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Isolation and characterization of a cell surface albumin-binding protein from vascular endothelial cells

(endothelial cell surface/gp60)

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ABSTRACT **Albumin-binding proteins identified in vascular endothelial cells have been postulated to contribute to the transport of albumin via a process involving transcytosis. In the present study, we have purified and characterized a 57- to 60-kDa (gp60) putative albumin-binding protein from bovine pulmonary microvessel endothelial cells. The endothelial cell membranes were isolated from cultured cells by differential centrifugation and solubilized with sodium cholate and urea. The solubilized extract was concentrated after dialysis by ethanol precipitation and reextracted with Triton X-100, and the resulting extract was subjected to DEAE-cellulose column chromatography. Proteins eluted from this column were further separated using preparative sodium dodecyl sulfate/polyacrylamide gel electrophoresis and used for immunizing rabbits. Fluorescence-activated cell sorter analysis using the anti-gp60 antibodies demonstrated the expression of gp60 on the endothelial cell surface. Affinity-purified anti-gp60 antibodies inhibited ≈90% of the specific binding of ^{125}I-labeled albumin to bovine pulmonary microvessel endothelial cell surface. The anti-gp60 antibodies reacted with gp60 from bovine pulmonary artery, bovine pulmonary microvessel, human umbilical vein, and rat lung endothelial cell membranes. Bovine anti-gp60 antibodies also reacted with bovine secreted protein, acidic and rich in cysteine (SPARC). However, bovine SPARC NH_2-terminal sequence (1–56 residues) antibodies did not react with gp60, indicating that the endothelial cell-surface-associated albumin-binding protein gp60 was different from the secreted albumin-binding protein SPARC. We conclude that the endothelial cell-surface-associated gp60 mediates the specific binding of native albumin to endothelial cells and thus may regulate the uptake of albumin and its transcytosis.**

The functions of albumin include the delivery of bound ligands such as hormones and fatty acids and the maintenance of vascular integrity and transvascular oncotic pressure gradient (1–4). A number of morphological studies have indicated that transcytosis of albumin in vascular endothelial cells can occur by a receptor-mediated process (5–8). Some studies have suggested that this is a predominant mode of albumin transport (6–8). Three major albumin-binding proteins (ABPs; 18, 31, and 56–60 kDa) have been identified in vascular endothelial cells using the ligand-blotting, photochemical cross-linking, and lectin-binding assays (8–10). Recent studies have shown that 18- and 31-kDa polypeptides present in vascular endothelial cells may be similar to the scavenger receptors identified on other cell types (11, 12). Schnitzer *et al.* (10) showed specific binding of native albumin with a 56- to 60-kDa (gp60) polypeptide in rat microvascular endothelial cells using lectin-binding analysis. Lectins such as *Limax flavus* agglutinin and *Ricinus communis* agglutinin (RCA) inhibited albumin binding and precipitated gp60 from rat microvascular endothelial cells (10). Studies from our laboratory showed that transendothelial albumin transport was inhibited by 40% in the presence of RCA but not by control lectins applied to bovine pulmonary artery endothelial cell (BPAEC) monolayers (13). RCA also precipitated gp60 from BPAECs (13). These results suggest a role for gp60 in albumin transport. Studies have shown that α-glycophorin (14) and secreted protein, acidic and rich in cysteine (SPARC), antibodies (15) also cross-react with gp60. We report the purification and characterization of gp60 from cultured vascular endothelial cells. Some of these observations have been elsewhere reported in an abstract (16).

MATERIALS AND METHODS

Materials. Dulbecco's modified essential medium (DMEM) and fetal bovine serum (FBS) were from GIBCO. Globulin-free bovine serum albumin (BSA), phenylmethylsulfonyl fluoride (PMSF), and protein A-Sepharose were from Sigma. Sodium cholate and Triton X-100 were from Calbiochem. DEAE-cellulose was from Whatman. All electrophoretic chemicals were from Bio-Rad. Polyclonal antibodies raised against BSA were from ICN. LF-BON-1 antiserum (anti-bovine SPARC) and LF-56 antiserum (against the NH_2-terminal amino acid residues 1–56 of bovine SPARC) were generous gifts from L. W. Fisher (National Institutes of Health, Bethesda, MD). Preparation of LF-56 antiserum has been described (17).

Cell Culture. Bovine pulmonary microvessel endothelial cells (BPMVECs), BPAECs, and rat pulmonary artery endothelial cells were isolated and cultured as described (18, 19). Human pulmonary artery endothelial cells (HPAECs) and human umbilical vein endothelial cells (HUVECs) were from Clonetics (San Diego). HUVECs were grown in RPMI 1640 medium supplemented with 10% FBS, 90 μg of heparin per ml, 2 mM L-glutamine, and 30 μg of endothelial cell growth factor per ml. HPAECs were grown in MDCB 131 medium supplemented with 10% FBS, 10 ng of human epidermal growth factor per ml, and 1 μg of hydrocortisone per ml. For isolating endothelial cell membranes, endothelial cells were cultured in 850-cm^2 roller bottles. To each roller bottle, 75 ml of culture medium was added and filled with an air/CO_2 mixture. The cells were then transferred to a 37°C roller bottle incubator and allowed to grow for 10–12 days.

Endothelial Cell Membrane Isolation. BPMVECs grown in roller bottles were washed twice with phosphate-buffered saline (PBS). Cells were scraped from roller bottles, suspended

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Abbreviations: BPMVEC, bovine pulmonary microvessel endothelial cell; BPAEC, bovine pulmonary artery endothelial cell; HPAEC, human pulmonary artery endothelial cell; HUVEC, human umbilical vein endothelial cell; ABP, albumin-binding protein; BSA, bovine serum albumin; SPARC, secreted protein, acidic and rich in cysteine; FACS, fluorescence-activated cell sorter; PVDF, poly(vinylidene difluoride).

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in buffer A (20 mM Hepes/Tris/0.15 M NaCl/0.1 mM PMSF, pH 7.4), and washed twice by centrifugation (700 × *g*, 10 min). Cells from six to eight roller bottles were suspended in 75 ml of buffer A and homogenized using a Polytron for 2 min at full speed. The homogenate was centrifuged (3000 × *g*, 10 min). The supernatant was collected and centrifuged (100,000 × *g*, 60 min). The pellet obtained was then suspended in buffer A and recentrifuged (100,000 × *g*, 60 min). The final membrane pellet was suspended in a small volume of buffer A containing 0.2 mM EDTA. The protein concentration of the membrane was determined; the preparation was stored at −70°C until further use.

Ligand Blotting. Endothelial cell membranes were preincubated with 1 mM PMSF/0.5 mM EDTA (20 min, 22°C), solubilized by mixing with 1.5 vol of solubilizing buffer (9 M urea/2% SDS/2% 2-mercaptoethanol/0.1 M Tris/0.02% bromophenol blue, pH 6.8), and incubated at 22°C for 30 min. The solubilized proteins were separated by SDS/PAGE according to Laemmli (20) using the slab-gel electrophoretic system (3% acrylamide in the stacking gel, 10% acrylamide in the separating gel). After electrophoresis, the proteins were transferred to either a poly(vinylidene difluoride) (PVDF) or a nitrocellulose membrane. The nonspecific binding was blocked by incubating the membrane with 5 mM $CaCl_2$ in TBS (20 mM Tris/0.5 M NaCl, pH 7.5) for 10 min and then with 0.5% Tween 20 in TBS overnight. After this step, the membrane was washed and cut into two strips. One strip was incubated with 0.6 mg of globulin-free BSA per ml in TBS containing 1.5% gelatin for 2 hr, and the other strip was incubated without BSA. The strips were washed and incubated with anti-bovine BSA antibodies for 60 min in TBS containing 1.5% gelatin. The membranes were then washed twice and incubated with a second antibody (goat anti-rabbit IgG) conjugated with alkaline phosphatase. The protein bands were localized after adding 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium salt.

We also used ^{125}I-labeled monomeric BSA (^{125}I-BSA) in ligand-blotting experiments to identify gp60. In this case, nonspecific binding was blocked with bovine γ-globulin (BSA free, 2 mg/ml in TBS) and then incubated with ^{125}I-BSA (50 μg/ml) for 2 hr. The strips were washed with TBS containing 0.05% Tween 20 and autoradiography was performed.

Purification of gp60. BPMVEC membranes (100 mg) were preincubated with 1 mM PMSF/0.5 mM EDTA (30 min, 22°C). The membranes were solubilized using final concentration of 2.5% sodium cholate and 4 M urea (4°C, 3 hr) with gentle stirring. The protein concentration was adjusted to 4 mg/ml during solubilization. After this treatment, the suspension was centrifuged (100,000 × *g*, 60 min). The supernatant was collected and dialyzed against 5 mM Hepes/Tris (pH 7.2). More than 80% of membrane proteins were recovered in the supernatant. The dialyzed suspension was concentrated by 60% ethanol precipitation at 4°C. The ethanol precipitate was collected by centrifugation (10,000 × *g*, 30 min, 4°C) and suspended in buffer A. This precipitate was solubilized with 2.5% Triton X-100 (overnight, 4°C) with gentle stirring. The suspension was centrifuged (100,000 × *g*, 60 min). The supernatant was collected and dialyzed against 4 liters of buffer B (50 mM Tris·HCl/0.2 mM EDTA/0.15% Triton X-100/0.1 mM PMSF, pH 8.0). The dialyzed extract was applied on a DEAE-cellulose column (10 × 13 cm), which was previously equilibrated with buffer B. The column was washed with 50 ml of buffer B and bound proteins were eluted from the column with 80 ml of a 0–500 mM linear NaCl gradient in buffer B at a flow rate of 15 ml/hr. The fractions from individual peaks were pooled separately and concentrated by 50% acetone precipitation; the precipitate was used for ligand blotting. Only peak 1 showed albumin-binding activity. The proteins present in peak 1 were further separated by using preparative SDS/PAGE (16 cm × 16 cm, 3-mm thick slab gel), and the gp60 eluted from the gel was used for further studies.

Antibody Production and Purification. The gp60 eluted from preparative SDS/PAGE was used to immunize rabbits. Approximately 50 μg of protein (per rabbit) was injected i.m. after mixing with an equal volume of Freund's complete adjuvant (21). A second injection was given after 4 weeks. Rabbits were bled at 2 weeks after the second injection, and the immune response was checked. The preimmune serum IgG and the anti-gp60-IgG were purified using a protein A-Sepharose column (21).

Fluorescence-Activated Cell Sorter (FACS) Analysis. Confluent endothelial cell monolayers were washed with serum-free medium and incubated with the same medium for 2 hr. After this incubation, cells were washed with PBS and detached by incubating with nonenzymatic cell dissociation medium (Sigma) (10–30 min, 37°C). Nonspecific binding was blocked by incubating the cells with 10% horse serum in PBS (60 min on ice). Cells (10^6 per tube) were incubated with either gp60 antiserum or preimmune serum (1:10 diluted) (60 min, 4°C), washed, and treated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG for 30 min. After washing, the cells were fixed with 1% paraformaldehyde and analyzed with an Ortho Cytoron Absolute Flow Cytometer (Ortho Diagnostic). The mean logarithmic fluorescence intensity for each sample was determined and converted into linear relative fluorescence units (ΔFL) by the formula: $\Delta FL = 10^{(E \times 0.0137)}$, where E is the mean channel fluorescence intensity (22).

Immunoblotting. Endothelial cell membranes were subjected to SDS/PAGE and electrophoretically transferred to nitrocellulose or PVDF membrane. Nonspecific binding was blocked with either 3% gelatin or 5% nonfat dry milk in TBS (5 hr, 22°C). The membrane was washed twice with 0.05% Tween-20 in TBS and incubated with antibodies diluted in TBS containing 1% gelatin. Incubation was carried out for 4–6 hr; the mixture was washed and then incubated for 60 min with the second antibody (goat anti-rabbit IgG conjugated to alkaline phosphatase). After incubation, the membranes were washed twice and the protein bands were localized as described above. Molecular masses of the proteins were determined using known marker proteins.

^{125}I-Labeled Albumin-Binding Studies. BPMVECs were seeded (3 × 10^5 per well) in six-well Corning tissue culture plates and grown to confluence. The monolayers were washed twice with DMEM and incubated with DMEM for 20–24 hr in a cell culture incubator. After incubation, the monolayers were washed twice with binding buffer (10 mM Hepes/DMEM, pH 7.4) and binding was initiated by adding 1 ml of 1 μg of ^{125}I-BSA in binding buffer. Incubation was carried out at 4°C for 60 min. Binding was terminated by washing the monolayer three times with the binding buffer. Radioactivity associated with the monolayer was determined after lysing the cells with 1 M NaOH (23). Nonspecific binding was determined by the inclusion of unlabeled BSA (40 mg/ml) during the binding procedure (10–12). The test components, preimmune-IgG and the anti-gp60-IgG, were preincubated 30 min with the monolayer prior to the addition of ^{125}I-BSA.

RESULTS

Identification of Native ABPs. We first isolated endothelial cell membranes from BPMVECs by differential centrifugation and the ABP present in this membrane fraction were identified using ligand blotting (see *Materials and Methods*). The BSA-binding regions were identified using polyclonal antibodies raised against native BSA. In the absence of exposure of the membrane strip to native BSA, the anti-BSA antibodies recognized only a 67-kDa polypeptide (Fig. 1*A*, lane 1), indicating that albumin binds to endothelial membranes. However, when the strip was treated with BSA, the anti-BSA antibodies reacted with three distinct polypeptides: 110, 57–60, and 18 kDa (Fig. 1*A*, lane 2). Of these, the antibodies reacted most

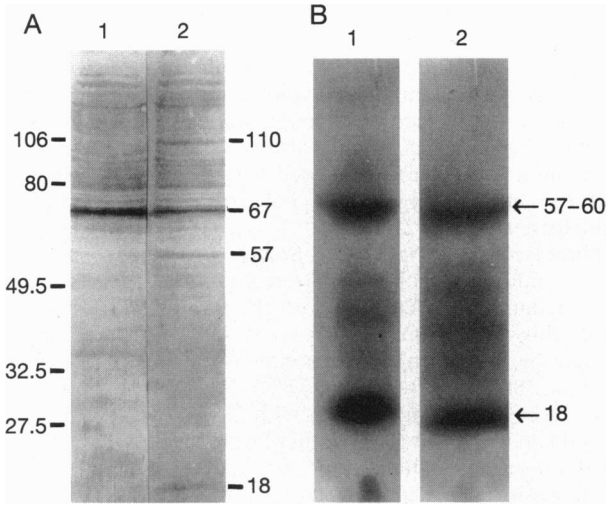

FIG. 1. (*A*) Identification of ABP in BPMVEC membranes using native albumin. BPMVEC membranes (100 μg of protein) were separated on SDS/PAGE and transferred to PVDF membrane strips. Lane 1, control (not treated with albumin); lane 2, incubation with albumin. Positions of known molecular mass markers are indicated in kDa. Results are representative of three separate experiments. (*B*) Identification of ABP in endothelial cell membranes using ^{125}I-labeled albumin. Lane 1, BPMVEC; lane 2, BPAEC.

intensely with the 57- to 60-kDa protein, indicating that gp60 is the major native ABP.

We also used ^{125}I-BSA to identify ABP in ligand-blotting experiments and found the specific interaction of ^{125}I-BSA with the 60- and 18-kDa polypeptides present in endothelial cell membranes (Fig. 1*B*).

Isolation of gp60. Since native albumin bound primarily to gp60, we next developed a method for the isolation of gp60 from BPMVEC membranes. Ligand blotting was employed to assess the presence of this protein during purification. BPMVEC membranes were initially solubilized with 2.5% sodium cholate/4 M urea, and the extract was dialyzed and concentrated by 60% ethanol precipitation. This precipitate was reextracted with Triton X-100. The Triton X-100-solubilized extract was chromatographed on the DEAE column, and the bound proteins were eluted with a linear NaCl gradient (0–500 mM). The proteins were eluted as three peaks. The fractions from each peak were pooled and screened for albumin binding using the ligand blotting assay. Only peak 1 showed albumin-binding activity (Fig. 2). Fig. 3 shows the SDS electrophoretic profile of proteins from native BPMVEC membrane (lane 1) and DEAE column peak 1 (lane 2) after staining with Coomassie brilliant blue R-250. The presence of 57- to 60-kDa protein corresponding to albumin binding was observed with ligand blotting in native membranes and DEAE peak I. SDS/PAGE was also performed under nonreducing conditions (in absence of 2-mercaptoethanol) and albumin binding was observed only with the 57- to 60-kDa region, suggesting that gp60 existed as a single polypeptide. We further purified gp60 using preparative SDS/PAGE, and the eluted protein from the gel was used for antibody preparation.

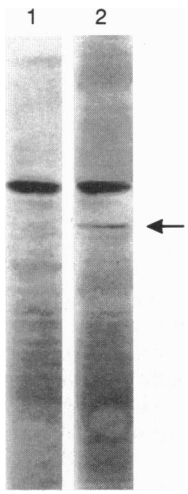

FIG. 2. Identification of gp60 from proteins eluted in the DEAE column. Proteins (50 μg) from peak 1 were subjected to SDS/PAGE and transferred to a PVDF membrane. Ligand blotting was performed using native albumin. Lane 1, control (not treated with albumin); lane 2, incubation with albumin. The arrow indicates the position of gp60. The wider band above the arrow is BSA eluted from the column.

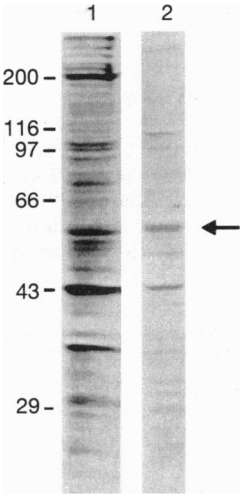

FIG. 3. SDS/PAGE profile of proteins. Lane 1, BPMVEC membrane; lane 2, DEAE peak 1. The arrow indicates the position of gp60. BPMVEC membranes (100 μg of protein) and DEAE peak 1 (50 μg of protein) were separated on SDS/PAGE and stained with Coomassie brilliant blue R-250. Molecular masses of the proteins were determined using known molecular mass marker proteins.

Cell Surface Expression of gp60. We nonenzymatically detached endothelial cells and incubated the cells with either gp60 antiserum or preimmune serum and carried out FACS analysis to investigate the cell surface expression of gp60 on endothelial cells. The patterns of preimmune and gp60 antiserum binding to different endothelial cell types are shown in Fig. 4. Data are plotted as log fluorescence intensity (in arbitrary units) against cell number. The gp60 antiserum-specific ΔFL for each cell type was calculated by subtracting preimmune serum ΔFL. The gp60-specific ΔFL values ranged from 10 to 12 units for BPMVEC and BPAEC; values ranged from 2 to 5.4 units for HUVEC and HPAEC.

Effect of Anti-gp60 IgG on Binding of ^{125}I-BSA to BPMVEC Monolayers. Preimmune serum IgG and anti-gp60 IgG were affinity purified using a protein A-Sepharose column. We investigated the influence of IgG fractions on binding of ^{125}I-BSA to BPMVEC monolayers at 4°C (Fig. 5). Endothelial cell monolayers were extensively washed with serum-free medium and then used for ^{125}I-BSA binding. Nonspecific

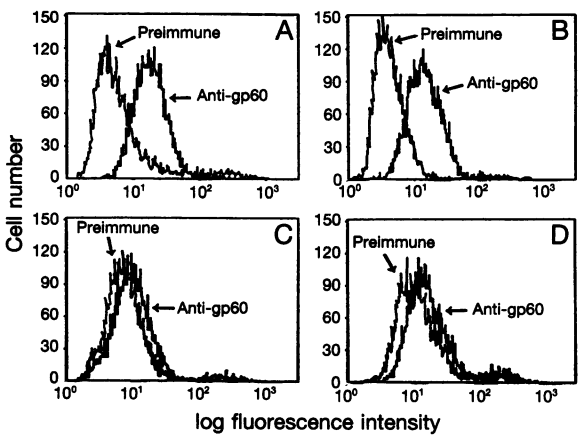

FIG. 4. FACS analysis of endothelial cells. Endothelial cells were detached nonenzymatically and incubated with either preimmune serum or gp60 antiserum and used for analysis. (*A*) BPMVECs; (*B*) BPAECs; (*C*) HUVECs; (*D*) HPAECs.

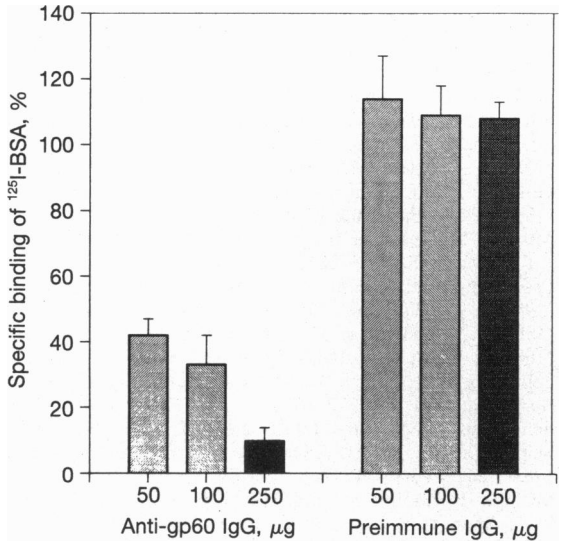

FIG. 5. Effect of anti-gp60 IgG and preimmune serum IgG on the binding of ^{125}I-BSA to BPMVEC monolayer at 4°C. Results are means ± SEM of three separate experiments carried out in a triplicate binding assay.

binding ranged from 30% to 40%. Preimmune serum IgG did not affect the specific binding of ^{125}I-BSA to the BPMVEC monolayers. In contrast, anti-gp60 IgG inhibited the specific binding of ^{125}I-BSA to BPMVEC monolayers in a dose-dependent manner. The inhibition was ≈90% at 250 μg of antibody per ml.

Immunoblotting of Endothelial Cell Membranes with Anti-gp60 Antibodies. BPMVEC and BPAEC membrane proteins were separated by using SDS/PAGE and transferred to nitrocellulose strips. The strips were immunoblotted with the gp60 antiserum (Fig. 6). Preimmune serum (Fig. 6, lanes 1 and 3) did not recognize any proteins from BPMVEC and BPAEC membranes. Anti-gp60 antibodies recognized two major proteins (57–60 and 36 kDa) and one minor protein (43 kDa) in both membrane preparations (Fig. 6, lanes 2 and 4). The anti-gp60 antibodies did not react with 18- and 31-kDa polypeptides, suggesting that 18- and 31-kDa ABPs are structurally different from gp60. To test the immunological cross-reactivity of bovine anti-gp60 antibodies with other species, we carried out immunoblotting experiments with human umbilical vein and rat lung endothelial cell membranes. The antibodies recognized human and rat endothelial cell membrane gp60 (Fig. 6, lanes 5 and 6).

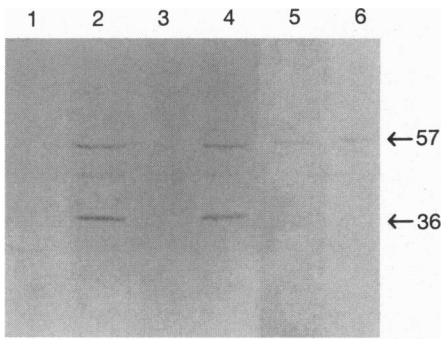

FIG. 6. Immunoblotting of endothelial cell membrane proteins with anti-gp60 antibodies. Endothelial cell membrane proteins (100 μg) were separated on SDS/PAGE and transferred to nitrocellulose membrane strips. Nonspecific binding was blocked with 5% nonfat dry milk in TBS. Antiserum and preimmune serum were diluted in blocking solution and incubated 3–4 hr at 4°C, washed, and treated with goat anti-rabbit IgG conjugated with alkaline phosphatase. Lanes 1 and 2, BPMVEC membranes; lanes 3 and 4, BPAEC membranes; lane 5, HUVEC membranes; lane 6, rat lung endothelial cell membranes. Lanes 1 and 3 were treated with preimmune serum; lanes 2, 4, 5, and 6 were incubated with gp60 antiserum. Positions of gp60 and 36-kDa protein bands are indicated by arrows. The protein band between gp60 and 36 kDa is 43-kDa protein. Results are representative of three separate experiments.

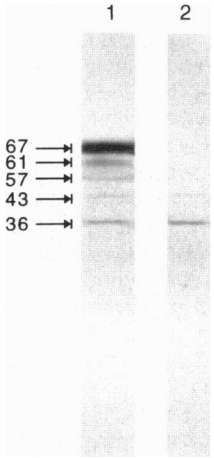

FIG. 7. Immunoblotting of BPMVEC membrane with anti-bovine SPARC and LF-56 antibodies. The antibodies, anti-bovine SPARC (1:1000) antiserum (lane 1) and LF-56 antiserum (1:300) (lane 2), were diluted and incubated with membrane strips for 5 hr. Other details are as described in the legend to Fig. 6. Results are representative of three separate experiments.

gp60 and SPARC. To study the proposed structural relationship between the endothelial membrane-associated and secreted ABPs, we carried out immunoblotting of BPMVEC membranes with the antibodies raised against purified bovine SPARC (Fig. 7). The antibodies raised against purified bovine SPARC recognized 67-, 61-, 57- to 60-, 43-, and 36-kDa polypeptides in BPMVEC membranes (Fig. 7, lane 1). The bovine anti-SPARC NH_2-terminal peptide antibodies reacted with 36- and 43-kDa polypeptides (Fig. 7, lane 2). We radiolabeled the endothelial cell surface with ^{125}I and immunoprecipitated the endothelial cell lysates using anti-gp60 antibodies (13) to study whether SPARC was associated with the endothelial cell surface. The anti-gp60 antibodies precipitated only the 57- to 60-kDa polypeptide (data not shown), suggesting that SPARC was not cell surface associated.

DISCUSSION

High-affinity binding sites for modified albumin (e.g., formaldehyde-treated albumin) have been reported in liver sinusoidal and renal plasma membrane preparations (24, 25). Receptors for these modified albumin molecules have been purified from liver plasma membrane preparations (26) and shown to bind specifically to the modified molecules but not to the native albumin (27). These ABPs may belong to a family of scavenger receptors identified in macrophages or macrophage-derived cells (27–29). This conclusion has recently been confirmed by a ligand-blotting experiment showing the existence of 18- and 31-kDa scavenger receptors for modified albumins on vascular endothelial cell surface (12). The receptors are also expressed in fibroblasts and smooth muscle cells. It has been suggested that the binding of modified albumins (i.e., formaldehyde- or maleic anhydride-treated albumin and albumin–gold complex) to these receptors may initiate endocytosis, and the ligand may be subsequently degraded by lysosomal proteases (11). The native monomeric albumin can also react with these binding proteins in endothelial cells (5, 9). Therefore, the ABPs are postulated to play a role in endocytosis and transcytosis of albumin in various cells. In the case of endothelial cells, ABPs may activate transcellular pathways and thereby contribute to albumin transcytosis (5, 9, 30).

In the present study, we isolated membranes from BPMVECs and used these for identification and isolation of gp60. Using the ligand-blotting technique, we demonstrated the direct interaction of the native albumin with gp60 in vascular

endothelial cell membranes (Fig. 1) and with the purified gp60 (Fig. 2). This finding is in close agreement with previous work using lectin-binding analysis, which showed that native albumin interacts with gp60 on rat endothelial cell surface (10, 31).

We prepared antibodies against the bovine gp60 to investigate characteristics of this protein in endothelial cells. We performed FACS analysis of endothelial cells using anti-gp60 antibodies. gp60 was expressed in vascular endothelial cells (Fig. 4) and the surface expression was greater in BPMVECs and BPAECs than in HUVECs and HPAECs. Affinity-purified anti-gp60 antibodies significantly reduced the specific binding of ^{125}I-BSA to BPMVEC monolayer, whereas preimmune serum IgG had no effect on the binding of ^{125}I-BSA to the cell monolayers (Fig. 5). These results demonstrate that the antibodies developed against gp60 specifically recognize the native albumin-binding sites on the endothelial cell surface.

We performed immunoblotting experiments with endothelial cell membranes to study the specificity of the anti-gp60 antibodies. The antibodies recognized two major polypeptides, 57–60 and 36 kDa, and one minor polypeptide, 43 kDa (Fig. 6), in endothelial cell membrane proteins. That the antibodies recognized only these proteins suggests that gp60 was purified to an apparent homogeneity. To test immunological cross-reactivity of bovine anti-gp60 antibodies with other species, we carried out immunoblotting experiments using human and rat endothelial cell membranes (Fig. 6). These results indicated that the bovine anti-gp60 antibodies reacted with human and rat endothelial cell membranes.

Studies have shown immunological cross-reactivity of anti-SPARC antibodies and SPARC peptide-based antibodies with the endothelial cell membrane-associated gp60 (15, 31). SPARC is secreted into the culture medium by endothelial cells and other cell types (32). SPARC is identical to the bone noncollagenous protein osteonectin (33, 34). The molecular size of this protein ranges from 36 to 43 kDa and it binds to albumin and extracellular matrix proteins (32–34). Recent studies have shown specific interaction of SPARC with endothelial cells (35). We carried out immunoblotting of bovine endothelial cell membranes using the antibodies raised against purified bovine SPARC and the NH_2-terminal sequence (1–56 residues) of bovine SPARC. The bovine anti-SPARC antibodies recognized many polypeptides, including gp60 in BPMVEC membranes (Fig. 7). The bovine NH_2-terminal SPARC peptide antibodies recognized only the 36- and 43-kDa polypeptides. It did not react with other endothelial membrane proteins, indicating that the membrane-associated gp60 was different from SPARC and that SPARC did not derive from gp60. The 36- and 43-kDa polypeptides identified (Fig. 6) in BPMVEC membranes using anti-gp60 antibodies may be due to the presence of SPARC associated with endothelial cell matrix proteins (35). The anti-gp60 antibodies also did not recognize the 18- and 31-kDa polypeptides [proposed as the scavenger receptors in endothelial cells (11, 12)], suggesting that scavenger receptors are structurally different from native albumin receptor, gp60.

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