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Isogenic Group B Streptococci Devoid of Capsular Polysaccharide or β-Hemolysin: Pulmonary Hemodynamic and Gas Exchange Effects during Bacteremia in Piglets

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ABSTRACT. Group B β-hemolytic streptococcus (GBS) causes thromboxane (Tx)-associated pulmonary hypertension and hypoxemia in neonatal animals and human infants. The components of GBS that induce these features of sepsis are incompletely characterized. The capsular polysaccharide has been implicated based on the effects of GBS extracts. We used isogenic mutants of a parent GBS strain (COH 31 r/s) devoid of capsular polysaccharide or β-hemolysin to determine if these components caused the acute features of GBS bacteremia. In neonatal piglets, we observed a similar increase in pulmonary vascular resistance (PVR, mm Hg/L/min) during a 1 h infusion at 5×10^8 colony-forming unit/kg/h of COH 31 r/s ($n = 5$, 11.6 ± 1.4 to 67.1 ± 17.9), an isogenic GBS mutant devoid of type III CP ($n = 5$, 12.5 ± 1.4 to 56.9 ± 5.0), and an isogenic GBS mutant devoid of β-hemolysin ($n = 4$, 11.0 ± 1.9 to 51.9 ± 7.9). All three GBS strains caused increases in blood TxB_2 levels, mild arterial hypoxemia, mild reduction in mixed venous Po_2, and a 30–40% reduction in cardiac output after a 1 h infusion. The Tx-synthase inhibitor, dazmegrel, completely reversed pulmonary hypertension, and partially reversed arterial hypoxemia and TxB_2 levels to baseline values for all GBS strains. In six additional piglets, infusion of polystyrene beads of similar size to GBS at a dose of 5×10^8 beads/kg/h caused no changes in gas exchange or blood TxB_2 levels, but a mild increase in PVR (13.3 ± 2.0 to 17.7 ± 3.5). This suggests a nonspecific response to circulating particulates is not the major cause of the acute features of GBS bacteremia in piglets. We conclude that type III capsular polysaccharide and β-hemolysin are not essential for type III GBS to cause acute Tx-associated pulmonary hypertension and hypoxemia in piglets. (*Pediatr Res* 26:241–245, 1989)

Abbreviations

cfu, colony forming unit
CO, cardiac output
COH 31 r/s, parent type III GBS strain
COH 31-15, GBS mutant devoid of capsular polysaccharide
COH 31C5, GBS mutant devoid of β-hemolysin
CP, capsular polysaccharide
DAZ, dazmegrel
GBS, group B streptococcus
P_{pa}, pulmonary artery pressure
PS, polystyrene
P_{sa}, systemic artery pressure
PVR, pulmonary vascular resistance
P_w, pulmonary capillary wedge pressure
TxB_2, thromboxane B_2
IV, intravenous
EU, endotoxin units

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GBS, a common gram-positive pathogen in neonatal sepsis, is associated with significant morbidity and mortality in septic human newborns (1, 2). GBS is classified into serotypes by the type-specific capsular polysaccharide; type III GBS cause the majority of neonatal infections (1). GBS bacteremia in human newborns and neonatal animals causes hypoxemia, pulmonary hypertension, and reduced cardiac output (3–7), and these acute changes are associated with increased blood TxB_2 levels (5–7). Gram-negative bacteremia causes similar features (8, 9), and the lipid A moiety of endotoxin is the bacterial component that induces arachidonic acid metabolites, hypoxemia, and pulmonary hypertension (10). Gram-positive organisms do not contain lipid A, and a toxin common to all gram-positive organisms has not been identified. However, multiple gram-positive organisms, including type III GBS, *Streptococcus faecalis*, and *Staphylococcus epidermidis*, cause acute Tx-associated pulmonary hypertension and hypoxemia during IV infusion in piglets (11). It is uncertain if each these gram-positive organisms contains a specific toxin that mediates the acute effects of bacteremia.

The type III GBS components that induce Tx-associated pulmonary hypertension and hypoxemia are incompletely characterized. Two different polysaccharide toxins, including an extracellular mannan polysaccharide and a GBS extract containing type III capsular polysaccharide, are reported to cause these features of GBS sepsis (12, 13). The type III capsular polysaccharide extract in these studies also contained group B polysaccharide and protein (12), and therefore the role of capsular polysaccharide in stimulating the acute hemodynamic and gas exchange alterations during GBS bacteremia remains uncertain.

Isogenic mutants of type III GBS devoid of only type-specific capsular polysaccharide expression have been isolated by transposon insertional mutagenesis (14). These mutants provide a powerful alternative method to GBS extracts for studying the role of type III capsular polysaccharide during experimental GBS bacteremia. We hypothesized that a type III GBS mutant devoid of capsular polysaccharide would not stimulate acute Tx-associ-

ated pulmonary hypertension and hypoxemia when infused in piglets. An isogenic mutant of type III GBS devoid of β-hemolysin was also used to test the role of β-hemolysin in the acute features of GBS bacteremia (15).

MATERIALS AND METHODS

Animal preparation. Twenty piglets, 16 ± 3 d of age, and weighing 3.7 ± 0.6 kg were anesthetized (30 mg/kg pentobarbital IV), paralyzed (0.3 mg/kg pancuronium bromide IV), anticoagulated with heparin (1000 IU IV), and mechanically ventilated via a tracheostomy tube with a large animal Harvard ventilator adjusted to deliver a tidal volume of 12 ± 2 mL/kg at a rate to maintain $Paco_2$ at 40 ± 5 mm Hg during baseline conditions. All animals were ventilated with room air throughout each experiment. As previously described (11), catheters were placed in *1*) the left external jugular vein for infusion of bacteria, *2*) the aorta to measure P_{sa} and sample arterial blood for pH, blood gas tensions, and TxB_2 measurements, and *3*) a branch of the left pulmonary artery (5 Fr Swan-Ganz thermodilution catheter) to measure P_{pa} and P_w, CO in triplicate by thermodilution using an Edwards 9520A cardiac output computer (Santa Ana, CA), and for sampling mixed venous blood. After instrumentation, anesthesia and muscle paralysis were maintained with pentobarbital (3 mg/kg IV q 1 h) and pancuronium bromide (0.3 mg/kg IV q 1 h), respectively. The piglets received sigh breaths to 30 cm H_2O every 20 min to minimize spontaneous development of atelectasis. Vascular pressures were measured using Hewlett-Packard 1280 transducers (Hewlett-Packard Co., Palo Alto, CA) and were referenced to midchest. Core temperature was maintained at 38.5 ± 0.5°C with an overhead radiant heat source.

GBS strains and preparation. The parent GBS strain is a type III clinical isolate that is tetracycline sensitive, but made rifampin and streptomycin resistant (COH 31 r/s). The isogenic mutants of type III GBS were derived previously from COH 31 r/s by transposon insertional mutagenesis (14, 15). Each GBS mutant tested is identical to the parent strain except for the absence of a specific bacterial component, and acquisition of tetracycline-resistance after transposon insertion. The GBS strain COH 31-15 is an isogenic mutant of the parent strain devoid of type III capsular polysaccharide. COH 31-15 contains no type III capsular polysaccharide on its surface based on a competitive ELISA assay or immune electron microscopy with type III GBS antiserum (14), but does express the group B polysaccharide. The GBS strain COH 31C5 is an isogenic mutant of the parent strain devoid of β-hemolysin (15), but expresses type III capsular polysaccharide and group B polysaccharide identical to the parent strain. The stability of the COH 31-15 mutation is demonstrated by the absence of a detectable revertance rate after serial passage of COH 31-15 on drug-free media or after isolation from infected animals (Rubens C, unpublished observations), and the persistence of tetracycline-resistance during this study. The stability of the COH 31C5 mutation is supported by the blood cultures from each piglet in this study yielding non-β-hemolytic colonies. The culture conditions, mode of resuspension in sterile nonbacteriostatic saline, tests of culture purity, measurement of bacterial concentrations, and quantitative blood cultures were performed as described (11). The GBS suspensions for infusion into piglets contained <0.03 EU/mL of endotoxin based on the limulus amebocyte lysate assay (5 EU/mL = 1 ng/mL) (Associates of Cape Cod, Woods Hole, MA).

Polystyrene beads. Uniform polystyrene beads 2.355 ± 0.026 μm in diameter (Seradyn, Particle Technology Div., Indianapolis, IN) were pelleted at 25000 × *g* and resuspended in 0.9% saline plus 0.02% Triton-X-100. The resuspended beads were probe sonicated at medium intensity for 20 s, and then inspected under light microscopy, which demonstrated no aggregation. The concentration of beads in a 1:100 dilution of the resuspension was measured in duplicate using a hemocytometer.

TxB_2 assays. Two-ml arterial blood samples were obtained under three experimental conditions for each protocol. The blood samples were drawn into cold inhibitor solution containing indomethacin and sodium EDTA, and centrifuged as previously described (6). The decanted plasma was frozen at −70°C until a RIA for TxB_2, the stable hydrolysis product of TxA_2, was performed as described (6). TxB_2 was assayed by measuring competitive inhibition of $[^3H]TxB_2$ to rabbit anti-TxB_2 binding as previously described (6). The average of duplicate assays was used to determine means for the different experimental conditions.

Experimental design. In each piglet infused with GBS, P_{pa}, P_w, P_{sa}, CO, arterial and mixed venous gas tensions, arterial blood samples for measurement of TxB_2, and quantitative blood cultures were obtained under three conditions: *1*) before GBS infusion (PRE), *2*) 60 min after the onset of the GBS infusion via a central venous catheter (0.1–0.2 mL/min) at a dose of 5×10^8 cfu/kg/h (60 min), and *3*) 90 min after the onset of the GBS infusion and 30 min after an IV infusion of 8 mg/kg of DAZ, a thromboxane synthase inhibitor (6). Five piglets received GBS strain COH 31 r/s, five piglets received GBS strain COH 31-15 (devoid of capsular polysaccharide), and four piglets received GBS strain COH 31C5 (devoid of β-hemolysin). Dazmegrel (UK 38,435 kindly provided by R. Urguilla, Pfizer Laboratories, Groton, CT) was dissolved in 1.5 mL of 0.1 N NaOH to which 0.9% saline was added to make a 7.5 mL solution.

Six piglets were infused with PS beads, similar in size to GBS, suspended in 0.9% saline plus 0.2% Triton-X-100 to serve as a particulate control. In parallel with the GBS experiments, the same measurements were obtained *1*) before to PS bead infusion (PRE), *2*) 60 min after the onset of 5×10^8 beads/kg/h (0.2–0.5 ml/min), and *3*) 90 min after the onset of bead infusion, and 30 min after DAZ administration. The infusion of 0.9% saline plus 0.02% Triton-X-100 alone at 1.0 mL/min caused no significant changes in hemodynamic or gas exchange parameters.

Statistical analysis. Analysis of variance was used to compare the 1 h GBS or bead values between the four experimental groups; paired Student's *t* tests were used to compare the 1 h GBS or DAZ values to PRE values for each experimental group (SPSS-PC+, Microsoft, Bellevue, WA). A p value of ≤ 0.05 was considered significant.

RESULTS

PVR significantly increased to a similar degree (4- to 6-fold) in piglets infused with either *1*) the parent type III GBS strain (COH 31 r/s), *2*) an isogenic GBS mutant devoid of type III capsular polysaccharide (COH 31-15) or *3*) an isogenic mutant devoid of β-hemolysin (COH 31C5) (Table 1). During the 1 h GBS infusion, P_{pa} increased 3-fold, P_w significantly increased, and CO decreased 30–40% for the three isogenic GBS strains (Table 1).

The three isogenic GBS strains caused a similar degree of arterial hypoxemia, and a similar decrease in mixed venous Po_2 1 h into the GBS infusion (Table 2). There were no significant changes in arterial Pco_2. Although there were small but statistically significant reductions in arterial pH after 1 h of infusion of all GBS strains, mean values remained in the normal range for piglets (Table 2).

Arterial TxB_2 levels significantly increased 1 h after the onset of bacterial infusion for all three GBS strains (Table 3). There were no statistically significant differences between the 60 min TxB_2 levels for the three GBS strains. However, the COH 31 r/s 60 min TxB2 levels (243, 118, 88, 74, and 143 pg/0.1 mL) were more variable than the COH 31-15 values (60, 67, 60, 70, and 62 pg/0.1 mL), and although there is no statistically significant differences between the two groups, the power of the test to demonstrate a difference is limited to 63%. Thirty min after DAZ infusion, with ongoing GBS infusion, pulmonary hypertension was reversed and TxB_2 levels were partially reversed to near preinfusion values for all GBS strains (Tables 1 and 3). Dazme-

Table 1. *Similar hemodynamic responses to three isogenic GBS strains in piglets**

	P_{pa} (mm Hg)	P_w (mm Hg)	C.O. (L/min)	PVR (mm Hg/L/min)
COH 31 r/s ($n = 5$) (parent)				
PRE	14.2 ± 1.1	2.4 ± 0.4	1.04 ± 0.10	11.6 ± 1.4
60 min	† 43.0 ± 1.8	† 5.7 ± 0.7	† 0.66 ± 0.13	† 67.1 ± 17.9
DAZ	14.0 ± 1.2	2.4 ± 0.5	0.83 ± 0.09	13.7 ± 2.0
COH 31-15 ($n = 5$) (capsule)				
PRE	14.4 ± 0.8	2.6 ± 0.7	0.98 ± 0.08	12.5 ± 1.4
60 min	† 39.1 ± 1.3	† 5.0 ± 1.1	† 0.62 ± 0.07	† 56.9 ± 5.0
DAZ	16.2 ± 1.0	3.2 ± 1.1	0.84 ± 0.06	15.7 ± 1.0
COH 31C5 ($n = 4$) (hemolysin)				
PRE	12.9 ± 1.0	2.6 ± 0.6	0.95 ± 0.04	11.0 ± 1.9
60 min	† 36.3 ± 2.3	† 3.9 ± 0.5	† 0.65 ± 0.06	† 51.9 ± 7.9
DAZ	12.0 ± 0.9	2.3 ± 0.7	0.86 ± 0.04	11.3 ± 1.3
Polystyrene beads ($n = 6$)				
PRE	15.3 ± 1.1	3.8 ± 0.3	0.93 ± 0.11	13.3 ± 2.0
60 min	† 18.0 ± 1.5	4.5 ± 0.3	0.85 ± 0.11	† 17.7 ± 3.5
DAZ	17.2 ± 0.8	4.5 ± 0.5	0.87 ± 0.10	15.4 ± 1.9

* Values are expressed as mean ± SEM. PRE, before GBS or PS bead infusion; 60 min, 60 min after the onset of the GBS or PS bead infusion; DAZ, 30 min after administration of DAZ with ongoing GBS or PS bead infusion.

† Denotes $p \leq 0.05$ compared to PRE value.

Table 2. *Similar blood gas changes in response to three isogenic GBS strains in piglets**

	pH	$Paco_2$ (mm Hg)	Pao_2 (mm Hg)	Pvo_2 (mm Hg)
COH 31 r/s (parent)				
PRE	7.42 ± 0.02	39 ± 2.0	94 ± 2.8	47 ± 1.4
60 min	† 7.36 ± 0.01	38 ± 2.2	† 64 ± 5.2	† 37 ± 2.6
DAZ	7.39 ± 0.02	37 ± 1.6	78 ± 2.0	44 ± 1.3
COH 31-15 (capsule)				
PRE	7.45 ± 0.01	39 ± 1.2	92 ± 4.0	41 ± 1.4
60 min	† 7.40 ± 0.01	40 ± 1.2	† 71 ± 5.5	† 31 ± 1.0
DAZ	7.41 ± 0.01	40 ± 1.1	80 ± 4.0	38 ± 2.0
COH 31C5 (hemolysin)				
PRE	7.42 ± 0.02	38 ± 2.0	95 ± 2.5	40 ± 2.7
60 min	† 7.38 ± 0.02	41 ± 1.7	† 66 ± 3.0	† 31 ± 4.0
DAZ	7.39 ± 0.01	40 ± 1.6	78 ± 1.1	36 ± 4.2
Polystyrene beads				
PRE	7.42 ± 0.01	38 ± 1.2	87 ± 2.4	42 ± 0.7
60 min	7.43 ± 0.01	39 ± 0.5	88 ± 3.4	42 ± 1.0
DAZ	7.42 ± 0.01	40 ± 1.2	82 ± 4.0	41 ± 1.0

* Values are expressed as mean ± SEM. PRE, before GBS or PS bead infusion; 60 min, 60 min after the onset of the GBS or PS bead infusion; DAZ, 30 min after administration of DAZ with ongoing GBS or PS bead infusion.

† Denotes $p \leq 0.05$ compared to PRE value.

Table 3. *Blood TxB_2 levels (pg/0.1 ml) increase during infusion of three isogenic GBS strains in piglets**

	COH 31 r/s (parent)	COH 31-15 (capsule)	COH 31C5 (hemolysin)	PS beads
PRE	<10	<10	17 ± 2	11 ± 1
60 min	† 133 ± 30	† 64 ± 5	† 71 ± 6	12 ± 2
DAZ	29 ± 10	30 ± 4	20 ± 6	<10

* Arterial blood was sampled under each of three experimental conditions and TxB_2 was assayed in duplicate by RIA as described in methods. Values are expressed as mean ± SEM. PRE, before GBS or PS bead infusion; 60 min, 60 min after the onset of the GBS or PS bead infusion; DAZ, 30 min after administration of DAZ with ongoing GBS or PS bead infusion.

† Denotes $p < 0.05$ compared to PRE value.

grel administration also resulted in partial return to baseline values for CO, Pao_2, and Pvo_2 (Tables 1 and 2).

Polystyrene beads (2.355 μm in diameter) of similar size to GBS were infused into piglets to test if the similar response to all three GBS strains was due to a nonspecific pulmonary vascular response to circulating particulates. The infusion of PS beads at 5×10^8 beads/kg/h caused mild, but consistent increases in Ppa and PVR, with no changes in cardiac output or gas exchange values (Tables 1 and 2). The degree of pulmonary vasoconstriction was significantly less in the piglets infused with PS beads compared to the three GBS strains (Table 1). In addition, arterial TxB_2 levels were not increased during PS bead infusion (Table 3).

Quantitative arterial blood cultures 1 h after the onset of GBS infusion found statistically similar colony counts for COH 31 r/s ($1.7 \pm 0.5 \times 10^4$ cfu/mL), COH 31-15 ($3.5 \pm 2.3 \times 10^4$ cfu/mL), and COH 31C5 ($10.5 \pm 6.6 \times 10^4$ non-β-hemolytic cfu/mL). There were no significant changes in blood colony counts after DAZ for all three GBS strains. All blood cultures were sterile before experimental GBS infusion.

DISCUSSION

An isogenic mutant of type III GBS devoid of only the type III capsular polysaccharide (COH 31-15) causes a similar degree of acute Tx-associated pulmonary hypertension, hypoxemia, and reduction in CO compared to the parent type III GBS strain (COH 31 r/s) in neonatal piglets. The increase in PVR, and in part the increase in TxB_2 levels induced by each GBS strain is

reversed by DAZ. These results provide evidence that type III capsular polysaccharide is not essential for type III GBS to induce thromboxane synthesis, pulmonary hypertension, hypoxemia, or reduce CO during the acute phase (<1 h) of type III GBS bacteremia in piglets. We are greater than 80% confident that we could have detected a 6 mmHg difference in the 1 h P_{pa} values for GBS 31 r/s and GBS 31-15. The similar results, including TxB2 levels, with COH 31-15 and the COH 31C5 strain, which lacks β-hemolysin but contains the same amount of capsular polysaccharide as the parent GBS strain, provides further evidence that capsular polysaccharide is not essential for the acute effects of GBS sepsis. There is a statistically insignificant trend toward increased 60 min TxB2 levels for COH 31 r/s compared to COH 31-15, but due to the limited power of the test we cannot exclude the possibility that the presence of type III capsular polysaccharide may influence the degree of TxB2 synthesis in piglets infused with GBS. The type III GBS strain COH-1 (nonisogenic to COH 31 r/s), contains approximately 10-fold more type III capsular polysaccharide than COH 31 r/s (Wessels M, Rubens C, unpublished data), and causes a similar degree of pulmonary hypertension and increased TxB_2 levels as COH 31 r/s in piglets (11). This is corroborating evidence that type III capsular polysaccharide is not essential for the acute effects of type III GBS bacteremia in piglets. However, type III capsular polysaccharide may play a role in the late responses (≥2 h) of GBS bacteremia, as extracts of type III GBS containing capsular polysaccharide can promote neutrophil adherence to endothelium *in vitro* (16), and induce lung inflammation when infused into sheep (17). Our experimental design did not evaluate this role of capsular polysaccharide in piglets.

Hemolysins are important virulence factors for some gram-positive neonatal pathogens, such as *Listeria monocytogenes* and *S. faecalis* (18, 19). However, the β-hemolysin of GBS is not a virulence factor in a neonatal rat model of sepsis based on the similar LD_{50} doses for COH 31 r/s and COH 31C5 (15). Our results with the isogenic mutant devoid of β-hemolysin (COH 31C5) provide evidence that β-hemolysin is not necessary to induce acute Tx-associated pulmonary hypertension and hypoxemia during GBS bacteremia in piglets. Although 1–2% of GBS isolated from infected human newborns are non β-hemolytic (15), there are no published data comparing the clinical features of non β-hemolytic strains to β-hemolytic strains to corroborate our findings.

An extract of GBS containing type III capsular polysaccharide induces acute pulmonary hypertension, hypoxemia, and hypotension during bolus infusions in sheep (12). These data differ from our results regarding the role of type III capsular polysaccharide in GBS bacteremia. Perhaps multiple GBS components are capable of inducing these features, but the reason for this discrepancy is uncertain. First, although type III capsular polysaccharide is not essential to induce the acute features of GBS bacteremia, the infusion of capsular polysaccharide may cause hemodynamic and gas exchange alterations in a dose-dependent manner. GBS strain COH 31 r/s contains 3 μg of type III capsular polysaccharide per 5×10^8 bacteria (Wessels M, Rubens C, unpublished data), and therefore we estimate piglets in our study were infused with the equivalent of 3 μg/kg/h of capsular polysaccharide. This is a 100 to 200-fold lower dose of capsular polysaccharide than the dose of GBS capsular polysaccharide extract that caused pulmonary vasoconstriction when infused into sheep (12). Second, the group B polysaccharide or protein components that contaminated the GBS extract may have caused the hemodynamic and gas exchange alterations. There are no data for the group B polysaccharide, but group B streptococcal peptidoglycan can induce complement consumption and inflammation in other animal models (20). Finally, the possible fragmentation and denaturation of the complex carbohydrate structure of the type III antigen during extraction may alter its biologic activity.

Polystyrene beads were infused in piglets to test if a nonspecific pulmonary vascular response to circulating particulates could explain why the three GBS strains caused similar hemodynamic and gas exchange alterations. Several species, including swine, contain pulmonary intravascular macrophages that are capable of ingesting circulating bacteria or particulates (21–23). Pulmonary intravascular macrophages can synthesize on array of arachidonic acid metabolites, including TxB_2, and therefore may play a role in the cardiopulmonary responses to bacteremia or circulating particulates (24, 25). The minimal hemodynamic response in piglets infused with polystyrene beads (5×10^8 beads/kg/h) suggest that a nonspecific pulmonary vascular response to circulating particulates is not the major cause of the acute features of GBS bacteremia in piglets.

Type III GBS capsular polysaccharide is a major virulence factor, and allows GBS to evade local or systemic phagocytic host defenses (14). Several studies indicate antitype III antibodies are protective against GBS infection in animal models and human infants (26–28). However, our data suggest that once host defenses are overwhelmed and significant bacteremia occurs, the type III capsular polysaccharide is not essential for inducing acute hemodynamic and gas exchange abnormalities of GBS sepsis.

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