Experimental Neonatal Group B Streptococcal Pneumonia: Effect of a Modified Porcine Surfactant on Bacterial Proliferation in Ventilated Near-Term Rabbits

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ABSTRACT

We studied bacterial proliferation in relation to surfactant treatment in a model of neonatal group B streptococcal (GBS) pneumonia. Surfactant (Curosurf) was isolated from pig lungs with a method preserving only polar lipids and hydrophobic proteins. Near-term rabbit fetuses were ventilated in a body plethysmograph system. At 15 min, a suspension of GBS strain 090 Ia LD (5 mL/kg, concentration $\sim 10^9$ /mL) was instilled intratracheally. At 30 min, surfactant ($n = 12$) or sterile saline ($n = 13$) was administered via the airways (2.5 mL/kg). A control group ($n = 12$) received the same volumes of saline. After 5 h the animals were killed, and samples for blood cultures and blood gases were taken from the heart. The left lung was aseptically removed, weighed, homogenized, serially diluted, and cultured on blood agar plates. The results were expressed as mean $\log_{10}$ colony forming units/g lung $\pm$ SD. Compared with animals ($n = 12$) killed immediately after GBS instillation (8.13 ± 0.54), there was a significant increase in

bacterial numbers in both groups ventilated for 5 h, but values for surfactant-treated animals (8.96 ± 0.38) were lower than those for animals receiving saline (9.46 ± 0.50 ; $p < 0.05$). After 5 h, 96% of GBS-infected animals had positive blood cultures. Light microscopic examination of the right lung of GBS-infected animals revealed inflammatory changes that tended to be less prominent in surfactant-treated rabbits. We conclude that intratracheal inoculation of near-term rabbits with GBS resulted in a significant bacterial proliferation during 5 h of ventilation and that bacterial growth was mitigated by treatment with surfactant. (*Pediatr Res* 36: 784–791, 1994)

Abbreviations

CFU, colony forming unit
GBS, group B streptococcus
RDS, respiratory distress syndrome

GBS is a frequently encountered pathogen in the neonatal period (1). Vaginal colonization with GBS is commonly found in pregnant women. Vertical transmission occurs in approximately 40% of deliveries, but only approximately 1% of neonates born to these mothers develop clinical signs of GBS infection (2). If diagnosis and treatment are delayed, the condition has a substantial mortality, especially in premature infants (2). Susceptibility to GBS infections depends on host defense factors (e.g. lack of type-specific opsonizing antibodies) and

virulence factors (e.g. polysaccharide capsule) of the infecting microorganism (3–6).

Animal models using rabbits, mice, and rats have been used for studies of experimental neonatal GBS infections (7–9). In these studies, the animals were either infected by injection (7, 9) or a bacterial suspension was nebulized via the airways in spontaneously breathing animals (8, 9). Our intention was to develop a model of GBS pneumonia in ventilated newborn rabbits infected by intratracheal injection of a bacterial suspension.

RDS and GBS pneumonia are difficult to differentiate on clinical grounds (10), and therefore neonates with GBS pneumonia might receive surfactant therapy. In a recent “rescue” study (11), about 10% of infants with a clinical diagnosis of RDS treated with surfactant demonstrated signs of systemic neonatal infection or pneumonia in the first days of life (12).

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From *in vitro* studies, there is serious concern that surfactant administration might interfere with pulmonary defense mechanisms (13, 14). Based on animal studies (15), it has been speculated that surfactant treatment might actually promote bacterial growth in premature infants with GBS pneumonia. Using our new model, we investigated the effect of a modified porcine surfactant (Curosurf) on bacterial proliferation, inflammatory changes, and lung function in experimental neonatal GBS pneumonia.

METHODS

Bacteria. A capsulated GBS strain found to be pathogenic to rabbits was used for the present experiments. It is a GBS Ia 90 LD serologic subtype supplied by Stellan Håkansson, Department of Pediatrics, Umeå, Sweden.

On the day before the experiments, the strain, which was stored in aliquots at -70°C until use, was inoculated into 3 mL of trypticase soy broth (TSB 3%, Becton Dickinson, Cockeysville, MD) as an overnight starter culture. After a 12-h incubation at 37°C , 0.3 mL of the suspension was inoculated into five separate tubes each containing 10 mL of fresh TSB 3% to shift bacterial growth to the "mid logarithmic growth phase" (16). The strain was then centrifuged at $900 \times g$ for 10 min at 4°C , washed in saline, and resuspended in sterile 0.9% NaCl. The OD of the suspension was measured spectrophotometrically at 595 nm and adjusted to a value corresponding to $\sim 10^9$ CFU/mL.

The exact number of viable CFU/mL in the GBS suspension used for every individual experiment was determined by doing a serial dilution of the bacterial suspension in sterile saline. Aliquots (100 μL) from the dilutions 10^{-5} to 10^{-10} were finally spread on blood agar plates (5% sheep blood agar) and incubated for 24 h at 37°C in air with 5% CO_2 to promote growth. GBS growth was recognizable by β -hemolysis on the blood agar plate. The number of CFU was determined using an electric colony counter. Each step was carried out in duplicate, and the average result from the two plates was used for further calculation. Autoclaved materials were used for all above procedures.

Surfactant. Curosurf is a modified natural surfactant isolated from minced pig lungs by chloroform-methanol extraction and liquid-gel chromatography. It is suspended in saline at a concentration of 80 mg/mL. This surfactant contains about 99% polar lipids (main component: dipalmitoylphosphatidylcholine) and 1% hydrophobic proteins (SP-B and SP-C) (17). The extraction procedure removes SP-A. To exclude bacterial contamination, Curosurf is sterilized using a high-pressure micropore filter system (first filter 1.0 μm , second filter 0.45 μm , and third filter 0.25 μm). The sterility of each batch is confirmed by bacteriologic examination of random vials. The physiologic effects of Curosurf have been confirmed *in vitro*, in animal experiments (18, 19), and in randomized clinical trials (11, 20). The standard dose used in these

trials was 200 mg/kg. The batch of surfactant used for the present experiments was tested in the pulsating bubble surfactometer. At a concentration of 5 mg/mL, it reduced minimum surface tension to 2 mN/m during 50% cyclic film compression. Its physiologic activity was proven in experiments on ventilated immature rabbits delivered at a gestational age of 27 d (21).

Animals. New Zealand White rabbit fetuses were delivered by cesarean section at 29.5 d gestation (term = 31 d). The animals were anesthetized, tracheotomized, and ventilated in individual boxes in a body plethysmograph system (21). Three mL/kg pentobarbital (Mebumal Vet 6 mg/mL, Nordvacc, Skärholmen, Sweden) and 3 mL/kg of the muscle relaxant pancuronium bromide (Pavulon 0.2 mg/mL, Organon, Göteborg, Sweden) were injected intraperitoneally every 2 h and additionally if needed. At 60 and 180 min, 5% glucose (Kabi Pharmacia, Stockholm, Sweden) was given intraperitoneally at a dose of 10 mL/kg. At the end of the ventilation period, the animals were killed by intracranial injection of 0.5 mL of lidocaine (Xylocaine 20 mg/mL, Astra, Södertälje, Sweden). The abdomen was opened with sterile instruments, and the diaphragms were inspected for evidence of pneumothorax. Then the thorax was opened and heart blood was aspirated from the right ventricle for culturing of bacteria (Sigma Oxoid blood culture system, Unipath, Basingstoke, UK); in addition, a heparinized sample was taken for blood gas analysis (ABL, Radiometer, Copenhagen, Denmark).

Both lungs were then removed aseptically and weighed. The right lung was fixed for histologic examination. The left lung was used for quantification of bacterial growth (22, 23). After processing in a nylon micro-chamber tissue homogenizer (Sorvall Omnimixer, Dupont Instruments, Newtown, CT), the lung homogenate was serially diluted and the number of CFU was calculated using blood agar plates by applying the same methodology as described above for determination of bacterial number in the GBS suspension. From the number of colonies detected and the dilution, we calculated the number of CFU per gram of lung tissue (wet weight). The design of the study corresponds to the Swedish animal protection law, and the trial was approved by the local committee for animal research.

Ventilator equipment and lung function measurements. The newborn animals were ventilated in warmed, sealed Plexiglas chambers as previously described (21). Up to 10 littermates were ventilated in parallel with a pressure-controlled Servo 900 B ventilator (Siemens-Elcoma, Solna, Sweden) delivering 100% O_2 at a frequency of 40 min^{-1} , an inspiration time of 0.75 s, and a working pressure of 50 cm H_2O . The peak pressure for the animals in the different boxes was adjusted individually to maintain a tidal volume of 8–10 mL/kg body weight (21). The insufflation pressure was recorded with a pressure transducer (EMT 34; Siemens-Elcoma). The tidal volume was measured with a modified "Fleisch-tube," a differential pressure transducer, an integrator, and an amplifier

(EMT 31, EMT 32, EMT 41; Siemens-Elema). The compliance of the respiratory system was calculated for each individual time point by dividing the tidal volume adjusted for body weight by the corresponding peak pressure. No positive end-expiratory pressure was applied, because this could have masked differences in compliance due to variations in surfactant function (24). Recordings including an ECG from subcutaneous needles were taken at 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min (Mingograf 81; Siemens-Elema). Animals with severe ECG abnormalities (bradycardia $< 100 \text{ min}^{-1}$, atrioventricular block) were excluded from the study.

In vitro studies of bacterial growth. *In vitro* growth of GBS was studied in the presence of different concentrations of surfactant. GBS (10^9 CFU) were suspended in 5 mL of sterile normal saline. Aliquots of this suspension were serially diluted and spread on blood agar plates for counting of CFU. After the bacterial suspension was incubated at 37°C for 4 h under agitation at 150 rpm (Incubator Shaker G 25, New Brunswick Scientific, Edison, NJ), another sample was processed as described above. The number of bacteria at time 0 was subtracted from that at 4 h, and the results were expressed as mean $\log_{10}$ CFU. A positive log difference indicated proliferation of GBS, and a negative log difference indicated a decrease in the number of viable bacteria. Similar experiments were performed after addition of varying amounts of Curosurf to the suspension (1, 10, and 20 mg/mL). Three or four experiments were carried out with each surfactant concentration.

General design of the animal studies. All fetuses were first connected to the ventilator for 15 min to allow stabilization after delivery and tracheotomy. At 15 min, the animals received via the tracheal cannula either saline or GBS suspension (5 mL/kg). Animals were allocated at random to the following groups.

Growth controls. These animals received 5 mL/kg GBS suspension at 15 min. They were ventilated for only 1 min after GBS inoculation to remove the instilled fluid from the central airways to the periphery of the lungs. The counts from this group were used to check viability and distribution of the bacteria immediately after inoculation.

Group I (GBS-Curosurf). Animals in this group received 5 mL/kg of the GBS suspension at 15 min. After another 15 min, 2.5 mL/kg of surfactant corresponding to 200 mg/kg body weight was administered by intratracheal bolus injection. The animals were killed after a 5-h ventilation period, and bacterial proliferation in the lungs and heart blood was quantified as described above.

Group II (GBS-NaCl). These animals were inoculated with 5 mL/kg GBS suspension at 15 min and received 2.5 mL/kg of sterile 0.9% NaCl at 30 min via the tracheal cannula. They were ventilated for 5 h, and blood cultures and counting of bacteria in lung tissue were performed as described above.

Group III (NaCl-NaCl). Animals in this "saline control" group received 5 mL/kg of sterile 0.9% NaCl at 15 min

and 2.5 mL/kg of NaCl at 30 min to check the influence of the intratracheal fluid load on lung function. Bacteriologic examination was performed in the same way as in the other groups to exclude contamination with GBS or other pathogens.

Histology. The lungs were fixed in 4% formaldehyde and embedded in paraffin. Longitudinal sections were cut from the middle of each paraffin block in a standardized fashion. The sections were stained with hematoxylin and eosin or with Gram's stain and examined under a light microscope with particular reference to inflammatory changes and presence of bacteria in the air spaces. The proportion (percentage) of total lung parenchyma with pneumonia, as indicated by the presence of inflammatory cells in the terminal air spaces, was estimated using a four-degree scale: 0, $<10\%$, 10–30%, and $>30\%$. The histologic evaluation was performed blindly, *i.e.* without knowledge of the experimental condition of individual animals.

Statistics. All data are expressed as means $\pm$ SD. Because bacterial growth follows a logarithmic curve (16), all figures for bacterial proliferation were transformed into decimal logarithms ($\log_{10}$). The CRISP statistical program (Crunch Software, San Francisco, CA) was used for data analysis. Between-group differences were calculated by analysis of variance followed by Student-Newman-Keuls' test. Differences in *in vitro* bacterial proliferation were evaluated with *t* test. Pearson's correlation and χ^2 test were used when appropriate. A *p* value <0.05 was regarded as statistically significant.

RESULTS

In vitro growth of streptococci. The number of viable GBS in saline remained virtually unchanged over a 4-h period of observation (log difference $+0.01$). At Curosurf concentrations of 1 or 10 mg/mL, there was a slight reduction in bacterial growth (log difference -0.18 and -0.48 , respectively). After addition of Curosurf at a concentration of 20 mg/mL, a significant decrease in bacterial number was observed (log difference -2.47 compared with time 0; $p < 0.05$).

Animal studies. Fifty-four liveborn fetuses from 10 litters were used for the experiments. Four animals (two in group I, one in group II, one in group III) died before the end of the experiment or had severe ECG abnormalities. One of these animals had pneumothorax. One animal in the growth control group was excluded because the blood culture showed growth of *Staphylococcus epidermidis* indicating contamination. Thus, 49 animals were available for final data analysis.

Growth controls. Mean birth weight of these 12 animals was 41.7 ± 9.2 g, *i.e.* similar to that of the other experimental groups (Table 1). The number of CFU (mean $\log_{10} \pm$ SD) per gram lung weight was 8.13 ± 0.54 . None of the blood cultures in this group were GBS positive.

Figure 1 shows the correlation between the number of bacteria given to each growth control rabbit and the

Table 1. Characterization of animals in different treatment groups*

Parameters	Group I: GBS-Curosulf (n = 12)	Group II: GBS-NaCl (n = 13)	Group III: NaCl-NaCl (n = 12)
Birth weight (g)	40.4 ± 6.7	38.2 ± 5.8	40.6 ± 6.0
Heart rate at 300 min (min ⁻¹)	234 ± 38	220 ± 37	244 ± 41
Final pH	7.31 ± 0.12	7.32 ± 0.11	7.32 ± 0.12
Final Pco ₂ (kPa)	4.57 ± 0.74	3.96 ± 0.81	5.18 ± 1.78
GBS-positive blood culture	11/12 (92%)	13/13 (100%)	0/12 (0%)
Left lung weight (g)	0.42 ± 0.11	0.37 ± 0.11	0.42 ± 0.10

*Values are given as means ± SD or ratios.

number of CFU detected in the left lung of the same animal after 1 min of ventilation. Although in individual animals maldistribution to either the right or the left lung seems to have occurred, it is justifiable to assume that approximately half of the instilled bacteria was distributed to each lung.

Animals subjected to prolonged ventilation (groups I-III). All these animals were ventilated for 5 h. Birth weights were nearly identical in the three groups (Table 1). Physiologic data at the end of the experiment including heart rate, blood gas values, and weight of the left lung were also similar (Table 1).

Bacterial proliferation. The number of GBS instilled into the airways was nearly identical in all infected groups (growth controls: 8.15 ± 0.52 ; GBS-Curosulf: 8.12 ± 0.62 ; GBS-NaCl: 8.14 ± 0.54). At the end of the 5-h period of ventilation, significant bacterial proliferation had occurred in both group I (GBS-Curosulf) and group II (GBS-NaCl) animals (Fig. 2). However, this proliferation was less marked in surfactant-treated rabbit neonates than in animals receiving saline (8.96 ± 0.38 versus

Figure 1. Correlation between the number of CFU injected intratracheally and the actual number of CFU detected in the left lung of growth control animals killed 1 min after inoculation. $r = 0.72$, $p < 0.01$; $y = 0.39 + 0.31x$.

Figure 2. Number of bacteria ($\log_{10}$ CFU/g lung tissue; mean ± SD) isolated from the left lung of rabbits after 5 h of ventilation in the two treatment groups in comparison with growth controls. ***, $p < 0.001$ vs growth controls; **, $p < 0.01$ vs growth controls; +, $p < 0.05$ vs GBS-Curosulf.

9.46 ± 0.50 ; $p < 0.05$). Assuming that half of the instilled bacteria reached the left lung, there was nearly a 10-fold increase in bacterial number in the Curosulf-treated and an approximately 40-fold increase in the number of CFU in saline-treated, GBS-infected animals. None of the rabbits in group III (NaCl-NaCl) showed bacterial growth in lung homogenate.

All blood cultures in the saline control group (group III) were sterile, whereas 24 of 25 GBS-infected rabbits showed GBS-positive blood cultures at the end of the experiment (Table 1).

Lung function. Average initial lung-thorax compliance values were compatible with lung maturity (21), but there was considerable individual variation as indicated by large SD (Table 2).

All animals showed a decrease in compliance after intratracheal instillation of 5 mL/kg of fluid at 15 min, irrespective of whether GBS or NaCl was administered. Instillation of 2.5 mL/kg surfactant or NaCl at 30 min did not result in any significant additional change in compliance. Animals receiving only saline at 15 and 30 min tended to have lower compliance values throughout the experiment. At some individual time points, this difference reached the 5% level of statistical significance because SD were small in the saline control group. During

Table 2. Lung-thorax compliance at different time intervals in various groups of animals*

Time (min)	Compliance (mL·cm H ₂ O ⁻¹ ·kg ⁻¹)		
	Group I: GBS-Curosurf (n = 12)	Group II: GBS-NaCl (n = 13)	Group III: NaCl-NaCl (n = 12)
15†	0.78 ± 0.22	0.91 ± 0.33	0.74 ± 0.15
30‡	0.59 ± 0.13	0.74 ± 0.22	0.55 ± 0.22
45	0.63 ± 0.10	0.73 ± 0.18	0.57 ± 0.09§
60	0.64 ± 0.08	0.65 ± 0.08	0.57 ± 0.06§
120	0.68 ± 0.12	0.67 ± 0.11	0.56 ± 0.07§
180	0.66 ± 0.18	0.75 ± 0.16	0.59 ± 0.11§
240	0.63 ± 0.09	0.63 ± 0.07	0.58 ± 0.09
300	0.61 ± 0.09	0.63 ± 0.11	0.56 ± 0.09

*All values are given as means ± SD.

†Before instillation of GBS suspension or 0.9% NaCl.

‡Before instillation of Curosurf or 0.9% NaCl.

§*p* < 0.05 vs group II.

||*p* < 0.05 vs group I.

the 5-h period of ventilation, mean lung-thorax compliance values decreased by 22% in group I (GBS-Curosurf), by 31% in group II (GBS-NaCl), and by 24% in group III (NaCl-NaCl). There were no statistically significant differences between the groups at the end of the experiment.

Histologic evaluation. Lungs from animals in the growth control group had no evidence of inflammation, but small

groups of Gram-positive cocci could be identified in terminal air spaces (Fig. 3A). Bacterial proliferation was a prominent feature in Gram-stained sections from groups I and II (Fig. 3B) after 5 h of ventilation.

Inflammatory changes characterized by intraalveolar accumulation of neutrophils and, to a lesser extent, macrophages were found in one of the 12 ventilated animals receiving only saline (group III) and in 22 of the 25 animals that were inoculated with GBS and ventilated for 5 h (groups I and II) (*p* < 0.001). In two surfactant-treated and one saline-treated, GBS-infected rabbit neonate, lung sections showed no evidence of pneumonia (Table 3). When present, the inflammatory changes (Fig. 4) were most prominent in the peripheral parts of the lungs and tended to be more severe in GBS-infected rabbits treated with saline, involving >30% of the parenchyma in seven animals (Table 3). A corresponding degree of inflammation was noted in only one of the infected animals treated with surfactant. However, this difference did not reach the limit level of statistical significance (χ^2 test).

DISCUSSION

Surfactant is an important component of the pulmonary defense system (25), and the effects of bronchoal-

Figure 3. Gram-positive cocci in alveolar spaces. Gram's stain $\times 250$. A, Growth control animal killed 1 min after GBS inoculation. B, Prominent bacterial proliferation in the lung of a GBS-infected animal treated with saline (group II) and ventilated for 5 h.

Table 3. Histologic grading of pulmonary inflammatory changes in rabbits of different treatment groups and in growth control animals

Percent of parenchyma involved	Group I: GBS-Curosurf (n = 12)	Group II: GBS-NaCl (n = 13)	Group III: NaCl-NaCl (n = 12)	Growth controls (n = 12)
0	2	1	11	12
<10	4	3	1	0
10-30	5	2	0	0
>30	1	7	0	0

veolar fluid and its cellular components on bacterial growth have been documented in several studies (25-28). Surfactant also stabilizes alveoli and small airways, preventing atelectasis (21), epithelial disruption (29), and protein leakage (30, 31) in the lungs of ventilated, premature animals. The formation of a surfactant monolayer on the alveolar surface probably contributes to antibacterial defense by acting as a mechanical barrier facilitating mucociliary clearance of inhaled particles.

Our present *in vitro* data indicate a significant negative impact of Curosurf on bacterial growth at a concentration of 20 mg/mL. This is 6-7 times higher than the "critical concentration" of surfactant phospholipids in the fetal lung fluid at birth but less than one fifth of the estimated phospholipid concentration of the alveolar lining layer in

adult animals (32). The phospholipid concentration of airway fluid resulting from instillation of 200 mg/kg surfactant lipids in a near-term animal 30 min after birth can be calculated only roughly. At birth, the volume of fetal lung fluid is about 30 mL/kg and the pool size of endogenous surfactant about 20 mg/kg (33); this corresponds to a concentration of 0.6-1 mg/mL. The concentration of endogenous surfactant in the airway fluid probably increases rapidly during the first few hours after birth due to accelerated release of lamellar bodies from type II cells and a concomitant resorption of fetal lung water. In our experimental animals, an additional amount of exogenous surfactant, about 10 times larger than the pool size of endogenous surfactant at birth, was administered after a period of artificial ventilation. This dose was probably not distributed homogeneously in the lungs, and the final concentration of surfactant must therefore be subject to considerable regional variation. Nevertheless, it seems likely that in many air spaces of the GBS-infected animals treated with Curosurf the concentration of surfactant in the airway fluid amounted to a level equivalent to that causing inhibition of bacterial growth *in vitro*. Thus, it is possible that the reduced proliferation of GBS in these animals was, at least in part, a direct effect of surfactant on the microorganisms, not mediated by phagocytic cells.

Figure 4. Histologic findings in a GBS-inoculated animal treated with saline (group II) and ventilated for 5 h. Hematoxylin and eosin. *A*, Widespread pneumonia involving more than 30% of total lung parenchyma. $\times 25$. *B*, Detail from the same section showing prominent intraalveolar accumulation of inflammatory cells, mainly neutrophils. $\times 250$.

Theoretically, treatment with surfactant could have also improved clearance of bacteria from the air spaces by counterbalancing the inhibitory effects of serum proteins leaking into the air spaces with the inflammatory exudate. Keeping the alveoli open throughout the ventilatory cycle facilitates surface flux from the alveoli to the mucociliary escalator and probably prevents local hypoxia and ischemia, which could interfere with phagocyte function. Total lung volumes were not measured in the present experiments, only tidal volumes. We therefore cannot draw any conclusions in regard to the possible recruitment of alveolar spaces in surfactant-treated animals. Lung-thorax compliance measurements indicated no significant differences between surfactant-treated animals and controls, and the absolute values for compliance were of the same order as those previously recorded with similar methods in healthy near-term animals (21). The exact mechanisms by which surfactant reduced bacterial growth in the present animal experiments thus remain to be clarified.

Pilot studies of surfactant treatment in neonates with severe respiratory failure due to pneumonia indicate that this therapy might be beneficial (34–37), although the therapeutic response (improvement in oxygenation) is slower than in infants with “idiopathic” RDS. So far, controlled trials of surfactant treatment for neonatal pneumonia have not been reported.

The surfactant used in our study lacks the hydrophilic surfactant proteins SP-A and SP-D. Both of these proteins are important stimulants of macrophage function (38, 39). In addition, SP-A plays a key role in regulating surfactant secretion and recycling (40), and it increases resistance to inhibition by serum proteins. Currently available surfactant preparations lacking SP-A and SP-D might therefore be suboptimal for treating infants with respiratory failure due to pneumonia. A decreased phagocytic capacity and a decreased release of cytokines such as tumor necrosis factor and IL-1 have been observed after *in vitro* exposure of phagocytic cells to different doses of Curosurf, suggesting that this surfactant might in fact down-regulate inflammatory reactions in the lung (13, 14).

A direct comparison of our results with data published in the literature is not possible, because different GBS strains at different infecting doses have been used. The serologic subtype Ia used for our studies and by other investigators (7, 8, 41) was isolated from neonates with GBS pneumonia and early onset septicemia, whereas type III GBS used by Hall and Sherman (42) is more commonly found in neonates beyond the first days of life (often in combination with meningitis). In addition, various surfactants seem to have different effects on bacterial growth (41), and fetuses of different gestational ages show distinct differences in their capacity to kill streptococci. Hall and Sherman (42) reported that mature rabbits were capable of reducing the number of inhaled GBS over an 8-h period of observation, whereas a significant increase in bacterial number was found in immature an-

imals. In these studies, GBS was administered as an aerosol, using specially designed cages by which a similar number of bacteria could be given simultaneously to many rabbits. However, in contrast to the present experiments in which the approximate number of instilled bacteria was known, the number of inhaled aerosolized bacteria had to be judged by counting the bacteria in the lungs of rabbits killed immediately after exposure to GBS (41, 42). In addition, the number of bacteria applied to the lungs in the aerosol studies (41, 42) was less than the number of GBS administered in our model.

Tracheotomy, needed for ventilation and for lung function measurements in our study, bypasses the upper airways and interferes with mucociliary clearance and may therefore be considered an additional factor promoting bacterial growth. Mechanical ventilation with intermittent positive airway pressure and high O₂ levels might also interfere with the pulmonary defense system and cause a more rapid proliferation of bacteria (43). Near-term rabbit fetuses born by cesarean section show a decreased capacity to kill invading microorganisms in comparison to animals that are born spontaneously, probably reflecting a delayed influx of phagocytes into the bronchoalveolar space (44). These circumstances might to some extent explain why we, in contrast to other investigators (42), could observe proliferation of GBS in near-term rabbits.

In our opinion, intratracheal injection of a liquid bolus mimics an ascending infection *in utero* more closely than does infection with aerosolized bacteria. Furthermore, the fetuses in our experimental setup were paralyzed and kept in a supine position, which might enhance bacterial growth in comparison with animals breathing spontaneously in an aerosolization chamber. With certain limitations (45), it could be argued that conditions similar to our experimental design exist in critically ill, immobilized, ventilated neonates with GBS pneumonia.

The model described in the present paper should allow further investigation of cellular and humoral factors determining bacterial proliferation and inflammatory response in neonatal GBS pneumonia and septicemia, also in more immature ventilated animals. We conclude that treatment of ventilated, near-term newborn rabbits with a modified porcine surfactant mitigated bacterial proliferation in experimental GBS pneumonia.

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