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Group B *Streptococcus* Capsular Serotype Alters Vaginal Colonization Fitness

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Background. Group B *Streptococcus* (GBS) remains a leading cause of infant morbidity and mortality. A candidate vaccine targets 6 GBS serotypes, offering a potential alternative to intrapartum antibiotic prophylaxis to reduce disease burden. However, our understanding of the contributions of specific capsule types to GBS colonization and disease remains limited.

Methods. Using allelic exchange, we generated isogenic GBS strains differing only in the serotype-determining region in 2 genetic backgrounds, including the hypervirulent clonal complex (CC) 17. Using a murine model of vaginal cocolonization, we evaluated the roles of the presence of capsule and of expression of specific capsular types in GBS vaginal colonization fitness independent of other genetic factors.

Results. Encapsulated wild-type strains COH1 (CC17, serotype III) and A909 (non-CC17, serotype Ia) outcompeted isogenic acapsular mutants in murine vaginal cocolonization. COH1 wild type outcompeted A909. Notably, expression of type Ia capsule conferred an advantage over type III capsule in both genetic backgrounds.

Conclusions. Specific capsule types may provide an advantage in GBS vaginal colonization in vivo. However, success of certain GBS lineages, including CC17, likely involves both capsule and noncapsule genetic elements. Capsule switching in GBS, a potential outcome of conjugate vaccine programs, may alter colonization fitness or pathogenesis.

Keywords. *Streptococcus agalactiae*; capsular polysaccharide; vaginal colonization.

Streptococcus agalactiae (group B *Streptococcus* [GBS]) remains a leading cause of neonatal disease [1–3]. The use of intrapartum antibiotic prophylaxis (IAP) has reduced the burden of early-onset GBS disease in newborns [4, 5]. However, rates of maternal GBS colonization and late-onset GBS disease remain nearly unchanged [5–8]. Furthermore, GBS disease burden remains high in regions where screening and IAP may not be widely available or feasible, particularly in sub-Saharan African nations [2, 7].

Maternal GBS vaccination may prove more feasible and effective than IAP in preventing GBS disease worldwide. The polysaccharide capsule is an important GBS virulence factor [9, 10]. Placental transfer of anticapsular maternal antibodies has been demonstrated to protect against early-onset GBS disease [11–13]. GBS6, a hexavalent capsular polysaccharide conjugate vaccine targeting serotypes Ia, Ib, II, III, IV, and V is well tolerated and immunogenic in healthy nonpregnant adults and has the potential to provide protection against most GBS isolates that cause invasive disease [8, 14–17]. However, it is possible that the implementation of a hexavalent polysaccharide vaccine may lead to serotype replacement with nonvaccine type strains, as was observed following pneumococcal vaccination in the United States [18, 19]. GBS capsular switching, including within the CC17 hypervirulent clone, has been documented [20–23].

Individual GBS serotypes vary in frequency of isolation from colonization or disease states [2, 3, 8, 16, 24]. Serotype III strains from clonal complex (CC) 17 are particularly common in late-onset GBS disease [8, 16, 25]. However, because serotype is nonrandomly associated with GBS genetic backgrounds as assessed by sequence type (ST) or CC assignment, it has been extremely challenging to determine whether there are independent effects of specific GBS serotypes on colonization or disease. Here, we used techniques to modify the GBS chromosome to generate otherwise isogenic bacterial strains differing only in capsular serotype. Employing these strains in a murine model of vaginal cocolonization probed the specific roles of type Ia and III capsule types in GBS fitness at the vaginal mucosal surface.

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METHODS

Bacterial Strains and Growth Conditions

GBS wild-type (WT) strains A909 (serotype Ia, ST7; American Type Culture Collection [ATCC] BAA-1138) and COH1 (serotype III, CC17; ATCC BAA-1176) were used. HY106, a capsule-deficient mutant of COH1, was the kind gift of Dr Kelly Doran. Strain HY106 has a deletion of a portion of the *cps* locus that abolishes capsule synthesis [26]. HY106, WT strains, and their derivatives were grown at 37°C under stationary conditions in tryptic soy broth (Fisher). Chemically competent *Escherichia coli* DH5α (New England Biolabs) was stored and transformed according to the manufacturer's instructions. *E. coli* growth was in LB medium at 37°C unless transformed with a temperature-sensitive plasmid, in which case the growth temperature was 28°C.

Murine Vaginal Colonization Model

All experimental procedures were reviewed and approved by the New York University Langone Institutional Animal Care and Use Committee. Female C57BL6/J mice, 6–8 weeks old, were purchased from Jackson Laboratories. Vaginal colonization was performed as previously described [27]. Animals were subcutaneously injected with 10 μg of water-soluble 17β-estradiol (Sigma-Aldrich) at 48 and 24 hours before colonization to synchronize the estrous cycle.

For the monocolonization model with WT A909 (Ia) or COH1 (III), bacterial cultures were grown overnight to stationary phase. Following overnight growth, the optical densities of the 2 strains were normalized to one another, and cultures were then centrifuged and resuspended in a 1:1 mixture of phosphate-buffered saline (PBS) and sterile 10% gelatin for a final concentration of 10^9 colony-forming units (CFU)/mL. Mice were anesthetized with 3%–5% isoflurane (Baxter), and 50 μL GBS suspension (total inoculum 5×10^7 CFU) was administered intravaginally using a sterile pipette. Mice were housed in separate cages for the remainder of the experiment. Vaginal swab specimens were collected using a sterile swab that was vigorously shaken into 300 μL of PBS. Serial dilutions were plated on CHROMagar (CHROMagar) for enumeration of CFU/mL.

For the cocolonization model, bacterial cultures were grown and centrifuged as described above. Cultures were resuspended in PBS, and a 1:1 mixture was made of the 2 resuspended strains to be competed. This mixture was then mixed 1:1 with sterile 10% gelatin. Mice were colonized, housed, and swabbed as described above. In addition to plating serial dilutions, dilutions were spread on CHROMagar plates with glass beads for determination of serotype by colony immunoblot.

Colony Immunoblotting

Following growth at 37°C, colonies were counted to identify plates with 20–200 colonies for subsequent immunoblotting. Plates were briefly overlaid with nitrocellulose membranes (Amersham) to allow adherence of GBS material to the membrane. Membranes were blocked in 3% bovine serum albumin (BSA) in PBS (blocking solution) for 1 hour. Blots were then transferred to type Ia or type III *Streptococcus* group B type antisera (Statens Serum Institut) and incubated for 30 minutes with gentle shaking. Type Ia antisera was diluted 1:2000 in blocking solution, and type III antisera was diluted 1:5000 in blocking solution. Blots were then washed 3 times in PBS. Blots were incubated in horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (Pierce) diluted 1:1000 in blocking solution for 2 hours. Blots were washed 3 times in PBS and stained using a 3,3'-diaminobenzidine tetrahydrochloride substrate kit (Abcam). Colonies positive for each capsule type were counted. In experiments comparing WT to acapsular mutants, nonreacting GBS colonies representing the acapsular mutants were also counted. Competitive index (CFU strain 1 recovered/CFU strain 1 inoculated)/(CFU strain 2 recovered/CFU strain 2 inoculated) was calculated and log-transformed for calculation of geometric mean and 95% confidence interval for each condition and time point.

Generation of Capsule Switch, Capsule Deficient, and Revertant Strains

Prior work using plasmid overexpression suggested that *cpsH* expression in *trans* was sufficient to mediate expression of either type Ia or type III capsule in a heterologous strain background with an intact native *cps* locus [28]. Comparison of the *cps* loci between GBS types Ia and III demonstrate a region of difference consisting of the 3′ end of *cpsG* and the full open reading frame of *cpsH* [29]. We generated capsule switch strains by constructing a cassette consisting of the heterologous region of difference flanked by 500 bp of homology to the target strain on each side. Each cassette was cloned into the temperature-sensitive plasmid pHY304 by Gibson assembly and transformed into electrocompetent *E. coli* DH5α. The insert was verified by polymerase chain reaction (PCR) and sequencing. Allelic exchange was performed essentially as described [30]. Briefly, the plasmid was transformed into electrocompetent WT A909 (Ia) or COH1 (III). Temperature-based selection was used to isolate single-cross and double-cross plasmid insertion and excision mutants, which were confirmed by PCR. A909 capsule switch mutants expressed type III capsule and COH1 capsule switch mutants expressed type Ia capsule, while revertant strains, which occur based on the direction of plasmid excision at the final step of the allelic exchange procedure, expressed their native capsule type. Capsule types of WT, capsule switch, and revertant strains were confirmed by latex agglutination with type Ia and type III sera (Immulex Strep-B kit; Statens Serum Institut) and by sequencing of the capsule locus.

CpsE catalyzes an essential step in the synthesis of the GBS polysaccharide repeating unit, and therefore inactivation of the *cpsE* gene leads to inactivation of capsule synthesis [31]. To generate capsule-deficient A909Δ*cpsE*, we amplified 500-bp homology arms to construct a cassette for in-frame deletion of the full open reading frame of *cpsE*. The cassette was

cloned into pMBsacB, a temperature-sensitive and sucrose-counterselectable mutagenesis shuttle vector [30] using Gibson assembly and transformed into electrocompetent *E. coli* DH5α. The insert was verified by PCR and sequencing. Allelic exchange was performed essentially as described [30]. A909Δ*cpsE* was verified by PCR and loss of latex agglutination with type Ia sera. Revertant strains continued to express type Ia capsule.

Enzyme-Linked Lectin Assay

An enzyme-linked lectin assay (ELLA) was used to assess the amount of capsule present in each strain. All GBS capsules possess a terminal α-2,3 sialic acid. Sialylation has been shown to be important for the full synthesis of the GBS capsule [32], and sialic acid has therefore been used as a means to recognize capsule expression [33]. A previously described protocol [33] was adapted for whole bacteria [34]. *Maackia amurensis* I lectin (MAL-I; Vector Labs), which recognizes sialic acid as Neu5Acα-2,3-Galβ-1,4-GlcNAc [35], was used for recognition of sialic acid on GBS type Ia and type III capsule.

GBS strains were grown in 10 mL of tryptic soy broth under stationary conditions at 37°C to an optical density at 600 nm of 0.6. Cultures were then centrifuged and resuspended in 10 mL of coating buffer (0.087 M $NaHCO_3$ and 0.015 M Na_2CO_3 in sterile water, pH 9.5). Prepared suspensions were diluted 1:10 in coating buffer and 100 µL was distributed in the wells of an enzyme-linked immunosorbent assay (ELISA) plate. This plate was incubated overnight at 37°C to allow the wells to dry.

The following day, samples were fixed with 50 µL of 100% methanol and allowed to dry for 40 minutes at 37°C. Wells were then washed with 200 µL of PBS for 15 minutes at room temperature. Samples were blocked with 200 µL/well of 1% BSA in PBS for 4 hours, covered, at 37°C. Following blocking, 50 µL of 10 µg/mL biotinylated MAL-I in PBS with 1% BSA and 0.05% Tween (PBSAT) was distributed in each well, and the plate was incubated at room temperature for 1 hour. Wells were then washed 3 times with 200 µL PBSAT for 5 minutes. Streptavidin-HRP (Pierce) was diluted 1:20 000 in PBSAT and 50 µL was distributed in each well. The plate was incubated at 37°C for 15 minutes. Wells were washed 3 times with PBSAT. 100 µL/well of TMB-ELISA substrate solution (Pierce) was added and the plate was incubated for 10 minutes at room temperature, protected from the light. The reaction was stopped with the addition of 50 µL/well of 1 N H_2SO_4. Finally, the absorbance was read at 450 nm in a plate reader.

Statistical Analyses

Persistence of vaginal monocolonization between WT A909 (Ia) and WT COH1 (III) was compared using the Log-rank (Mantel-Cox) test, and CFUs recovered from the vagina at each day were compared by 2-way analysis of variance with Sidak multiple comparison test. ELLA absorbance outcomes between strains were compared using Kruskal-Wallis test (Prism, GraphPad Software).

RESULTS

COH1 Outcompetes A909 in a Mouse Model of Vaginal Cocolonization

We vaginally colonized mice with WT A909 (Ia) or WT COH1 (III) and monitored colonization persistence and bacterial burden with vaginal swabbing (monocolonization model). Both A909 and COH1 were able to establish persistent vaginal colonization under conditions of monocolonization (Figure 1A). There was no significant difference in duration of colonization (Figure 1A) or in recovered CFU between WT A909 (Ia) and COH1 (III) strains (Supplementary Figure 1).

In clinical settings, multiple GBS serotypes are frequently found to cocolonize the vagina [36–38]. We therefore used a competition model to more carefully assess differences in establishment and persistence of GBS vaginal colonization by simultaneously colonizing the vagina with 2 GBS strains. Geometric mean competitive index was greater than 7 by 2 days

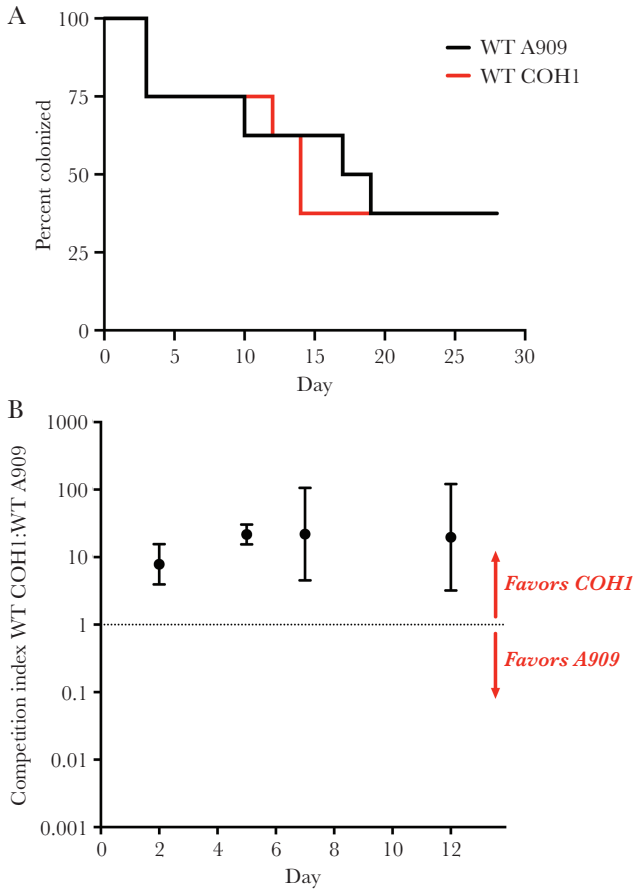

Figure 1. COH1 outcompetes A909 in a mouse model of vaginal cocolonization. *A*, Adult nonpregnant C57/BL6J mice were vaginally colonized with wild-type (WT) COH1 or A909 (n = 8 for each strain). Kaplan-Meier curve represents colonization persistence, as determined by vaginal swabs every 2–3 days (*P* = not significant, Log-rank test). *B*, Adult nonpregnant C57/BL6J mice were simultaneously vaginally colonized with a 1:1 mixture of WT A909 + WT COH1 (n = 5). Samples collected by vaginal swabs at 4 time points postcolonization were spread on Chromagar plates. Immunoblot with type Ia and type III primary antibody was used to differentiate between A909 and COH1. Data points represent geometric mean competition indices and error bars represent 95% confidence intervals.

postcolonization, and exceeded 20 by 5 days postcolonization, suggesting a competitive advantage for WT COH1 (III, CC17) over WT A909 (Ia, ST7) strain in vaginal colonization fitness (Figure 1B).

Presence of Type Ia or Type III Capsule Confers an Advantage Over Capsule-Deficient Strains

The GBS capsule is an important virulence factor. We therefore sought to assess the role of capsule presence versus absence as well as potential effects of specific capsule types in vaginal colonization fitness. We created a capsule-deficient A909 strain, A909Δ*cpsE*, and utilized a capsule-deficient mutant of COH1, HY106. We confirmed the abolition of type Ia or type III capsule production in these mutant strains by latex agglutination with type Ia sera for A909 strains and type III sera for COH1 strains (Figure 2A). We then performed an ELLA, which confirmed that these capsule-deficient mutants did not produce any detectable level of capsule, in contrast to their WT counterparts (Figure 2B).

In the murine vaginal colonization model, we compared the relative fitness of capsule-producing and capsule-deficient strains of the same strain background (ie, encapsulated A909 vs A909Δ*cpsE* and WT COH1 vs HY106). In the setting of cocolonization with the encapsulated revertant A909 versus A909Δ*cpsE*, the geometric mean competitive index exceeded 4 at 2 days postcolonization and exceeded 75 by 7 days postcolonization, suggesting a robust competitive advantage of encapsulated A909 over the capsule-deficient A909Δ*cpsE* (Figure 2C). Likewise, encapsulated COH1 outcompeted the acapsular HY106 mutant, with a mean competitive index exceeding 10 by 5 days postcolonization and 30 by 7 days postcolonization (Figure 2D). Encapsulated A909 and COH1 strains therefore efficiently outcompeted isogenic acapsular

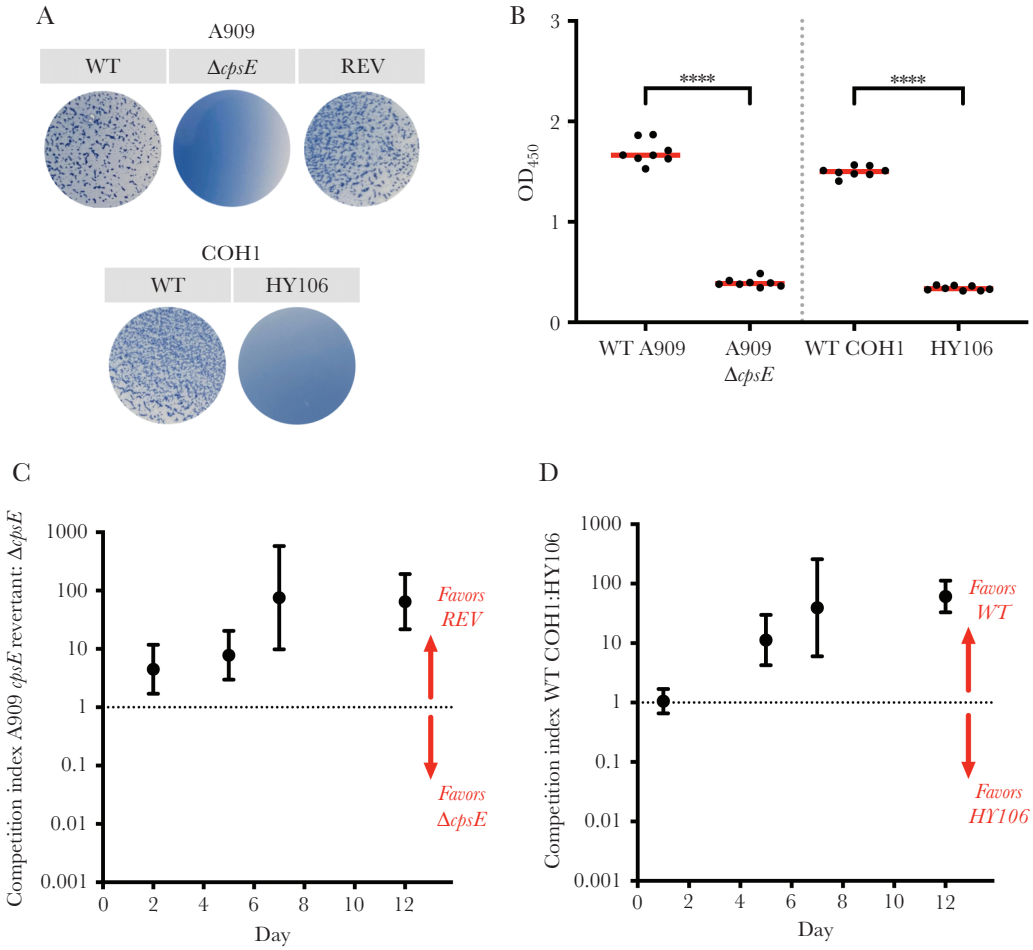

Figure 2. Presence of type Ia or type III capsule confers an advantage over capsule-deficient strains. *A*, Latex agglutination was used to confirm production of capsule. Type Ia antisera was used for wild-type (WT) A909, A909 Δ*cpsE*, and revertant (REV) strains. Type III antisera was used for WT COH1 and HY106. *B*, Enzyme-linked lectin assay using *Maackia amurensis* (MAL-I) lectin was used to estimate capsule amount in WT and capsule-deficient A909 and COH1 strains. Data points represent technical replicates (n = 8 for each strain), and median optical density (OD) was determined. Data shown are representative of 3 experiments. (**** $P < .0001$, Kruskal-Wallis test). *C* and *D*, Adult nonpregnant C57/BL6J mice were vaginally colonized with a 1:1 mixture of a capsule-producing and capsule-deficient strain (*C*, n = 8; *D*, n = 6). Samples collected by vaginal swabs at 4 time points postcolonization were spread on Chromagar plates. Immunoblot with type Ia (*C*) or type III (*D*) primary antibody was used to differentiate between encapsulated and capsule-deficient strains. Data points represent geometric mean competition indices and error bars represent 95% confidence intervals.

strains, suggesting that GBS capsule likely provides an advantage in the maintenance of vaginal colonization.

Characterization of Capsule Switch and Revertant Strains

Capsule switch strains, specifically, A909 expressing type III capsule and COH1 expressing type Ia capsule, were created by allelic exchange of a fragment including the 3′ end of *cpsG* and the full open reading frame of *cpsH* (Figure 3A). Latex agglutination confirmed capsular switching of mutants, and revertant strains were identified as strains that had undergone plasmid insertion but reverted to their native capsule type based on the direction of plasmid excision (Figure 3B). Revertant strains were used for

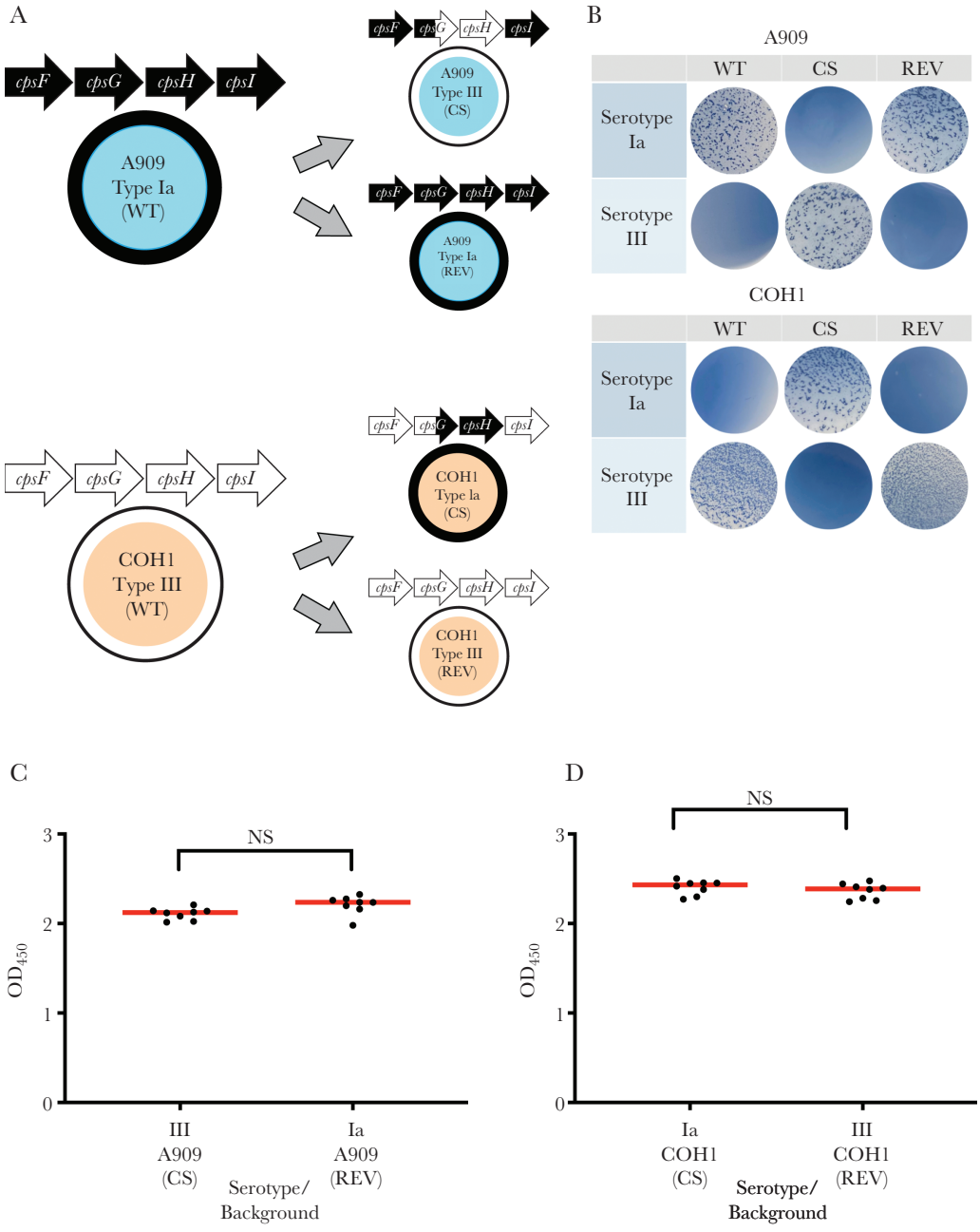

Figure 3. A909 and COH1 capsule switch strains produce similar amounts of capsule. *A*, pMBsacB mutagenesis was used for allelic exchange of *cpsG* and *cpsH* of the *cps* locus from a type III strain to a type Ia strain and vice versa. Capsule switch (CS) strains, A909 type III and COH1 type Ia, differ from the wild type (WT) in only these sections of the *cps* locus, but produce a different capsule type. Revertant strains (REV), A909 type Ia and COH1 type III, have undergone pMBsacB mutagenesis, but revert back to the native *cpsG* and *cpsH*, therefore producing their native capsule type. *B*, Latex agglutination with type Ia and type III antisera was used to confirm capsular switching in A909 and COH1 mutant strains. *C* and *D*, Enzyme-linked lectin assay using *Maackia amurensis* (MAL-I) lectin was used to estimate capsule amount in CS and REV A909 and COH1 strains. Data points represent technical replicates (n = 8 for each strain) and median optical density (OD) was determined. The x-axis label indicates the expressed serotype (either Ia or III), the strain background (either A909 or COH1), and either CS or REV to indicate the specific strain type. Data shown are representative of multiple individual experiments (*P* = not significant [NS], Kruskal-Wallis test).

comparison to control for alterations in phenotype that could have been introduced by the allelic exchange procedure.

ELLA was used to compare the amount of capsule produced by capsule switch and revertant strains of the same genetic background. The amount of capsule produced by capsule switch and revertant strains did not differ in either the A909 or COH1 background (Figure 3C and 3D).

Strains Producing Type Ia Capsule Outcompete Strains Producing Type III Capsule Independent of Genetic Background

Following confirmation that matched capsule switch and revertant strains produce similar amounts of capsule, we competed these strain pairs in the murine vaginal cocolonization model. The A909 revertant strain expressing native type Ia capsule outcompeted the capsule-switch A909 strain expressing type III capsule, with geometric mean competitive index reaching approximately 50 by 12 days postcolonization (Figure 4A). Point estimates of competitive index at days 5 and 7 indicated an approximately 6-fold advantage for A909 expressing capsule type Ia, but the 95% confidence intervals crossed 1 at those time points. Notably, the COH1 capsule switch strain expressing type Ia capsule outcompeted the revertant strain expressing its native type III capsule. Geometric mean competitive index was approximately 0.18 (>5-fold difference) by 5 days postcolonization, and reached approximately 0.03 (>40-fold difference) by 12 days postcolonization, indicating a strong competitive advantage of the COH1 capsule switch expressing type Ia capsule over COH1 expressing its native type III capsule (Figure 4B). Taken together, these data suggest that the production of type Ia capsule may confer an advantage over type III capsule in vaginal colonization, even in a heterologous genetic background.

DISCUSSION

GBS vaginal colonization plays a critical role in pathogenesis. Certain GBS capsular serotypes are more commonly isolated from vaginal colonization or invasive disease conditions [3, 16, 24], but it remains unclear whether capsular serotype itself drives differences in colonization success among GBS strains, particularly because capsular serotype and strain genetic background are nonrandomly associated. Prior work has suggested that GBS serotype may affect the magnitude of host inflammatory responses as well as colonization success [39, 40]. However, these studies have generally used clinical GBS isolates and thus cannot control rigorously for the effect of capsule versus noncapsule genetic features.

Rates of maternal GBS colonization have remained unchanged since the 1970s [5], and therefore understanding of the contribution of GBS capsule to vaginal colonization may help us understand the impact of the introduction of a GBS hexavalent vaccine. Furthermore, humoral immunity has been shown to enhance GBS clearance from the vaginal tract [41], suggesting that GBS vaccination strategies targeting vaginal colonization may aid in the control of neonatal GBS disease. Vaccination with a pneumococcal conjugate vaccine led to serotype replacement, or an increase in nonvaccine type strains among both asymptomatic carriers and strains causing invasive disease, likely due to both unmasking of nonvaccine type strains and recombination events leading to capsular switching and vaccine escape [18, 19]. Serotype replacement following the implementation of a conjugate vaccine program poses a threat to the success of vaccination in reducing GBS disease burden, and therefore continued monitoring of both vaginal colonization and disease burden will be important.

Here, we demonstrated that the presence of type Ia or type III capsule confers a fitness advantage in GBS vaginal colonization over acapsular strains of the same genetic background. This finding establishes a role for common GBS capsule types

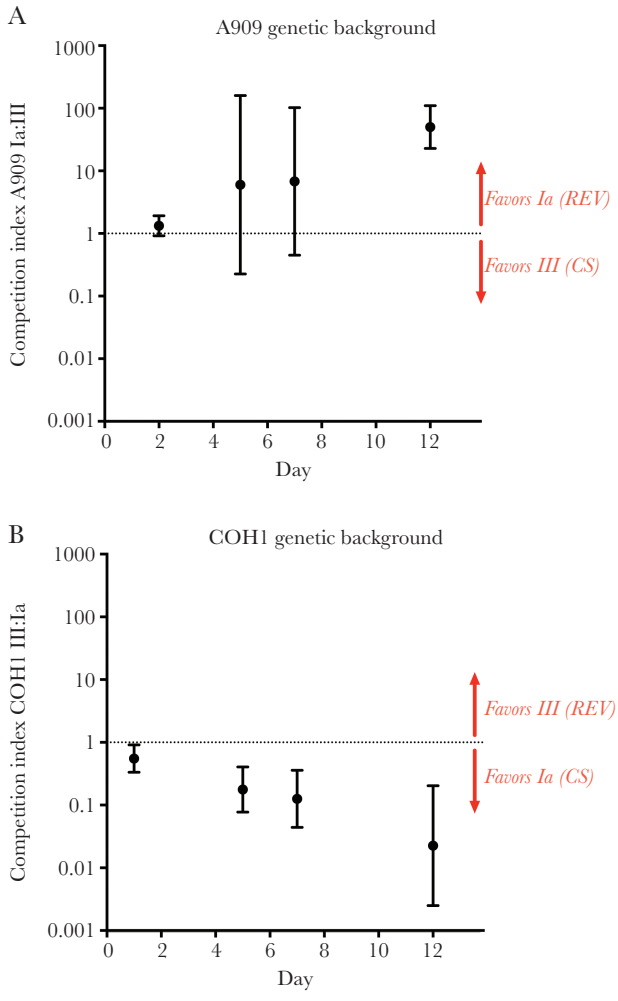

Figure 4. Strains producing type Ia capsule outcompete strains producing type III capsule independent of genetic background. *A* and *B*, Adult nonpregnant C57/BL6J mice were vaginally colonized with a 1:1 mixture of a capsule switch (CS) and revertant (REV) strains, as in Figure 1 (*A*, n = 8; *B*, n = 6). Data points represent geometric mean competition indices and error bars represent 95% confidence intervals. The graph title indicates the strain genetic background (either A909 or COH1), and the right-side labels indicates the expressed serotype (either Ia or III) and either CS or REV to indicate the specific strain type.

at a critical stage of host-pathogen interaction. The interruption of the expression of type III capsule has been shown to result in a mutant that is avirulent in a neonatal rat model of sepsis [9]. Furthermore, the type III capsule has been shown to prevent C3 deposition on the bacteria, which blocks phagocytosis of GBS [42]. A recent study showed a role for GBS capsule in ascending infection during pregnancy using a murine model [43]. Capsular sialic acid has been shown to contribute to the resistance of serotype III GBS to opsonophagocytosis [44]. GBS sialic acid mimics host sialoglycans, leading to attenuated host immune responses, such as neutrophil responses and platelet activation, by engaging inhibitory Siglecs [10, 45, 46]. The specific mechanisms by which capsule enhances GBS colonization fitness at the vaginal mucosal surface remain unclear.

We created isogenic capsule switch and revertant strains to assess the specific role of type Ia and type III capsule in GBS vaginal colonization. In the A909 background, the native type Ia capsule-expressing strain outcompeted the capsule switch (type III) strain. Notably, the COH1 capsule switch strain (expressing type Ia capsule) outcompeted the revertant expressing native capsule type III, suggesting that type Ia capsule confers an advantage over type III capsule in vaginal cocolonization, independent of strain background. This finding was surprising, given that strains expressing type III capsule are more commonly isolated in vaginal colonization as well as early-onset and late-onset GBS disease than type Ia and that type III strains make up the majority of the hypervirulent CC17 strains [24, 47]. Furthermore, we observed that WT COH1, which expresses its native type III capsule, outcompeted WT A909, expressing type Ia capsule, consistent with the hypothesis that both capsule-dependent and capsule-independent factors contribute to colonization fitness. CC17 strains are highly associated with late-onset GBS disease, possibly due to the expression of a CC17-specific hypervirulent GBS adhesin, HvgA [48]. Other CC17-specific factors, including the serine-rich repeat adhesin Srr2, have also been implicated in colonization and pathogenesis [49]. The overall advantage of COH1 (III) over A909 (Ia), despite the fact that A909 expresses a type Ia capsule, indicates that these adhesins or other noncapsule features likely play an important but nonexclusive role in vaginal colonization fitness, consistent with prior findings [50]. Taken together, these findings also highlight the importance of using carefully constructed strains to assess the independent contribution of bacterial factors, including capsular serotype, to the outcome of host-pathogen interactions.

We note that there are technical limitations that may limit applicability of our findings. Importantly, we only tested 2 GBS strain backgrounds, A909 (Ia) and COH1 (III). At this point, we are unable to generalize beyond these strains, and construction of capsule switch and revertant strains in other backgrounds, as well as with other capsule types, will be necessary to fully understand the specific impact of capsule type on colonization and disease. The lectin-based technique to detect capsule amounts is only semiquantitative, and we are unable to precisely quantify capsule amounts produced by each strain. For that reason, the quantity of capsule produced by each strain may be a contributing factor in the outcomes of our experiments. In addition, we relied on a murine model of vaginal colonization for our findings. One advantage of this approach is that it occurs in complex tissues in the presence of an immune system. However, there are important differences between murine and human anatomy, as well as in host-pathogen interactions. In addition, the cellular composition of the murine vaginal mucosal surface may change in response to estrogen, which we use to synchronize estrus cycles. Ascending infection of the uterus was not explored in these experiments. We recognize the limitations of the murine model and caution against direct application of the findings reported here to considerations of human epidemiology.

GBS6, a candidate hexavalent capsular polysaccharide conjugate vaccine, targets the most prevalent GBS serotypes—Ia, Ib, II, III, IV, and V. Capsule switching has been documented in the CC17 hypervirulent clone from a type III to type IV capsule [20–23], and switching events involving other capsule types and genetic backgrounds are likely, particularly if vaccination becomes widespread. Our results suggest that capsule switch events may alter vaginal colonization fitness, in addition to their potential for mediating vaccine escape. Surveillance for such events and further studies to understand the role of GBS specific capsule types in colonization and disease are warranted.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. A. J. R. has acted as a consultant for Pfizer and on a compassionate use advisory board for CompAC (NYU/Janssen). T. A. H. has received consulting fees from Techspert.io. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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