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Research Article

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A Human Endothelial Cell Membrane Protein that Binds *Staphylococcus aureus* In Vitro

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Abstract

We have investigated *S. aureus* adherence to human endothelial cells utilizing an in vitro model. Staphylococcus binding to confluent endothelial cell monolayers was saturable in both dose and time response studies suggesting that the binding interaction was specific. We have developed a technique, based on the pH dependent affinity of iminobiotin for streptavidin, for the isolation of an endothelial cell membrane component that binds *S. aureus*, in vitro. A 50-kD membrane component was isolated and purified using this approach. This component was trypsin sensitive, periodate insensitive, and did not label with [^{3}H]glucosamine. [^{35}S]Methionine and [^{125}I]iodine labeling confirmed that the protein was synthesized by and expressed on the endothelial cell surface. Functional binding studies demonstrated that staphylococci, but not endothelial cells, bound to the protein when immobilized on microtiter wells. Preincubation of staphylococci with the purified protein significantly ($P < 0.001$) reduced staphylococcal binding to cultured endothelial cells. The capacity of *S. aureus* to colonize and invade endovascular surfaces may in part be a consequence of staphylococcal interaction with this endothelial cell membrane protein. (*J. Clin. Invest.* 1990. 85:1248–1254.) *Staphylococcus aureus* • endothelial cells • adherence • endocarditis

Introduction

Staphylococcus aureus is well recognized as an invasive pathogen capable of causing such devastating diseases as endocarditis, shock, or metastatic suppurative collections (1–2). The morbidity and mortality of endovascular staphylococcal infections remains significant despite the availability of effective antimicrobial agents (1–4). The basis for this invasive potential is not clearly understood. One important pathogenetic mechanism that has received limited attention is the ability of staphylococci to colonize host tissue surfaces. Several studies have demonstrated the capacity of *S. aureus* to adhere to connective tissue glycoproteins such as fibronectin, collagen, and laminin. These components have been recognized as potentially important receptors for bacterial adhesion in the presence of tissue injury (5–8). However, in the absence of antecedent injury, as is often the case in acute infective endocarditis or staphylococcal bacteremia, the initial adhesion interaction is with the endothelial cell surface (3, 9). Staphylococci are capable of adhering to and penetrating endothelial cells in greater numbers than other bacterial species (10–15). This affinity for endothelial cell tissue may be a consequence of adhesin-receptor interactions that occur between the bacteria and the endothelial cell surface (10, 12, 15).

We have developed a procedure for the identification and isolation of endothelial cell surface components that bind *S. aureus*. This approach involves the adherence of staphylococci to detergent-solubilized, 2-iminobiotin-labeled endothelial cell membranes and their subsequent purification by streptavidin agarose affinity chromatography. In the present study, we describe the isolation of a 50-kD endothelial cell membrane component which binds and facilitates *S. aureus* attachment to endothelial cells, in vitro.

Methods

Endothelial cell tissue culture conditions

Human endothelial cells were harvested from umbilical veins and maintained in tissue culture as previously described (12, 16). The culture media contained Medium 199 (M199, Gibco Laboratories, Grand Island, NY), supplemented with 15 mM hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes; Calbiochem-Behring, La Jolla, CA), 25 μg/ml of heparin (Sigma Chemical Co., St. Louis, MO), 10% human and 20% newborn calf serum (Gibco), 1.5 mM glutamine (Sigma), and 3 mg/100 ml partially purified acidic fibroblast growth factor extracted from bovine brain (16). The cells were maintained at 37°C and 5.5% CO_2 on 100 mm plates. Cell type was confirmed by the typical cobblestone morphology observed in tissue culture and by the presence of von Willebrand factor antigen. Confluent human endothelial cell monolayers were also prepared in either 24 well tissue culture plates (Corning Glassware, Corning, NY) or in 96 well microtitre plates (Linbro, Flow Laboratories, McLean, VA) coated with 1.5% gelatin (Sigma) substrate.

Bacterial storage and preparation

The primary staphylococcal strain (Wb) used in these studies was a blood culture isolate from a patient with acute infective endocarditis. Other strains used were also blood culture isolates from clinically documented infections. Bacteria were stored and prepared for use as previously described (12). Before a study, the bacteria were resuspended in M199 containing 15 mM Hepes, pH 7.4. The bacterial concentration was initially adjusted by optical density and the actual inoculum determined by viability counts. For the binding assay, bacteria were iodinated ([^{125}I]iodine, Amersham Corp., Arlington Heights, IL) by the lactoperoxidase technique using Enzymobeads (Bio-Rad Laboratories, Richmond, CA) (17).

Infection assay

The infection assay is a modification of a previously described technique (12). Bacteria were added to confluent monolayers of endothe-

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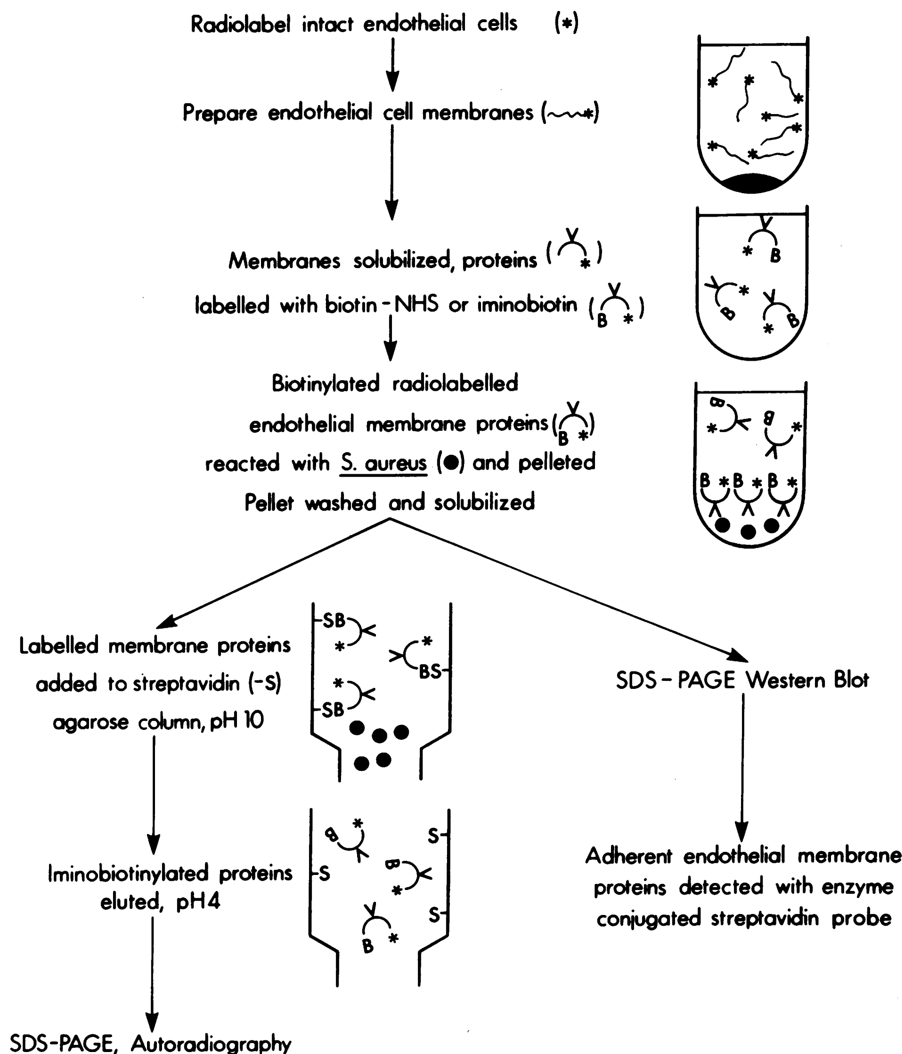

Figure 1. Procedure for the identification and isolation of the endothelial cell membrane protein that binds *S. aureus*.

lial cells in 24 well tissue culture sets and incubated at 4°C. The supernate was then decanted, the monolayer washed and the remaining adherent bacteria removed with the endothelial cells by trypsin. The trypsinate was added to distilled water to disrupt the endothelial cells, serially diluted and plated into heart infusion or Todd Hewitt agar (Difco Laboratories, Detroit, MI). After a 48-h incubation at 37°C the number of colonies were counted. The experiment was performed at 4°C to measure adherence in the absence of endocytosis. The number of endothelial cells/well was determined for each well set to assure uniformity of numbers. The results were expressed as the number of adherent bacteria/well or bacteria/endothelial cell.

Identification and isolation of the endothelial cell membrane protein

The methods for this isolation procedure are illustrated in Fig. 1.

Preparation of solubilized endothelial cell membranes. Confluent human umbilical vein endothelial cells were detached from the substrate after a single wash with 15 mM Hepes, pH 7.4, using a rubber policeman. They were then washed, pelleted, and resuspended in 5 mM Hepes, pH 7.4, containing 250 mM sucrose and mechanically disrupted on ice. The completeness of cellular disruption was monitored microscopically. Nuclei were collected by a brief spin (200 *g*, 30 s) and the remaining membrane enriched supernatant transferred to a Corex tube and centrifuged (27,000 *g*, 20 min, 4°C). The enriched membrane pellet was resuspended in a solubilization buffer consisting of 10 mM sodium borate, pH 7, containing 1% Triton X-100, 10 mM benzamidine, 1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM iodoacetamide, and 1 mM PMSF, and left overnight at 4°C. The next morning the sample was centrifuged (15,000 *g*, 4°C, 20 min) and the supernatant collected. Protein determination (Bicinchoninic acid protein assay reagent, Pierce Chemical Co., Rockville, IL) of an aliquot from the supernatant and the initial membrane preparation demonstrated 60–80% solubilization.

Biotinylation of endothelial cell membrane proteins. The solubilized membrane proteins were biotinylated with 50 mM Biotin-*N*-hydroxy-succinimide ester (NHS),[1] (Calbiochem-Berhing Diagnostics) in dimethysulfoxide. The proteins were incubated with biotin-NHS (final concentration 5 mM) for 2 h on ice. The excess biotin was removed by membrane dialysis (18).

Identification of biotinylated endothelial cell membrane proteins which bind S. aureus. The staphylococcal strain (Wb) was adjusted to a density of 10^9 CFU/ml in M199, pH 7.4 containing 15 mM Hepes, 1 M NaCl, 0.1% bovine serum albumin (BSA), 1 mM PMSF, 1 mM iodoacetamide, 1 mM EDTA, and 10 mM benzamidine. Biotinylated membranes were incubated with bacteria for 2 h on ice. The suspension was centrifuged and washed two times. The washed pellet, which contained bacteria and adherent biotinylated endothelial membrane proteins, was dissolved in 2% SDS in 10 mM Tris, pH 6.8, 20% glyc-

1. *Abbreviations used in this paper:* ECM, extracellular matrix; NHS, *N*-hydroxy-succinimide; PVDF, polyvinylidene difluoride.

erol, 0.004% bromophenol blue. The solubilized proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (19). The membrane proteins were blotted to polyvinylidene difluoride (PVDF; Millipore Corp., Bedford, MA) using a Trans-Blot Cell (Bio-Rad Laboratories) (20) and the excess binding sites blocked with 10% milk (Carnation Co., Los Angeles, CA) in 10 mM Tris, pH 7.4, 0.9% NaCl for 2 h at 37°C. The biotinylated membrane proteins that bound to *S. aureus* were identified with a streptavidin-alkaline phosphatase conjugate (Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, MD) and the color developed with the chromogen 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt and nitroblue tetrazolium chloride (BRL).

Isolation of the endothelial cell membrane protein which binds S. aureus. In the isolation procedure, 2-iminobiotin-NHS (50 mM; Pierce Chemical Co.) which has a pH dependent affinity for streptavidin, was used to label endothelial cell membranes (21, 22). The solubilized iminobiotinylated membrane proteins were incubated with staphylococci for 2 h on ice as described for biotin-NHS. The suspension was centrifuged and the pellet containing *S. aureus* and the endothelial cell membrane protein was solubilized in either 4% Triton X-100, 50 mM borate buffer, pH 8 containing 1 mM PMSF, 10 mM benzamidine, 1 M NaCl and 1 mM EDTA or a 2% SDS buffer overnight. The material was then centrifuged at 10,000 *g* for 20 min and the supernatant collected. The pH of the supernatant was raised to 10 and then added to a 2-ml streptavidin-agarose column (Pierce). The column was washed with 10 column volumes of 50 mM sodium borate, pH 10 containing 1 M NaCl, 0.1% Triton X-100 and 1 mM PMSF to remove any contaminating bacterial material. The bound iminobiotin-labeled material was then eluted with 50 mM ammonium acetate, pH 4, containing 1 M NaCl, 0.1% Triton and 1 mM PMSF.

Characterization of the endothelial cell membrane component. Endothelial cells were metabolically labeled for 48 h in the presence of either [^{35}S]methionine (New England Nuclear, DuPont, Wilmington, DE), 167 μCi/100 mM plate or [^{3}H]glucosamine (Amersham Corp.), 125 μCi/100 mM plate.

Cells were also iodinated to demonstrate that the protein was a surface component. For these studies, the cells were detached from the substrate with 1 mM ethyleneglycol-bis (β-amino-ethyl ether) *N,N'*-tetra acetic acid (EGTA) (Fisher Scientific Co., Fair Lawn, NJ) in Hepes buffer to avoid cellular disruption. The harvested cells were pelleted and then iodinated (Amersham) by the lactoperoxidase technique with Enzymobeads. Enzymatic treatment of the solubilized membrane component was performed in order to determine if it was sensitive to trypsin or periodate. Iodinated membranes were incubated for 1.5 h at 37°C with 0.02 M sodium periodate (Sigma) or 1 mg/ml trypsin (Sigma) following incubation with staphylococci in the isolation procedure.

Autoradiography of radiolabeled endothelial cell membrane proteins was performed using Kodak X-Omat AR film and a DuPont Lightening Plus intensifying screen at −70°C.

The purified protein was probed with anti-fibronectin (BRL) and anti-bovine serum albumin (Cappel Laboratories, Westchester, PA) antibody by slot blot analysis. The fractions collected from the affinity column were applied to nitrocellulose membranes (BA 85; Schleicher & Schuell, Inc., Keene, NH) in a 96-well Minifold II apparatus (Schleicher & Schuell) (23). Control dilutions of purified fibronectin (10 μg/ml) and BSA (7.5 μg/ml) were applied. After 0.5 h, the nitrocellulose was removed from the apparatus and remaining sites blocked with 2% milk. The antibodies (1:1,000 dilution) were then added for 1.5 h. The blot was washed and probed with anti-rabbit, alkaline phosphatase-conjugated antibody (BRL) which was developed using a chromogenic substrate (BRL).

Preparation of endothelial cell conditioned, complete serum-free medium and complexes. To determine if the protein that binds *S. aureus* was released into the media, endothelial cell conditioned, serum free media was prepared as previously described (24). 4–6 d after the umbilical vein endothelial cells were split, the proliferating cells were washed twice with M199, 15 mM Hepes. The cells were then incubated in M199 containing 1.6 mM glutamine, 15 mM Hepes, 5 μg of the partially purified growth factor, 100 U/ml penicillin, and 50 μg/ml streptomycin (complete serum-free medium) for 20 h at 37°C.

Complexes were isolated from the conditioned serum free media as described (24). The medium was removed and centrifuged for 10 min at 500 *g* to remove cells, for 20 min at 25,000 *g* to remove cell debris, and for 3 h at 150,000 *g* to precipitate the complexes. The pelleted complexes were resuspended in 10 mM sodium borate, pH 7 containing 1% Triton X-100, 10 mM benzamidine, 1 mM EDTA, 1 mM iodoacetamide, and 1 mM PMSF. The complexes were biotinylated and reacted with staphylococci as previously described. The serum free medium, following collection of the complexes was precipitated with 90% ammonium sulfate, the precipitate dissolved in solubilization buffer, biotinylated and reacted with staphylococci.

Preparation of endothelial cell extracellular matrix. Endothelial cell extracellular matrix (ECM) was prepared as described previously (25, 26). After removal of medium, cell monolayers were washed twice with 15 mM Hepes buffer, pH 7.6, at 37°C. Cells were removed with 1 mM EGTA in Hepes buffer and ECM isolated with 0.5% Triton X-100 and 0.02 M NH_4OH. The plates were then washed three times with H_2O to remove cell debris. ECM proteoglycans were then extracted under dissociative conditions with 4 M guanidine HCl buffer, pH 6.3, containing 2.0% Triton X-100, 0.1 M EDTA, 0.05 M sodium acetate and 2 mM iodoacetamide at 4°C for 48 h. The extraction was clear and centrifugation at 30,000 *g* for ½ h at 4°C did not produce a pellet. This extract was then biotin-labeled and reacted with bacteria.

Binding assay with the purified endothelial cell membrane protein

Binding of staphylococci to the purified protein was determined using a modification of the method of Froman et al. (27). Removable microtiter wells (Immulon 2 Removawell strips, Dynatech Microtiter System, Chantilly, VA) were coated with the purified protein (300 μl) for 18 h at 4°C. The remaining liquid was aspirated off and the wells were then incubated for 1 h at 37°C with 10% milk in 10 mM Tris, pH 7.5, containing 0.9% NaCl. The surface was washed three times and incubated with [^{125}I]iodine labeled *S. aureus* (200 μl, 10^7 CFU/ml) suspended in M199, pH 7.4 containing 15 mM Hepes and 0.5% BSA at 37°C. Wells coated with BSA (10 mg/ml) in 50 mM ammonium acetate, pH 7, were used as negative controls. Fibronectin (75 μg/ml) was used as a positive control in these studies. The surface was washed five times following incubation before removal of the wells and determination of the number of adherent staphylococci. The number of counts/well was determined by placing the wells in the gamma counter. The binding assay was performed with additional bacterial strains as well as in the presence of physiologic concentrations of human serum albumin and fibrinogen.

A similar procedure was followed to determine whether the protein increased endothelial cell adhesion. Endothelial cells were labeled with [^{125}I]iodine after collection using EGTA. They were suspended in M199, 15 mM Hepes, pH 7.4, containing 0.5% BSA at a density of 10^7 cells/ml. Aliquots (200 μl) were added to the protein prepared wells and then incubated at 37°C in 5.5% CO_2. After incubation, the wells were washed, then removed and the number of counts determined.

All points in these studies were done in triplicate and experiments were repeated on at least three separate occasions.

Competition assay with the purified endothelial cell membrane protein

A modification of the infection assay was used to evaluate the effect of the purified endothelial cell protein on the binding of *S. aureus* to endothelial cells in vitro. Staphylococci were grown overnight in Todd Hewitt broth, pelleted, washed, and then suspended in M199, pH 7.0, containing 15 mM Hepes to an optical density of 0.1 at 620 nM. Aliquots were then incubated with an equal volume of either purified protein, M199 or 50 mM ammonium acetate, pH 7.0 containing 1 M NaCl, 0.1% Triton X-100. After incubation, the bacteria were pelleted, washed, and resuspended in M199. The suspensions were vigorously

vortexed and aspirated through a 25-gauge syringe to break up bacterial aggregates. Colony counts of the initial bacterial inoculum assured comparable bacterial density. The mixtures were then incubated for ½ h at room temperature. Aliquots of each mixture (100 μl/well) were added to confluent endothelial cells in 96-well microtiter plates. The plates were incubated at 4°C for ½ h, decanted, washed and the remaining bacteria removed with the endothelial cells by trypsin. The number of bacteria/endothelial cell was determined (12). All points were done in triplicate and all studies repeated on 3 separate occasions. Additional controls for these studies included preincubation of staphylococci with human serum albumin (35 mg/ml) or human fibrinogen (2.5 mg/ml) in 50 mM ammonium acetate buffer, pH 7.0 containing 1 M NaCl, 0.1% Triton X-100.

Results

Staphylococcal infection assay. Adherence of the Wb strain to the endothelial cell monolayer was both time and dose dependent. Saturation was demonstrable with an initial bacterial inoculum of 10^6 CFU/ml (Fig. 2). Binding was rapid and appeared virtually complete by 5 min.

Identification of an endothelial cell membrane component that binds S. aureus. The biotin-NHS labeled, solubilized endothelial cell membranes were incubated with *S. aureus* and the complexes isolated by centrifugation. After detergent solubilization, the complexes were resolved by SDS-PAGE and blotted onto PVDF. A major 50-kD band was identified on the PVDF using alkaline phosphatase conjugated streptavidin (Fig. 3 *a*). The solubilized staphylococci alone did not react with the streptavidin (not shown). The 50-kD component was also identified with two other adherent staphylococcal strains (not shown).

Purification and characterization of the endothelial cell binding protein. An endothelial cell membrane binding protein was purified by iminobiotin-streptavidin affinity chromatography. Approximately 5 μg of purified protein was isolated from 1.6×10^7 cells. The component was similar in molecular weight to the 50-kD band identified using biotin-NHS labeled membranes (Fig. 3 *b*).

[^{125}I]Iodine labeled intact cells and [^{35}S]methionine metabolically labeled cells were used to confirm the surface location and the endothelial cell origin of the 50-kD component, respectively. In both studies the labeled component was eluted from the streptavidin column and demonstrated by autoradiography to be 50 kD (Fig. 3 *c* and *d*). Thus, the purified endothelial cell protein was synthesized by, and expressed on the surface of, the endothelial cell.

Enzymatic digestion of the iodinated membrane component demonstrated smaller fragments with trypsin but no change in size with periodate when analyzed by SDS-PAGE. The purified membrane component was not labeled with [^{3}H]glucosamine. The molecular weight was unchanged after reduction with dithiothreitol and separation by SDS-PAGE. The membrane component therefore appeared to be a protein.

Conditioned serum free media, collected from confluent endothelial cells, was separated into complexes by high speed centrifugation and assayed for the presence of the binding protein. The precipitated complexes (24) were biotinylated and exposed to staphylococci. The 50-kD band was detectable by Western blot (not shown). The supernatant that remained following centrifugation did not contain the 50-kD protein. The protein was not demonstrable in the solubilized extracellular matrix using the previously described isolation technique (Fig. 1). In addition, the purified protein was not recognized by anti-BSA or fibronectin antibodies by slot blot analysis.

Binding assay. Binding studies were performed with the purified endothelial cell membrane protein adsorbed onto microtiter wells. The binding of [^{125}I]iodine labeled *S. aureus* was greater and showed an incremental rise with time when compared with controls (Fig. 4). Differences in binding were significant at 30, 60, and 90 min ($P < 0.01$) by the *t* test. The presence of human serum albumin (35 mg/ml) or fibrinogen (2.5 mg/ml) did not effect these results.

The ability of other adherent and nonadherent (Table I) bacterial strains to bind to the purified membrane protein in the binding assay was studied. Both strains of *S. aureus* (Wb and Am) exhibited approximately equal degrees of binding to protein coated wells (Table I). Binding of strains found to be nonadherent in the infection assay was not significantly different than binding to BSA coated control wells. The two *Escherichia coli* strains exhibited low binding to protein coated wells.

Binding of ^{125}I-labeled endothelial cells to the purified

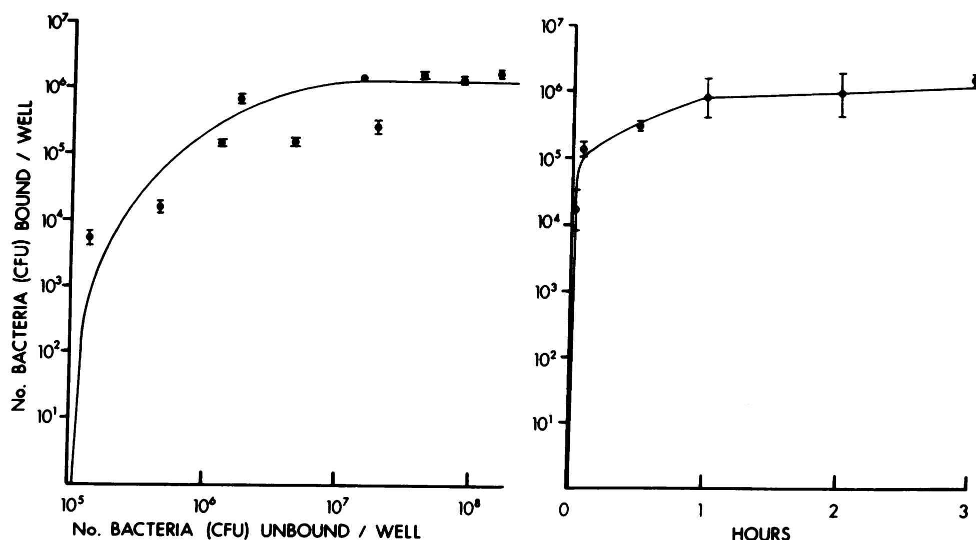

Figure 2. Dose response infection assay using Wb strain (*left*). Bacteria were incubated for ½ h at 4°C with confluent human umbilical vein endothelial cells. Time response infection assay with Wb strain using an initial bacterial inoculum of 7.4 × 10^6 CFU/ml (*right*). Results represent the mean±SD of three separate studies.

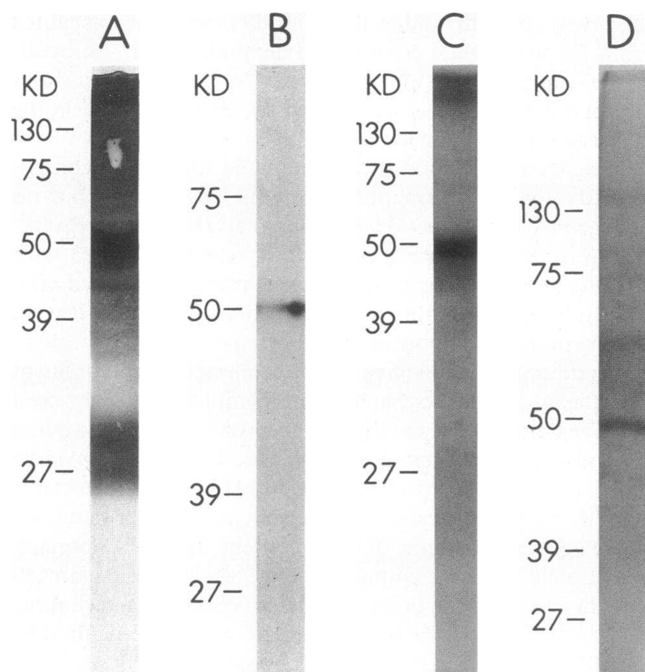

Figure 3. Solubilized, biotin-labeled, endothelial cell membrane components that bound S. aureus after a 2-h incubation were separated by SDS-PAGE. (A) Western blot of biotin-NHS labeled endothelial cell membranes; (B) Western blot of iminobiotin-NHS labeled endothelial cell membranes following passage over streptavidin affinity column; (C) autoradiogram of endothelial cell membranes labeled with [^{35}S]methionine and iminobiotin and eluted from the streptavidin affinity column; (D) autoradiogram of endothelial cell membranes labeled with [^{125}I]iodine and iminobiotin and eluted from the streptavidin affinity column.

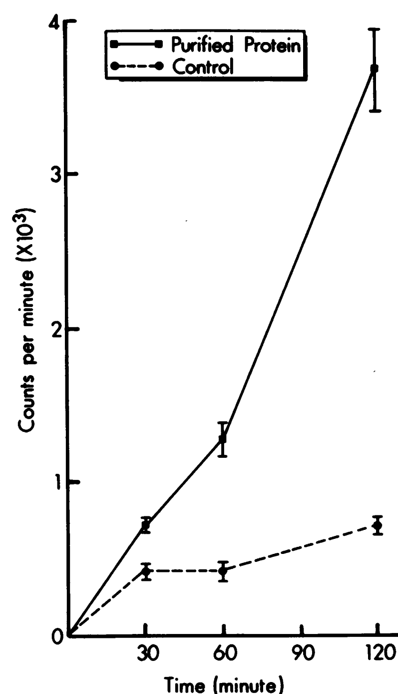

Figure 4. Binding of [^{125}I]iodine labeled *S. aureus* (5 × 10^7 CFU/ml) to endothelial cell membrane protein coated or uncoated microtiter wells. Each point represents the mean±SD of three determinations. Differences in binding between protein-coated and control wells at 30, 60, and 90 min are significant ($P < 0.01$).

membrane protein was investigated to determine if the protein had bivalent properties. Endothelial cell adherence was not increased above controls.

Competition assay. The effect of the partially purified membrane protein on staphylococcal adherence to human endothelial cells was evaluated. Preincubation of *S. aureus* for ½ h with the protein significantly decreased ($P < 0.001$, *t* test) adherence to the endothelial cells when compared with M199 and ammonium acetate buffer controls (Table II). Adherence was not diminished by preincubation of bacteria with physiologic concentrations of human fibrinogen (2.5 mg/ml) or serum albumin (35 mg/ml). The number of bacteria/endothelial cell was 18.1±5.3 for fibrinogen, 23.8±9.6 for human serum albumin versus 18.5±7.2 for elution buffer (mean±SD of four separate studies).

Discussion

Staphylococcal bacteremia is often complicated by endocarditis or metastatic suppurative collections (1–4). The frequency with which these complications occur is greater with *S. aureus* than with most other bacterial pathogens. While the basis for this virulence is incompletely understood, it appears in part to result from the organisms ability to colonize endovascular surfaces and to elaborate proteolytic toxins, which facilitate bacterial migration to adjoining tissues. A classic example of this process is acute infective endocarditis where previously normal valves are colonized and subsequently destroyed (3, 9).

We, and others, have demonstrated that *S. aureus* adhere more avidly to human endothelial cells in tissue culture and to endovascular tissue than other bacterial species (10–15). Investigators have speculated that this bacterial tropism may be the result of specific adhesin-receptor interactions (10). The present study supports this hypothesis, since staphylococcal binding to the cultured human endothelial cells was saturable in both a dose and time dependent fashion (Fig. 2).

While specific eucaryotic cell surface receptors for *S. aureus* have not been previously isolated, cellular receptors for other bacteria have been identified and purified. A glycolipid found on the surface of uroepithelial cells mediates *E. coli* attachment (28) and may contribute to the pathogenesis of urinary tract infections. The glycoprotein fibronectin coats buccal epithelial cells and thereby mediates group A streptococcal adherence (29). Sanford et al. (30, 31) have investigated the basis for increased staphylococcal adherence to viral infected cells. They have identified several membrane polypeptides present on virus infected and uninfected Madin-Darby canine kidney cells which bind *S. aureus* surface proteins. They speculate that these components may play a role in the increased binding of *S. aureus* to influenza infected cells.

We have developed and utilized a novel approach for the isolation and purification of host tissue receptors or bacterial ligands. This technique relies on the pH dependent affinity of iminobiotin-NHS for streptavidin (21, 22). Potential receptors can be reacted with the intact bacterial cell, solubilized and then purified by streptavidin affinity chromatography. The host receptors can easily be labeled with [^{125}I]iodine or [^{35}S]-methionine.

A 50-kD endothelial cell membrane component that binds *S. aureus* has been purified using this technique. Its surface location was confirmed using [^{125}I]iodine labeled intact endo-

Table I. Binding of [^{125}I] Iodine Labeled Bacteria to Endothelial Cell Membrane Protein-coated Wells

	Infection assay		Binding assay	
Bacterial strain	Initial bacterial (CFU) inoculum (×10^7)	No. bacteria/endothelial cell±SD	Initial bacterial inoculum (×10^7)	Binding as percentage of control*
S. aureus				
1) Wb	3.1	14.8±0.5	2.1	181‡
2) Am	4.1	17.1±3.3	3.7	171§
S. epidermidis	11.0	0.9±0.1	2.5	102
E. coli				
1) 32542	5.6	1.9±0.2	4.8	100
2) 3110	4.0	0.6±0.3	1.1	96
S. sanguis	6.0	2.2±0.5	1.7	127

* Results are expressed as the percent change from binding to the BSA-coated control wells. ‡ $P < 0.05$ when compared with BSA controls. § $P < 0.005$ when compared with BSA controls.

thelial cells and its endothelial cell origin demonstrated by metabolic labeling with [^{35}S]methionine. Trypsin sensitivity and methionine incorporation confirmed the protein nature of this component and the lack of glucosamine incorporation and insensitivity to periodate digestion suggest that it is not a glycoprotein. A 50-kD component was demonstrated in complexes isolated by ultracentrifugation from the conditioned serum-free media. These complexes are endothelial cell products that are released into the media. The significance of these cellular complexes is unknown (24). Conversely, incorporation of the protein into the solubilized extracellular matrix was not demonstrated.

The purified protein had functional activity when immobilized on plastic and when used in competitive binding studies. Preincubation of *S. aureus* with the purified endothelial cell membrane protein resulted in a marked decrease in the number of bacteria binding to endothelial cells. The inability of albumin or fibrinogen to inhibit binding support the specificity of the 50-kD protein in *S. aureus* adherence. This is also suggested by the limited binding of other bacterial species to the immobilized protein. These studies therefore suggest that this component may function as a receptor for *S. aureus*.

The relationship of this *S. aureus* binding protein to known endothelial membrane proteins remains to be investigated.

*Table II. Staphylococcal Adherence to the Endothelial Cell Monolayer after Incubation with the 50-kD Protein**

	Initial bacterial inoculum	
Treatment group	No. bacteria (×10^8) + SD	No. bacteria/ endothelial cell + SD‡
M199, 15 mM Hepes buffer	0.5±0.1	21.7±3.9
50 mM ammonium acetate buffer	1.4±1.5	19.9±4.7
Purified protein	1.3±0.3	4.6±1.6

* Staphylococci were preincubated with the 50-kD protein in the 50-mM ammonium acetate elution buffer, M199-15 mM Hepes buffer, or the 50-mM ammonium acetate buffer for 1/2 h before performance of the infection assay.
‡ The results represent the mean±SD of three separate studies with each point performed in triplicate. Differences between the protein group and either control group are significant ($P < 0.001$).

The endothelium is a dynamic cell type that plays an important role in vascular function. Many of these functions are mediated by a variety of surface molecules that have been identified on the endothelial cell. These include receptors that determine sites for cell binding and transit (ICAM-I, LFA-3), as well as binding sites for a variety of macromolecules (32–34). Most of the previously identified membrane proteins and glycoproteins are larger than 50 kD. The purification of the *S. aureus* binding protein was performed at 4°C in the presence of protease inhibitors in order to reduce the likelihood that the isolated component was a degradation fragment of a larger molecule.

Matrix glycoproteins such as fibronectin, laminin, and collagen have been shown to bind *S. aureus* (5–7). Fibronectin, in particular, has been identified as an important mediator of staphylococcal adhesion in the presence of tissue injury. Its presence in the nonbacterial thrombus encountered on traumatized valvular surfaces in subacute infective endocarditis results in increased bacterial adhesion (6). Fibronectin does not appear to have a major role in the present binding interaction since it is not expressed on the luminal surfaces of endothelial cells. In addition, the purified protein did not react with antifibronectin antisera. It is likely that *S. aureus* colonization of tissue surfaces is a complex process and, as with other bacterial-tissue interactions, involves more than a single adhesin-receptor interaction.

Our investigation has shown that *S. aureus* binding to endothelium is saturable with both dose and time supporting the specificity of binding. The purified endothelial cell membrane protein binds staphylococci. It also competitively inhibits bacterial attachment to endothelial cells. The presence of this binding site or endothelial cell receptor for staphylococci may contribute to the capacity of these organisms to colonize and invade endovascular surfaces. Factors that influence expression of this endothelial component may play a role in determining the sites for bacterial seeding. The identity and functional role of this membrane component remains to be established.

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