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Group B Streptococci Invade Endothelial Cells: Type III Capsular Polysaccharide Attenuates Invasion

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Group B streptococci (GBS) are the most common cause of neonatal sepsis and pneumonia. The pathogenesis of GBS disease is not completely defined. GBS-induced endothelial cell injury is suggested by histological findings at autopsy and in animal studies. We hypothesized that (i) type III GBS (COH-1) invade and injure human umbilical vein endothelial (HUVE) cells and (ii) isogenic mutations in GBS capsule synthesis would influence HUVE invasion. Confluent HUVE monolayers were infected for 0.5, 2, or 6 h. Media with penicillin plus gentamicin were added and incubated for 2 h to kill extracellular bacteria. Cells were washed and lysed, and the number of live intracellular bacteria was determined by plate counting. COH-1 invaded HUVE cells in a time-dependent manner at levels 1,000-fold higher than those of the noninvasive *Escherichia coli* strain but significantly lower than those of *Staphylococcus aureus*. There was no evidence for net intracellular replication of GBS within HUVE cells. COH-1 infection of HUVE cells caused the release of lactate dehydrogenase activity. GBS invasion was inhibited by cytochalasin D in a dose-dependent manner; GBS-induced lactate dehydrogenase release was attenuated by cytochalasin D. The isogenic strains COH 1-11, devoid of capsular sialic acid, and COH 1-13, devoid of all type III capsule, invaded HUVE cells three- to fivefold more than the parent COH-1 strain. The type III capsular polysaccharide and particularly the capsular sialic acid attenuate GBS invasion of HUVE cells. Electron micrographs of lung tissue from a GBS-infected newborn *Macaca nemestrina* also showed GBS within capillary endothelial cells. We conclude that endothelial cell invasion and injury are potential mechanisms in the pathogenesis of GBS disease.

Group B streptococci (GBS) are the most common cause of bacterial sepsis and pneumonia in the neonatal period (4). The pathogenesis of GBS disease is primarily inferred from epidemiologic, clinical, and pathologic data but is not completely defined. GBS presumably enter the alveolar space after the fetus aspirates infected amniotic fluid (4, 6, 8, 13, 22, 35); then, GBS presumably bypass and/or injure the lung endothelium as one route to enter the circulation. GBS-induced endothelial cell injury is suggested by the presence of intra-alveolar and interstitial hemorrhage and the presence of protein-rich pulmonary edema in human infants and animal models with GBS sepsis (1, 12, 16, 24, 35). There are no published reports of GBS invasion of endothelial cells in vivo or in vitro.

The ability of GBS to invade the endothelial cell barrier would provide one mechanism for GBS to access the vascular space from the alveolar space or to enter the interstitial space from the vascular compartment. Intravenous infusion of *Streptococcus viridans* into rabbits resulted in bacterial invasion and injury of the aortic endothelium in vivo (18). Cultured endothelial cells ingest *Staphylococcus aureus* into intracellular phagosomes (11, 33). The degree of *S. aureus* invasion and injury of the endothelial cell monolayer was dependent upon time of incubation, inoculum size, and bacterial strain.

There are limited studies on the role of the bacterial capsule on endothelial cell invasion. The less-encapsulated Re mutant of *Salmonella minnesota* is ingested in greater numbers than the wild-type strain by pulmonary artery endothelial cells, and phagocytosis is associated with increased superoxide anion production (28, 29). The type III capsular polysaccharide is a major virulence factor in animal models of GBS sepsis (3, 4). Complete ablation of capsular polysaccharide biosynthesis by transposon mutagenesis results in loss of GBS virulence in a neonatal rat model of sepsis (26). The type III GBS capsule protects the organism from opsonophagocytosis and clearance by alveolar macrophages and neutrophils (20). The influence of type-specific capsular polysaccharide in GBS-endothelial cell interactions is unknown.

We hypothesize (i) that type III GBS can invade and injure human umbilical vein endothelial (HUVE) cells and (ii) that isogenic mutations in GBS capsule synthesis will influence endothelial cell invasion by this organism. This report describes the invasion of HUVE cells by GBS and the attenuation of invasion by type III capsular polysaccharide.

MATERIALS AND METHODS

Endothelial cell culture. Cell Systems Corporation (Kirkland, Wash.) supplied fully characterized HUVE cells on the basis of cobblestone monolayer morphology, direct polyclonal immunofluorescence staining for VWF/Factor VIII, and uptake of fluorescent acetylated low-density lipoprotein. Cell Systems tests the HUVE cultures for sterility with aerobic and anaerobic cultures and mycoplasma contamination by immunofluorescent staining with Hoechst bisbenzimide H33342, and the pooled umbilical cord sera were tested for human immunodeficiency virus antigen (Genetic Systems assay kit). The cells were isolated from fresh, pooled umbilical cords by using collagenase solution by a modification of the method of Schwartz (31). The HUVE cells (passages, <3) were passaged in defined CS-1 medium

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with 50 μg of heparin and heparin binding growth factor per ml in the absence of serum (Cell Systems). One hour before inoculation, the cells were washed with RPMI medium plus 1% fetal calf serum (FCS) and then refed with 0.5 ml of RPMI medium plus 1% FCS per well.

Bacteria. COH-1 is a highly encapsulated type III beta-hemolytic GBS clinical isolate (19). Isogenic mutants of COH-1 were derived by transposon-insertional mutagenesis as described previously (26, 38) and include (i) COH 1-13, devoid of type III capsular polysaccharide, and (ii) COH 1-11, devoid of capsular sialic acid. A noninvasive laboratory strain of *Escherichia coli*, DH5α, was used as a negative control (10); a clinical isolate of *S. aureus* from the sputum of a patient with cystic fibrosis was used as a positive, invasive control. All bacteria were grown to stationary phase in Todd-Hewitt broth (THB) overnight. On the day of experimentation, an aliquot of stationary-phase broth culture was reinoculated into THB to an optical density at 600 nm of less than 0.1 (0.4 ml of stationary-phase culture to 10 ml of THB). The cultures were then incubated at 37°C in 5% CO_2 until the optical density at 600 nm was ~0.4 (mid-log phase or equivalent to ~10^8 CFU/ml). The inocula were centrifuged and washed with RPMI medium and then resuspended in the same volume of RPMI medium plus 1% FCS. The concentration and purity of each inoculum were determined by quantitative culture on Todd-Hewitt agar plates.

HUVE cell invasion assays. GBS invasion of HUVE cells (passages, <3) was assayed by a modification of the technique of Isberg and Falkow (15). COH-1, COH 1-13, and COH 1-11 strains of GBS, *S. aureus*, and DH5α were added at 10^6 CFU per well (10^5 cells per well) in 24-well tissue culture plates (Corning) with confluent HUVE monolayers in 0.5 ml of RPMI medium containing 1% FCS (Cell Systems). After the bacteria were added, the cultures were incubated at 37°C in 5% CO_2 for 0.5, 2, or 6 h. The supernatants were removed by aspiration, and the cell monolayers were washed three times with media. Tissue culture media containing 5 μg of penicillin per ml and 100 μg of gentamicin per ml were added, and the cells were incubated as described above for an additional 2 h to kill extracellular bacteria. In two experiments comparing the effects of 100 μg of gentamicin per ml alone with penicillin plus gentamicin, we observed no significant difference in the number of intracellular CFU for both *S. aureus* and GBS (data not shown). Penicillin and gentamicin provided increased killing of extracellular bacteria compared with gentamicin alone in control wells without cells (data not shown). There are no specific data on penicillin entry into HUVE cells, but extracellular penicillin is not reported to enter other phagocytic eukaryotic cells, alveolar macrophages, at significant levels (~1%) (40). The similar findings with penicillin plus gentamicin or gentamicin alone also suggest no significant entry of penicillin into HUVE cells at the concentrations used in this study.

After removal of the antibiotic-containing media, the cells were washed as described above and treated with 0.25% trypsin without EDTA for 5 min, and the monolayers were lysed with Triton X-100 at a final concentration of 0.025% (vol/vol) in sterile distilled water. The lysate was vortexed, and aliquots of the lysate were plated on Todd-Hewitt agar to quantify the number of live intracellular bacteria. In pilot studies, sonication of the lysates prior to plating did not result in increased measured numbers of intracellular CFU. The data are expressed as the number of intracellular CFU divided by the inoculum CFU times 100.

Identical concentrations of bacteria were inoculated into wells of 24-well plates containing RPMI medium plus 1% FCS without HUVE cells as a control for nonspecific binding of bacteria and to ensure that the antibiotics killed extracellular bacteria. For all conditions, there were fewer than 20 CFU present to demonstrate that the concentrations of antibiotics used were sufficient to kill extracellular bacteria in 2 h (data not shown). Pilot experiments (n = 2) using COH-1 and DH5α showed similar degrees of invasion for COH-1 in the absence or presence of 1% FCS, but in the absence of serum there was a 10-fold increase in nonspecific binding of both COH-1 and DH5α to tissue culture plastic controls. Killing curves of 0.025% Triton X-100 for GBS show no effect on the organism viability after a 45-min exposure (25a). All experimental wells were plated onto Todd-Hewitt agar in less than 45 min after exposure to Triton X-100.

The initial studies evaluated the time course of COH-1 invasion compared with those of DH5α and *S. aureus*, with each condition performed in triplicate for each experiment (n = 4 experiments). In experiments utilizing the isogenic GBS mutants, each condition was repeated five times for each experiment (n = 6 experiments for 6-h assays; n = 3 simultaneous experiments for 2-h assays).

Assay for endothelial cell injury. Endothelial cell injury was assessed by (i) exclusion of trypan blue (0.04% in RPMI medium) and (ii) the release of lactate dehydrogenase (LDH) activity into the culture media at 8 h after inoculation. Trypan blue exclusion was quantified by counting 100 cells per well by using inverted phase-contrast microscopy. The LDH activity in media and HUVE cell lysates was determined by a colorometric assay for pyruvate, with the amount of residual pyruvate being inversely proportional to the amount of LDH activity (Sigma Chemical Co., procedure 500). For each condition, the total LDH activity was measured in two sister wells in which the monolayers were lysed with 0.025% Triton X-100 in distilled water. The LDH release for each condition was expressed as a ratio of the LDH in supernatants of experimental wells to the LDH in cell lysates and supernatants of sister wells times 100.

Assay for intracellular replication of GBS. The assays for intracellular replication of GBS were performed by a modification of the invasion assay described above. After 2 h of incubation with GBS (10^6 CFU per well), the HUVE monolayers (24-well plates) were washed, and antibiotics were added as described above. The HUVE monolayers were then incubated in the presence of antibiotics for 2, 4, and 8 h. The monolayers were washed, treated with Triton X-100 and trypsin, lysed, and plated on agar for quantitative measurement of live intracellular CFU as described above. The experiment was performed four times, with each condition in triplicate.

Effect of cytochalasin D on GBS invasion and LDH release. HUVE monolayers (24-well plates) were preincubated for 1 h with RPMI medium plus 1% FCS without and with serial dilutions of cytochalasin D (Sigma) at final concentrations of 0.01, 0.1, 1, 5, and 10 μg/ml. The HUVE monolayers were then inoculated with COH-1 (10^6 CFU per well) and incubated for 2 h in the presence of cytochalasin D. The remainder of the protocol was identical to the invasion assay described above. This experiment was performed four times, with each condition in triplicate. To test the effect of cytochalasin D on GBS-induced LDH release, the above protocol was also performed with an 8-h infection, and LDH activity was measured in the culture media (n = 4 experiments, with each condition performed in triplicate).

Electron microscopy. HUVE cell monolayers in 24-well

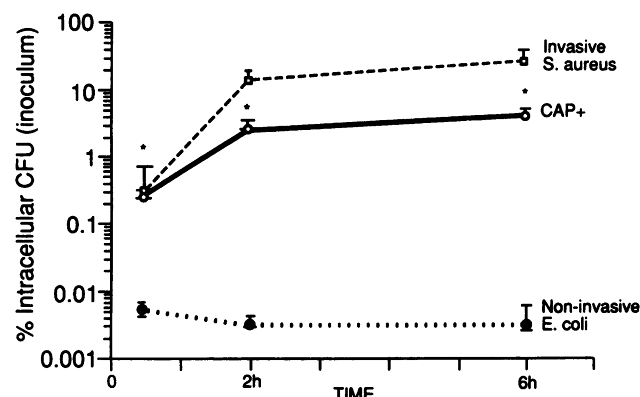

FIG. 1. Time course of HUVE cell invasion by GBS, *S. aureus*, and *E. coli* DH5α. A plot of the ratio of intracellular CFU/inoculum to CFU times 100 (log scale) versus time for the three bacteria is shown. Data are presented as means ± SEMs. CAP+, COH-1, the type III GBS parent strain containing capsular polysaccharide; *, $P < 0.05$ (compared with noninvasive *E. coli*).

plates were inoculated with COH-1 and treated identically to those used in the invasion assays described above. After 6 h of incubation in the absence of antibiotics, monolayers were incubated for 2 h with penicillin and gentamicin as described above. Monolayers were washed with RPMI medium without serum and fixed for 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) (25). Monolayers were then washed in buffer, postfixed in 2% OsO_4 plus 1% potassium ferricyanide in cacodylate buffer, and dehydrated through a graded alcohol series. The HUVE cells were then embedded in Epon, thin sectioned, and stained with uranyl acetate and lead citrate. To corroborate our in vitro studies, archival lung tissue from a newborn *Macaca nemestrina* infected with COH-1 in utero was reexamined for evidence of GBS invasion of endothelial cells in vivo (27). Specimens were examined by E. Chi with a JEOL-100B electron microscope.

Statistics. All values are expressed as means ± standard errors of the means (SEMs). The data for each experiment were analyzed by analysis of variance with Student-Neuman-Kuuls correction for multiple comparisons (SPSS-PC+, version 3.0; SPSS, Inc., Chicago, Ill.). A *P* value of <0.05 was considered significant.

RESULTS

GBS invasion of endothelial cells. The time course of bacterial invasion of HUVE cells is shown in Fig. 1. This invasion assay was based on the inability of extracellular penicillin and gentamicin to enter eukaryotic cells (15, 40); the lack of significant intracellular antibiotic accumulation allows the quantification of intracellular bacteria after extracellular bacteria were killed. To ensure that the number of intracellular CFU was not affected by the presence of both antibiotics, two control experiments were performed with gentamicin alone. No significant differences were observed between wells treated with penicillin plus gentamicin and wells treated with gentamicin alone (data not shown). Data for assays performed with penicillin plus gentamicin are presented as a ratio of intracellular CFU to inoculum CFU times 100 (percent invasion). The highly encapsulated type III GBS strain, COH-1, showed significant invasion of HUVE cells at 0.5, 2, and 6 h, at levels 1,000-fold higher than those of DH5α. Both COH-1 and *S. aureus* showed increased invasion at 6 h compared with that at 0.5 or 2 h. The *S. aureus* strain showed significantly more invasion than COH-1 at all time points. DH5α was noninvasive, as was observed for epithelial cells (10).

Electron microscopy. To document that COH-1 was entering HUVE cells in vitro, transmission electron microscopy was performed on equivalent monolayers from a 6-h invasion assay. Transmission electron micrographs in Fig. 2 show (i) COH-1 attachment to the endothelial surface with early invagination (Fig. 2A), (ii) intracellular COH-1 within a membrane-bound vacuole (Fig. 2B), and (iii) dividing COH-1 within three membrane-bound vacuoles (Fig. 2C). We observed no evidence of intracellular DH5α (data not shown). To corroborate the in vitro invasion data, we examined the pulmonary capillary endothelium of archival lung tissue from a primate model of neonatal GBS infection (27). Figure 3 shows histological evidence that COH-1 can invade pulmonary capillary endothelial cells in a subhuman primate model of GBS disease. The pulmonary capillary endothelial cell shows increased vacuolization that suggests cellular injury.

Assay for intracellular replication. The ability of GBS to survive within HUVE cells was tested by a modification of the invasion assay (Fig. 4). COH-1 showed no significant net increase in intracellular CFU between 2 and 8 h of infection in the presence of antibiotics. These data suggest that GBS remain viable within HUVE cells but do not replicate.

Effect of cytochalasin D on GBS invasion and GBS-induced endothelial injury. The effect of cytochalasin D on GBS invasion of HUVE cells is shown in Fig. 5. Cytochalasin D caused a dose-dependent inhibition of COH-1 invasion of HUVE cells. The inhibition was significant at a dose of 0.1 μg/ml and increased to a 1,000-fold inhibition at a dose of 5 to 10 μg/ml. These data suggest an active role for HUVE cell microfilaments in the uptake of GBS.

Table 1 summarizes data on GBS-induced LDH release and the potential effect of cytochalasin D on this measure of GBS-induced endothelial cell injury. GBS causes a significant increase in LDH release from HUVE cells compared with both the control medium and the noninvasive *E. coli*, DH5α (Table 1, COH-1 vs. DH5α). GBS-induced LDH release was partially attenuated by cytochalasin D at doses of >0.01 μg/ml (Table 1, COH-1 + cytochalasin D). There was greater than 95% trypan blue exclusion for all conditions at the end of the 8-h infection. These data suggest that endothelial cell injury, but not cell death, occurs as a consequence of GBS infection and that GBS invasion of HUVE cells is an important cause of cell injury.

Effect of capsular polysaccharide on GBS invasion. Figure 6 summarizes data on HUVE cell invasion with COH-1 and two isogenic mutants, namely, COH 1-11 (devoid of capsular sialic acid residues) and COH 1-13 (devoid of all type III capsular polysaccharide). The *E. coli* strain, DH5α, was not invasive in these experiments (<0.005% of inoculum). The COH 1-11 and COH 1-13 isogenic strains of COH-1 invaded HUVE cells significantly more than the parent strain at both 2 and 6 h of infection. There was no significant difference in HUVE cell invasion between the mutant strains. Therefore, the type III capsule and particularly the capsular sialic acid attenuate GBS invasion of HUVE cells.

The three isogenic GBS strains showed similar growth rates in RPMI medium plus 1% FCS (data not shown). Therefore, the growth rates of the three isogenic GBS strains do not explain the differences in HUVE cell invasion. Although DH5α grew slower than all GBS strains, this noninvasive strain did reach stationary phase by 6 h of infection (data not shown).

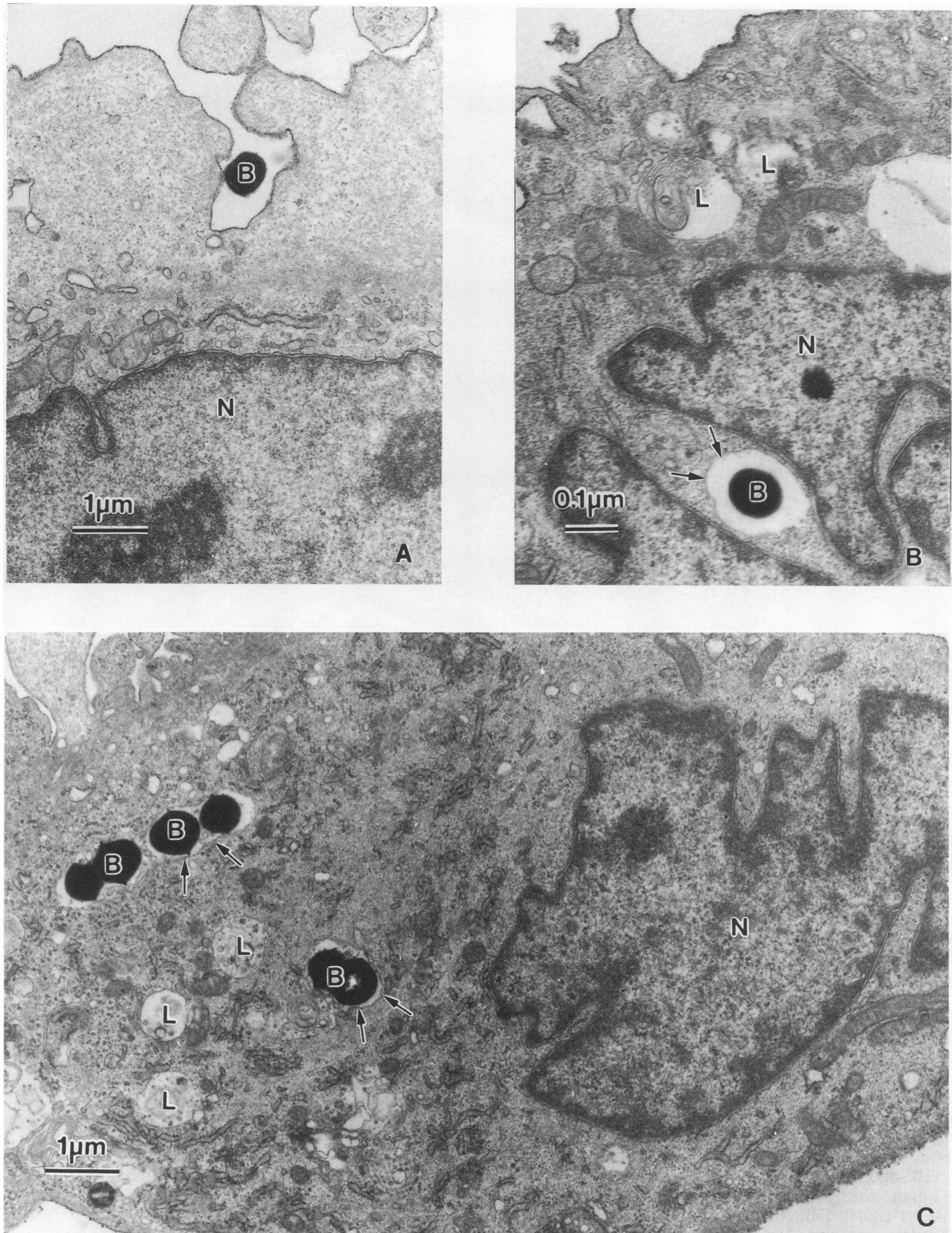

FIG. 2. Electron micrographs of GBS within HUVE cells. (A) A single COH-1 bacterium (labeled B) attached to the endothelial cell surface and in the early stages of endocytosis; (B) a single COH-1 bacterium (labeled B) within a membrane-bound vacuole (arrows); (C) dividing COH-1 bacteria (labeled B) within membrane-bound vacuoles (arrows). N, nucleus of HUVE cell; L, lysosomal vacuole within HUVE cell.

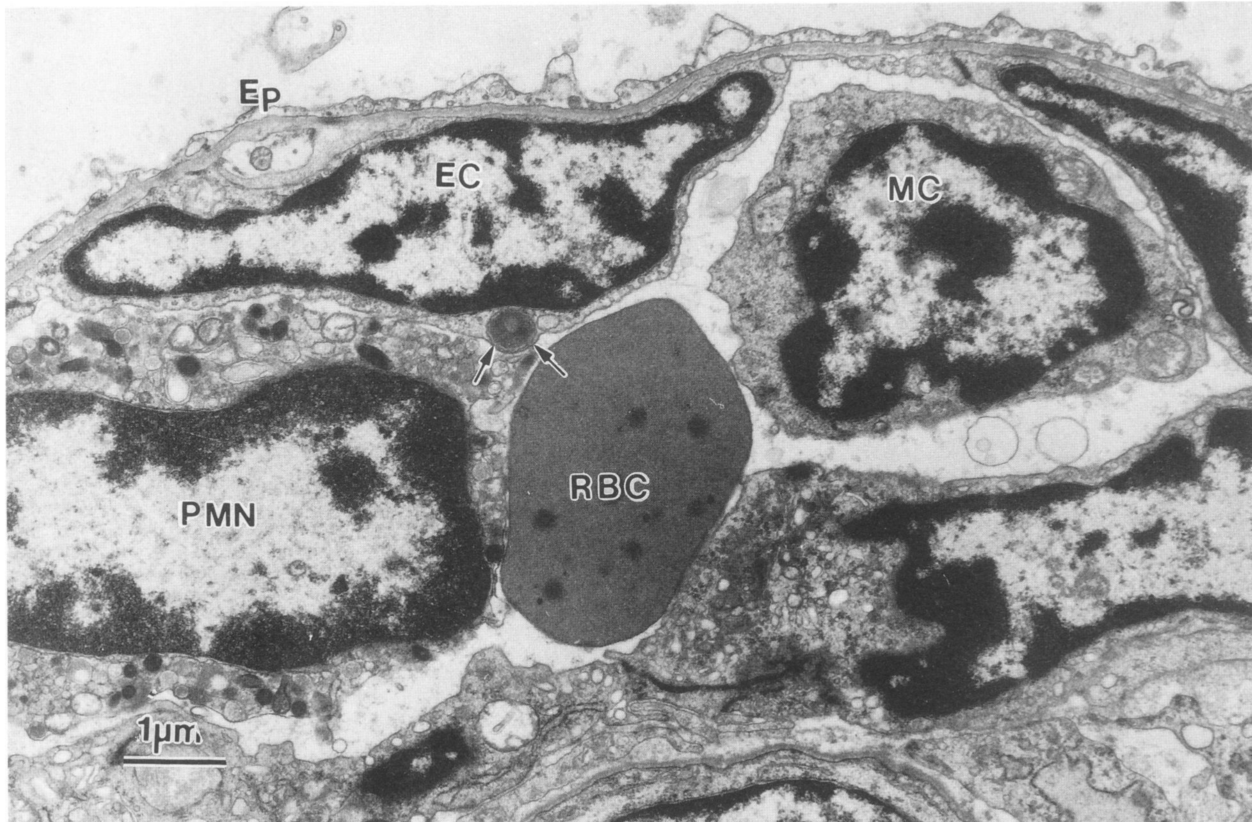

FIG. 3. Electron micrograph of pulmonary capillary of a GBS-infected *Macaca nemestrina*. The arrows denote a COH-1 organism within a membrane-bound vacuole of a pulmonary capillary endothelial cell (EC). The endothelial cell shows increased vacuolization as evidence of cell injury. Ep, alveolar epithelial cell; MC, mononuclear cell; PMN, polymorphonuclear neutrophil; RBC, erythrocyte within the capillary lumen.

DISCUSSION

Type III GBS were shown to invade and injure lung capillary endothelial cells in vivo, and GBS were shown to invade HUVE cells in vitro on the basis of direct visualization by transmission electron microscopy and the presence of CFU resistant to extracellular antibiotics. GBS invade HUVE cells significantly more than a noninvasive strain of *E. coli* but less than a clinical isolate of *S. aureus*. As shown previously for other pathogens (11, 14, 25, 37), the entry of GBS into HUVE cells is inhibited by cytochalasin D in a dose-dependent manner. We conclude that microfilaments are necessary for endothelial uptake of GBS. Electron micrographs of HUVE cells showed some dividing GBS within membrane-bound vacuoles, but we observed no net intracellular replication. This was similar to data on *S. aureus* in bovine aortic endothelium (33). Despite the lack of intracellular replication, *S. aureus* was observed to cause progressive endothelial cell injury with increasing duration of intracellular residence (33). The *S. aureus* alpha-hemolysin is an important virulence factor in endothelial cell injury (34). GBS did not cause significant endothelial cell death in vitro but did cause endothelial cell injury as indicated by the release of LDH. Cytochalasin D attenuated GBS-induced LDH release at concentrations that inhibited GBS invasion of HUVE cells. These data suggest that endothelial cell invasion is in part responsible for GBS-induced endothelial cell injury. The GBS virulence factors that contribute to invasion and injury of the endothelium are unknown, but

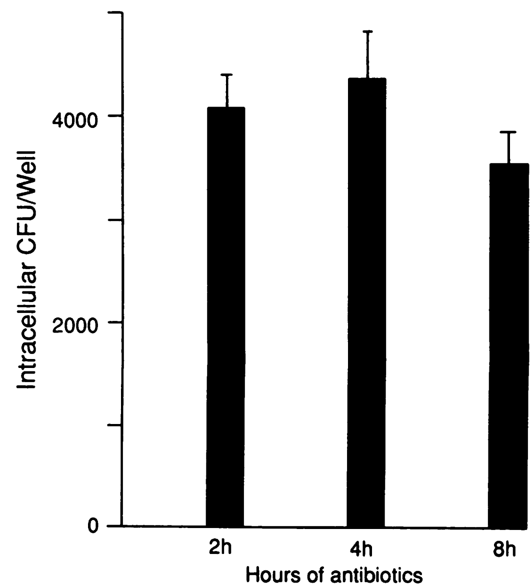

FIG. 4. GBS survival within HUVE cells. A plot of intracellular GBS CFU per well within HUVE cells versus time of incubation in the presence of penicillin and gentamicin is shown. This is an assay for intracellular replication or killing of GBS. Data are presented as means ± SEMs.

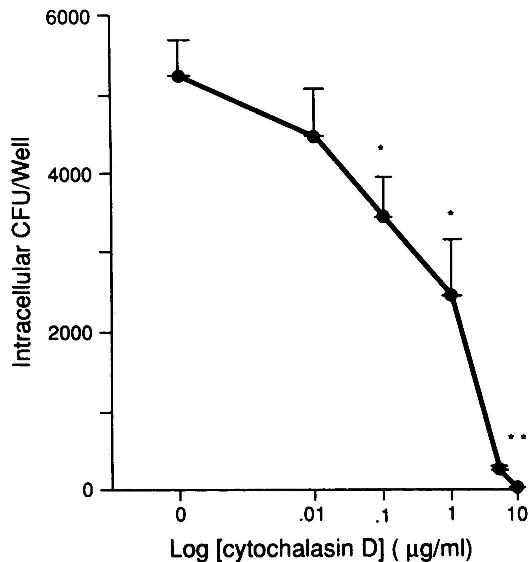

FIG. 5. Effect of cytochalasin D on GBS invasion. A plot of intracellular GBS CFU per well in HUVE cells versus the concentration of cytochalasin D (log scale) is shown. Data are presented as means ± SEMs. *, $P < 0.05$ (compared with the absence of cytochalasin D).

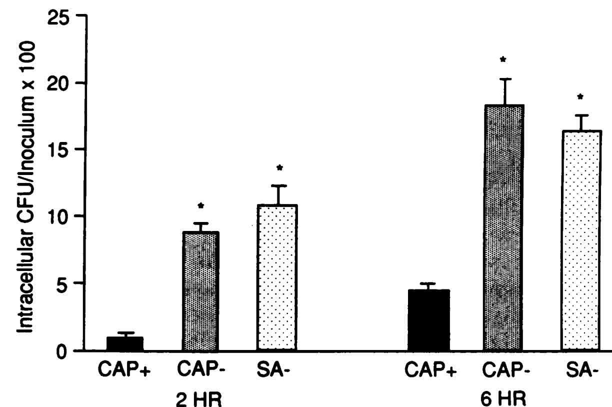

FIG. 6. Effect of capsular polysaccharide on GBS invasion. A histogram of percent intracellular CFU divided by inoculum CFU for three isogenic strains of GBS at both 2 and 6 h of infection is shown. Data are presented as means ± SEMs. CAP+, COH-1 (parent strain of GBS containing type III capsular polysaccharide); CAP−, COH 1-13 (isogenic mutant devoid of type III capsular polysaccharide); SA−, COH 1-11 (isogenic mutant devoid of capsular sialic acid); *, $P < 0.05$ (compared with CAP+).

may include beta-hemolysin, proteases, and other products (9). We speculate that GBS invasion and residence within the lung endothelium may contribute to lung injury and the pathophysiology of GBS pneumonia and sepsis.

Bacterial-host cell interactions resulting in adherence and invasion probably involve both specific and nonspecific processes. There are no data for specific GBS components or endothelial cell ligands involved in GBS-endothelial cell interactions. Specific endothelial cell surface proteins are important in the adhesion of *Plasmodium falciparum* (intercellular adhesion molecule-1) and *S. aureus* (50-kDa protein) (7, 32). *S. aureus* can bind to several additional glycoproteins on the surface of cells, including fibronectin, vitronectin, fibrinogen, and laminin (23). *Salmonella minnesota* binding to C1q enhances endothelial cell phagocytosis of this pathogen (29). Besides these specific adhesin-ligand interactions, bacterial-surface interactions depend in part upon the relative surface tensions of three phases: the bacteria, the liquid medium, and the cellular substrate (2). Hydrophobic bacteria adhere to all surfaces to a greater extent than hydrophilic organisms and were phagocytosed by neutrophils to the greatest extent (2). The type-specific polysaccharide of GBS is hydrophilic, and this may explain in part the increased invasion of HUVE cells by isogenic GBS mutants devoid of capsular polysaccharide.

Polysaccharide capsules of other microorganisms have been shown to influence endothelial cell invasion (25, 28, 29). Our data suggest that type III capsular polysaccharide, and particularly capsular sialic acid, attenuates but does not prevent type III GBS entry into HUVE cells. It is uncertain if type III GBS can alter the expression of capsular sialic acid or capsular polysaccharide in response to local in vivo environments. There is preliminary evidence for modulation of GBS capsule expression between maternal and infant strains of GBS to suggest that the external milieu may influence capsule expression (17). The growth phase of GBS and nutrients in the environment are reported to influence the amount of GBS surface capsule expression (5, 36). Perhaps in vivo modulation of type III GBS capsular sialic acid or polysaccharide may contribute to virulence by promoting intracellular invasion with reduced expression or evasion of phagocytic host defenses with increased expression.

Sialic acid residues act as biological masks in several biological systems (5). The type III GBS capsular polysaccharide consists of pentasaccharide repeating units containing terminal sialic acid residues (36). The capsular polysaccharide, and specifically the sialic acid residues, may provide an electronegative charge and steric hindrance to attenuate GBS adherence and invasion of endothelial cells. It is unknown if type III capsular polysaccharide is partially masking specific components of GBS that are critical for adherence or invasion of eukaryotic cells. We have prelim-

TABLE 1. Percent LDH activity released from HUVE cells: effect of cytochalasin D

Culture conditions	% LDH release[a]
COH-1 vs. DH5α	
Medium alone	15.3 ± 3.6
Medium + COH-1	41.0 ± 4.3[b]
Medium + DH5α	12.2 ± 3.5
COH-1 + cytochalasin D	
Medium alone	9.7 ± 1.8
Medium + COH-1	37.0 ± 3.2[b]
Medium + COH-1 + cytochalasin D at:	
0.01 μg/ml	34.1 ± 4.8
0.1 μg/ml	24.3 ± 3.9[c]
1.0 μg/ml	28.0 ± 5.2
5.0 μg/ml	20.2 ± 2.2[c]
10.0 μg/ml	17.5 ± 2.3[c]

[a] Data are expressed as means ± SEMs. The data are presented as the ratio of LDH in supernatants of experimental wells to LDH in cell lysates and supernatants of sister wells lysed with Triton X-100 times 100.
[b] $P < 0.05$ (compared with media alone).
[c] $P < 0.05$ (compared with COH-1 in the absence of cytochalasin D).

inary evidence that less-encapsulated type III GBS strains are more adherent to HUVE monolayers (25a). The increased HUVE cell invasion by strains COH 1-11 and COH 1-13 may be due in part to increased adherence. Studies are ongoing in our laboratory to identify specific GBS adhesins or "invasins" by using transposon-insertional mutagenesis.

We have observed GBS invasion of alveolar epithelial cells, interstitial fibroblasts, and now lung capillary endothelium in a newborn *Macaca nemestrina* in an in utero model of GBS disease (27). Our in vitro data suggest that GBS can invade the apical (luminal) surface of the endothelium. The current study does not address the ability of GBS to invade the basolateral surface of endothelial cells. Invasion of the basolateral surface of endothelial cells could be an important mechanism for GBS to access the vascular space from the alveolar space in the pathogenesis of GBS pneumonia and sepsis. In addition, the current study does not address whether the site of endothelial cell origin (i.e., organ or vessel size) can influence GBS invasion or GBS-induced endothelial cell injury. Other investigators have observed that lung microvascular endothelial cells are more resistant to endotoxin-induced injury than endothelial cells from the main pulmonary artery (21). GBS invasion of both the pulmonary epithelial and endothelial cell barriers may contribute to the pathogenesis of GBS disease by direct cellular injury and/or the bypassing of local host defenses.

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