

Endothelial Cells Use $\alpha_2\beta_1$ Integrin as a Laminin Receptor

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Abstract. Human umbilical vein endothelial cells attach and spread on laminin-coated substrates. Affinity chromatography was used to identify the attachment receptor. Fractionation of extracts from surface-iodinated endothelial cells on human laminin-Sepharose yielded a heterodimeric complex, the subunits of which migrated with molecular sizes corresponding to 160/120 kD and 160/140 kD under non-reducing and reducing conditions, respectively. The purified receptor bound to laminin and slightly less to fibronectin and type IV collagen in a radioreceptor assay. This endothelial cell laminin receptor was classified as an $\alpha_2\beta_1$ integrin because monoclonal and polyclonal antibodies directed against the α_2 and β_1 subunits immunoprecipitated the receptor. Cytofluoro-

metric analysis and immunoprecipitation showed that the α_2 subunit is an abundant integrin α subunit in the endothelial cells and that the α subunits associated with laminin binding in other types of cells are expressed in these cells only at low levels. The $\alpha_2\beta_1$ integrin appears to be a major receptor for laminin in the endothelial cells, because an anti- α_2 monoclonal antibody inhibited the attachment of the endothelial cells to human laminin. These results define a new role for the α_2 subunit in laminin binding and suggest that the ligand specificity of the $\alpha_2\beta_1$ integrin, which is known as a collagen receptor in other types of cells, can be modulated by cell type-specific factors to include laminin binding.

ADHESIVE properties of endothelial cells play an important role in the maintenance of the vessel wall integrity and in diseases such as thrombosis and arteriosclerosis (Gimbrone, 1986). On the luminal side these cells form a nonthrombogenic surface, whereas on the basal side they rest on a basement membrane to which they adhere. The endothelial basement membrane contains laminin, type IV collagen, type V collagen, and proteoglycans (Madri et al., 1980; Palotie et al., 1983; Fujiwara et al., 1984; Siebold et al., 1988; for review see Timpl, 1989). Many of these basement membrane components mediate endothelial cell adherence. Laminin, in particular is thought to be important in this regard (Form et al., 1986; Madri and Williams, 1983; Herbst et al., 1988).

Various types of cells have been shown to have laminin receptors that are members of the integrin family. Integrins are heterodimeric proteins that consist of an α and a β subunit; each integrin is, therefore, defined by its subunits (Pytela et al., 1986; Buck and Horwitz, 1987; Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Hemler et al., 1987; Takada et al., 1987). Integrins $\alpha_3\beta_1$ (Wayner and Carter, 1987; Takada et al., 1988; Gehlsen et al., 1988; Gehlsen et al., 1989) and $\alpha_6\beta_1$ (Sonnenberg et al., 1988), as well as an integrin similar to $\alpha_1\beta_1$ (Tomaselli et al., 1988; Ignatius and Reichardt, 1988) have each been implicated as laminin receptors. Endothelial cells are known to express the $\alpha_5\beta_1$,

$\alpha_3\beta_1$, and $\alpha_2\beta_1$ integrins (Albelda et al., 1989; Conforti et al., 1989; Giltay et al., 1989). The $\alpha_5\beta_1$ integrin is a fibronectin receptor (Argraues et al., 1987); $\alpha_3\beta_1$ is known as a laminin, fibronectin, and collagen receptor (Wayner et al., 1988); while $\alpha_2\beta_1$ has previously been identified as a collagen receptor in platelets and in other cells (Wayner and Carter, 1987; Kunicki et al., 1988; Staatz et al., 1989). In addition, endothelial cells also express the vitronectin receptor ($\alpha_v\beta_3$) (Suzuki et al., 1987; Cheresch, 1987). Endothelial cells attach to laminin through an integrin-mediated mechanism; that is suggested by the inhibition of endothelial cell attachment to laminin by an antibody directed against the β_1 integrin subunit (Cheng, Y. F., C. Damsky, and R. H. Kramer, unpublished observations). However, endothelial cells have also been suggested to interact with laminin through a 69-kD protein (Yannariello-Brown et al., 1988) that is not an integrin.

To characterize the endothelial cell receptor for laminin, we have used affinity chromatography procedures similar to those applied to other types of cells (Pytela et al., 1985a,b; Gehlsen et al., 1988), as well as antibody probes. Our results show that the endothelial cells bind laminin through an integrin composed of α_2 and β_1 . Since the $\alpha_2\beta_1$ integrin in other types of cells binds only to collagens, this finding reveals cell type-specific functional heterogeneity of the $\alpha_2\beta_1$ integrin.

Materials and Methods

Chemicals

PMSF, soybean trypsin inhibitor, 2-mercaptoethanol, BSA, Triton X-100, EDTA, and protein A-Sepharose were purchased from Sigma Chemical Co. (St. Louis, MO). Fluorescein-conjugated goat anti-mouse or anti-rat IgG and IgM were obtained from Tago Inc. (Burlingame, CA). Rabbit anti-mouse IgG antiserum was obtained from Cappel Laboratories (Malvern, PA). ^{125}I -Na was from Amersham Corp. (Arlington Heights, IL). Molecular size markers were from Pharmacia Fine Chemicals (Piscataway, NJ); pre-stained size markers were from Bethesda Research Laboratories (Gaithersburg, MD).

The peptide GRGDSP (Pierschbacher and Ruoslahti, 1984) modeled after the cell attachment site in fibronectin was synthesized by solid-phase synthesis in a peptide synthesizer (Applied Biosystems Inc., Foster City, CA) and purified by ion exchange HPLC. Cell culture reagents were from Irvine Scientific (Santa Ana, CA).

Proteins

Mouse type IV collagen and mouse laminin isolated from the Engelbreth-Holm-Swarm murine tumor were from Bethesda Research Laboratories, bovine type I collagen from Collaborative Research (Lexington, MA), and human type I and type IV collagens were from Telios Pharmaceuticals (La Jolla, CA). Vitronectin was purified by monoclonal antibody affinity chromatography (Hayman et al., 1983). Pepsin-extracted laminin was isolated from human placental tissue by using monoclonal antibody affinity chromatography as described (Wewer et al., 1983; Engvall et al., 1986). In some experiments, the human laminin preparations were further fractionated by FPLC on a Mono Q column (Pharmacia Fine Chemicals) as described (Dillner et al., 1988). Human plasma fibronectin was purified by affinity chromatography on gelatin-Sepharose (Engvall and Ruoslahti, 1977).

Antibodies

Mouse monoclonal antibody 2E8 has been described (Engvall et al., 1986). Mouse monoclonal anti β_1 antibody LM442 was prepared against the fibronectin receptor purified from placenta in collaboration with Dr. David Cheresch, Department of Immunology, Research Institute of Scripps Clinic. Mouse monoclonal antibodies A1A5, against the β_1 integrin subunit, and TS2/7, against the α_1 subunit, were obtained from Dr. Martin Hemler (Dana-Farber Cancer Institute, Boston, MA). Monoclonal antibody AP3 to the β_3 subunit was obtained from Dr. Tom Kunicki (The Blood Center, Milwaukee, WI). Mouse monoclonal antibodies to the α_2 subunit (PIH5 and P4B4), the α_4 subunit (P4G9) and the integrin complexes $\alpha_3\beta_1$ (PIB5), and the $\alpha_5\beta_1$ (PID6) have been described (Wayner and Carter, 1987; Wayner et al., 1988; Wayner et al., 1989). PIH5 and PID6 inhibit fibroblast and platelet adhesion to collagen- and fibronectin-coated substrates, respectively (Kunicki et al., 1988; Wayner et al., 1988). A monoclonal antibody P4H9 to the β_2 (CD18) integrin subunit was also used. Rat monoclonal antibody GoH3 against the α_6 integrin subunit was obtained from Dr. Arnoud Sonnenberg (Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). For some experiments IgG was purified from culture supernatants on protein A-Sepharose as described (Ey et al., 1976).

Rabbit antisera against the human placental fibronectin receptor have been described (Pytela et al., 1987). Rabbit antisera prepared against peptides from the cytoplasmic domains of the α_3 and α_5 subunits were obtained from Drs. Eugene Marcantonio and Richard O. Hynes (Massachusetts Institute of Technology, Boston, MA) (Plantefaber and Hynes, 1989; Hynes et al., 1989). Rabbit anti α_2 antiserum was from Dr. M. Hemler (Dana Farber Cancer Institute, Boston, MA) (Takada and Hemler, 1989). Rabbit antiserum against human laminin has been described (Wewer et al., 1983).

Cells

Endothelial cells were isolated from human umbilical cords and cultured as described previously (Languino et al., 1989). The cells were used between the 1st and 10th passages. Some of the cell adhesion assays and the flow cytometric analyses were performed with human umbilical cord endothelial cells obtained from Cell Systems (Seattle, WA). Cells from each source were characterized as endothelial cells based on their cobblestone morphology and their expression of FVIII-associated antigen (Hoyer et al., 1973).

MG-63 human osteosarcoma cells were obtained from the American Type Culture Collection (Rockville, MD) and grown in MEM with 10% FBS.

Flow Cytofluorometric Analysis of Receptor Expression

Expression of integrin α and β subunits on endothelial cells was quantitated by flow cytometry on an EPICS 752 dual laser cell sorter (Coulter Electronics, Inc., Hialeah, FL) with an MDADS II data processor. Relative fluorescence intensity (log fluorescence intensity) was determined on a three-cycle log scale. Fluorescence histograms were derived by plotting cell number (y axis) as a function of log fluorescence intensity (x axis) from 0 to 255 (channel number). Background fluorescence was determined with a nonimmune mouse IgG control.

Endothelial cells incubated in Hanks' balanced salt solution (HBSS) supplemented with 20 $\mu\text{g}/\text{ml}$ purified goat IgG, 2% BSA, and 0.02% sodium azide for 30 min at 4°C to block Fc receptors. The cells ($2 \times 10^5/\text{ml}$) were washed and incubated with the primary antibodies (10 $\mu\text{g}/\text{ml}$) in HBSS/sodium azide for 60 min at 4°C. Then they were washed twice and incubated with FITC-conjugated goat anti-mouse (1:250 dilution) or FITC-conjugated goat anti-rat (for GoH3) antibodies (1:100 dilution) for 60 min at 4°C. At the end of this incubation the cells were washed once, fixed in cold 2% paraformaldehyde, and analyzed on the flow cytometer.

Endothelial Cell Adhesion Assay

Endothelial cell attachment to protein-coated substrates was measured in a cell adhesion assay as described (Ruoslahti et al., 1982). The coating efficiencies for the extracellular matrix proteins used in the assay have been shown to be similar (Dillner et al., 1988; Hautanen et al., 1989). The endothelial cells for these assays were detached from culture flasks with 0.08% trypsin and 0.2 mM EDTA in PBS. In antibody inhibition experiments cells were incubated in MEM supplemented with (a) 1% BSA, and (b) various dilutions of the antiserum, preimmune rabbit serum, or hybridoma culture media containing monoclonal antibodies or IgGs purified from culture supernatants, for 1 h at 4°C, and then transferred to the adhesion assay.

Nonadherent cells were removed after 2 h by washing with PBS and attached cells were fixed with 3% paraformaldehyde followed by staining with crystal violet. The attached cells were then dissolved in 1% SDS and the relative number of cells in each well was evaluated measuring the absorbance at 600 nm in a Multiscan ELISA reader (Flow Laboratories Inc., McLean, VA).

Alternatively, endothelial cells were labeled with $\text{Na}_2^{51}\text{CrO}_4$ (50 $\mu\text{Ci}/\text{ml}$) for 4 h, washed, and allowed to adhere to the substrates as described (Wayner and Carter, 1987). Attached cells were dissolved in SDS/NaOH and radioactivity was quantitated in a gamma counter.

Affinity Chromatography

Human laminin-Sepharose and 110-kD fibronectin fragment-Sepharose affinity matrices were prepared and used for receptor isolation as described previously (Pytela et al., 1985a, 1987; Gehlsen et al., 1988; Gailit and Ruoslahti, 1988).

Electrophoresis

Proteins were boiled in 3% SDS (wt/vol), 10% glycerol, 0.001% bromophenol blue, and 50 mM Tris-HCl, pH 6.8, with or without added 5% 2-mercaptoethanol. The samples were run on a 7.5% polyacrylamide gel in SDS (Laemmli, 1970) and the protein bands were visualized by Coomassie blue staining or by autoradiography.

Radioreceptor Assay

The binding of receptors to various protein ligands was examined in a radioreceptor assay as previously described (Hautanen et al., 1989). Microtiter wells were coated with 2 $\mu\text{g}/\text{ml}$ of the proteins examined. The coating efficiency for various proteins has been previously shown to be ~60% under the conditions used (Dillner et al., 1988; Hautanen et al., 1989). ^{125}I -labeled laminin receptors isolated from surface-iodinated cells were allowed to bind to the coated wells for 2 h in TBS (150 mM NaCl, 50 mM Tris-HCl, pH 7.5) containing 50 mM octyl- β -glucopyranoside, 1 mM PMSF, 1 mM CaCl_2 , MgCl_2 , with or without 1 mM MnCl_2 . The amount of receptor bound to the wells was measured after solubilization in 1% SDS in TBS by

counting the bound radioactivity. The total binding was 5–10% of the added radioactivity.

Immunoprecipitation

Endothelial cells or MG-63 cells were surface labeled and proteins were immunoprecipitated as described (Wayner and Carter, 1987); the buffer used for the immunoprecipitation contained 50 mM Tris-HCl (pH 7.5), 1 mM PMSF, 1 mM CaCl₂, 1 mM MgCl₂, and 0.5% Triton X-100 (vol/vol). Iodinated receptors were immunoprecipitated using the same buffer but with 50 mM octyl- β -glucopyranoside instead of Triton X-100.

Results

Laminin Promotes Endothelial Cell Adhesion

Pepsin-extracted human laminin and mouse laminin promoted endothelial cell adhesion to a similar extent (Fig. 1). Both laminin preparations were somewhat less active than fibronectin. Moreover, as observed earlier for other types of cells (Dillner et al., 1988), the cell morphology was different on these substrates; the cells appeared more elongated on the laminin-coated substratum than on fibronectin (not shown). A further difference between the fibronectin and laminin-induced attachment was that the cell attachment-promoting peptide, GRGDSP, inhibited the attachment of the endothelial cells to fibronectin by 90% at a concentration of 1 mg/ml but affected only marginally (19% inhibition) their attachment to human laminin.

Polyclonal antilaminin antibodies inhibited endothelial cell attachment to laminin but not to type IV collagen or fibronectin (Fig. 2). Preimmune rabbit serum used at the same dilutions as the antilaminin did not inhibit the attachment of the cells to any of these ligands. These results show that the pepsin-extracted human laminin specifically promotes the attachment of endothelial cells. We next used this

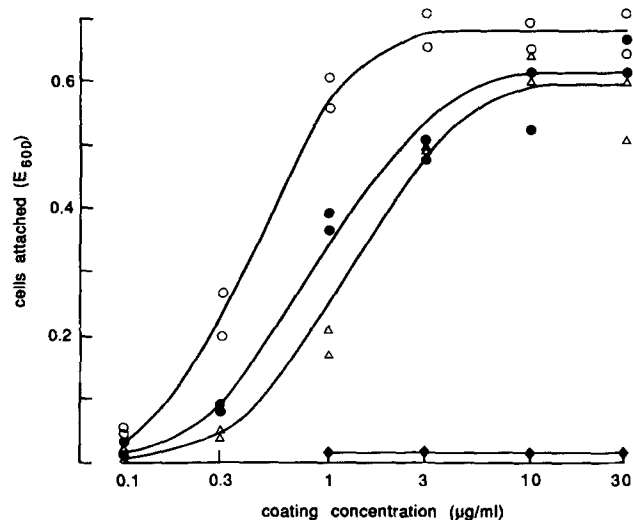


Figure 1. Endothelial cell adhesion to human laminin, mouse laminin, and fibronectin. Endothelial cells were trypsinized and allowed to adhere for 2 h to microtiter wells coated with increasing concentrations of pepsin-extracted human laminin that had been FPLC purified (●), mouse laminin (Δ), and human fibronectin (○). BSA coated wells (◆) were used as a control. Attached cells were fixed and stained, and the number of cells attached were recorded using a multiscan ELISA reader after staining the cells with crystal violet.

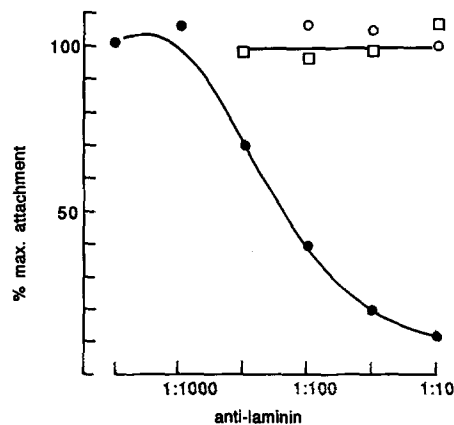


Figure 2. Inhibition of endothelial cell adhesion to laminin by antilaminin polyclonal antibodies. Cells were allowed to adhere to pepsin-extracted human laminin (●), human fibronectin (○), and human type IV collagen (□) in the presence of various dilutions of the polyclonal antibody prepared against pepsin-extracted human laminin. Coating concentrations were 5 µg/ml for laminin and type IV collagen, and 1 µg/ml for fibronectin. The assay was performed as in Fig. 1.

laminin to identify laminin receptors by affinity chromatography.

Affinity Chromatography of Endothelial Cell Extracts on Human Laminin

Affinity chromatography was performed by applying extracts from surface iodinated endothelial cells to laminin-Sepharose. Since integrin-type receptors bind to their ligands in a divalent cation-dependent manner (e.g., Gailit and Ruoslahti, 1988) and since the attachment of endothelial cells to human laminin was inhibited by EDTA (result not shown), EDTA was used to elute the proteins bound to the affinity matrix. This procedure yielded an apparent integrin type receptor with an α and a β subunit (Fig. 3). This integrin showed two bands of 160 and 140 kD under reducing conditions, while the fibronectin receptor, purified on 110-kD fibronectin-Sepharose affinity matrix from the same cells and shown for comparison in lanes 2 of Fig. 3, had the previously observed mobility of 140 kD. Under nonreducing conditions, the laminin-binding integrin and fibronectin receptor subunits migrated with the same approximate mobilities (160 and 120 kD). In some preparations of the laminin-binding integrin, the intensity of labeling in the α subunit was low, suggesting that the availability of this subunit for iodination on the endothelial cell surface may vary.

The Endothelial Cell Laminin-binding Integrin Is the $\alpha_2\beta_1$ Integrin

Polyclonal and monoclonal antibodies against integrins were used to identify the subunits of the endothelial cell laminin-binding integrin. The purified integrin bound to a polyclonal antibody that reacts with both the α (α_5) and β (β_1) subunits of the placental fibronectin receptor (Fig. 4, lane 1). It also bound to monoclonal antibody PIH5, directed against the α_2 subunit (Fig. 4, lane 8), to monoclonal antibody LM442 directed against the β_1 subunit (Fig. 4, lane 12) that reacts also with the endothelial cell fibronectin receptor (Fig. 4,

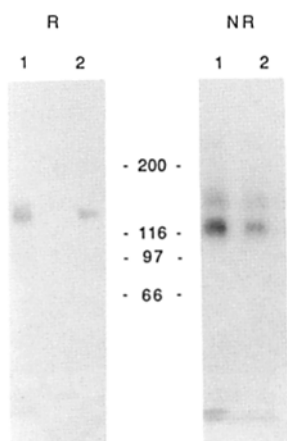


Figure 3. Electrophoretic analysis of endothelial cell laminin-binding integrin. Surface-iodinated endothelial cells were fractionated on a laminin-Sepharose or a 110-kD fibronectin fragment-Sepharose column, and the columns were eluted with 20 mM EDTA. The peak fractions from each fractionation were analyzed on a 7.5% acrylamide gel in SDS under reducing (*R*) and non-reducing (*NR*) conditions. The laminin-binding integrin (lanes 1) and fibronectin receptor (lanes 2) subunits were visualized by autoradiography. The positions of molecular size markers in kD are shown.

lane 13) and to a polyclonal antibody prepared against the platelet α_2 subunit (not shown). A variable amount of an 80-kD band was immunoprecipitated with the receptor; it may be a degradation product of either of the receptor subunits.

Exhaustive immunoprecipitations with the anti- α_2 polyclonal antibody and with the LM442 anti- β_1 monoclonal antibody cleared all the material reactive with the antifibro-

nectin receptor antiserum, showing that the $\alpha_2\beta_1$ integrin is the only significant component among the proteins obtained by the affinity chromatography on laminin (not shown). Moreover, the PIH5 antibody (anti- α_2) immunoprecipitated from surface-labeled endothelial cells two polypeptides with mobilities identical to those seen in the laminin bound preparation (not shown). The endothelial cell laminin-binding integrin did not react with polyclonal antibodies prepared against the cytoplasmic domains of the α_3 (Fig. 4, lane 4) or α_5 (Fig. 4, lane 5) subunits, nor did it immunoprecipitate with monoclonal antibodies against $\alpha_3\beta_1$ or $\alpha_6\beta_1$ integrin complexes (not shown).

A laminin receptor isolated from the human MG-63 osteosarcoma cells was used for comparison in the immunoprecipitations. The MG-63 cell laminin receptor was also immunoprecipitated by the antifibronectin receptor antiserum (Fig. 4, lane 2). However, unlike the endothelial cell laminin-binding integrin, it reacted with the antiserum directed against the cytoplasmic domain of the α_3 subunit (Fig. 4, lane 3) but not with the anti- α_2 monoclonal antibody PIH5 (Fig. 4, lane 9).

Taken together, these results show that the endothelial cell laminin-binding integrin is a heterodimeric molecule consisting of an α subunit antigenically indistinguishable from the α_2 subunit, and a β subunit antigenically indistinguishable from the integrin β_1 subunit. The results also show that the endothelial cell laminin-binding integrin is different from the MG-63 osteosarcoma cell laminin receptor.

Binding Specificity of the Endothelial Cell Laminin-binding Integrin

The interaction of the purified laminin-binding integrin with laminin and other potential ligands was evaluated in a radioreceptor assay. In the presence of Mn^{2+} , the laminin-binding integrin bound to human laminin, fibronectin, and human type IV collagen in a concentration-dependent manner (Fig. 5). Saturation could not be studied, because the amounts of the laminin-binding integrin available became a limiting factor. Assays performed in the presence of Ca^{2+} and Mg^{2+} without Mn^{2+} , showed similar specificity except that in the absence of Mn^{2+} the laminin binding was consistently higher than the binding to fibronectin or type IV collagen. The integrin also interacted slightly with bovine type I collagen (22% of the laminin binding) as well as with human type I collagen. The fibronectin receptor isolated from endothelial cells showed significant binding only to fibronectin among these proteins in the radioreceptor assay (not shown).

To exclude the possibility that contaminants in the laminin preparation could be responsible for the observed laminin binding by the isolated integrin, we compared the binding of this integrin to different laminin preparations. There was no significant difference in the binding of this integrin to human laminin before (100%) or after (90%) FPLC purification, and it also bound well to mouse laminin (90%).

These results confirm the ability of the purified $\alpha_2\beta_1$ -related integrin to bind laminin to show that it can also bind to fibronectin, type IV collagen, and possibly type I collagen.

Expression of Integrin Subunits by Endothelial Cells

The integrin surface phenotype of endothelial cells was determined by flow cytometry (Fig. 6). High levels of α_1 , α_2 ,

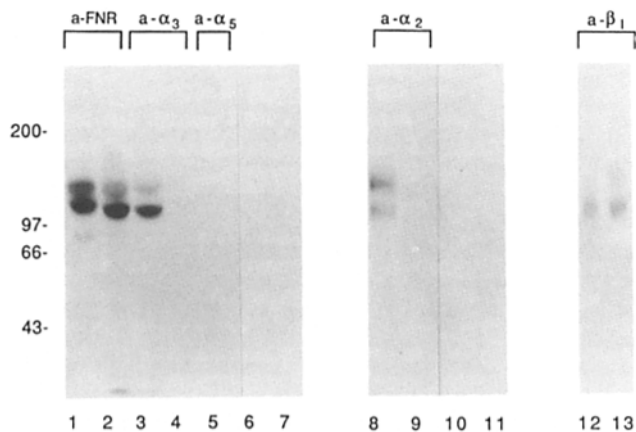


Figure 4. Immunoprecipitation of laminin-binding integrins purified from endothelial cells and MG-63 cells. The laminin-binding integrins purified by laminin-Sepharose affinity chromatography from surface-iodinated endothelial cells (lanes 1, 4, 5, 6, 8, 10, and 12) or MG-63 cells (2, 3, 7, 9, and 11), and fibronectin receptor purified by chromatography on 110-kD fibronectin fragment-Sepharose from endothelial cells (lane 13), were immunoprecipitated with antibodies against integrins. The antibodies were polyclonal antifibronectin receptor antibody (lanes 1 and 2), a polyclonal antibody against the α_3 cytoplasmic domain (lanes 3 and 4), a polyclonal antibody against the α_5 cytoplasmic domain (lane 5), a mouse anti- α_2 monoclonal antibody (PIH5) (lanes 8 and 9), and a mouse anti- β_1 monoclonal antibody (LM442) (lanes 12 and 13). Nonimmune rabbit serum (lanes 6 and 7) and 2E8 antilaminin monoclonal antibody (lanes 10 and 11) were used as controls. A positive control for the anti- α_5 antibody is shown in Fig. 7. The immunoprecipitates were analyzed on a 7.5% SDS-PAGE under nonreducing condition and visualized by autoradiography. The positions of prestained marker proteins in kilodaltons are shown.

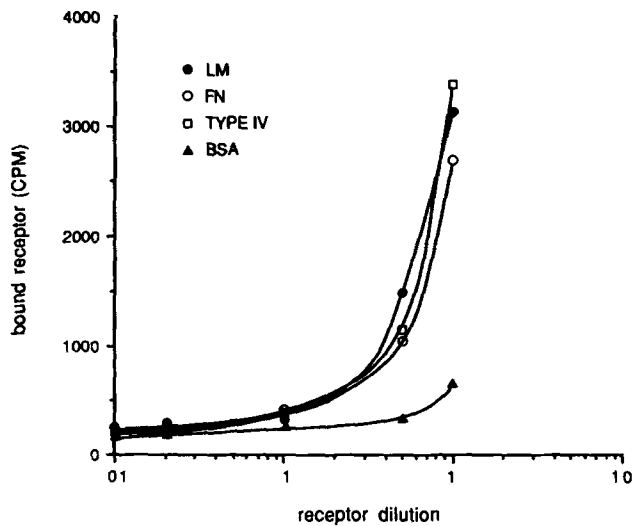


Figure 5. Binding of endothelial cell laminin-binding integrin to extracellular matrix proteins in radioreceptor assay. Microtiter wells were coated with pepsin-extracted human laminin (LM), human plasma/fibronectin (FN), human type IV collagen (TYPE IV), or BSA, and the binding of the laminin-binding integrin isolated from surface-iodinated endothelial cells to the wells was assayed. Values are the mean of duplicate observations.

and α_5 were found, α_3 was present at low levels, while α_4 and α_6 were present at trace levels (α_6) or undetectable (α_4). β_1 and β_3 were expressed at high levels and no β_2 was detected by flow cytometry.

Immunoprecipitation of detergent extracts from surface-labeled endothelial cells confirmed the flow cytometry

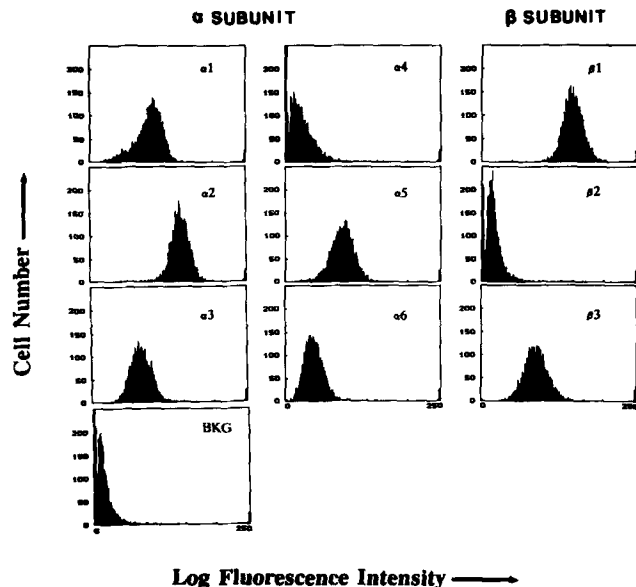


Figure 6. Expression of the integrin α and β subunits on cultured endothelial cells. Endothelial cells were stained in suspension for the presence of six α and three β subunits with the monoclonal antibodies TS2/7 (α_1), P1H5 (α_2), P1B5 (α_3), P4G9 (α_4), P1D6 (α_5), GoH3 (α_6), A1A5 (β_1), P4H9 (β_2), and AP3 (β_3), and analyzed by flow cytometry. The results are expressed as histograms with cell number (y axis) plotted as a function of relative fluorescence intensity (x axis) as determined on a three-cycle log scale. The fluorescence intensity is expressed in arbitrary units. Background fluorescence with nonimmune mouse IgG control is also shown (BKG).

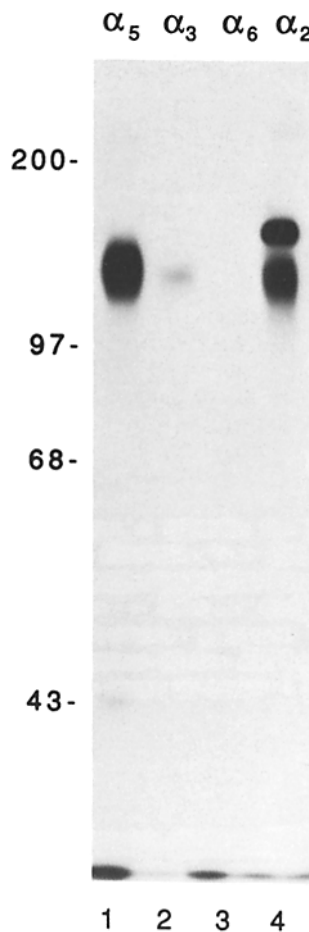


Figure 7. Electrophoretic analysis of the integrins expressed on endothelial cell surface. Detergent extracts of surface-iodinated endothelial cells were immunoprecipitated with a polyclonal antibody against α_5 cytoplasmic domain (lane 1) or mouse monoclonal anti- α_3 (P1B5), anti- α_6 (GoH3), and anti- α_2 (P4B4) antibodies (lanes 2, 3, and 4, respectively). The immunoprecipitates were run on 7.5% SDS-PAGE in the presence of 2-mercaptoethanol and visualized by autoradiography.

results (Fig. 7). Among the potential laminin receptor α subunits, high levels of $\alpha_2\beta_1$ were observed (Fig. 7, lane 4) whereas $\alpha_3\beta_1$ (Fig. 7, lane 2) and $\alpha_6\beta_1$ (Fig. 7, lane 3) were very low or undetectable. Also in agreement with the flow cytometry results, high levels of $\alpha_5\beta_1$ were detected by immunoprecipitation with an α_5 -specific antibody (Fig. 7, lane 1). Taken together, these data show that $\alpha_2\beta_1$ is a major integrin expressed by human umbilical vein endothelial cells.

Role of $\alpha_2\beta_1$ Integrin in Endothelial Cell Adhesion to Laminin

To evaluate the contribution of the $\alpha_2\beta_1$ integrin in the attachment of the endothelial cells to laminin, we assayed various monoclonal antibodies for their ability to inhibit this attachment. The P1H5 monoclonal antibody, which is an anti- α_2 , inhibited endothelial cell adhesion to human laminin by up to 80% (Fig. 8). Inhibitory monoclonal antibodies to α_3 and α_5 (P1B5 and P1D6) had no effect, but an inhibitory antibody against α_6 (GoH3) partially inhibited the laminin attachment. However, as shown above (Figs. 6 and 7), the endothelial cells expressed only small amounts of this potential laminin receptor, and the purified laminin-binding integrin was not immunoprecipitated by the anti- α_6 antibody. Therefore, if the $\alpha_6\beta_1$ integrin plays a role in the laminin attachment of endothelial cells, such a role is likely to be only a minor one under the conditions we have exam-

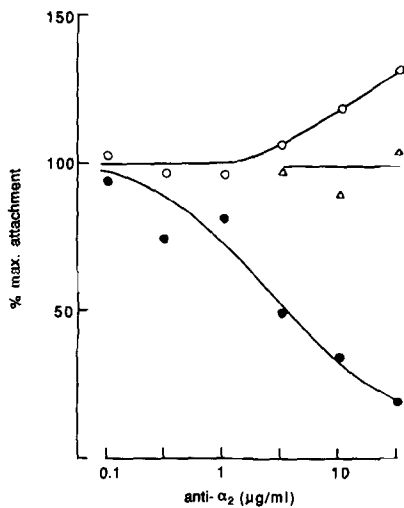


Figure 8. Inhibition of endothelial cell adhesion to laminin by monoclonal anti α_2 . Endothelial cells (6×10^3 cells/well) were allowed to adhere to microtiter wells coated with pepsin-extracted human laminin (\bullet , $5 \mu\text{g/ml}$), fibronectin (\circ , $1 \mu\text{g/ml}$), or vitronectin (Δ , $3 \mu\text{g/ml}$), in the presence of increasing concentrations of purified anti α_2 -monoclonal antibody PIH5, and the number of adherent cells was quantitated as in Fig. 1. Values are means of duplicates.

ined. Controls showed that the PIH5 antibody did not affect endothelial cell attachment to fibronectin or vitronectin even at the highest concentrations used (Fig. 8). However, it did inhibit their adhesion to type I collagen by 90% at a concentration of $10 \mu\text{g/ml}$. MG-63 cell attachment to laminin, which is mediated by an α_3 integrin (Gehlsen et al., 1988; Gehlsen et al., 1989) was not inhibited by PIH5.

Discussion

The results reported here show that a laminin receptor from human umbilical vein endothelial cells is immunologically indistinguishable from the $\alpha_2\beta_1$ integrin. This is a surprising finding for several reasons: the $\alpha_2\beta_1$ integrin has previously been identified as a collagen receptor (Wayner and Carter, 1987; Kunicki et al., 1988), three other integrins have been found to function as laminin receptors in other cells (Ignatius and Reichardt, 1988; Gehlsen et al., 1988; Sonnenberg, 1988; Gehlsen et al., 1989), and the endothelial cell laminin receptor has been reported to be a 69-kD protein unrelated to integrins (Yannariello-Brown et al., 1988).

The role of the $\alpha_2\beta_1$ integrin as a laminin receptor in endothelial cells is supported by a number of observations reported here. First, chromatography of endothelial cell extracts on a laminin matrix yielded an integrin that was essentially quantitatively immunoprecipitable with monoclonal antibodies directed against the α_2 or the β_1 subunit. Secondly, the isolated receptor bound to both human and mouse laminins in a radioreceptor assay. Thirdly, a monoclonal anti- α_2 antibody inhibited the attachment of endothelial cells to laminin. These results show that the endothelial cells express a laminin receptor that is an integrin not previously associated with laminin binding, and they therefore define a new role for the $\alpha_2\beta_1$ integrin, in addition to its previously known role as a collagen receptor.

The $\alpha_2\beta_1$ integrin has been considered a collagen receptor because (a) functional antibodies directed against the α_2 subunit inhibit the binding of cells to collagen (Wayner and Carter, 1987) and (b) the $\alpha_2\beta_1$ integrin isolated from platelets binds to various types of collagens but has no affinity for laminin (Staatz et al., 1989). In fact, the laminin receptor of platelets is reported to be the $\alpha_6\beta_1$ integrin (Sonnenberg et al., 1988). In contrast to the platelet $\alpha_2\beta_1$ integrin, the integrin we have isolated from endothelial cells binds to laminin and, somewhat less efficiently, to collagens and fibronectin.

The molecular basis of the apparent specificity difference among the $\alpha_2\beta_1$ integrins is unclear. The endothelial cell receptor subunits reacted with each of the monoclonal and sequence-specific polyclonal anti- α_2 and anti- β_1 antibodies we tested. This result argues for extensive similarity of the endothelial cell $\alpha_2\beta_1$ integrin with $\alpha_2\beta_1$ integrin from other cells. This similarity raises the exciting possibility that the already impressive diversity of the integrin system could be further augmented by cell type regulation of the specificities of individual integrins. Possible molecular mechanisms for such regulation include alternative mRNA splicing, post-translational modification of the receptor, or the association of the receptor with some modifying component that differs among different cell types. One alternative splicing for integrin subunits has already been found (van Kuppevelt et al., 1989), and gangliosides would be candidates for modulating factors, since they are known to be associated with integrins (Cheresh et al., 1987).

The α_2 -containing integrin we have identified appears to be the main receptor on endothelial cells for laminin. This result does not agree with the recent claim that the endothelial cell laminin receptor is the laminin-binding 67-69-kD nonintegrin protein previously identified in several other cell lines (Yannariello-Brown et al., 1988). Recent data from a number of laboratories have shown that cells other than endothelial cells have integrin type laminin receptors, making it likely that this would also be the case with endothelial cells. Moreover, the sequence of the 67-69-kD protein is that of a typical cytoplasmic protein, in that it has no clearly discernible signal sequence or transmembrane sequence (Makrides et al., 1988; Hunt and Barker, 1988). The evidence therefore, strongly favors the integrin as the endothelial cell laminin receptor.

The $\alpha_2\beta_1$ integrin we describe here as the laminin receptor is the fourth laminin-binding integrin described in the literature. It is difficult to see why so many different receptors would be needed for laminin. There is redundancy in the integrin system such that a single matrix protein can have more than one receptor. This is best documented with fibronectin which binds at least to the $\alpha_5\beta_1$ and gp IIb/IIIa integrins (Pytela et al., 1986), the integrin studied here, and possibly by others (Gehlsen et al., 1988; Cheresh et al., 1989). Laminin may have more than one cell attachment site (reviewed in Timpl, 1989) and some receptor heterogeneity may, therefore, be needed to provide receptors for such alternative sites. However, recent evidence shows that laminin is not a single protein but a mixture of proteins with shared and unique subunits and tissue-specific distributions (Hunter et al., 1989; Ehrig et al., 1989). It is an exciting possibility that the various laminin-binding integrins might recognize only one form of laminin each, and that this would endow a cell with the ability to recognize the appropriate basement membranes.

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