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Cell Biology

Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor

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ABSTRACT **Human umbilical vein endothelial cells express a heterodimeric adhesion receptor complex consisting of noncovalently associated α and β subunits that under reducing conditions have molecular masses of 135 kDa and 115 kDa, respectively. This complex can be isolated in pure form from an affinity matrix consisting of an Arg-Gly-Asp-containing heptapeptide and is specifically immunoprecipitated with monoclonal antibodies (mAbs) directed against the vitronectin receptor of human melanoma cells. These data suggest that this complex is one member of a large family of cell adhesion receptors. One of the mAbs, LM609, inhibits the attachment of human endothelial cells to fibrinogen, von Willebrand factor, and vitronectin yet has no effect on the attachment of these cells to fibronectin, collagen, or laminin. In addition, mAb LM609 inhibits attachment of endothelial cells to an immobilized synthetic peptide containing the Arg-Gly-Asp sequence. This adhesion receptor appears structurally similar to the IIb/IIIa glycoprotein complex expressed on platelets yet is antigenically distinct, since mAb LM609 fails to recognize IIb/IIIa glycoproteins. This receptor organizes in clusters on endothelial cells during their attachment to von Willebrand factor, vitronectin, or the Arg-Gly-Asp-containing heptapeptide. The data presented in this report suggest that Arg-Gly-Asp recognition may play a significant role in biological events associated with vascular proliferation.**

The molecular interactions that contribute to the proliferation, adhesion, and motility of endothelial cells are undoubtedly critical events associated with vessel wall repair in injured tissues and vascular proliferation in tumors. To this end it is important to understand the mechanism(s) involved in endothelial cell–matrix interaction.

A number of factors are known to potentiate an interaction between endothelial cells and the surrounding matrix. Among these, fibrinogen and its derivative fibrin were shown to influence endothelial cell motility and growth during events associated with blood coagulation and wound repair (1, 2). It has been postulated that endothelial cells specifically bind fibrinogen in a reversible and saturable manner, suggesting the presence of a cell surface receptor (1). Another important molecule potentially involved in endothelial cell–matrix interactions is von Willebrand factor (vWF), a multimeric glycoprotein produced by these cells that is incorporated into the subendothelial matrix (3). Although much is known about the biological function of these and other matrix proteins recognized by endothelial cells, little is known about their adhesion receptor(s).

Recent work has defined a class of cell adhesion receptors that recognize a number of extracellular matrix components including fibronectin, vitronectin, collagen, fibrinogen, and vWF. These receptors are composed of two noncovalently associated subunits and are capable of recognizing the sequence Arg-Gly-Asp present in each of the above matrix proteins (4–6). A variety of normal and transformed cell types in species ranging from slime mold to man (4–12) have been shown to express one or more of these matrix receptors. One member of this family is the IIb/IIIa glycoprotein expressed on platelets, whose recognition of fibronectin, vitronectin, fibrinogen, and vWF can be inhibited by Arg-Gly-Asp-containing peptides (13, 14). A IIb/IIIa-like glycoprotein identified on endothelial cells (15, 16) may serve as a matrix receptor.

This report describes an Arg-Gly-Asp-directed adhesion receptor expressed by human endothelial cells that is structurally similar to the IIb/IIIa complex on platelets but antigenically and functionally distinct. This receptor is directly involved in endothelial cell attachment to fibrinogen, vWF, and vitronectin, but not to fibronectin, collagen, or laminin and is specifically recognized by monoclonal antibodies (mAbs) directed against the vitronectin receptor expressed by human melanoma cells (5). The endothelial cell adhesion receptor described here may be one member of a large family of cell adhesion receptors that may play a key role in mechanism(s) involved in vascular proliferation and wound healing.

MATERIALS AND METHODS

Cells. Human endothelial cells were isolated from collagenase digestion of fresh human umbilical veins and propagated as described (17).

Monoclonal Antibodies. mAbs LM142 (IgG1) (5) and LM609 (IgG1) (unpublished results), reactive with the M21 human melanoma cell adhesion receptor, and mAb 9.2.27 (IgG2a), directed against a chondroitin sulfate proteoglycan core glycoprotein (18), were produced in this laboratory. mAb W6/32 (IgG2a), reactive with a framework determinant on human class I histocompatibility antigens, was provided by P. Parham (Stanford University). All mAbs were purified on protein A-Sepharose and stored frozen.

Adhesive Proteins. Both synthetic peptides used in this study were provided by R. Houghton (Scripps Clinic). Human fibronectin, vitronectin, and both synthetic peptides were also provided by M. Pierschbacher (La Jolla Cancer Research Foundation). Fibrinogen was a gift from E. Plow (Scripps Clinic), and vWF (pool I, containing large multimers of $>5 \times 10^3$ kDa, and pool III, consisting of multimers of $2–5 \times 10^3$ kDa) was provided by Z. Ruggeri and T. Zimmerman (Scripps Clinic).

Functional Isolation of an Arg-Gly-Asp Receptor from Human Endothelial Cells. For metabolic labeling, human umbilical vein endothelial cells were seeded into two T-150 tissue culture flasks (Corning), each containing 20 ml of growth medium. After the cells reached semiconfluence, each flask was supplemented with 2 μCi of [^{35}S]methionine (1295 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq). Cells were then allowed to incorporate radiolabel for 72 hr at 37°C.

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Abbreviations: mAb, monoclonal antibody; vWF, von Willebrand factor.

The adhesion receptor was isolated by affinity chromatography on Gly-Arg-Gly-Asp-Ser-Pro-Lys-Sepharose from octyl β-D-glucoside extracts of endothelial cells (100 μl packed cell volume), metabolically labeled with [^{35}S]methionine, as previously described for M21 human melanoma cells (19). This procedure resulted in the isolation of two radiolabeled polypeptides (135 kDa and 115 kDa) by NaDodSO$_4$/PAGE (20) in 7.5% gels under reducing conditions (0.3 M 2-mercaptoethanol), after exposure of gels to x-ray film as described (5).

Immunoprecipitation of Endothelial Cell Adhesion Receptor. Receptor was isolated from metabolically labeled endothelial cells as above, and eluted material was further extracted with an equal volume of 2× RIPA lysis buffer [1× is 0.1 M Tris Cl, pH 7.2/0.15 M NaCl/1% (wt/vol) deoxycholate, 1% (wt/vol) Nonidet P-40/0.1% (wt/vol) NaDodSO$_4$/1% (wt/vol) aprotinin/2 mM phenylmethylsulfonyl fluoride] as previously described (5). Immunoadsorbants were prepared by covalently coupling 10 mg of purified mAb to 1 ml of CNBr-activated Sepharose (Pharmacia) according to the manufacturer's procedure. Immunoadsorbant beads containing ≈20 μl of Sepharose were incubated overnight at 4°C either with cell lysate (10^6 cells per ml of lysate) or with eluted fractions from the Arg-Gly-Asp-peptide affinity matrix. At this point immunoadsorbants were washed extensively, boiled in Laemmli sample buffer (20), analyzed by NaDodSO$_4$/7.5% PAGE under reducing conditions, and subjected to fluorography as described (5).

Adhesion Assay. Endothelial cells were grown as monolayers in tissue culture flasks and metabolically labeled for 72 hr with [^{32}H]leucine (50 μCi/ml; 100 Ci/mmol, ICN). The cells were detached with a trypsin/EDTA solution (M. A. Bioproducts, Walkersville, MD) and washed with growth medium containing 1% fetal bovine serum. Prior to the adhesion assay, cells were allowed to react with appropriate purified mAb (0.5 mg/ml in growth medium) for 1 hr at 4°C, washed free of excess antibody, and resuspended in growth medium containing 1% fetal bovine serum. Endothelial cells (5×10^3) were added to individual wells of a polystyrene (non-tissue culture) microtiter plate that were previously coated either with appropriate adhesive protein [10–20 μg/ml in phosphate-buffered saline (PBS: 0.15 M NaCl/0.01 M phosphate, pH 7.2)] or with covalently coupled Gly-Arg-Gly-Asp-Ser-Pro-Cys peptide as described (21). Attachment of cells was allowed to occur for 5–120 min at 37°C, after which microtiter wells were washed twice with PBS to remove unattached cells. The remaining attached cells were harvested with an automated cell harvester (Scatron, Sterling, VA) as described (22). In the absence of adhesive protein, <200 cpm of cell-binding activity was observed after 120 min at 37°C. Attached cells were counted by microscopic examination of random fields, and the results were correlated directly with those obtained by the automated harvesting procedure.

Indirect Immunofluorescence. Endothelial cells were allowed to attach for 1 hr to glass coverslips that had been coated with various adhesive proteins at 10 μg/ml in PBS. After attachment, the cells were fixed with 3% paraformaldehyde for 15 min at room temperature. Each coverslip was individually washed several times in Hanks' balanced salts solution (HBSS) (GIBCO), followed by an additional wash in HBSS/0.1 M glycine. In some cases, cells were then permeabilized for 1 min with 0.2% Triton X-100 in 50 mM Tris Cl, pH 7.5/0.15 M NaCl/0.1% NaN$_3$. The coverslips were overlaid with an appropriate mAb (5 μg/ml) for 1 hr at room temperature, washed, and overlaid for 1 hr at room temperature with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Bio-Rad), diluted 1:50 in HBSS containing 1% bovine serum albumin. After three washes the coverslips were inverted and mounted on slides in a drop of Fluoromount-G (Fisher). The stained cells were observed through a Zeiss epifluorescence microscope and photographed.

RESULTS

Human Umbilical Vein Endothelial Cells Synthesize a Heterodimeric Complex That Specifically Recognizes an Arg-Gly-Asp-Containing Heptapeptide. Experiments were performed to determine whether human endothelial cells contain a potential Arg-Gly-Asp-directed adhesion receptor. For this purpose an octyl glucoside lysate from metabolically labeled endothelial cells was allowed to bind to a Sepharose column containing the covalently coupled heptapeptide Gly-Arg-Gly-Asp-Ser-Pro-Lys. After the binding of lysate, the column was washed and then bound material was eluted with either Gly-Arg-Gly-Glu-Ser-Pro (inactive cell adhesion peptide) or Gly-Arg-Gly-Asp-Ser-Pro (active cell adhesion peptide) at 1 mg/ml. When individual fractions of the eluate were examined by NaDodSO$_4$/PAGE under reducing conditions, the inactive peptide (fractions 1–10) failed to elute significant material from the column, whereas the active peptide (fractions 11–20) specifically eluted two polypeptides of 135 kDa and 115 kDa (Fig. 1). Polypeptides of identical size were specifically immunoprecipitated from metabolically labeled endothelial cells with mAbs [LM142 (5) and LM609 (data not shown)] directed to the vitronectin receptor.

Arg-Gly-Asp-Binding Polypeptides Isolated from Human Endothelial Cells Are Antigenically Related to an Adhesion Receptor Expressed by Human Melanoma Cells. To determine whether Arg-Gly-Asp-binding proteins of endothelial cells that were eluted from the affinity matrix with Arg-Gly-Asp peptide were antigenically related to an Arg-Gly-Asp-directed receptor from human melanoma cells (5), the 135/115-kDa proteins thus eluted were subjected to immunoprecipitation with mAbs LM142 and LM609 (Fig. 2). mAb LM142 (lane A) and LM609 (lane B) directly immunoprecipitated the 135/115-kDa complex from the Arg-Gly-Asp-eluted fraction, whereas mAb 9.2.27 (lane C), directed to an antigen expressed only on the melanoma cells, failed to immunoprecipitate these polypeptides. These results demonstrate that mAbs LM142 and LM609 specifically recognize an Arg-Gly-Asp-directed heterodimeric complex that is antigenically similar to the 130/105-kDa vitronectin receptor complex expressed on human melanoma cells (5).

Although mAbs LM142 and LM609 recognize common antigens shared between human melanoma and endothelial cells, these reagents fail to react with the structurally similar IIb/IIIa glycoprotein complex on platelets. Specifically, these mAbs do not react with purified IIb/IIIa by ELISA and have no effect on platelet adhesion to fibrinogen (L. Frelinger, Scripps Clinic, personal communication). Moreover, several antibodies directed to the IIb/IIIa complex failed to react with the surface of human endothelial cells or the vitronectin receptor (data not shown). Specifically, mAbs LJCP10, LJCP3, and LJP9 (provided by Z. Ruggeri, Scripps Clinic) failed to react with endothelial cells (data not shown). In fact, mAb LJP9, directed to the IIb/IIIa complex, was shown to block platelet interaction with vWF and fibrinogen and inhibit platelet aggregation (23).

mAb LM609 Specifically Blocks Endothelial Cell Attachment to vWF, Fibrinogen, Vitronectin, and an Arg-Gly-Asp-Containing Peptide. Cell adhesion assays were performed to determine whether the antigens recognized by mAbs LM142 or LM609 are involved in endothelial cell attachment. As shown in Fig. 3, cells preincubated in the presence or absence of various mAbs were allowed to attach to microtiter wells coated with fibronectin (*A*), collagen (*B*), laminin (*C*) vitronectin (*D*), vWF (*F* and *G*), fibrinogen (*H*), or an Arg-Gly-Asp-containing heptapeptide (*E*). Endothelial cells pretreated with either growth medium alone or mAb W6/32 (anti-HLA) attached identically to all adhesive proteins in a time-dependent manner, with maximal binding

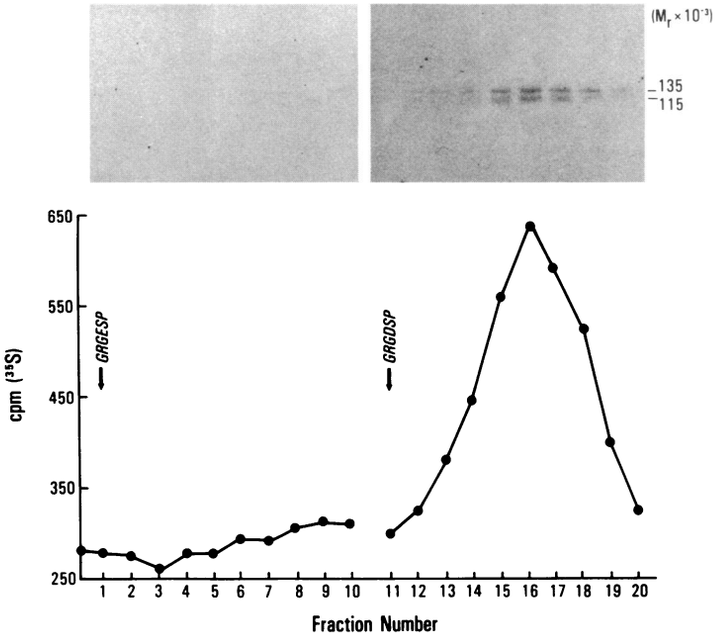

FIG. 1. Isolation of Arg-Gly-Asp-directed receptor from human endothelial cells by affinity chromatography on Gly-Arg-Gly-Asp-Ser-Pro-Lys-Sepharose. An octyl glucoside extract of human umbilical vein endothelial cells metabolically labeled with [^{35}S]methionine was allowed to bind an affinity matrix of Gly-Arg-Gly-Asp-Ser-Pro-Lys-Sepharose as described in *Materials and Methods*. The matrix was washed, and bound material was eluted with Gly-Arg-Gly-Glu-Ser-Pro (GRGESP, 1 mg/ml, fractions 1–10) followed by Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP, 1 mg/ml; fractions 11–20). (*Upper*) Aliquots (50 μl) of each fraction (200 μl) were analyzed by NaDodSO$_4$/7.5% PAGE under reducing conditions, followed by exposure of gels to x-ray film. (*Lower*) Radioactivity of a 50-μl aliquot of each fraction was determined in a liquid scintillation counter.

occurring at 60–90 min. In contrast, mAb LM609 (directed to the 135/115-kDa heterodimer) significantly inhibited endothelial cell attachment to various-sized multimers of vWF, as well as fibrinogen, vitronectin, and an Arg-Gly-Asp-containing heptapeptide, yet failed to reduce attachment to fibronectin, collagen, or laminin. It is apparent from these results that mAb LM609 does not affect a generalized mechanism of endothelial cell adhesion. Overall, the attachment of endothelial cells reached a maximum after 90 min, at which time cells treated with mAb LM609 showed a 75–80% reduction in attachment to vWF, fibrinogen, and the Arg-Gly-Asp-containing heptapeptide, while attachment to vitronectin was inhibited ≈50%. Although mAb LM142 reacts with the same 135/115-kDa complex on the endothelial cells, it failed to significantly inhibit the attachment of endothelial or melanoma cells to all of the above-mentioned adhesive proteins, suggesting that it recognizes a determinant that is not involved in Arg-Gly-Asp recognition (data not shown).

To assess whether mAb LM609 could perturb endothelial cells that were preattached, such cells were allowed to adhere and spread on vitronectin, vWF, or fibrinogen, overlaid with mAb LM609 (500 μg/ml), and examined microscopically for 1–4 hr. Under these experimental conditions, mAb LM609 failed to cause cell-rounding or detachment of cells from any of the adhesive proteins tested.

The 135/115-kDa Adhesion Receptor Organizes on Endothelial Cells after Their Attachment to Specific Adhesion Proteins. The distribution of the endothelial cell adhesion receptor was examined by indirect immunofluorescence using mAb LM142 as a primary antibody, since once cells are attached and spread on a substrate, the epitope recognized by mAb LM609 becomes mostly unavailable, as demonstrated by the relatively minimal staining with this mAb (data not shown). In addition, optimal staining could only be observed on cells that were permeabilized by mild detergent treatment (Fig. 4 *A–D*). Endothelial cells attached to immobilized vitronectin expressed receptor in discrete foci (Fig. 4*A*). These foci appear to be distributed at cell–substratum contact points, since they were only observed in the focal plane at the cell–substratum interface. Cells attached to vitronectin that were not permeabilized showed markedly reduced staining (Fig. 4*E*), indicating that the permeabilization step exposed antigenic sites that may normally be inaccessible at the cell–substratum interface. In contrast, permeabilized cells spread on a fibronectin substrate and stained in an identical manner did not show the same degree of receptor clustering in the focal plane at the cell–substratum interface (Fig. 4*B*). Due to the permeabilization step, diffuse staining could also be observed near the center of the cell.

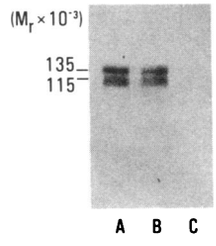

FIG. 2. mAbs immunoprecipitate the 135/115-kDa Arg-Gly-Asp-binding proteins isolated from human endothelial cells. The 135/115-kDa polypeptides isolated from the Arg-Gly-Asp affinity matrix (above) were further extracted with a RIPA lysis buffer and subjected to immunoprecipitation using mAb LM609 (lane A), LM142 (lane B), or 9.2.27 (lane C) as described in *Materials and Methods*. Immunoprecipitated material was analyzed by NaDodSO$_4$/7.5% PAGE under reducing conditions, followed by exposure to x-ray film.

Endothelial cells attached and spread on the Arg-Gly-Asp-containing heptapeptide (Fig. 4*D*) had a receptor staining pattern essentially identical to that observed on cells attached to vitronectin (Fig. 4*A*). Cells attached to vWF showed partial receptor organization (Fig. 4*C*)—somewhat less than that observed for vitronectin and Arg-Gly-Asp-containing peptide, but more than that for fibronectin. In control experiments where endothelial cells were stained with mAb W6/32 (anti-HLA), organized staining patterns were not observed when the cells were spread on any of the adhesion proteins (Fig. 4*F*).

DISCUSSION

Endothelial cells can interact with a number of proteins present in plasma and in the subendothelial matrix. These interactions are critical for events associated with wound healing, coagulation, lymphocyte infiltration at sites of inflammation, and tumor cell hematogenous spread. For this reason it is important to identify the endothelial cell receptors for components present in plasma and the subendothelial matrix.

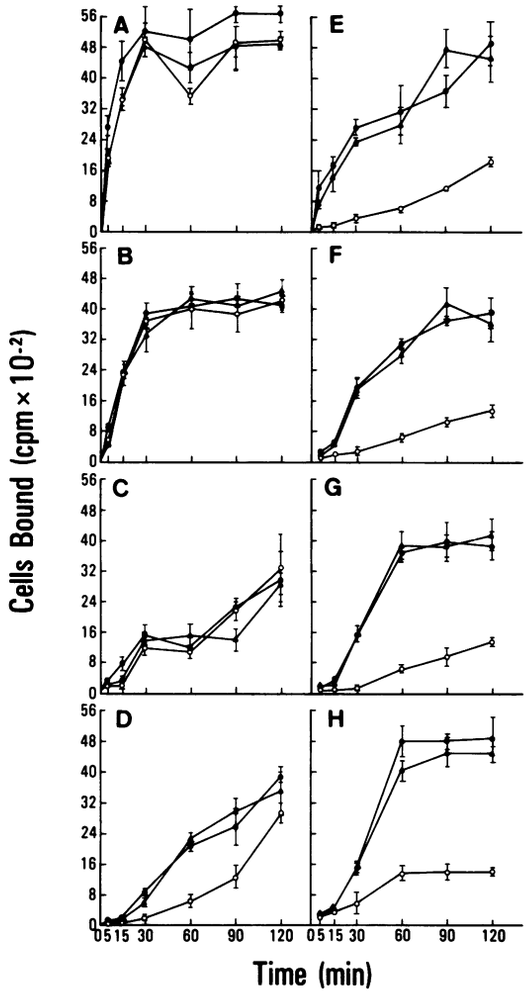

FIG. 3. Effects of mAb LM609 on the kinetics of human endothelial cell attachment to immobilized substrates. Human umbilical vein endothelial cells (5×10^3) metabolically labeled with [^{3}H]leucine were allowed to attach for 5–120 min to microtiter wells coated with fibronectin (*A*), collagen IV (*B*), laminin (*C*), vitronectin (*D*), Gly-Arg-Gly-Asp-Ser-Pro-Cys (*E*), vWF pool I (*F*), vWF pool III (*G*), or fibrinogen (*H*). Before addition to substrate-coated wells, the cells were allowed to react with mAb LM609 (○) or W6/32 (●) at 50 μg/ml or with growth medium alone (▲) for 1 hr at 4°C and then were washed free of excess antibody. Data are expressed as the total number of cells (cpm bound) at designated times. Each point represents the mean ± SD of five replicates. When microtiter wells were not coated with adhesive protein, cell binding could not be detected during the course of this assay.

A heterodimeric glycoprotein receptor complex is described here that is expressed on the surface of human umbilical vein endothelial cells and appears to be directly involved in their attachment to and spreading on vWF, fibrinogen, and vitronectin. Several lines of evidence suggest that this glycoprotein complex is one member of a family of cell adhesion receptors that are capable of recognizing the Arg-Gly-Asp sequence present in a number of adhesive proteins. First, the endothelial cell adhesion receptor, like other members of this family, is composed of two noncovalently associated polypeptides that under reducing conditions have apparent molecular masses of 135 and 115 kDa, which under nonreducing conditions change to 145 and 100 kDa, respectively (data not shown). These data suggest that the larger subunit possesses a disulfide-linked light chain, whereas the smaller subunit contains intrachain disulfide bridges as previously demonstrated (5, 6, 8, 10, 11, 15, 16). Second, this receptor can be isolated on an affinity matrix composed of an

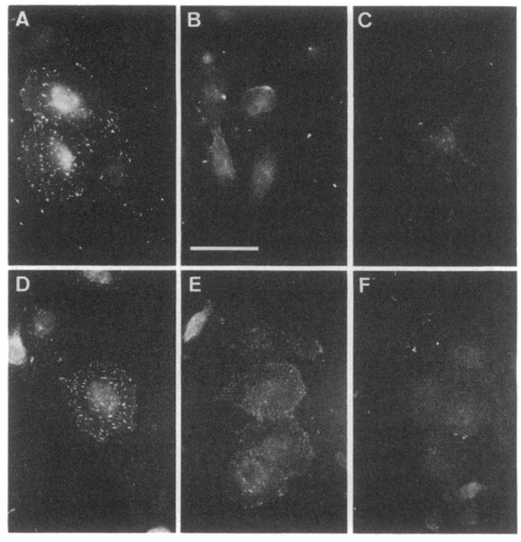

FIG. 4. Immunofluorescence localization of the 135/115-kDa receptor on the endothelial cells spread on a substrate-coated surface. Human endothelial cells were allowed to attach to coverslips coated with either vitronectin (*A*, *E*, and *F*), fibronectin (*B*), vWF (*C*), or Gly-Arg-Gly-Asp-Ser-Pro-Cys (*D*) for 1 hr, fixed, permeabilized (*A–D*), and stained by indirect immunofluorescence using mAb LM142 (*A–E*) or mAb W6/32 (*F*) as a primary antibody. Representative cells spread on each matrix protein were photographed through a Zeiss microscope equipped for epifluorescence. (Bar = 25 μm.)

Arg-Gly-Asp-containing heptapeptide. Third, the 135/115-kDa receptor is specifically recognized by mAbs LM142 (5) and LM609 (unpublished results), which are directed to the vitronectin receptor that is expressed on human melanoma cells. In addition, these mAbs also show a high degree of reactivity with endothelial cells present in certain normal and malignant tissues when examined by the immunoperoxidase technique (data not shown). Most significantly, one of these mAbs, LM609, specifically inhibits endothelial cell attachment to vitronectin, vWF, fibrinogen, and a synthetic heptapeptide containing Arg-Gly-Asp. A kinetic analysis of the effects of mAb LM609 on endothelial cell attachment revealed that the most significant effects occurred within the first 60 min. This suggests that either (*i*) mAb LM609 inhibits primarily the initial phase of cell attachment or (*ii*) after 60–90 min at 37°C, endothelial cells begin to express additional receptors that allow them to overcome the effects of antibody pretreatment. mAb LM142 failed to significantly inhibit endothelial cell attachment to any of the adhesion proteins tested, suggesting that its antigenic epitope is not involved in receptor function and that mere binding of an anti-receptor antibody is not sufficient to inhibit receptor function. Although endothelial cells that were preincubated with mAb LM609 were inhibited in their attachment to vitronectin, vWF, and fibrinogen, preattached cells revealed no significant loss in attachment or spread appearance when incubated with this antibody for up to 4 hr. These data suggest that the antigen recognized by this antibody may not be adequately exposed on substrate-attached endothelial cells. Alternatively, it is possible that once cells are attached and spread, they develop multiple adhesive interactions, some of which are not affected by mAb LM609.

The fact that this adhesion receptor is functionally active on the surface of endothelial cells prompted an analysis of its distribution on these cells. Indirect immunofluorescence analysis indicated that once endothelial cells were attached to vitronectin or the Arg-Gly-Asp-containing heptapeptide, the adhesion receptor assumed a distinct focal staining pattern. This was evident only on the focal plane of the cell–

substratum interface on cells that were permeabilized with mild detergent. The requirement of mild permeabilization for optimal staining suggests that mAb LM142 recognizes a receptor epitope close to the lipid bilayer on either an extracellular or cytoplasmic domain. Complete cell removal from the substrate by either EDTA or harsh detergent treatment left behind focally stained, substrate-attached material (data not shown), providing further evidence that the receptor localized at the cell–substratum interface. Receptor clustering also occurred on endothelial cells attached to the Arg-Gly-Asp peptide, suggesting that receptor organization does not require an intact matrix protein. In contrast, cells spread on fibronectin failed to demonstrate the focally organized staining pattern, suggesting that cell contact leading to receptor organization is substrate-dependent as previously observed in studies with human melanoma cells (unpublished results). This observation is not completely unexpected, since previous studies have shown that cell attachment to vitronectin and fibronectin is mediated by distinct receptor complexes whose functional activity is inhibited by the addition of soluble Arg-Gly-Asp-containing peptides (4). It is of interest that partial receptor clustering could be observed when endothelial cells were allowed to attach and spread on vWF and little was detected on cells attached to fibrinogen, suggesting the possibility that mechanism(s) other than Arg-Gly-Asp recognition may account for adhesion to these substrates. In fact, the γ chain of fibrinogen, which does not contain an Arg-Gly-Asp sequence, has been shown to contain cell attachment-promoting activity (24, 25). Alternatively, endothelial cells may utilize these adhesive proteins to migrate on the substratum rather than to establish firm contact. In such a case the substrate may not promote receptor organization on the cell surface.

Arg-Gly-Asp-directed cell adhesion receptors have been described on many normal and malignant cell types (4–13). Human endothelial cells have been shown to express a glycoprotein IIb/IIIa-like receptor (15). In fact, Fitzgerald *et al.* (26) recently demonstrated that human umbilical vein endothelial cells express a polypeptide virtually identical to platelet glycoprotein IIIa, by showing that a translated endothelial cell cDNA contained five sequences that corresponded to the platelet protein. Functionally, IIb/IIIa on platelets was shown to be involved in interactions with fibronectin, vitronectin, vWF, and fibrinogen, and these interactions could be inhibited with Arg-Gly-Asp-containing peptides (13). Evidence provided in this report indicates that the endothelial cell receptor differs to some degree from glycoprotein IIb/IIIa. Specifically, mAbs LM142 and LM609 failed to react with the IIb/IIIa platelet glycoproteins, and anti-IIb/IIIa mAbs failed to recognize the 135/115-kDa complex on endothelial cells. mAbs LM142 (5) and LM609 (unpublished results) were shown to recognize antigen epitopes including the larger (α) subunit of the human melanoma vitronectin receptor. Functionally, the IIb/IIIa glycoproteins can recognize fibronectin, whereas the receptor described in this report has no apparent interactions with this molecule. Thus, the 135/115-kDa receptor on endothelial cells appears functionally and antigenically distinct from the IIb/IIIa receptor on platelets and probably is more closely related to the vitronectin receptor expressed by human melanoma cells, even though previous studies (26) demonstrated that both receptors share a common β subunit.

In summary, human endothelial cells synthesize and express a 135/115-kDa heterodimeric receptor complex that is a member of a large family of cell adhesion receptors capable of recognizing the sequence Arg-Gly-Asp. This receptor appears to be directly involved in the attachment and spreading of human endothelial cells on vWF, fibrinogen, and vitronectin. Further studies on the molecular interactions between this receptor complex and these adhesive proteins may lead to a better overall understanding of events leading to blood coagulation, wound healing, and vascular proliferation.

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