1979; Daneker et al., 1989). The RKO cell line derived from a human rectal carcinoma was provided by M. Brattain (Boyd et al., 1988). NIH:OVCAR-3 cells which were derived from a human ovarian carcinoma were obtained from the American Type Tissue Collection (Rockville, MD). Cells were grown in RPMI-1640 supplemented with 10 mM Hepes, 10% FCS, 2 mM L-glutamine, 100 IU/ml penicillin, and 50 µg/ml streptomycin and then maintained at 37°C in a 5% CO2 atmosphere. All media components were purchased from Gibco Laboratories (Grand Island, NY).

Laminin

Laminin was purified from the Engelbreth-Holm-Swarm (EHS) murine sarcoma following a published protocol (Kleinman et al., 1982). Protoplasts from EHS laminin (Nurcombe et al., 1989) were a generous gift of Ruppert Timpl (Max-Planck Institute, Martinsried, Germany).

Antibodies

The rat mAb GoH3 (anti-α6; Sonnenberg et al., 1987) was purchased from Amac (Westbrook, ME) or the Central Lab of the Netherlands Red Cross (Amsterdam). The mouse mAb UM-A9 (anti-β4; Van Wae et al., 1991) was provided by T. Carey (University of Michigan, Ann Arbor, MI). The mouse mAb 3E1 (anti-β4) was purchased from Telios (San Diego, CA). The rat mAb A1B2 (anti-β1; Werb et al., 1989) was provided by C. Damacky (University of California, San Francisco, CA). The mouse mAb P1H5 (anti-α2; Wayner and Carter, 1987) was a gift of E. Wayner. The ICAM-1 specific mouse mAb CBRC1, provided by T. Springer (Harvard Medical School, Cambridge, MA), was used as an IgG2a control for the experiments involving antibody inhibition of laminin adhesion. Rabbit antiuramin specific for the COOH terminus of the β1 integrin subunit was provided by R. Hynes (Massachusetts Institute of Technology, Cambridge, MA) (Marcantonio and Hynes, 1988).

Adhesion Assays

The adhesion assays (shown in Fig. 1) were performed as described previously (Lotz et al., 1990). The antibody inhibition assays (shown in Fig. 2) were done as follows. Microwell plates (48 well; Costar, Cambridge, MA) were coated overnight with either laminin (10 µg/ml) or collagen I (40 µg/ml). Cells were detached from tissue culture flasks with EDTA (0.5 mM) in PBS and resuspended in RPMI-H containing 1% BSA. Detached cells were pre-incubated with specific mAbs for 30 min at room temperature with gentle agitation. Subsequently, cells (10⁵) were plated in the protein-coated microwell wells, and the plates were incubated for 20 min at 37°C. The wells were then washed three times with RPMI-BSA and the adherent cells were detached using a solution of trypsin (0.5%) and EDTA (0.5 mM) in PBS. A Coulter Counter (Coulter; Hialeah, FL) was used to count the number of adherent cells.

Cell Surface Labeling

Tissue culture dishes (150 mm) containing confluent cells were detached using 0.5 mM EDTA in PBS. The cells (1-2 x 10⁶) were washed three times with PBS containing 40 mM D-glucose. The pellet was resuspended in 2 ml of the PBS/glucose buffer and the cells were then surface radiolabeled using the lactoperoxidase method at 4°C as described previously (Lotz et al., 1990). Radiolabeled cells were solubilized in 50 mM Tris-HCl buffer, pH 7.4, containing 200 mM octyl-β-D-glucopyranoside (Boehringer Mannheim Biochemicals, Mannheim, Germany), 2 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM each of aprotinin, leupeptin, pepstatin, and 5 mM of the appropriate divalent cation. After 10 min, the extract was centrifuged at 14,000 g for 15 min and the supernatant was either used for immunoprecipitation directly or loaded onto ligand Sepharose columns.

Affinity Chromatography

Columns were prepared by conjugating Sepharose 4B to purified laminin (Kleinman et al., 1982) or collagen type I (Upstate Biotechnologies, Lake Placid, NY) at a ratio of 4 mg protein/ml Sepharose as previously described (Woo et al., 1990). The columns were equilibrated at 4°C with running buffer (50 mM Tris-HCl, 50 mM octyl-β-D-glucopyranoside, 2 mM PMSF, and the appropriate concentration of divalent cations). Cell extracts were loaded on the columns and allowed to interact with the matrix for a minimum of 6 h at 4°C. The columns were then washed extensively with the running buffer. Subsequently, the columns were washed sequentially with running buffer containing 0.2 M lactose, 0.2 M NaCl, 10 mM EDTA, and 1 M NaCl. One column volume fractions (1 ml) were collected, acetone precipitated, and analyzed by 8% SDS-PAGE under reduced conditions followed by autoradiography.

Immunoprecipitations

Selected fractions from affinity chromatography separations were immunoprecipitated with integrin antibodies. Briefly, aliquots (0.5 ml) were "precleared" for 2 h at 4°C with either goat anti-β1 IgG agaroase (Sigma Chemical Co., St. Louis, MO), protein G-agaroase (Pharmacia Fine Chemicals, Piscataway, NJ), or protein A-agaroase (Boehringer Mannheim Biochemical). After removal of the nonspecifically bound immune complexes by centrifugation, the integrin antibodies were added to the supernatant and incubated overnight at 4°C. For these experiments, 4 µg of purified GoH3 were added to 0.5 ml of pre-cleared extract, and A1B2 hybridoma supernatant was used at a dilution of 1:10. Subsequently, anti-γIgG was added for 2 h at 4°C. The agaroase beads were then washed four times with 10 mM Tris-HCl, pH 8.0, containing 0.1% Triton X-100, and 0.15 M NaCl, once with 0.05 M Tris-HCl, pH 6.8, and finally resuspended in Laemmli sample buffer and incubated at 100°C for 5 min, with 5% 2-mercaptoethanol. After separation of the polypeptides by 8% SDS-PAGE, the dried gels were exposed to X-Omat RP film (Eastman Kodak Co., Rochester, NY). For immunoprecipitation using the A9 antibody, protein A-agaroase (Boehringer Mannheim Biochemicals) was used to capture the immune complexes and for 3E1 precipitations, protein G-agaroase (Pharmacia Fine Chemicals) was used.

Immunoblotting

EDTA eluted samples (2.0 ml) of clone A cell extracts that had been fractionated on laminin Sepharose were immunoprecipitated with GoH3 (16 µg) or A1B2 (0.1 ml dilution) as described above. The polypeptides were resolved by 8% SDS-PAGE and transferred to nitrocellulose. The immunoreaction was carried out by incubating with a 1:100 dilution of the polyclonal antibody for β1 integrin and the bound antibodies were visualized using a 1:300 dilution of peroxidase-conjugated goat anti-rabbit IgG (Boehringer Mannheim Biochemicals).
Results

Behavior of Clone A Cells on Laminin

Clone A cells, which were derived from a poorly differentiated human colon adenocarcinoma (Dexter et al., 1979), adhere avidly to laminin substrata (Daneker et al., 1988). When plated on laminin-coated dishes, ~45% of the cells adhere within 5 min and by 30-60 min maximal adhesion (~90% of total cells) is seen (Fig. 1 a). In contrast, these cells adhere poorly, if at all, to tissue culture plastic (Fig. 1 a) or fibronectin, although they do adhere well to collagen I (Lotz et al., 1990). This rapid adhesion of clone A cells to laminin is quite distinct from that which we observed for less invasive carcinoma cell lines (Daneker et al., 1989), as well as for other cell types including 3T3 fibroblasts, bovine endothelial cells, PC12 cells, and mouse macrophages (not shown).

The ability of clone A cells to adhere to the major cell-binding fragments of laminin (Nurcombe et al., 1989) was also examined. As shown in Fig. 1 b, clone A cells adhere only to the E8 fragment of laminin and not to the PI fragment. The PI fragment, however, did promote the adhesion of OVCAR cells, a human ovarian carcinoma cell line (Fig. 1 b).

Antibodies to the α6 and β4 Integrin Subunits Inhibit Laminin Adhesion

Previously, we reported that antibodies specific for the α6, α2, and β1 integrin subunits blocked clone A adhesion to laminin (Lotz et al., 1990). In this study, we examined the ability of the β4 specific mAb A9 (Kimmel and Carey, 1986; van Waes et al., 1991) to inhibit laminin adhesion. This β4 antibody was obtained using an invasive squamous carcinoma cell line (UM-SSC-1) that is similar to clone A cells in its behavior on laminin and pattern of integrin expression (van Waes et al., 1991). A9 inhibited laminin adhesion by ~30% compared to the mouse IgG2a control (Fig. 2 a). In contrast, the A9 antibody had no inhibitory effect on clone A adhesion to collagen I. The ability of GoH3 to block adhesion to laminin is shown in Fig. 2 b for comparison. This α6 antibody yielded a 60% inhibition of laminin adhesion in agreement with our previous study (Lotz et al., 1990).

α6β4 Integrin Binds to Laminin Affinity Columns

Affinity chromatography was performed to assess the laminin binding function of α6β4. Fig. 3 shows a representative elution profile of surface radiolabeled clone A extracts fractionated on a laminin-Sepharose column. Little, if any, protein was eluted from the column with 0.2 M NaCl. However, elution of the column with 10 mM EDTA yielded a distinct protein band at 200 kD and a broad band that migrated at 130 to 160 kD.

The surface proteins that bound to laminin-Sepharose were identified by immunoprecipitation of the column fractions (Fig. 4). For these experiments, laminin-Sepharose columns were eluted sequentially with 0.2 M lactose, 0.2 M NaCl, 10 mM EDTA, and finally 1 M NaCl. Lactose was included in the elution buffers because of the report that α6β1 can interact with laminin through a carbohydrate-dependent mechanism (Chammas et al., 1991). Using the α6 specific antibody GoH3, no proteins were detected in the lactose or NaCl fractions, but in the EDTA fraction a major protein band was evident at 200 kD and other bands were seen at 180, 150, and 125 kD. This immunoprecipitation pattern is identical to the pattern obtained after GoH3 precipitation of total cell extracts (see below). The intense band at 200 kD corresponds to intact β4 subunit, and the minor bands at 180 and 150 are proteolytic products of this subunit, an observation made ini-
tially by other labs (Hemler et al., 1989; Kajiji et al., 1989). The faint band at 125 kD corresponds to the α6 subunit that is recognized by GoH3. The presence of β4 in this fraction was confirmed using 3E1, a β4 specific antibody that precipitated the major 200-kD β4 subunit as well as the minor 180- and 150-kD proteolytic products. The β1 specific antibody AIIB2 precipitated two distinct proteins (155 and 130 kD) from the EDTA fraction indicative of the α2β1 heterodimer and in agreement with our previous study (Lotz et al., 1990).

We examined the specificity of α6β4 for laminin by using Figure 3. Laminin-Sepharose chromatography of radiolabeled clone A extracts. Clone A cells were surface radiolabeled and detergent extracts were fractionated on a laminin-Sepharose column as described in Materials and Methods. Aliquots of the three 0.2 M NaCl washes (lanes 1–3), the three 10 mM EDTA washes (lanes 4–6), and the final three 1 M NaCl washes (lanes 7–9) were precipitated with acetone and analyzed by SDS-PAGE (8%) under reducing conditions and detected by autoradiography. Molecular weight markers are shown in left hand margin.

The Journal of Cell Biology, Volume 117, 1992

collagen-Sepharose. As shown in Fig. 5, most of the protein that bound collagen eluted with EDTA and not with 0.2 M NaCl. Immunoprecipitation with GoH3 revealed that no α6-containing integrins bound to collagen. However, using AIIB2 and P1H5 we observed that the α2β1 integrin binds very well to collagen-Sepharose (Fig. 5) confirming the results of our previous study (Lotz et al., 1990).

Because these data provide the first demonstration that α6β4 can bind to laminin, we thought it important to compare this binding to that of α6β1. Previous studies had reported that α6β1 binding to laminin requires Mn²⁺ and that little, if any, binding is observed in the presence of Mg²⁺ (Kramer et al., 1990). To determine the divalent cation specificity of α6β4, clone A extracts were fractionated on laminin-Sepharose in buffers containing either Mg²⁺ or Mn²⁺, and the EDTA eluants were immunoprecipitated with GoH3. As shown in Fig. 6, α6β4 binds laminin in either Mg²⁺- or Mn²⁺-containing buffers.

**Clone A Cells Express α6β4 and No Detectable α6β1**

The immunoprecipitation profiles shown in Fig. 4 indicate that the α6β4 integrin binds to laminin in the absence of any detectable α6β1 in agreement with our previous data on the lack of α6β1 expression in clone A cells (Lotz et al., 1990). In the present study, this finding was substantiated by two different methods. GoH3 and AIIB2 immunoprecipitates of EDTA-eluted samples from the laminin-Sepharose column were immunoblotted with a β1 polyclonal antisera (Fig. 7). For this experiment, ~5 × 10⁶ cells were used for each immunoprecipitation to maximize detection of any β1 in the GoH3 immunoprecipitates. In this immunoblot, however, a β1 band is seen only in the AIIB2 precipitate and not in the GoH3 precipitate (Fig. 7).

The second approach to detecting α6β1 in clone A cells involved immunodepleting or “pre-clearing” a sample of ¹²⁵I-labeled cells with the β4 antibody A9. This aliquot was immunoprecipitated seven times with A9. This process re-
moved all of the \( \beta 4 \) integrins as evidenced by the fact that no bands were evident after the fifth A9 precipitation (Fig. 8). Subsequently, this sample was immunoprecipitated with either AIIB2 or GoH3. If \( \alpha 6 \beta 1 \) were present under these conditions, it should have been detected in the GoH3 precipitation of the \( \beta 4 \) pre-cleared sample. However, as seen in Fig. 8, there is no evidence of any bands in the GoH3 precipitate even after prolonged exposure. It is important to note that the \( \alpha 2 \beta 1 \) and \( \alpha 0 \beta 1 \) integrins were immunoprecipitated with AIIB2 from the same pre-cleared sample. Thus, the lack of \( \alpha 6 \beta 1 \) expression cannot be attributed to a non-specific depletion of \( \beta 1 \) integrins by the exhaustive \( \beta 4 \) pre-clearing.

**RKO Cells Express Only \( \alpha 6 \beta 1 \)**

In our survey of the adhesive properties of various colon carcinoma cell lines, we found that RKO cells (Boyd et al., 1988), adhere poorly to laminin (Fig. 9a). This is particularly evident when their adhesion to laminin is compared to that of clone A cells (Fig. 9a). This finding prompted us to examine their expression of \( \alpha 6 \) integrins. Surface radiolabeled RKO cells were immunoprecipitated with either an \( \alpha 6 \) specific (GoH3) or a \( \beta 4 \) specific antibody (A9). As shown in Fig. 9b, GoH3 immunoprecipitated the \( \alpha 6 \beta 1 \) heterodimer with no evidence of any \( \alpha 6 \beta 4 \). The absence of \( \beta 4 \) expression in RKO cells was confirmed by the A9 immunoprecipitation because no detectable bands were precipitated with this antibody (Fig. 9b). RKO cells do express \( \alpha 2 \beta 1 \) (not shown).

**Discussion**

The data presented in this report establish that laminin is a ligand for the \( \alpha 6 \beta 4 \) integrin and that this integrin functions as a laminin receptor on clone A cells. Because the laminin binding ability of this integrin had not been demonstrated previously, its ability to function as a laminin receptor had been seriously questioned (reviewed in Quaranta and Jones, 1991). Our use of a cell line that has a strong avidity for laminin and that expresses relatively high levels of the \( \alpha 6 \beta 4 \) integrin probably facilitated the demonstration of its laminin binding function. We also found that binding is very dependent on the use of freshly prepared laminin-Sepharose suggesting that the physical state or conformation of laminin is critical for \( \alpha 6 \beta 4 \) binding. Although we cannot extend the
Figure 6. Divalent-cation dependency of α6β4 laminin binding. Laminin-Sepharose chromatography of radiolabeled clone A extracts was performed in the presence of either 5 mM Mg" or 5 mM Mn". Aliquots of the peak EDTA eluted fractions were immunoprecipitated with GoH3, resolved by SDS-PAGE (8%) under reducing conditions, and detected by autoradiography. The typical α6β4 electrophoretic pattern is seen in both Mg"- and Mn"-containing buffers.

The possibility that the α6-dependent adhesion of clone A cells to laminin is mediated entirely by α6β1 and not α6β4 is remote. The laminin affinity chromatography data in conjunction with the inhibition of laminin adhesion by α6 and β4 specific antibodies establish the laminin receptor function of α6β4. We found no evidence for α6β1 expression in clone A cells either in this study or in a previous publication (Lotz et al., 1990), and conclude that α6 associates exclusively with β4 in these cells, although it is possible that trace amounts of α6β1 are present in clone A cells that were not detected by our experiments. In addition to this biochemical evidence, a role for α6β1 in clone A adhesion to laminin is...
diminished by comparative data from other cell lines. We have characterized colon carcinoma cell lines that express both α6β4 and α6β1 or exclusively α6β1 (e.g., RKO cells in Fig. 9) and that adhere to laminin with much slower kinetics than clone A cells (Daneker et al., 1989). In fact, RKO cells adhere poorly to laminin even though they express both α6β1 and α2β1. These findings suggest that the avidity of colon carcinoma adhesion to laminin is determined by the expression of the α6β4 integrin. Indirect support of this possibility comes from several studies that have correlated β4 expression with the invasive and metastatic behavior of tumor cells (Falcioti et al., 1986; Kimmel and Carey, 1986; Wolf et al., 1990). In particular, it is worth noting the compelling report that β4 expression (A9 antigen) is a predictive expression with the invasive and metastatic behavior of tumor cells (Falcioti et al., 1986; Kimmel and Carey, 1986; Wolf et al., 1990). In vitro studies by the same group have implicated α6β4 as a squamous carcinoma laminin receptor based on GoH3 inhibition of laminin adhesion and the lack of detectable α6β1 expression (Van Waes et al., 1991).

The above observations raise several interesting questions about the molecular basis of α6 and β4 integrin expression. Most importantly, why does the α6 subunit associate exclusively with β4 in some colon carcinoma cell lines and with both β4 and β1 in other cell lines? One possibility is that quantitative differences in β4 expression regulate α6 subunit association. If β4 expression is in excess, then α6β4 is seen exclusively. However, if β4 expression is limiting, then both α6β4 and α6β1 are observed. This possibility is supported by the finding that α6 associates preferentially with β4 compared to β1 (Hemler et al., 1989) and by the observation that clone A and related cell lines express high levels of β4 (Hemler et al., 1989; Lee et al., unpublished). Another possibility is that structural differences in either the β4 (Tamura et al., 1990; Suzuki and Naitoh, 1990; Hogervorst et al., 1990) or α6 (Tamura et al., 1990; de Curtis et al., 1991) subunits account for the different patterns of association observed. Alternative splicing has been demonstrated for both the α6 (Cooper et al., 1991) and β4 subunits (Tamura et al., 1990). It will be informative to compare these sequences in cell lines that express solely α6β4 to those that express both α6β4 and α6β1.

Clone A adhesion to laminin requires not only the α6β4 integrin but also a β1 integrin, α2β1. The reason for at least two distinct integrin laminin receptors is not apparent at present. Because antibodies to either one of these integrins will inhibit adhesion significantly, it may be that these two integrins do not function in tandem but rather act sequentially. All of the colon carcinoma cell lines that we have examined express α2β1, but differ in their relative expression of α6β4 and α6β1. This suggests that the α6 subunit in association with the appropriate β subunit plays a dominant role in determining the ability of these cells to adhere to laminin. In this scenario, the α2β1 integrin could stabilize adhesion initiated by the α6 heterodimer. It is also possible that ligation of the α6 heterodimer "activates" the laminin binding function of α2β1. Though speculative, these possibilities suggest strategies for studying adhesion that is mediated by multiple integrins, a situation that appears to be the rule rather than the exception (reviewed in Mercurio and Shaw, 1991).

Finally, an important issue that needs to be addressed is why α6β4 has not been shown to bind to laminin in other cell types. Although it remains a likely possibility that other ligands exist for this integrin, it is unlikely that α6β4 functions as a laminin receptor only on invasive carcinoma cells. To explain this apparent discrepancy, it is worth considering the possibility that the laminin binding function of α6β4 is regulated. Several studies have concluded that the laminin binding function of α6β1 is regulated by physiological stimuli (Shaw et al., 1990; L. M. Shaw and A. M. Mercurio, J. Cell Biol. 115:131a; Shimizu et al., 1990) or during embryonic development (de Curtis et al., 1991). This mode of regulation that occurs in the absence of quantitative changes in surface expression has been termed "post-translational" regulation (de Curtis et al., 1991). In the case of macrophages, post-translational regulation of α6β1 function requires protein phosphorylation and may, in fact, involve phosphorylation of the α6 cytoplasmic domain (Shaw et al., 1990; L. M. Shaw and A. M. Mercurio, J. Cell Biol. 115:131a). If similar mechanisms were involved in the regulation of α6β4 function, it could be argued that the highly tumorigenic clone A cells, which are known to have up-regulated kinase activities, constitutively activate the laminin binding function of this integrin. The observation that the β4 integrin is constitutively phosphorylated in clone A cells (Lotz, M. M., and A. M. Mercurio, unpublished results) supports this possibility. In marked contrast to the aggressive interaction of clone A cells and other invasive carcinoma cells with laminin, keratinocytes, for example, require relatively long periods of time to form adhesive contacts with the basement membrane. This adhesion, which involves the formation of complex cytoskeletal structures, appears to be tightly regulated (Carter et al., 1990; Quaranta and Jones, 1991; Sonnenberg et al., 1991; Stepp et al., 1990). Perhaps, this regulation involves the latent activation of the laminin binding function of α6β4. Along these lines, it is worth mentioning that, in macrophages, α6β1 will not bind to laminin affinity columns unless the cells are physiologically stimulated (L. M. Shaw and A. M. Mercurio, J. Cell Biol. 115:131a). These possibilities suggest that the regulation of α6β4 ligand binding should be examined carefully in specific cell types before a laminin receptor function is excluded.

We thank Thomas Carey for his generous gift of the A9 antibody and Rupert Timpl for providing us with proteolytic fragments of laminin. Many helpful discussions were had with Leslie Shaw. Cynthia Korzelius provided expert technical assistance.

This work was supported by National Institutes of Health Grants CA44704 and CA42276. M. Lotz was funded by an NIH National Research Service Award and A. Mercurio is the recipient of an American Cancer Society Faculty Research Award.

Received for publication 6 August 1991 and in revised form 5 February 1992.

References


