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Effect of Type III Group B Streptococcal Capsular Polysaccharide on Invasion of Respiratory Epithelial Cells

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Group B streptococcal (GBS) capsular polysaccharide is an important virulence factor, and its role in invasion of cultured respiratory epithelial cells was investigated. A type III GBS clinical isolate, COH1, and asialo and unencapsulated isogenic transposon capsule mutants of it were compared in an in vitro invasion assay. The results demonstrated that capsule attenuated the invasion process. Invasion was not affected when the A549 epithelial cells were preincubated with purified type III GBS capsular polysaccharide. Polyclonal type III GBS capsule antibody inhibited invasion by COH1 but did not affect invasion by the capsule mutants. Serotypes Ia, Ib, Ia/c, II, and III all invaded respiratory epithelial cells but demonstrated some strain variation in magnitude of invasion. These observations led us to conclude that type III capsular polysaccharide was not essential for invasion of respiratory epithelial cells by GBS and that bacterial factors other than capsule were responsible for respiratory epithelial cell invasion.

In the late 1960s, the group B streptococci (GBS) were recognized as a neonatal pathogen, and currently they are the most common cause of infectious neonatal morbidity and mortality in the United States (1). GBS are facultative, beta-hemolytic, gram-positive cocci possessing a distinct polysaccharide capsule that is responsible for the serotype specificity. While all serotypes have been associated with human infection, type III GBS is the isolated most frequently serotype from septic infants in the United States (1, 9).

The type III capsular polysaccharide is a repeating pentasaccharide subunit consisting of a glucose, galactose, and *N*-acetylglucosamine backbone with a disaccharide side chain composed of a terminal sialic acid residue attached to a galactose. The structure of the capsular polysaccharide is important for its function as a virulence factor. It has been shown that the terminal sialic acid residues on the capsular polysaccharide inhibit the activation of the alternative complement pathway in the absence of capsule-specific antibody, allowing the organism to evade opsonophagocytosis, a major host defense mechanism (3, 4).

We previously used transposon mutagenesis to derive asialo and unencapsulated isogenic capsule mutants of COH1, a type III GBS strain isolated from a septic infant that produces a large amount of capsule. The asialo isogenic capsule mutant was designated COH1-11, and the unencapsulated isogenic capsule mutant was designated COH1-13. Virulence studies with neonatal rats demonstrated a significant decrease in virulence of both capsule mutants compared with COH1 (19). These data confirmed that the capsule was an important virulence factor and that the terminal sialic acid moiety was critical for capsule function.

Previous studies in a nonhuman primate model of neonatal GBS pneumonia demonstrated the presence of organisms within respiratory epithelial cells by electron microscopy (14). Subsequently, in an in vitro invasion assay adapted from that of Isberg et al. (7), COH1 was shown to invade immortalized respiratory epithelial cells by quantitative culture and electron microscopy (15). When COH1 and COH31r/s, a less-virulent type III clinical isolate that produces a small amount of capsule (15), were compared in the in vitro invasion assay, the intracellular recovery of COH1 was four- to fivefold higher than that of COH31r/s. Since COH1 produces more capsule than COH31r/s, this suggested that the capsule might be important for invasion. These data, coupled with our prior observations showing decreased in vivo virulence for the COH1 capsule mutants, led to our hypothesis that the capsule played a role in entry of epithelial cells by GBS. This report describes our investigation of the effect of type III capsular polysaccharide on invasion of respiratory epithelial cells by GBS.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. The GBS strains were stored at −70°C in Todd-Hewitt broth (THB) with 25% glycerol after isolation on 5% sheep blood agar. Working aliquots of each strain were made by inoculating THB from the freezer stock, incubating it overnight at 37°C, and storing it at −70°C in 1-dram vials with 25% (vol/vol) glycerol. *Escherichia coli* DH5α was kept at −70°C in 25% glycerol–THB.

Tissue culture reagents and methods. The A549 cell line was derived from a human lung alveolar carcinoma and obtained from the American Type Culture Collection. RPMI 1640 tissue culture medium (Cellgro) with 10% fetal calf serum (Hyclone Laboratories, Inc.), 1% L-glutamine (200 mM; GIBCO Laboratories), penicillin G (50 U/ml), and streptomycin (50 μg/ml; GIBCO Laboratories) was used to maintain the cell line.

Epithelial cell invasion assay. The invasion assay has been described (5, 15). Briefly, A549 monolayers were inoculated with 10^5 mid-log-phase GBS (giving a multiplicity of infection of 1:1) and incubated at 37°C in 5% CO_2 for 2, 4, or 6 h. Stationary-phase organisms were used in the invasion assays to study the effect of purified capsular polysaccharide and anti-native type III antibody on invasion because data on the invasive ability of GBS in various stages of growth showed that optimal invasion occurred during stationary- and early-

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TABLE 1. Bacterial strains

Strains	Relevant characteristics[a]	Reference(s)
GBS		
COH1	Type III, Tc^r	10
COH1-11	Type III, Tn*916*ΔE, asialo, Tc^r Em^r	12, 19
COH1-13	Type III, Tn*916*ΔE, cap$^-$, Tc^r Em^r	12
M732	Type III, Tc^r	19
COH46	Type Ia	This study
COH58	Type Ia	This study
COH50	Type Ib	This study
COH9	Type Ib	This study
COH8	Type Ic	This study
COH19	Type Ic	This study
COH28	Type II	This study
COH21	Type II	This study
E. coli DH5α	F$^-$ ϕ80*dlacZ*ΔM15 *recA1 endA1 gyrA96 thi-1 hsdR17* (r_k^- m_k^-) *supE44 relA1 deoR* Δ(*lacZYA argF*) *U169*	17

[a] Tc^r, tetracycline resistant; Em^r, erythromycin resistant.

log-phase growth (15). After incubation with the bacteria, the cells were washed with sterile phosphate-buffered saline (PBS) and incubated for 2 h with fresh tissue culture medium containing penicillin and gentamicin to kill remaining extracellular organisms. After being washed with sterile PBS as above, the monolayers were trypsinized, lysed, and plated on Todd-Hewitt agar (THA) for quantitation of intracellular CFU. The initial bacterial dilution inoculated onto the epithelial cells was confirmed by quantitative plate counting on THA. The degree of bacterial replication in the supernatant during the 2-, 4-, and 6-h incubation periods was determined by plating serial dilutions of the infected medium from one well on THA at each time point. The data are presented as intracellular CFU recovered per well and represent the mean for three wells ± the standard error of the mean.

Electron microscopy. For electron microscopy, bacteria were prepared as for the invasion assay described above. Ninety-six-well, flat-bottomed tissue culture plates with A549 monolayers were used. An inoculum of 10^7 CFU/ml was added to the monolayers for each bacterial strain, and infection was carried out for 2, 4, and 6 h. The larger inoculum used in this experiment was optimal for the observation of intracellular organisms. At each time point, the monolayer was fixed with 2.5% glutaraldehyde and prepared for electron microscopy as described before (15).

Effect of purified type III capsular polysaccharide. The effect of purified capsule on bacterial uptake was analyzed by a modification of the invasion assay. Purified type III GBS capsular polysaccharide (<1% protein) from GBS strain M732 was kindly provided by M. Wessels, Channing Laboratories, Boston, Mass. The capsular polysaccharide was dissolved in sterile distilled water and frozen at −20°C. A working solution of 10 μg/ml was made in sterile PBS just prior to use. The working solution was diluted to the appropriate concentration with RPMI 1640 tissue culture medium containing 10% fetal calf serum for use in the assay. Wessels et al. (19) quantitated the amount of capsular polysaccharide on COH1. They found the equivalent of 1.1 μg of type 14 pneumococcal capsular polysaccharide per 10^9 CFU of COH1. From this quantification, a 500- to 10,000-fold excess of purified capsular polysaccharide was used in these experiments.

A549 cells were prepared for the invasion assay as described above. Prior to inoculation with bacteria, A549 cells were incubated with 0.05, 0.1, or 1.0 μg of type III capsular polysaccharide per well for 30 min at 37°C. The medium containing the capsular polysaccharide was removed, and the A549 cells were washed once with sterile PBS and inoculated with the appropriate bacterial suspensions. Bacteria were allowed to infect the cell monolayers for 1 h at 37°C. The remainder of the invasion assay was carried out as described above. Control wells were preincubated with tissue culture medium without free capsular polysaccharide.

Effect of antibody to native type III GBS capsule. Antibody to native type III GBS capsular polysaccharide was kindly provided by M. Wessels, Channing Laboratories, Boston, Mass. The antibody was raised by intravenous injection of rabbits with purified type III capsular polysaccharide from GBS strain M732 conjugated to tetanus toxoid. Antibody was stored undiluted in serum at −20°C. Dilutions for the invasion assay experiments were made just prior to use in RPMI 1640 tissue culture medium containing 10% fetal calf serum as the diluent and ranged from 1:1,000 to 1:200,000.

The bacterial strains were prepared as for the usual invasion assay except that the organisms were grown to the stationary phase. Antibody dilutions were incubated with 10^5 CFU for 30 min at 25°C. Two controls for each bacterial strain were included; one contained bacteria preincubated in tissue culture medium containing preimmune rabbit serum, and the second contained bacteria preincubated in tissue culture medium without antibody. A549 monolayers were inoculated with the antibody-bacteria suspension and incubated at 37°C in 5% CO_2 for 1 h. After removal of the antibody-bacteria suspension, the A549 cells were washed with sterile PBS four to five times, and the remainder of the invasion assay was performed as described above. Gram stains were performed on antibody-bacteria dilutions of 1:1,000, 1:10,000, and 1:20,000 to check for aggregation of bacteria.

RESULTS

Invasion of A549 cells by type III GBS. The parent strain, COH1, was allowed to infect A549 monolayers for 2, 4, and 6 h before the addition of penicillin and gentamicin to kill extracellular GBS. The number of intracellular CFU recovered after 4 and 6 h was 4- and 18-fold higher, respectively, than after 2 h (Fig. 1). *E. coli* DH5α did not invade the cells significantly. These data confirmed our previous observation of continued invasion by GBS over time (15).

Invasion assays comparing COH1 with its capsule mutants COH1-11 and COH1-13 were performed. Higher numbers of intracellular CFU of the capsule mutants were recovered at all time points (Fig. 1). The number of intracellular CFU recovered was three- and fourfold higher for COH1-11 and COH1-13, respectively, at 2 h, four- and eightfold higher, respectively, at 4 h, and two- and fourfold higher, respectively, at 6 h than for COH1. From 2 to 4 h of incubation, the number of intracellular CFU recovered increased remarkably, observed as an increase in the slope of the line, suggesting that enhancement of the bacterial entry process was occurring. *E. coli* DH5α did not invade the cells significantly at any time point during these assays. The fewer intracellular CFU of COH1-11 than of COH1-13 recovered was also significant. These data suggest that capsule attenuates bacterial uptake and that decreases in the amount of

capsule on the surface of the mutants may expose and promote a surface factor(s) that is important for invasion.

We previously demonstrated that active bacterial growth was essential for GBS invasion of A549 respiratory epithelial cells. An increase in intracellular CFU was observed when the initial bacterial inoculum was increased at least 10-fold. Additionally, organisms in the stationary or early lag phase invaded A549 cells in greater numbers (15). To determine whether differences in growth rate were contributing to the greater invasion by the capsule mutants, growth curves were performed with THB and RPMI 1640 tissue culture media.

The growth curve in THB demonstrated that COH1 grew slightly faster but to an optical density similar to that of the capsule mutants (Fig. 2). Similar observations were made for COH1 grown in RPMI 1640 tissue culture medium (data not shown). The small differences in growth rates between COH1 and its capsule mutants were not thought to contribute to the differences observed in invasion by these GBS strains.

We evaluated extracellular replication in the supernatant fluid by quantitatively culturing the supernatant of one well at each time point for each bacterial strain tested in four separate experiments. We observed a $10^{2.5}$-fold increase for *E. coli* DH5α and 10^4-fold increase in GBS bacterial density during the 6 h of incubation (Table 2). The increase in intracellular CFU of the GBS strains recovered was 10^2-fold during the same time period that DH5α numbers increased 10-fold. These data demonstrated that the dramatic increase in bacterial density in the supernatant was not proportional to the increase in intracellular GBS and was probably not responsible for the differences observed between the mutants and COH1. A finite number of GBS appear to enter A549 cells over time despite continued, significant extracellular bacterial replication.

Previously, we demonstrated that COH1 did not replicate inside cells (15). The ability to replicate intracellularly was evaluated for each capsule mutant. The invasion assay was performed as above, and 2 h after infection of the monolayers, the supernatants were removed, the cells were washed, and fresh medium containing antibiotics to kill extracellular organisms was added for 2 or 6 h. The results demonstrated that there was no intracellular replication after 2 or 6 h for COH1 or the mutants. However, as demonstrated above, more CFU were observed in cells infected with either mutant than in those infected with COH1.

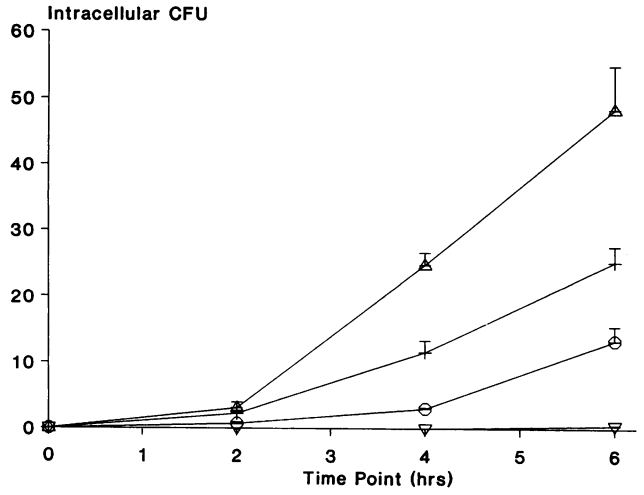


FIG. 1. Invasion of A549 monolayers by COH1 and its capsule mutants, COH1-11 and COH1-13. *E. coli* DH5α was the negative control strain. Intracellular CFU were recovered after 2, 4, and 6 h of infection. The data (CFU in thousands) presented are from a single experiment performed in triplicate and are representative of three experiments. In addition to the increase in intracellular CFU recovered over time, there is a remarkable increase in the slope of the line after 2 to 4 h of incubation of the GBS strains, suggesting an increased rate of invasion. ○, COH1; △, COH1-13; +, COH1-11; ▽, DH5α.

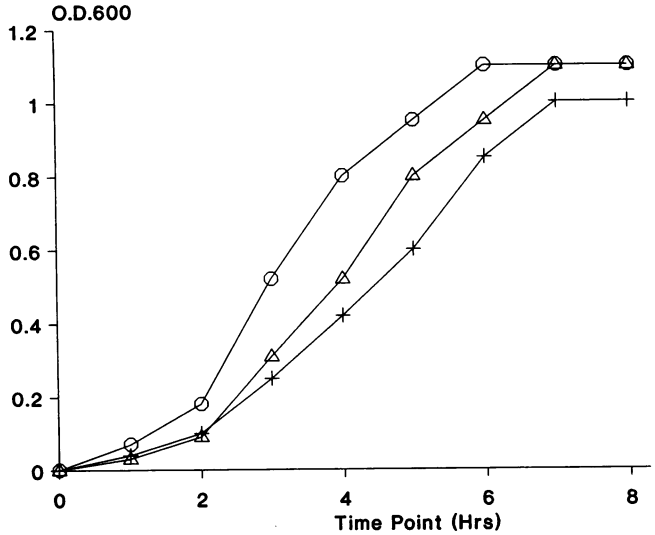


FIG. 2. Growth curves in THB culture medium. Optical density (O.D.) readings were taken at 30-min intervals. COH1 grew slightly faster but to an optical density similar to that of the capsule mutants. ○, COH1; △, COH1-13; +, COH1-11.

Electron microscopy. Transmission electron microscopy was used throughout the experiments to confirm the presence of intracellular GBS. We observed GBS strains COH1, COH1-11, and COH1-13 within membrane-bound vacuoles inside A549 epithelial cells at all time points (Fig. 3). More bacteria were observed within A549 cells infected with the mutant strains than with COH1, supporting our invasion results. The cellular architecture appeared normal, without indication of cell death or loss of cell membrane integrity at any time point.

Effect of purified type III capsular polysaccharide. To confirm our findings with the capsule mutants, we analyzed the effect of purified type III capsule on epithelial cell invasion by type III GBS. The same GBS strains were used, and *E. coli* DH5α served as the negative control. Preincubation of the A549 cells for 30 min with a 500- to 10,000-fold excess of purified type III capsular polysaccharide had no significant effect on the number of intracellular CFU recovered compared with the number recovered from control wells containing epithelial cells that were not preincubated with the capsule (Fig. 4). These data show that free type III capsular polysaccharide did not enhance or block GBS invasion of A549 epithelial cells.

Effect of anti-native type III capsular antibody. The same strains were used in experiments evaluating the effect of rabbit monospecific anti-type III capsule antibody on invasion of respiratory epithelial cells. Incubation of 10^5 stationary-phase bacteria with high-titer rabbit anti-native type III capsule antibody at dilutions of 1:200 to 1:200,000 was done

TABLE 2. Extracellular replication in the well supernatant and corresponding intracellular CFU recovered[a]

Time postinfection (h)	COH1		COH1-11		COH1-13		DH5α	
	Growth	Recovery	Growth	Recovery	Growth	Recovery	Growth	Recovery
0	9.7×10^4		9.0×10^4		9.7×10^4		4.6×10^4	
2	9.4×10^6	740	5.3×10^5	2,233	4.4×10^6	3,070	1.2×10^5	17
4	1.4×10^8	3,000	4.4×10^6	11,600	2.6×10^7	24,700	7.7×10^5	0
6	4.8×10^8	13,300	1.5×10^7	25,100	9.2×10^7	48,300	1.6×10^6	470

[a] All values are CFU.

before inoculation of A549 cells for the invasion assay. Figure 5 demonstrates that the number of intracellular CFU of COH1 recovered was reduced 70 to 80% after incubation of the organism with a 1:1,000 dilution of the antibody. The same degree of inhibition was seen for COH1 at lower antibody dilutions. The inhibitory effect of the antibody was titratable up to a dilution of 1:100,000, and antibody dilutions of greater than 1:100,000 did not affect intracellular CFU recovery for COH1. Invasion by COH1-11 was reduced by 30% at the 1:1,000 dilution of antibody, and COH1-13 and DH5α were not affected at any antibody dilution. Control wells containing bacteria preincubated with preimmune rabbit serum did not differ significantly in the number of intracellular CFU from control wells containing bacteria preincubated with tissue culture medium alone. The antibody-associated inhibition of invasion by COH1 and COH1-11 was probably due to steric hindrance caused by the antibody-capsule complex.

To assess whether agglutination of bacterium-antibody complexes contributed to the lower recovery of COH1, suspensions containing 10^4 bacteria in tissue culture medium with antibody at dilutions of 1:1,000, 1:10,000, and 1:20,000 were evaluated by light microscopy after Gram staining. None of the Gram stains demonstrated agglutination. The remarkable inhibition of COH1 invasion by the antibody and the lack of inhibition of capsule mutant invasion suggest that other surface components of GBS are important for bacterial entry into epithelial cells.

Invasion by different GBS serotypes. Our prior studies (15) and those above were performed strictly with type III GBS strains. To determine whether other GBS serotypes could invade A549 respiratory epithelial cells, clinical isolates representing serotypes Ia, Ib, Ia/c, II, and III were compared in the invasion assay (Fig. 6). All GBS serotypes invaded A549 cells. Invasion varied between strains of the same serotype as well as between serotypes. These data demonstrated that strains of all GBS serotypes were capable of invading respiratory epithelial cells independent of the specific capsular serotype.

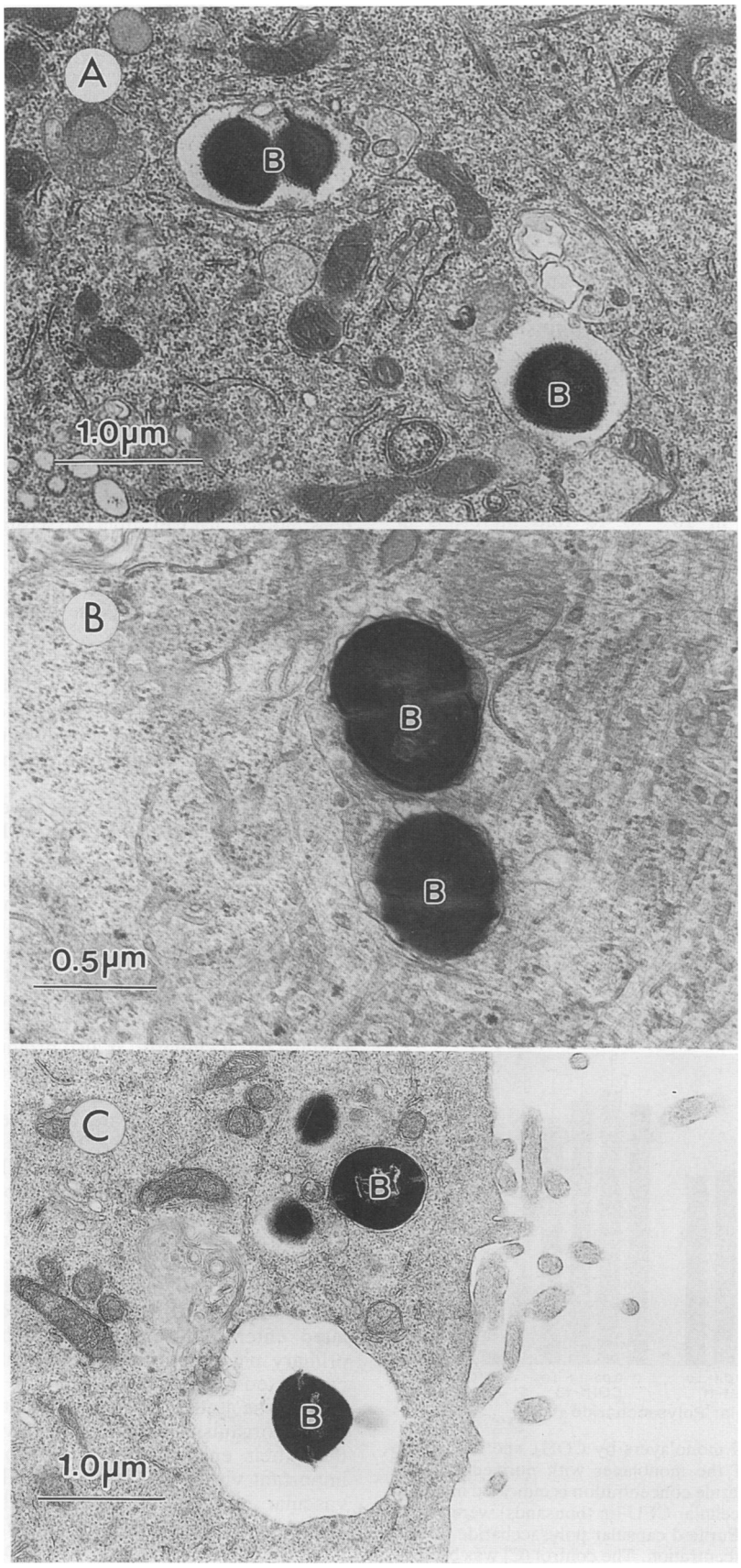
DISCUSSION

This study investigated the role of the type III capsule in GBS invasion of respiratory epithelial cells. One of the main portals of entry into the neonatal host is the respiratory tract (10), an epithelialized mucosal surface. Dissemination of infection from the respiratory tract requires penetration of the respiratory epithelium, the pulmonary interstitium, and the pulmonary endothelium. We have previously shown that type III GBS enter respiratory epithelial cells in vivo and in vitro and pulmonary interstitial cells in vitro (11, 14, 15). Gibson et al. (5) demonstrated higher levels of invasion of human umbilical vein endothelial cells by the capsule mutants COH1-11 and COH1-13 than by COH1 and concluded that capsule appeared to attenuate bacterial entry into endothelial cells. While they demonstrated that GBS enter endothelial cells in vitro, extrapolation of the results of invasion assays done with endothelial cells cannot be extended to epithelial cells.

Very little is known about the eukaryotic and bacterial mechanisms involved in infection by GBS, and while it appeared that capsule was not important for invasion of endothelial cells, it was unknown whether the capsule played an important role in invasion of epithelial barriers. Previously, we demonstrated that COH1, which produces a large amount of capsule, was significantly more invasive than COH31r/s, which produces a small amount of capsule. These results suggested that there was strain-to-strain variation in the ability to invade respiratory epithelium or that capsule might be important for invasion (15). The previous derivation of the isogenic transposon capsule mutants (COH1-11 and COH1-13) of the clinical type III GBS strain COH1 provided a unique opportunity to define the role of type III GBS capsule during invasion of epithelial cells.

The data comparing invasion by the encapsulated parent strain, COH1, with that by its capsule mutants demonstrated that the capsule mutants were two- to eightfold more invasive within 6 h of infection of A549 cells. These data suggested that capsule was interfering with but not preventing bacterial uptake. The asialo mutant, COH1-11, has a smaller capsule in addition to lacking the sialic acid residues on the capsule backbone (19). Additionally, significant conformational changes in the capsular polysaccharide may take place when the terminal sialic acid residues are removed (8). The reduction in capsule size, changes in conformation, and loss of the negative charge could all contribute to higher CFU recovery from respiratory epithelial cells by enhancing exposure of a surface moiety important for invasion and/or changing the bacterium-host cell interaction to favor invasion. COH1-13, the unencapsulated mutant, has no capsular polysaccharide, so conformational changes in the capsular polysaccharide play no role in its invasive ability. However, other surface factors important for invasion would be more exposed, resulting in a more favorable interaction between the bacteria and the epithelial cells. Although capsule associated with the bacteria appeared to attenuate invasion of epithelial cells, probably by virtue of steric hindrance or

FIG. 3. Electron micrographs demonstrating organisms within membrane-bound vacuoles (marked B) at all time points, with retention of normal cytoskeletal architecture. (A) COH1 after 4 h of incubation; (B) COH1-11 after 2 h of incubation; (C) COH1-13 after 4 h of incubation.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charge effects, no effect was observed when free purified type III GBS capsular polysaccharide was incubated with A549 cells before the invasion assay. These data suggested that capsule does not have a role in a receptor-ligand interaction involved in the mechanism of GBS entry into respiratory epithelial cells.

In addition to the quantitative increase in intracellular CFU recovered, we also observed a dramatic increase in the rate of intracellular CFU recovery over time, which suggested that the bacterial entry process was enhanced as the infection progressed. The increase in recovery rate could be due to induction and increased production of a bacterial product that stimulated bacterial uptake. Alternatively, as GBS enter the stationary phase, they may invade cells more efficiently (15).

We also considered factors other than changes in encapsulation which may have influenced the invasion assay results. We demonstrated that the growth rates of COH1 and its capsule mutants were not significantly different and thus unlikely to influence the results of the assay. Extracellular replication of COH1 and its capsule mutants during the invasion assay resulted in 10^1- to 10^4-fold increases in bacterial numbers for all of the strains, an increase which was not proportional to the increase in intracellular CFU recovery. It is possible that there may be a finite number of epithelial cell surface receptors available to the organism for invasion, a finite number of bacteria within the population which are capable of entry, or a limited increase in epithelial cell susceptibility to bacterial invasion. Finally, COH1 does not replicate inside A549 epithelial cells (15), and neither did the COH1 capsule mutants during this study. These results confirmed that the differences observed in the invasion assay results for COH1 and the capsule mutants reflected their differences in encapsulation rather than altered growth characteristics.

Inhibition of COH1 and COH1-11 invasion of epithelial cells by anti-native type III capsule antibody was most likely caused by antibody binding to the capsular epitope, with subsequent steric hindrance of the bacterial-epithelial cell

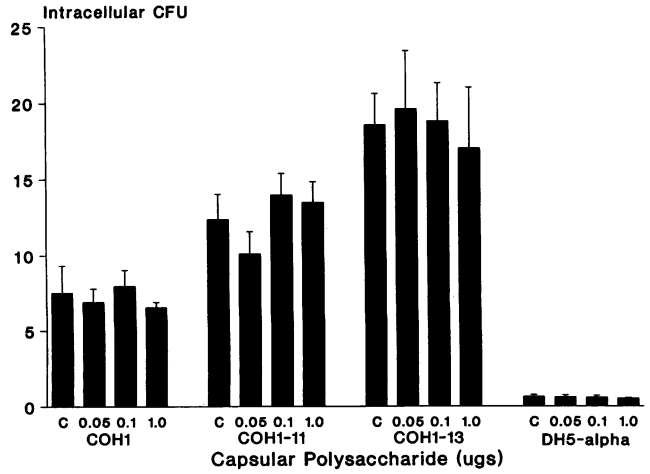

FIG. 4. Invasion of A549 monolayers by COH1 and its capsule mutants after incubation of the monolayer with purified type III capsule. Capsular polysaccharide concentration is indicated in micrograms added per well. Intracellular CFU (in thousands) were recovered after 1 h of infection. Purified capsular polysaccharide had no effect on invasion at any concentration. The control (C) was bacteria preincubated in medium without free capsular polysaccharide.

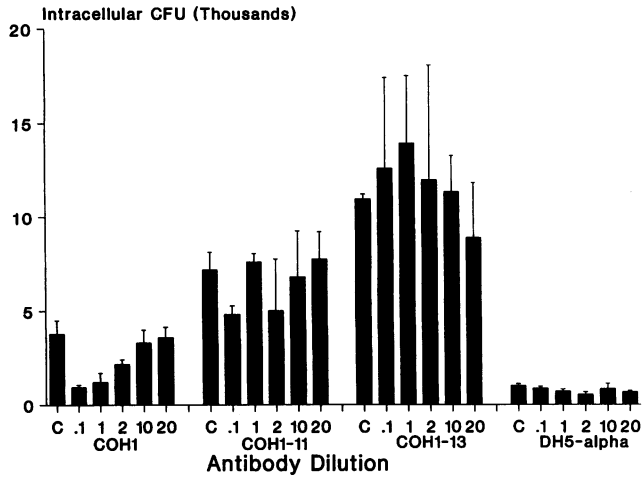

FIG. 5. Invasion of A549 monolayers by COH1 and its capsule mutants after incubation of the bacteria with polyclonal antibody to native type III capsular polysaccharide at the indicated 10^4 dilutions. Intracellular CFU from two separate experiments were determined after 1 h of incubation. Anticapsular antibody inhibited invasion by COH1 by 70 to 80%. COH1-11 was inhibited 30% at the 1:1,000 antibody dilution. The antibody did not affect invasion by COH1-13 or *E. coli* DH5α. The control (C) was bacteria preincubated without antibody.

interactions. COH1-13 would not be expected to bind the antibody because it lacks capsule. Reduced or lack of antibody binding to the bacterial surface resulted in minimal or no inhibition of entry into respiratory epithelial cells by these mutants.

These data demonstrate that type III GBS capsular polysaccharide attenuates the ability of but does not prevent this organism from invading a respiratory epithelial cell monolayer. These observations agree with those made for GBS invasion of endothelial cells (5). The differences in invasion of A549 respiratory epithelial cells by COH1 and COH31r/s (15) are consistent with the strain-to-strain variability observed in this study among GBS of the same serotype as well as those of different serotypes. This variation may represent differences in ability to adhere and invade or ability to survive intracellularly. It also provides further evidence that factors other than the capsular polysaccharide are important for invasion.

These observations are consistent with those made in studies of epithelial cell adherence and invasion with other encapsulated bacterial pathogens. For example, the encapsulated *Haemophilus influenzae* type b parent strain was less adherent and invasive than its unencapsulated isogenic mutants in a cultured human respiratory epithelial cell line (18). Encapsulated clinical isolates of *Neisseria meningitidis* were less adherent to human buccal epithelial cells than unencapsulated variants of several laboratory strains (2). Encapsulated enterotoxigenic *E. coli* adhere poorly to cultured primary pig intestinal epithelial cells compared with unencapsulated mutants (16).

As these studies and our own data demonstrate, unencapsulated organisms adhere to and invade epithelial cells better than their encapsulated counterparts, yet capsule is an important virulence factor and is known to enhance intravascular survival of several pathogens, including GBS, *Streptococcus pneumoniae*, and *H. influenzae* type b (3, 4, 6, 18). Spontaneous recombination at the *capB* locus of *H. influenzae* type b results in loss of encapsulation, occurs at

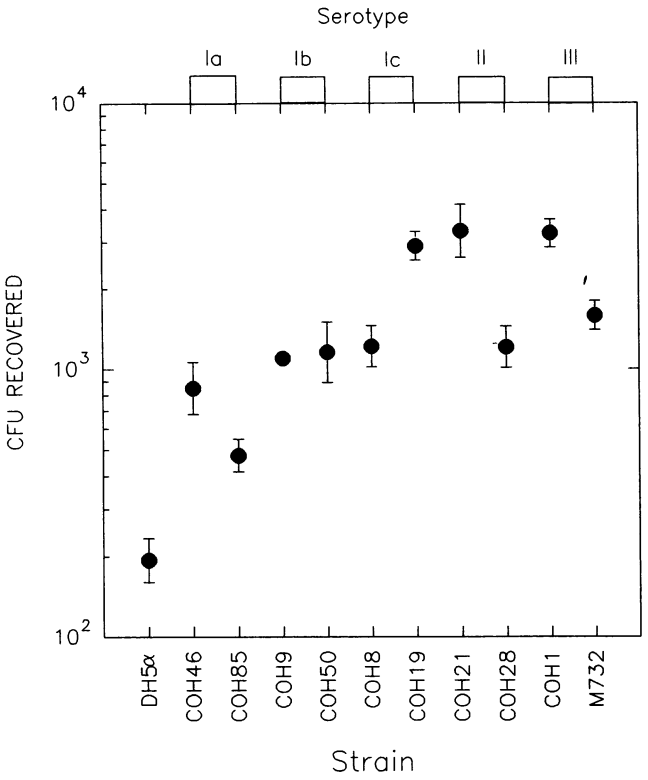

FIG. 6. Invasion of A549 monolayers by different GBS serotypes. The data are presented as the geometric means for a minimum of 10 experiments ± the 95% confidence interval and demonstrate that all GBS serotypes invade A549 cells better than the negative control strain DH5α.

high frequency, and results in organisms that adhere better to the nasopharyngeal mucosa and survive longer at that site (18).

Just as genetic events affect *H. influenzae* capsule production, infection, and survival, we postulate that genetic control of capsule production may be important in the pathogenesis of infection by GBS. Decreased production of capsular polysaccharide by GBS may allow colonization and invasion of gastrointestinal, genitourinary, and respiratory tract mucosa, whereas increased synthesis may be important once GBS invade to promote tissue and intravascular survival by protecting the organism from killing by the host's professional phagocytes. Whether GBS regulates capsule biosynthesis in response to different environmental and host conditions remains to be determined.

We will continue to characterize invasion of epithelial cells by GBS by deriving isogenic invasion mutants. Identification and characterization of the genes and gene products involved in invasion will provide further definition of this complex process in the pathogenesis of neonatal infections caused by this pathogen.

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