Pneumonia and lung injury are hallmarks of early-onset neonatal group B streptococcal (GBS) infections. Production of a β-hemolysin/cytolysin (β-h/c) encoded by the cylE gene is associated with GBS virulence in vivo. To elucidate the contribution of the β-h/c toxin to lung injury, the interactions of GBS wild-type strains and isogenic cylE mutants with A549 lung epithelial cells were examined. Compared with wild-type GBS strains, cylE mutants did not produce cytolytic injury, even at high inocula, and exhibited decreased cellular invasion. Additionally, cylE mutants induced less A549 cell release of the neutrophil chemoattractant interleukin (IL)–8. GBS invasion and IL-8 induction were significantly reduced in the presence of dipalmotyl phosphatidylcholine, a major constituent of lung surfactant and a known inhibitor of β-h/c activity. These data indicate that the GBS β-h/c contributes to invasion and immune activation of lung epithelial cells and may represent a multifunctional virulence factor in the early pulmonary stages of GBS infection.

Group B streptococcal (GBS) infection is the leading cause of serious bacterial infections in human newborn infants [1]. Early-onset GBS disease is heralded by respiratory signs, and 80% of autopsy cases show histological evidence of lobar or multilobar pneumonia [2, 3]. GBS pneumonia is characterized by evidence of severe lung injury, including dense bacterial and neutrophilic infiltrates, alveolar hemorrhage, proteinaceous exudate, and hyaline membrane formation [4, 5]. Premature, low–birth weight infants are at especially high risk for acquiring GBS pneumonia and invasive disease [6].

The lung is the apparent portal of entry for group B streptococci (GBS) into the bloodstream, after which septicemia may ensue [1, 7]. To access the circulation, the bacteria must first breach 2 host cellular barriers—the alveolar epithelium and the pulmonary capillary endothelium. A relevant virulence mechanism involves the ability of GBS to penetrate and to survive intracellularly within these host cells. GBS invasion of alveolar epithelial cells and pulmonary endothelial cells has been clearly demonstrated in human tissue culture lines and in the primate model of early-onset infection [8–10]. In severe pulmonary infections, direct cytotoxicity from bacterial factors, along with byproducts of the neutrophil inflammatory response, would likely further compromise the integrity of the neonatal mucosal barrier [11].

Most GBS clinical isolates exhibit a β-hemolytic phenotype when plated on blood agar [12]. The GBS β-hemolysin is a pore-forming membrane toxin capable of injuring a broad range of eukaryotic cell types [13, 14]. Because of the notable direct correlation between the level of GBS β-hemolysin production and cytolytic injury to human alveolar epithelial [15] and pulmonary capillary endothelial cells [16], the designation β-hemolysin/cytosin (β-h/c) has been applied to the toxin. GBS β-h/c–induced injury of lung epithelial cells is blocked by the phospholipid dipalmotyl phosphatidylcholine (DPPC) [15], the major component, by weight, of human pulmonary surfactant [17]. This observation provides a theoretical rationale by which the β-h/c toxin may contribute to the increased susceptibility of premature surfactant-deficient neonates to severe GBS disease [14, 15]. Animal model studies that have used GBS transposon mutants have correlated GBS β-h/c expression with disease severity. When compared with the corresponding wild-type parent strains, β-h/c–negative mutants were less virulent in adult mice after intranasal [18] or intravenous [19] inoculation, in neonatal rats after transtracheal inoculation [20], and in adult rabbits after intravenous administration [21].

The genetic basis for the GBS β-h/c phenotype has very recently been elucidated [22, 23]. A single gene, cylE, which encodes a novel 78-kD predicted protein product, is both necessary for GBS β-h/c production and sufficient to confer a β-hemolytic phenotype to Escherichia coli [23]. Discovery of GBS β-h/c gene cylE facilitates precise in vitro and in vivo analysis of the specific virulence role(s) of the β-h/c toxin during key steps in the infectious process. In the present study, we used targeted GBS cylE knockout mutants to study the effects of β-h/c ex-
pression on the bacterium’s ability to invade lung epithelial cells and trigger release of the chemokine IL-8.

Materials and Methods

Bacterial strains. COH1 (serotype III), A909 (serotype Ia), and NCTC 10/84 (serotype V) are well-characterized GBS clinical isolates from the blood or cerebrospinal fluid of neonates with sepsis [24–26]. These strains are representative of the 3 most common GBS serotypes associated with early-onset infection [1]. The isogenic β-h/c–deficient COH1:cylE.KO, A909:cylE.KO, and NCTC: cylE.KO are products of targeted plasmid integration mutagenesis of the β-h/c structural gene, cylE [23]. COH1-13, which lacks the type III surface polysaccharide capsule, is a Tn916ΔE mutant of parent strain COH1 [27]. By use of plasmid integration [23], COH1-13:cylE.KO was created for these studies, to provide an unencapsulated, β-h/c–deficient double mutant. Bacteria were grown in Todd-Hewitt broth to log-phase (OD600, 0.4, ~10^8 cfu/mL), washed twice in PBS, and then resuspended and diluted in cell culture medium for use in the cytotoxicity, cellular invasion, and IL-8 release assays.

Lung epithelial cell culture. A549 cells (American Type Culture Collection), a human type II alveolar epithelial carcinoma cell line, were maintained and passaged in RPMI 1640 tissue culture medium that contained 10% fetal calf serum. Monolayers were seeded into 96- or 24-well tissue culture plates (Corning) and were used experimentally within 24 h of establishing confluence. Monolayers were washed 3 times with PBS, and fresh culture medium was added immediately prior to assays.

Cytotoxicity assay. Cytotoxicity of GBS strains was determined by measuring release of the intracellular enzyme lactate dehydrogenase (LDH) from A549 monolayers in a 96-well microtiter plate assay, as described elsewhere [15].

Cellular invasion and adherence assays. Intracellular invasion of A549 monolayers GBS was measured by using an antibiotic protection method developed by Rubens et al. [9]. Companion assays were performed to measure total A549 cell–associated bacteria (invading and surface adherent) [28]. In these assays, the initial incubation period was shortened from 2 h to 30 min. Then, in place of the 2-h exposure to extracellular antibiotics, monolayers were washed 6 times with PBS before monolayer lysis and release of bacteria. In both cellular invasion and adherence assays, dilutions of the lysate were plated for counts of bacteria. Assays were performed in triplicate and were repeated 4 times. In phospholipid inhibition experiments, A549 medium with 500 µg/mL of DPPC (Sigma P-0763) was prepared by sonication and was used for the duration of the assay.

IL-8 induction assay. Log-phase GBS (1 x 10^4 to 1 x 10^6 cfu) were added to A549 cell monolayers in 24-well plates, which were centrifuged at 800 g for 10 min to place bacteria on the monolayer surface and then were incubated at 37°C in 5% CO2 for the duration of the assay. For comparison of wild-type versus mutant bacteria, a standard inoculum of 1 x 10^6 cfu (MOI, 1:1) and an assay time of 4 h were used. The nonhemolytic, noninvasive Lactococcus lactis strain NZ9000 [29] and medium alone were used as negative controls; tumor necrosis factor (TNF)–α at 5 ng/mL was used as a positive control. In phospholipid inhibition experiments, 500 µg/mL of DPPC was added to the A549 cell medium. At the end of the exposure period, cell culture supernatants were collected, and bacteria were removed by centrifugation and stored at 4°C until IL-8 measurement. IL-8 from cell culture supernatants was measured by ELISA, as described elsewhere by Eckmann et al. [30]. IL-8 induction assays were performed in triplicate and were repeated 3 times.

Reverse-transcription polymerase chain reaction (RT-PCR) for IL-8 transcript. The standard IL-8 induction assay was performed as described above, with use of strain A909 and TNF-α as a positive control. At 0, 1, and 4 h, the supernatant was removed, and the A549 cell monolayers from each of 3 replicate wells were solubilized in 300 µL of Trizol reagent (Gibco/BRL) for RNA extraction, according to the manufacturer’s protocol. Total cellular RNA (1 µg) was reverse transcribed at 42°C for 50 min in 20 µL of buffer that contained 50 mM Tris-HCl (pH 8.3); 75 mM KCl; 3 mM MgCl2; dATP, dCTP, dGTP, and dTTP, each 1 mM; 10 mM dithiothreitol; 330 ng of random primers (pd[N]6; Amersham Pharmacia Biotech); and 200 U of SuperScript II (Gibco/BRL). Reactions were stopped by heating at 70°C for 15 min. The cDNA products (2 µL) were amplified by PCR in 50 µL of 20 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, each dNTP at 200 µM, and each primer at 0.5 µM. The reactions were heated to 95°C for 5 min and 55°C for 3 min before the addition of 2.5 U of Taq DNA polymerase (AmpliTaq Gold; Perkin Elmer). Cycling parameters were 72°C for 30 s, 94°C for 30 s, and 58°C for 1 min, for a total of 30 cycles. The primers and resulting PCR products for IL-8 (289 bp) and β-actin (661 bp) have been described elsewhere [31]. PCR products were analyzed by running reactions on 1% agarose/ethidium bromide gels. Negative controls for RT-PCR detection were performed by omission of reverse transcriptase from the cDNA synthesis reaction, to exclude amplification of contaminating DNA.

Statistical analyses. Percentage of invasion, percentage of total cell-associated bacteria, and IL-8 measurements are reported as the mean of all observations ± SE. To compare among strains or culture conditions, t tests were performed, using a statistical software package (Excel; Microsoft). P < .05 was considered to be significant.

Results

Cytolytic activity of GBS against A549 cells. The 3 wild-type GBS clinical isolates showed significant differences in A549 cytoltyc activity, as measured by LDH release. After 4 h of incubation, complete monolayer lysis followed the initial MOI of 0.25:1 for the strongly hemolytic strain NCTC 10/84 (serotype V), 16–32:1 for the moderately hemolytic strain A909 (serotype Ia), and 128:1 for the weakly hemolytic strain COH1 (serotype III; figure 1). In contrast, no LDH release above baseline was observed for subcytolytic bacterial inocula used in the quantitative cellular invasion and IL-8 assays to follow.

β-h/c expression contributes to GBS invasion of and adherence to A549 cells. The effect of eliminating β-h/c production
on the ability of GBS to intracellularly invade lung epithelial cells was studied in a quantitative assay. Invasion studies were performed at an MOI of 1:1 for 2 h, below the threshold for monolayer cytolysis by the GBS wild-type strains. All 3 wild-type strains grew equally well through the logarithmic phase in Todd-Hewitt broth (THB). However, after transfer to A549 cell tissue culture media under conditions mimicking those used in the invasion, adherence, and IL-8 assays, strain COH1 (III) grew at 125% of the rate of strain NCTC10/84 (V) and at 200% of the rate of strain A909 (Ia). The growth of the \( \beta \)-h/c mutants was similar to that of their respective parent strains, both in THB and after transfer to the tissue culture medium (data not shown). As shown in figure 2A, each isogenic-deficient \( \beta \)-h/c mutant exhibited a significant decrease in its ability to invade A549 cells, compared with that of the parent strain. To determine whether the decrease in intracellular invasion was due to a decrease in the ability of \( \beta \)-h/c mutants to adhere tightly to the A549 monolayer surface, a 30-min assay with stringent washing was performed to quantify total cell-associated (surface-adherent plus intracellular) bacteria. Figure 2B shows that significant decreases (20%–45%) in total cell-associated bacteria were observed with all \( \beta \)-h/c mutant cells, compared with the wild-type parent strains, which closely mirrored the intracellular invasion findings. As predicted by the LDH release assays, strain NCTC10/84 at an MOI of 1:1 produced evidence of monolayer cytotoxicity during the course of the adherence and invasion assays. We speculate that this cytotoxicity may have led to an underestimation of the wild-type NCTC10/84 invasion and adherence potential. Overall, we conclude that GBS strains that lacked \( \beta \)-h/c were attenuated in their ability to invade and adhere to A549 epithelial cells.

**GBS stimulate IL-8 production by A549 cells in a time- and dose-dependent fashion.** Because neutrophilic infiltration is a hallmark of GBS pneumonia, we hypothesized that exposure to GBS would trigger lung epithelial cells to release the neutrophil chemoattractant IL-8. Figure 3A shows the time course of IL-8 release from A549 cells exposed to 1 × 10^6 cfu of wild-type GBS strain A909 (MOI, 10:1). IL-8 release peaked at 4–6 h and then reached a plateau as bacterial cytotoxicity to the mono-

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**Figure 1.** Group B streptococcal (GBS) injury to A549 lung epithelial cells, as measured by lactate dehydrogenase (LDH) release. Three different wild-type GBS strains of varying hemolytic activity were tested, along with their corresponding isogenic \( \beta \)-hemolysin/cytolysin mutants, by increasing MOI. Monolayers were exposed to the bacterial inoculum for 4 h before collection of supernatants for LDH determination by colorimetric assay. Arrows depict which MOI was used for subsequent experiments. IL, interleukin.

**Figure 2.** Effect of group B streptococcal \( \beta \)-hemolysin/cytolysin (\( \beta \)-h/c) expression on invasion of and adherence to A549 lung epithelial cells. A, Percentage of intracellular bacteria after 2 h of incubation followed by 2 h of antibiotic exposure to kill extracellular bacteria; B, percentage of total cell-associated bacteria after 30 min of incubation and stringent washing. MOI is 1:1 in both cases. Experiments were performed in triplicate wells. Data shown represent mean ± SE of 4 separate experiments. *P < .05; **P < .01.
layer became readily evident on microscopic examination. IL-8 released by A549 cells on exposure to GBS was ~50% the level observed with the TNF-α–positive control stimulus. Maximal IL-8 release was noted at an inoculum of $1 \times 10^7$ cfu (MOI, 100:1) for strain COH1 and at an inoculum of $1 \times 10^6$ cfu (MOI, 10:1) for strain A909 (data not shown). At a higher MOI for each strain, the level of IL-8 release decreased in association with β-h/c–induced monolayer injury. Our results indicate that subcytolytic inocula of GBS induce IL-8 production by A549 cells in a time- and dose-dependent fashion. RT-PCR was used to determine whether IL-8 was being induced at the mRNA expression level. Figure 4 shows that, although TNF-α induced immediate IL-8 mRNA expression, the specific increase in IL-8 mRNA levels after exposure to GBS was barely detectable at 1 h and maximal at 4 h.

**GBS β-h/c promotes IL-8 induction.** We sought to test the specific requirement of β-h/c expression in IL-8 induction by comparing isogenic β-h/c–deficient mutants with wild-type GBS parent strains. To avoid confounding effects of cytotoxicity on data interpretation, a standard bacterial inoculum (MOI, 10:1) and a 4-h exposure were selected for comparative analyses of IL-8 induction in response to GBS wild-type versus mutant strains. This inoculum resulted in complete monolayer destruction with the strongly hemolytic NCTC10/84 (figure 1); consequently, this strain was not used in IL-8 studies. As shown in figure 5, the β-h/c–deficient mutants A909:cyIE.KO and COH1:cyIE.KO elicited significantly lower levels of A549 cell IL-8 release than their respective β-h/c–producing parent strains. Because gram-positive bacterial cell wall components (e.g., lipoteichoic acid and peptidoglycan) have been shown to activate host cytokine responses, we hypothesized that the polysaccharide capsule of GBS could itself be an activator (or, alternatively, could cloak more potent cell wall–stimulating factors). However, the isogenic capsule–deficient mutant COH1-13 did not differ from its parent strain COH1 in the level of IL-8 induction (figure 5). Consistent with earlier observations, the double capsule and β-h/c–deficient mutant COH1-13:cyIE.KO induced markedly less IL-8 release than that induced by the capsule-deficient but β-h/c–producing COH1-13. Used as a control, the nonhemolytic and noninvasive L. lactis did not elicit significantly more IL-8 release than media alone. Thus, it appears that β-h/c production or bacterial invasion may be more important factors in the ability of GBS to induce IL-8 release from A549 cell monolayers than capsule or other cell wall components.

**Surfactant phospholipid DPPC inhibits GBS A549 cell invasion and IL-8 induction.** DPPC is the major constituent of human surfactant and is a known inhibitor of GBS hemolytic and cytolytic activity [14, 15]. Because we observed that GBS β-h/c activity was associated with increased lung epithelial cell invasion, adherence, and IL-8 release, we hypothesized that DPPC might inhibit these processes. Using a concentration (500 µg/mL) that approximates the DPPC level in the alveolar fluid of a term neonate, we added this phospholipid to standard cellular invasion and IL-8 induction assays, using wild-type...
GBS and the corresponding isogenic \( \beta\)-h/c–negative mutant. The results of these studies are summarized in figure 6. We found that DPPC inhibited wild-type GBS invasion of A549 cells by 50% and IL-8 induction from A549 cells by 85%. In contrast, DPPC had no effect on initial GBS adherence to A549 cells, as measured by the total cell-associated bacteria after 30 min of exposure. DPPC also had significant inhibitory effects on invasion and IL-8 induction by the \( \beta\)-h/c mutant, although the absolute magnitude of these decreases was not as great as that seen with the more invasive and stimulatory parent strain. Control experiments indicated that the presence of DPPC did not affect growth of GBS in A549 culture media, the degree of IL-8 release stimulated in response to TNF-\( \alpha \), or the ability to detect IL-8 in the ELISA assay (data not shown). Thus, we found that DPPC inhibited GBS invasion of A549 cells and the amount of IL-8 released from these cells, but the magnitude of this inhibition was only partially explained by neutralization of \( \beta\)-h/c activity.

Discussion

GBS production of a \( \beta\)-h/c toxin has been correlated with cellular injury in vitro and with virulence after respiratory tract or bloodstream infection in vivo. In the present study, we probed mechanisms by which this toxin could contribute to the early pulmonary stages of GBS infection, using isogenic GBS \( cylE \) gene mutants that are \( \beta\)-h/c deficient. Our results confirmed that \( \beta\)-h/c is directly cytolytic to lung epithelial cells at a high bacterial MOI. At a lower bacterial MOI, we found that the \( \beta\)-h/c contributes to GBS intracellular invasion of lung epithelial cells and stimulates their release of the neutrophil chemoattractant IL-8. These findings are relevant to the pathogenesis of GBS pneumonia, which is characterized by diffuse lung cell injury, neutrophilic inflammation, and bacterial penetration into the bloodstream that produces septicemia.

GBS invasion of the neonatal lung epithelium is considered to be a key step in the establishment of systemic infection [11]. GBS isolates from the blood of infected neonates are significantly more invasive to respiratory epithelial cells than are isolates from vaginal carriers or from colonized neonates without clinical symptoms [32]. On microscopic examination, GBS are found in close association with the epithelial cell surface or intracellularly within membrane-bound vacuoles [8, 9]. This suggests that the organism first binds to the host cell surface and then, in a process resembling phagocytosis, elicits its own engulfment. Here we observed that GBS \( \beta\)-h/c expression promoted lung epithelial adherence and invasion. One hypothesis to explain this finding could be that the \( \beta\)-h/c, which normally associates with the GBS membrane [33], possesses adhesin domains for eukaryotic epithelial surface components within its native protein structure. Alternatively, or in addition, the lung epithelial cells may be “activated” in response to the effects of the hemolysin toxin to bind and to engulf more bacteria. The latter notion would be con-

Figure 5. Effect of group B streptococcal (GBS) \( \beta\)-hemolysin/cytolysin (\( \beta\)-h/c) expression on induction of interleukin (IL)–8 release from A549 lung epithelial cells. A549 cells were exposed to a bacterial MOI of 10:1 for 4 h, at which time the supernatant was collected for IL-8 determination by ELISA. A nonhemolytic and noninvasive strain of *Lactococcus lactis* was used as a control. Experiments were performed in triplicate wells. Data shown represent the mean \( \pm \) SE of 3 separate experiments. The media baseline IL-8 release was subtracted from all values. *\( P < .0002.\)

Figure 6. Effect of surfactant phospholipid dipalmityloyl phosphatidylcholine (DPPC) on interactions of group B streptococci with A549 lung epithelial cells. DPPC was added at 500 \( \mu \)g/mL (+DPPC). A, Two-hour cellular invasion assay that used strain COH1 and its isogenic \( \beta\)-h/c mutant at an MOI of 1:1. B, Thirty-minute total cell-associated bacteria (adherence) assay that used strain COH1 and its isogenic \( \beta\)-h/c mutant at an MOI of 1:1. C, Four-hour interleukin (IL)–8 release assay that used strain A909 and its isogenic \( \beta\)-h/c mutant at an MOI of 10:1. All experiments were performed in triplicate wells. Data shown represent the mean \( \pm \) SE of 3 separate experiments. The media baseline IL-8 release was subtracted from all values. *\( P < .001. \) –DPPC, DPPC absent.
sistent with the observation that structurally diverse groups of hemolytic toxins from a variety of bacterial species have been shown to promote eukaryotic cell invasion—for example, *Proteus mirabilis* [34], *E. coli* [35], *Haemophilus ducreyi* [36], and *Streptococcus suis* [37].

IL-8 is a potent neutrophil chemotactic stimulus [38] that further acts to up-regulate the expression of adhesion molecules on the neutrophil surface [39], to promote their transendothelial migration [40], and to stimulate oxidative burst and release of lysosomal enzymes [41]. An elevated circulating IL-8 level is a strong predictor of bacterial infection in the newborn period [42], and marked increases in IL-8 levels have been detected in sputum or bronchoalveolar lavage fluid from patients with acute pulmonary infections [43, 44]. IL-8 is also the major neutrophil chemoattractant produced by lung epithelial cells after experimental infection [45]. In the present study, we found that GBS induced IL-8 release from A549 lung epithelial cells in a dose- and time-dependent fashion and that β-h/c activity contributed to this induction. The levels of IL-8 produced by the A549 cells in response to GBS were similar to those seen after live challenge with the related pulmonary pathogen *S. pneumonaeia* [46].

GBS disease is uncommon in healthy adults, and <1% of newborns who are colonized at birth with GBS from vertical transmission go on to develop invasive infection [1]. Therefore, the ability of human lung epithelial cells to recognize and respond to GBS and GBS toxins with IL-8 production is likely to be adaptive in the majority of all host-pathogen encounters. In addition to promoting a potent and dose-dependent neutrophil recruitment [47], IL-8 augments the ability of neutrophils to kill a variety of bacterial and fungal pathogens [44, 48–50]. IL-8 release by A549 cells may be intimately linked to GBS intracellular invasion, given that both invasion and IL-8 release are correlated to β-h/c expression. Human intestinal and cervical epithelial cells secrete IL-8 in response to intracellular invasion by *Salmonella* species and *Listeria monocytogenes* [51]. This finding suggests a common pattern by which IL-8 secreted by epithelial cells may be the initiating signal for the innate immune response after bacterial invasion of mucosal surfaces [52]. The chemotactic signal could be augmented in vivo by alveolar macrophages, which generate factors, such as IL-1 and TNF-α, that are potent stimuli for the induction of IL-8 by the alveolar epithelial cells [45, 53]. The cascade may be further amplified as neutrophil adherence to lung epithelial cells stimulates IL-8 release [54] and nonopsonic binding of GBS induces the neutrophils themselves to produce IL-8 [55].

In the special circumstance of severe early-onset GBS infection, the overwhelming bacterial inoculum to the fetal lung might transform an adaptive IL-8–mediated neutrophil recruitment into a destructive acute inflammatory process. In utero infection appears to account for the 40%–60% of newborns with early-onset infection who have poor Apgar scores and develop pulmonary symptoms within a few hours of birth [56, 57]. Deficiencies in alveolar macrophage clearance by the newborn host allow multiply GBS to attain very high concentrations within the alveolar spaces [5]. Indeed, when experimental GBS pneumonia is induced in newborn primates after intra-amniotic inoculation, bacterial densities reach 10⁷–10¹² cfu/g of lung tissue [8]. Under such circumstances, high levels of neutrophil recruitment and activation by IL-8 could aggravate pulmonary injury through release of proteases and reactive oxygen metabolites. High IL-8 concentrations in bronchoalveolar lavage fluid are, in fact, correlated with the incidence of respiratory failure in at-risk patients [58], and elevations of IL-8 have been observed in respiratory secretions of newborns who later developed chronic lung disease [59]. The high bacterial density of early-onset pneumonia may also well exceed the threshold for direct GBS β-h/c–mediated cytotoxicity to lung epithelium and endothelium, thus leading to extensive breakdown of the mucosal barrier and access for the bacteria into the bloodstream [10, 15].

In earlier studies, we have demonstrated that the phospholipid DPPC, the major component of human surfactant, inhibits GBS β-h/c–induced injury to A549 lung epithelial cells [15]. In the present study, we found that DPPC also inhibits the ability of GBS to intracellularly invade these cells and to stimulate their production of IL-8. Because GBS β-h/c appears to be a membrane pore–forming toxin, the protein would be expected to show affinity for the phospholipid components of the eukaryotic cellular membrane. Free extracellular phospholipid (i.e., DPPC) present in lung surfactant might act as a “sink,” diminishing GBS injury of lung epithelial cells by competitive binding of the β-h/c toxin. Yet significant levels of DPPC inhibition of A549 cell invasion and IL-8 release were observed, even with β-h/c–negative cylE mutants. Thus, in addition to blocking β-h/c action, the presence of DPPC may interfere with other stimulatory GBS factors and/or modulate the pattern of host cellular response. DPPC inhibition of GBS induced lung cell injury, invasion, or inflammatory activation may be reflected in the increased incidence of severe GBS pneumonia and septicemia in premature surfactant-deficient neonates.

Our observations also provide in vitro data to suggest that administration of DPPC in surfactant preparations could be of therapeutic benefit in infants with severe early-onset GBS pneumonia. Indeed, in studies in which preterm rabbits undergoing mechanical ventilation were administered GBS intratracheally, surfactant treatment reduced histological evidence of lung inflammation, mitigated bacterial proliferation, and improved lung compliance [60–62]. Several clinical reports have also described improved gas exchange parameters or clinical outcome in preterm or full-term infants with GBS pneumonia who receive surfactant therapy [63–66]. The potential benefits of complete surfactant preparations against GBS lung infection are not limited to the β-h/c neutralizing effects of DPPC—for example, surfactant protein A is capable of binding to GBS and promoting opsonophagocytosis by alveolar macrophages [67].

In summary, the β-h/c toxin of GBS may contribute to neonatal disease pathogenesis by direct cytotoxicity, increased lung...
epithelial cell invasion, and stimulation of IL-8 release. The
availability of specific β-h/c mutants will facilitate further study
of the role of this toxin in newborn infection and the potential
therapeutic applications of blocking β-h/c activity in patients
with invasive GBS disease.

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