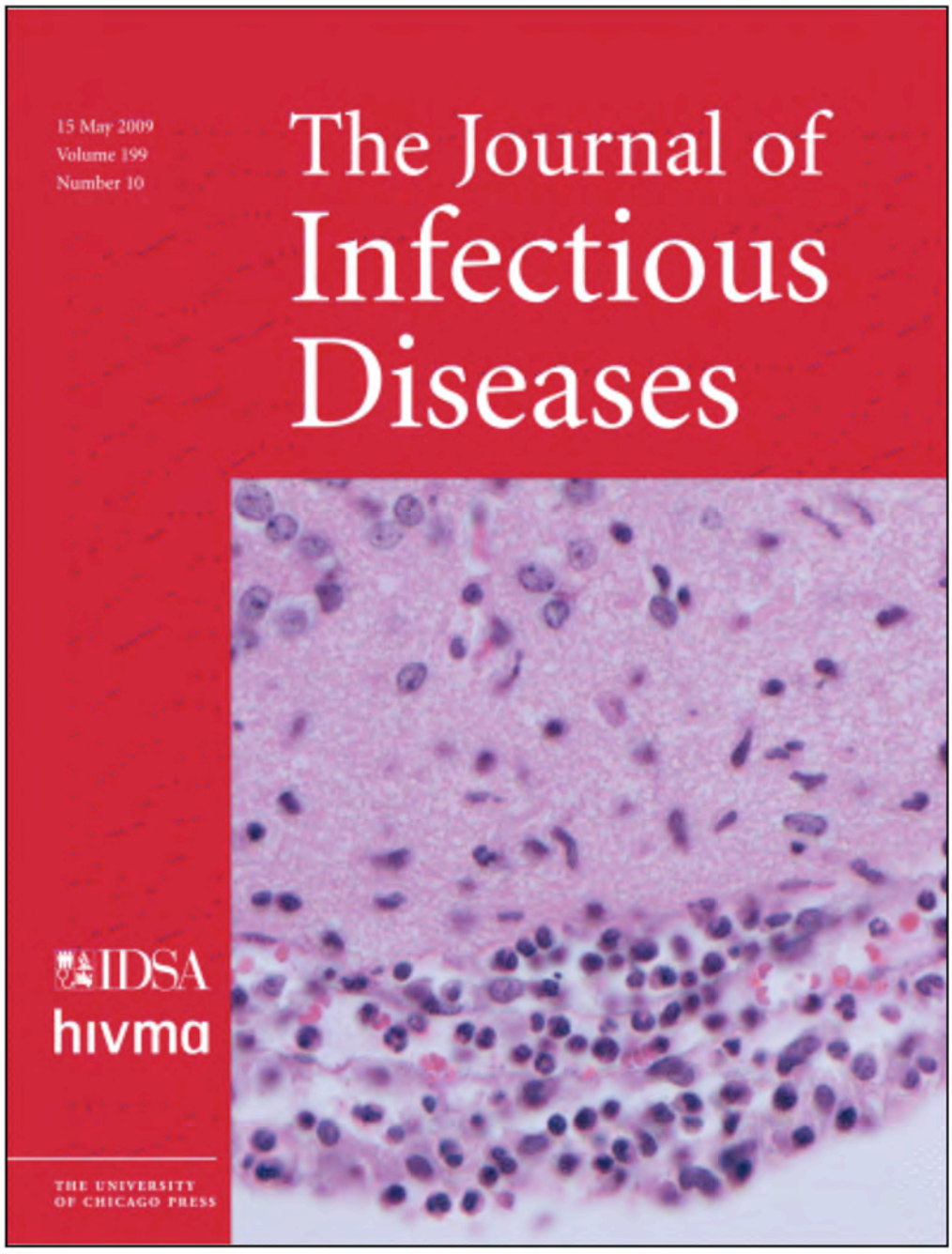


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On the cover: Histopathological analysis of a brain sample obtained from a mouse infected with an invasive group B *Streptococcus* strain reveals meningeal thickening and cellular infiltration. (See van Sorge et al., on pp. 1479–87)

# The Group B Streptococcal Serine-Rich Repeat 1 Glycoprotein Mediates Penetration of the Blood-Brain Barrier

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***Background.*** Group B *Streptococcus* (GBS) is the leading cause of bacterial meningitis in newborn infants. Because GBS is able to invade, survive, and cross the blood-brain barrier, we sought to identify surface-expressed virulence factors that contribute to blood-brain barrier penetration and the pathogenesis of meningitis.

***Methods.*** Targeted deletion and insertional mutants were generated in different GBS clinical isolates. Wild-type and mutant bacteria were analyzed for their capacity to adhere to and invade human brain microvascular endothelial cells (hBMECs) and to penetrate the blood-brain barrier using our model of hematogenous meningitis.

***Results.*** Analysis of a GBS (serotype V) clinical isolate revealed the presence of a surface-anchored serine-rich protein, previously designated serine-rich repeat 1 (Srr-1). GBS Srr-1 is a glycosylated protein with high molecular weight. Deletion of *srr1* in NCTC 10/84 resulted in a significant decrease in adherence to and invasion of hBMECs. Additional mutants in other GBS serotypes commonly associated with meningitis showed a similar decrease in hBMEC invasion, compared with parental strains. Finally, in mice, wild-type GBS penetrated the blood-brain barrier and established meningitis more frequently than did the Δ*srr1* mutant strain.

***Conclusions.*** Our data suggest that GBS Srr glycoproteins play an important role in crossing the blood-brain barrier and in the development of streptococcal meningitis.

Group B *Streptococcus* (GBS), also called *Streptococcus agalactiae*, is the leading cause of bacterial meningitis in newborns [1]. Associated mortality remains high, despite antibiotic therapy, and 25%–50% of surviving infants experience permanent neurological sequelae, including cognitive deficits, cerebral palsy, blindness, deafness, and seizures [2]. To gain access to the central nervous system (CNS), GBS must cross the blood-brain barrier (BBB), which is primarily composed of a single layer of specialized brain microvascular endothelial cells (BMECs).

Penetration of the BBB by a bacterial pathogen is multifactorial and reflects a complex interplay between host endothelium and microbial products. Studies to better understand this interaction have become feasible through the availability of tissue culture models of human BMECs (hBMECs) [3, 4]. The hBMEC model system has been used to identify virulence genes in different bacterial species that promote cellular invasion [5–10]. Although GBS adheres to and invades hBMECs [11], the factors that contribute to the penetration of the BBB and the development of meningitis are still being elucidated. For GBS, only 2 specific virulence factors have been shown to contribute to the pathogenesis of meningitis in vivo: the pore-forming β-hemolysin/cytolysin (β-h/c) toxin [12] and the glycosyltransferase, *iagA*, which is required for proper anchoring of lipoteichoic acid to the cell wall [5]. However, neither of these proteins function directly to mediate GBS attachment to BBB endothelium, which is the critical first step in CNS invasion and disease progression.

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Members of the streptococcal and staphylococcal serine-rich repeat (Srr) protein family have been described to function as adhesins in different model systems [13–15], including endothelial cells [16]. Recently, it was shown that GBS strains carry 1 of 2 *srr* gene alleles, designated *srr1* [14] and *srr2* [17], which are similar in architecture but show only limited homology (<20% identity) [17]. Interestingly, expression of the Srr-2 protein seems to be restricted to serotype III-3 and multilocus sequence type (ST) 17 strains [17], which represent >90% of the serotype III invasive neonatal disease isolates. In addition to serotype III, serotypes Ia, Ib, and V are also commonly isolated from neonates, children, and adult patients with meningitis [18], suggesting that both Srr-1 and Srr-2 could be relevant for the pathogenesis of meningitis.

Because of the described role of Srr homologues in host interactions, we hypothesized that GBS Srr proteins could play a role in the interaction with brain endothelium. In this study, we identified and characterized Srr-1 in the serotype V clinical isolate NCTC 10/84. Using targeted mutagenesis, we showed that the NCTC 10/84 *srr1,* as well as other *srr* genes in GBS serotypes III, Ia, and Ib, contributes directly to hBMEC invasion in vitro. Finally, we provided evidence that Srr-1 promotes BBB penetration and the development of GBS meningitis in a mouse meningitis model.

## METHODS

***Bacterial strains and growth conditions.*** The following wild-type (WT) GBS clinical isolates were used in this study: NCTC 10/84 (1169-NT1; ATCC 49447) (serotype V) [19], COH1 (serotype III) [20], 515 (serotype Ia) [21], and H36B (serotype Ib) [22]. GBS was grown in Todd-Hewitt broth (Difco, BD Diagnostics) at 37°C. For antibiotic selection, 5 μg/mL erythromycin or 2 μg/mL chloramphenicol was used. *Streptococcus gordonii* strain M99 and the GspB mutant (PS846; M99 Δ*gspB*::pEVP3) have been described elsewhere [13, 23]. *S. gordonii* WT and GspB mutant strains were grown in Todd-Hewitt broth, with 5 μg/mL chloramphenicol added when appropriate, at 37°C.

***Cell lines.*** The hBMEC line was obtained from Kwang Sik Kim (Johns Hopkins University, Baltimore, Maryland) and maintained as described elsewhere [3, 4, 24]. A549 cells (a human type II alveolar epithelial carcinoma cell line) and HeLa cells (a human cervical carcinoma cell line) were maintained and passaged in RPMI 1640 tissue culture medium that contained 10% fetal bovine serum. Cultures were incubated at 37°C in a humid atmosphere of 5% carbon dioxide.

***Targeted mutagenesis and complementation vector construction.*** Using the Basic Local Alignment Search Tool (BLAST), polymerase chain reaction (PCR), and sequence analysis of GBS clinical isolate strain NCTC 10/84, we identified a putative cell wall–anchored protein as a member of the streptococcal and staphylococcal Srr protein family known as Srr-1 [17]. PCR was used to generate in-frame substitution of the *srr1* gene with the chloramphenicol acetyltransferase *(cat)* gene with use of a method described elsewhere [5]. In brief, 752-bp and 835-bp regions immediately upstream and downstream, respectively, of *srr1* were amplified with the primers *srr1*upF (5'-CCGCTCGAGGGCATCTTCCTGAGTAAGTT-3', and *srr1*upR+cat, 5'-GGTGGTATATCCAGTGATTTTTTTCTCCATGTTTCCTCCATATATAAA-TAT-3') and the primers *srr1*downF+cat (5'-TACTGCGATGAGTGGCAGGGCGGGGCGTAATAAACCTACTTTGAATCCTTA-3') and *srr1*downR (5'-GTACTGCAGGTAGGTAGAATAACAACATCCG-3'). The *srr1*upR+cat and *srr1*downF+cat primers were constructed with 25-bp 5' extensions corresponding to the 5' and 3' ends of the *cat* gene, respectively. PCR was used to generate a fragment containing an in-frame substitution of *srr1* with *cat,* which was subcloned to vector pHY304. Allelic exchange mutagenesis in NCTC 10/84 was performed as described elsewhere [25], to generate the mutant NCTC 10/84Δ*srr1*. Allelic replacement of *srr1* with *cat* in the NCTC 10/84Δ*srr1* chromosomal was confirmed by PCR and sequence analysis. For complementation analysis, *srr1* plus flanking DNA was PCR amplified from the NCTC 10/84 chromosome using *srr1*FLF (5'-CGTGGAATTCGTATCTACGTGCTTAACGG-3') and *srr1*FLR (5'-GCGGGATCCCAAAGTAGGTTTAGTCTTTATC-3') and cloned into expression vector pDCerm [26], yielding plasmid pSrr1.

Additional *srr* mutants were generated by plasmid insertional mutagenesis, as described elsewhere [25]. PCR was used to amplify a 590-bp fragment of the *srr2* gene using primers 5'- CGTGAAGCTTGCAGTTTGGAAACTTTGGTG-3' and 5'-TCAGCTGCAGGTTGAACTCTAGCGGTCGTTGC-3' containing *Hin*dIII and *Pst*I restriction sites, respectively. The PCR product was cloned into pTOPO and subcloned to pVE6007 [27]. The construct was transformed into electrocompetent COH1, and plasmid integration was confirmed by PCR analysis using primers homologous to plasmid sequences and to sequences upstream of the point of insertion. A similar strategy was used to generate *srr-1* mutants in GBS strains 515 and H36B.

***Western blot and lectin blot analyses of GBS Srr-1.*** To determine the cross-reactivity of GBS Srr-1 with antiserum raised against Srr-1 homologue GspB in *S. gordonii*, total bacterial lysates were prepared for Western blot analysis. Bacteria cultured overnight were washed and incubated for 3 h at 37°C in Tris buffer (pH 7.0) containing mutanolysin (1 U/μL), DNase I (0.1 U/μL), and complete protease inhibitor (Roche), before the addition of 3 × sample buffer. Samples were separated by SDS-PAGE performed with the use of 3%–8% Tris-acetate gels (Invitrogen) under reducing conditions and then were transferred to nitrocellulose membranes. Membranes were blocked using Tris buffer (pH 7.5) containing 4% bovine serum albumin (BSA; Roche). Next, membranes were incubated overnight with goat polyclonal anti-GspB serum (1:500) [28] in Tris-buffered saline Tween 1% BSA at 4°C, followed by detection with peroxidase-conjugated donkey antigoat IgG (1:25,000; Jackson Immunore-

search). Lectin blot analysis with the *N*-acetylglucosamine–specific lectin wheat germ agglutinin (WGA; Vector Labs) was performed as described elsewhere [29]. Western blots were developed with Supersignal West Pico chemiluminescent substrate (Pierce Biotechnology).

***Adherence and invasion assays.*** GBS adherence and invasion assays were performed as described elsewhere, with minor modifications [11]. In brief, confluent hBMEC monolayers were incubated with log-phase grown bacteria at an MOI of 1, unless indicated otherwise. Plates were centrifuged at 800 *g* to synchronize the infection and were incubated at 37°C with 5% carbon dioxide. After 2 h, the monolayers were washed, and 1 mL of RPMI 10% fetal bovine serum containing 100 $\mu$g of gentamicin and 5 $\mu$g of penicillin G was added for 2 additional hours. After washing, monolayers were disrupted, and the number of invasive bacteria was quantified by serial dilution plating. To assess the level of surface-adherent (total cell-associated) bacteria, bacteria were quantified from hBMEC monolayers prior to the addition of extracellular antibiotics after 30 min of incubation. Parallel invasion experiments were performed in A549 lung epithelial cells and HeLa epithelial cells. All cellular adherence and invasion assays were performed in triplicate and repeated at least 3 times.

***Mouse infection studies.*** All animal experiments were approved by the committee on the use and care of animals and performed according to accepted veterinary standards. A murine model of hematogenous GBS meningitis has been described elsewhere [12]. In brief, outbred 6- to 8-week-old male CD-1 mice (Charles River Laboratories; 10 mice per group) were injected via the tail vein with $7.5 \times 10^7$ cfu WT GBS or the Δ*srr1* mutant. Blood was collected by retro-orbital puncture at indicated times and was plated to determine the level of bacteremia. At the experimental end point (day 4 after injection), samples of blood, brain, and spleen were collected aseptically from mice after euthanasia. Bacterial counts in blood and tissue homogenates were determined by plating serial dilutions. Bacterial counts in brain and spleen samples were corrected for differences in organ weight.

***Statistical analysis.*** SPSS software (version 12.0.02 for Windows; SPSS) was used for statistical analysis. Student's 2-tailed *t* test was used to study differences in adherence and invasion in hBMECs between WT and mutant strains, as well as differences in the bacterial load in mice. Statistical significance was defined as $P < .05$.

## RESULTS

***Genetic characterization of highly-conserved Srr-1 in GBS strain NCTC 10/84.*** To characterize new GBS virulence factors that are critical for penetration of the BBB, we focused on

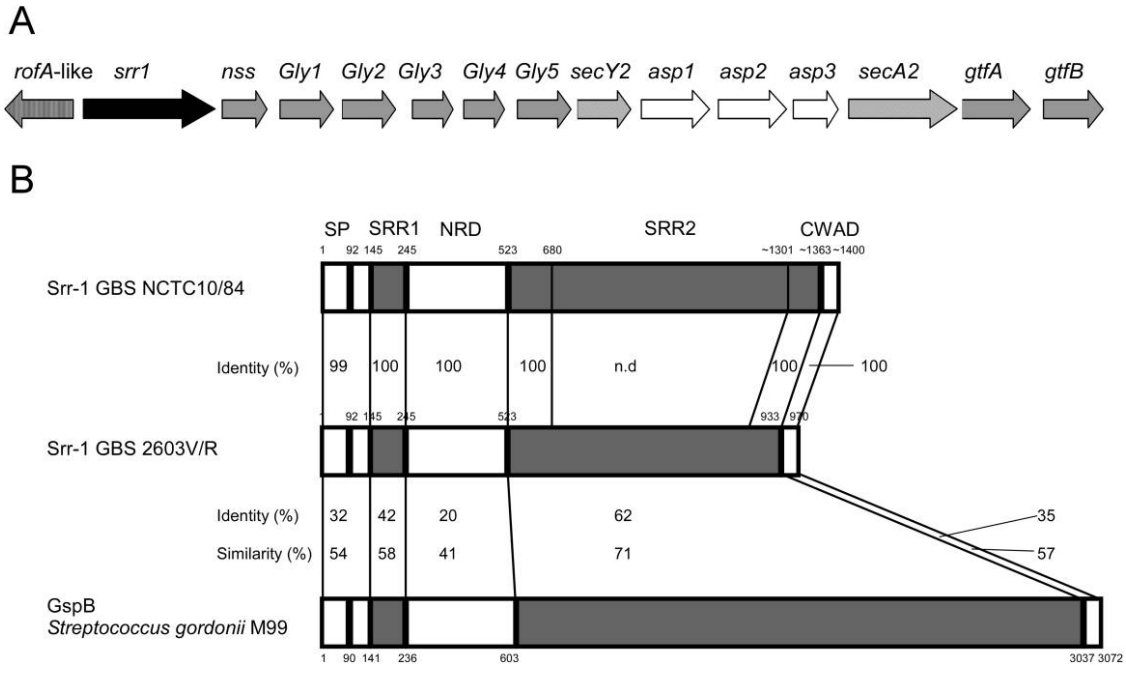


**Figure 1.** *A,* Schematic diagram of the group B *Streptococcus* (GBS) *srr1* locus. *RofA*-like is a putative transcriptional regulator, *srr1* is a putative cell surface–anchored serine-rich repeat (Srr) protein, *nss* is similar to nucleotide sugar synthetases, *asp* are accessory secretory proteins, *Gly* and *gtf* are putative glycosyltransferases, and *secA2/Y2* is a putative sec translocation system. *B,* Protein identity between Srr-1 in serotype V GBS strains NCTC 10/84 (deduced from a determined nucleotide sequence) and 2603V/R and between GBS Srr-1 and GspB homologue in *Streptococcus gordonii.* Alignment was performed using the COFFEE-T alignment program. CWAD, cell wall–anchoring domain; NRD, nonrepeat domain; SP, signal peptide; SRR1, serine-rich repeat domain 1; SRR2, serine-rich repeat domain 2.

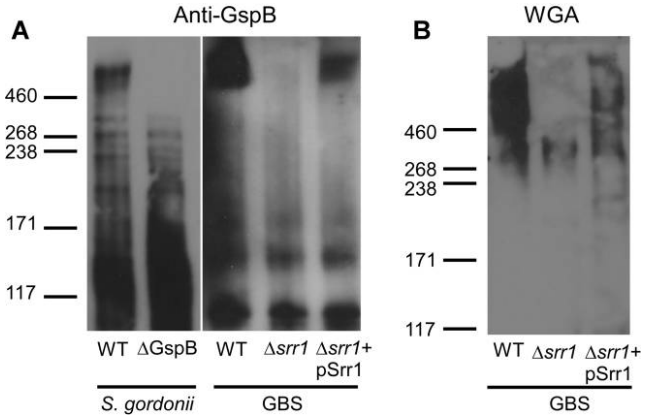


**Figure 2.** *A,* NCTC 10/84 serine-rich repeat 1 (Srr-1) is cross-reactive with GspB-specific antiserum (anti-GspB). NCTC 10/84 Srr-1 migrates as a >460-kD protein. Specificity was determined using Δ*srr1* mutant and Δ*srr1*+pSrr1 strains as controls for the protein band in NCTC 10/84 wild-type (WT) protein lysate, whereas *Streptococcus gordonii* WT and Δ*gspB* strains were used as positive and negative controls for staining with anti-GspB, respectively. *B,* Detection of carbohydrate modifications on NCTC 10/84 Srr-1 protein by use of the *N*-acetylglucosamine–specific lectin wheat germ agglutinin (WGA). Proteins extracts were separated by gel electrophoresis performed using 3%–8% Tris-acetate gradient gels. GBS, group B *Strepotoccus.*

GBS genes predicted to encode surface-anchored proteins. Through BLAST analysis of published GBS genome sequences, we identified a locus encoding a putative RofA-like transcriptional regulator protein, followed by a protein containing a LP(X)TG cell wall–anchoring motif, a SecY2/A2 secretion system, accessory secretion proteins, and multiple glycosyltransferases (figure 1*A*). This locus represents one of a group of "genomic islands" that are highly conserved and identical among different GBS strains [30]. Also, similar loci have been identified in other gram-positive streptococcal and staphylococcal species [31], including *S. gordonii* [28], *Streptococcus pneumoniae* [32], and *Staphylococcus aureus* [15]. PCR and sequence analyses confirmed the presence of this locus in serotype V GBS clinical isolate NCTC 10/84, which is a highly virulent strain in a mouse model of hematogenous meningitis [12].

The putative cell wall–anchored protein was identified as a member of the streptococcal and staphylococcal Srr protein family known as Srr-1 [14, 17]. Gene amplification showed that the NCTC 10/84 *srr1* gene, which is ~4300 bp, is larger than other *srr1* genes in sequenced GBS strains, which range from 2730 bp to 3981 bp. DNA sequence analysis of the NCTC 10/84 *srr1* gene revealed >99% sequence identity between the N- and C-terminal serine-rich regions (SRR1 and SRR2), compared with another serotype V GBS strain, 2603V/R (figure 1*B*). We found the same repeat pattern—SAS(T/M)—in the long SRR2 domain, which is present in the majority of sequenced GBS strains and is responsible for the observed size variation among GBS Srr-1 proteins [14]. In addition, protein alignment demonstrated a high sequence similarity between GBS Srr-1 and Srr homologues in other species, such as GspB in oral *S. gordonii* (figure 1*B*).

***GBS Srr-1 is a high-molecular-weight glycoprotein.*** To characterize Srr-1 in GBS strain NCTC 10/84, we analyzed bacterial cell lysates by Western blot analysis and lectin staining. With use of an antiserum reactive to the native form of *S. gordonii* GspB [28], we observed multiple cross-reactive proteins in the WT GBS NCTC 10/84 sample, including a protein with a molecular weight (>460 kD) similar to that of GspB (figure 2*A*). In contrast, cross-reactive species were not observed with antiserum raised against a recombinant (nonglycosylated) Srr1-family protein, SraP from *S. aureus* [15] (data not shown). Western blot analysis of the isogenic Δ*srr1* mutant revealed loss of the >460-kD glycoprotein band reactive to *S. gordonii* GspB antiserum (figure 2*A*); complementation of the Δ*srr1* mutant with the WT GBS *srr1* gene on a plasmid expression vector (pSrr1) restored expression of the glycoprotein (figure 2*A*).

Because of extensive glycosylation, proteins of the Srr family usually have a much higher molecular weight than that predicted by amino acid content [15, 28, 33]. Similarly, NCTC 10/84 Srr-1 was impressively larger than the expected size of ~140 kD. We performed lectin blot analysis using the *N*-acetylglucosamine–specific lectin WGA, which has been shown to bind *S. gordonii* GspB [34]. As shown in figure 2*B*, WGA reacted with the same high-molecular-weight protein in the WT GBS strain, which was not present in the Δ*srr1* mutant but was restored in the complemented strain. Taken together, these results demonstrate that GBS Srr-1 is posttranslationally modified by glycosylation.

***Srr-1 promotes hBMEC adherence and invasion.*** GBS Srr-1 and Srr-1 homologues function as adhesins in different cell model systems [14, 15, 28]. Therefore, we addressed the role of Srr-1 in the GBS-BBB interaction, using our well-characterized in vitro hBMEC model [11, 12]. WT GBS bacteria efficiently adhered to and invaded hBMECs, whereas the GBSΔ*srr1* mutant exhibited up to a 50% reduction in adherence and a 70% reduction in invasion (figures 3*A* and 3*B*). Growth kinetics and susceptibility to the antibiotics used in our invasion assays did not differ between the WT and Δ*srr1* mutant strains (data not shown). In addition, levels of WT invasion were restored by complementation of the GBS Δ*srr1* mutant with Srr-1 cloned on expression vector pSrr1 (figure 3*C*). We performed additional invasion assays using the human lung epithelial A549 cell line and the human cervical HeLa cell line. Invasion in these cells was significantly attenuated in the Δ*srr1* mutant (figure 3*D*), demonstrating that GBS Srr-1 contributes to penetration of different host cell barriers.

***Contribution of Srr-1 and Srr-2 in other GBS serotypes to hBMEC adherence and invasion.*** GBS strains contain either of 2 *srr* genes, *srr-1* or *srr-2*. To address the potential role of Srr proteins in other GBS serotypes associated with meningitis, we generated plasmid insertional mutations in *srr-2* in COH1 (se-

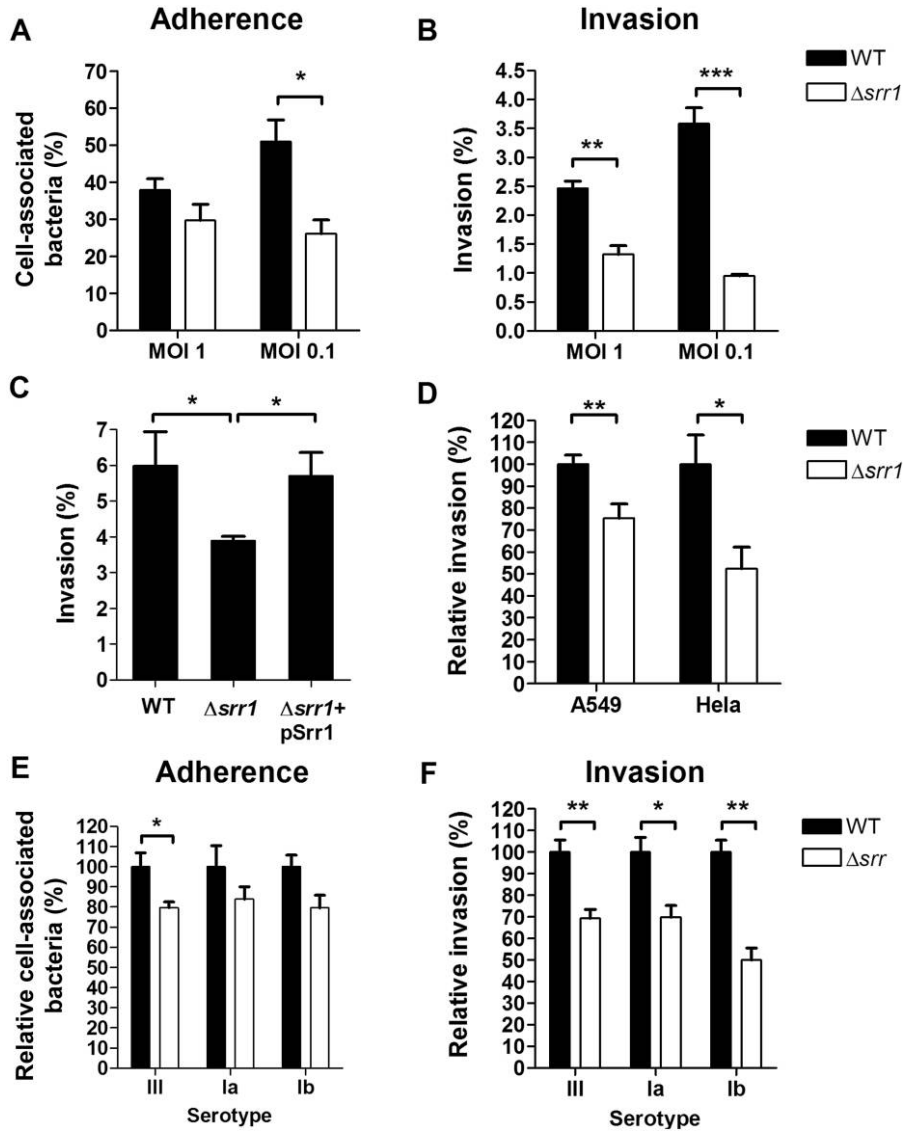


**Figure 3.** Contribution of serine-rich repeat 1 (Srr-1) to adherence *(A)* and invasion *(B)* of human brain microvascular endothelial cells (hBMECs). *C,* Attenuation of invasion is rescued by expressing Srr-1 on expression vector pSrr1 (MOI of 1). *D,* NCTC 10/84 Srr-1 contributes to invasion in A549 and HeLa epithelial cells (MOI of 1). Contribution of Srr proteins to adherence *(E)* and invasion *(F)* of hBMECs in different group B *Streptococcus* (GBS) serotypes. Serotype III expresses Srr-2, whereas serotypes Ia and Ib express the Srr-1 protein. For adherence, bacteria were enumerated after 30 min of incubation, whereas invasion was quantified after 2 h of incubation with hBMECs and 2 h of incubation with penicillin-gentamicin to kill extracellular bacteria. All experiments were repeated at least 3 times; data from a representative experiment are shown. The error bars denote 95% confidence intervals of the mean values for 3 wells. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .005$; WT, wild type.

rotype III-3) and *srr-1* in 515 (serotype Ia) and H36B (serotype Ib). All of these isogenic mutants exhibited decreased hBMEC invasion, compared with the parental strain (figure 3*E* and 3*F*), demonstrating that Srr proteins generally contribute to interaction with brain endothelium.

***Lack of contribution of GBS Srr-1 to survival in whole blood.*** Several studies have reported an association between the levels of bacteremia and the development of meningitis [35–37]. To reach this critical bacterial threshold, bacteria must subvert host defense mechanisms to proliferate and survive in the bloodstream [38]. We therefore tested whether Srr-1 affected bacterial survival in human blood. WT GBS or Δ*srr1* bacteria were incubated with human blood samples obtained from a healthy donor and were sampled for surviving bacteria at multiple time points. Both the WT GBS and Δ*srr1* strains were killed at a similar rate, indicating that Srr-1 does not contribute to GBS survival in whole human blood (data not shown).

***Role of Srr-1 in BBB penetration in a murine model of GBS meningitis.*** To test whether our in vitro phenotype translated into a diminished ability to breach the BBB in vivo, we used a murine model of hematogenous GBS meningitis [12]. Groups of CD-1 mice (10 mice per group) were infected intravenously with

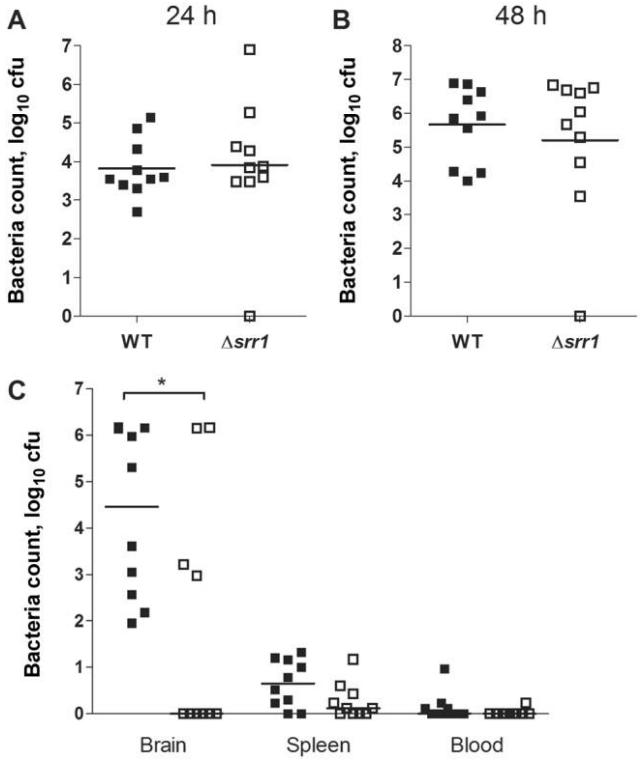


**Figure 4.** Deletion of *srr1* does not affect survival of group B *Streptococcus* (GBS) in mouse blood at 24 h *(A)* or 48 h *(B)* after infection, compared with survival of wild-type (WT) bacteria. *C,* Bacterial counts (expressed in colony-forming units) in the brain, spleen, and blood of mice infected with NCTC 10/84 WT or Δ*srr1* mutant bacteria, determined on on day 4 after infection. The horizontal lines denote the median number of bacteria in each group of 10 mice. Each square denotes 1 mouse. Filled squares denote mice infected with WT bacteria, and open squares denote mice infected with Δ*srr1* bacteria. $^*P < .05$.

WT GBS or the Δ*srr1* mutant strain. Similar to observations made with bactericidal assays in human whole blood, mice developed similar levels of bacteremia at 24 h and 48 h after infection (figures 4*A* and 4*B*). However, mice infected with the WT GBS strain had significantly higher bacterial counts in the brain than did mice infected with the Δ*srr1* mutant strain at the experimental end point (figure 4*C*). Few bacteria were recovered from blood or spleen samples at the experimental end point (figure 4*C*), indicating that the bacteria recovered from the brain were not attributable to blood present in the microvasculature of the brain.

To confirm the development of meningitis in these GBS-infected mice, the brains were examined by microscopy. Representative findings of histopathological examination of brains from infected mice are shown in figure 5. In mice with a high bacterial load in the brain, histopathological examination revealed clear evidence of GBS meningitis, with associated neutrophil infiltration in the meninges and CNS (figures 5*B* and 5*C*) accompanied by tissue destruction (figures 5*D* and 5*E*). Mice without bacterial penetration did not show signs of inflammation (figure 5*A*), although occasionally hemorrhage was observed. Histopathological findings did not differ between mice that developed meningitis as a consequence of WT GBS and those that developed meningitis as a consequence of Δ*srr1* bacteria. These data demonstrate that Srr-1 significantly contributes to the penetration of the BBB and the development of GBS meningitis in vivo.

## DISCUSSION

Our studies indicate that the surface-anchored GBS Srr glycoproteins contribute to hBMEC invasion. Furthermore, Srr-1 represents the first invasin known to directly promote GBS BBB penetration in vivo. Additional in vitro studies have described a role for GBS surface-expressed proteins in promoting hBMEC interactions, including PilB (which is the major backbone protein for pilus formation) [6], fibrinogen-binding protein FbsA [39], and laminin-binding protein Lmb [9]; however, none of these proteins have been examined for their potential contribution to GBS meningitis in vivo.

Srr family proteins were first characterized in oral streptococci as serine-rich (>35%) high-molecular-mass glycosylated proteins that are transported across the membrane by a dedicated SecA2/Y2 secretion system [28, 40, 41]. In GBS, 2 types of Srr proteins, known as Srr-1 and Srr-2, have been identified [17]. Expression of Srr-1 seems to be widespread among GBS isolates [14], whereas expression of Srr-2 seems to be restricted to serotype III-3 and the ST-17 strain [17]. Srr-1 is surface exposed [14] and highly conserved (>85% nucleotide identity and amino acid identity) among published genomes of GBS strains belonging to different serotypes. In contrast, there is <20% sequence identity with Srr-2. GBS Srr-1 proteins contain ~30% serines, showing 48% identity and 60% similarity to *S. gordonii* GspB. Comparison of protein profiles of WT GBS and Δ*srr1* mutant bacteria demonstrated that Srr-1 runs as a protein of >460 kD and reacts with the glycan-specific lectin WGA, which confirms the posttranslational modification of the protein. In addition, GBS Srr-1 cross-reacts with GspB-specific antiserum, which is known to recognize the carbohydrate moieties on GspB (data not shown). This suggests that the posttranslational modification of Srr-1 may share similarities with the glycosylation of GspB. Although glycosylation was a suggested modification of Srr-2 [17], this is, to our knowledge, the first experimental proof of Srr-1 glycosylation in GBS. The pathway responsible for this extensive glycosylation of GBS Srr-1 is currently unknown, but it likely involves several genes encoding putative glycosyltransferases in the *srr1* locus.

GBS Srr homologues have been described as functioning as adhesins in different model systems. GspB in *S. gordonii* mediates binding to platelets interacting with sialylated moieties of the platelet glycoprotein Ibα [42], and Fap1 in *Streptococcus parasanguis* is required for expression of long fimbriae mediat-

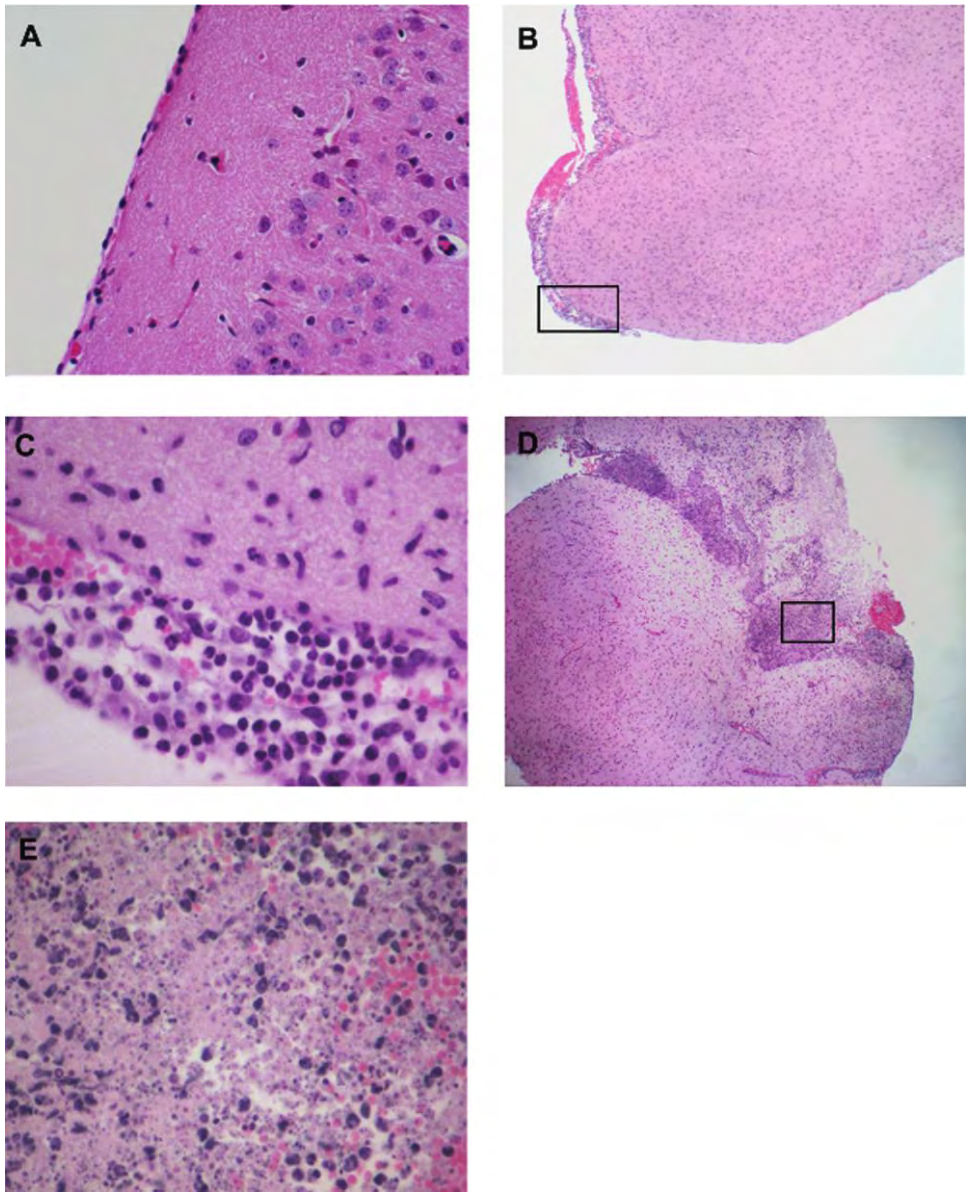


**Figure 5.** Hematoxylin and eosin stains of brain tissue from group B *Streptococcus* (GBS)–infected mice, including a sample from a mouse infected with the Δ*srr1* mutant, which showed normal brain histopathological characteristics *(A)* (original magnification, ×10), and samples from mice infected with wild-type GBS, which showed meningeal thickening *(B)* (original magnification, ×4), neutrophil infiltration *(C)* (original magnification, ×4), and tissue destruction *(D* and *E)* (original magnification, ×400). *C* and *E* are inserts of *B* and *D*, respectively.

ing bacterial attachment to saliva-coated hydroxyapatite [33, 43]. In the present study, we demonstrated a role for Srr glycoproteins in different GBS serotypes in the invasion of hMBECs and a role for Srr-1 in the pathogenesis of meningitis. This mechanism could also be relevant for other meningeal pathogens, such as *S. pneumoniae*, which is the leading cause of meningitis after the neonatal period and in the elderly population, because *S. pneumoniae* encodes a GBS Srr protein homologue, PsrP [44]. Interestingly, PsrP resides in 1 of 2 *S. pneumoniae* pathogenicity islands whose presence appears to correlate with an invasive phenotype [32]. Although a recent study did not show a role for PsrP in adherence to hBMECs, the strains were not tested for differences in their capacity for invasion [45]. Additional studies of the role of PsrP in the interaction of *S. pneumoniae* with the BBB are required but may reveal a common pathway for the pathogenesis of streptococcal meningitis in humans.

Invasion genes often mediate entry and passage through a number of host cellular barriers, and evidence indicates that adherence to and invasion of other cell types, such as chorion epithelial cells [46] and lung epithelial cells [47], are likely to be important for earlier stages of GBS neonatal disease pathogenesis. Deletion of GBS *iagA*, for example, not only affects invasion of hBMECs but also results in attenuated invasion of lung and chorion epithelial cells [5]. Similarly, we found that deletion of

Srr-1 attenuates GBS invasion in multiple cell types, including both endothelial and epithelial cell lines. Samen et al. [14] have also recently reported that Srr-1 in another GBS clinical isolate strain contributes to adherence to Hep-2 cells. It will be of interest to determine the role of carbohydrate modification in the ability of Srr-1 to mediate host cell adherence and invasion.

The human BBB endothelium serves as a critical barrier to protect the CNS against microbial invasion, and penetration of the BBB is likely to be a primary and essential step in the pathogenesis of neonatal meningitis [38]. Although it is conceivable that the neonatal BBB displays unique features that render it more susceptible to penetration by neonatal pathogens, such as GBS, previous studies that have compared this hBMEC cell line with hBMECs derived from humans and BMECs derived from rats at different ages (ranging from fetal to geriatric rats) have demonstrated no differences in susceptibility to bacterial infection and invasion [48]. These results corroborate the usefulness of this in vitro BBB model in the study of host-pathogen interactions—specifically, the identification and characterization of GBS genes and gene products that are responsible for the interaction of GBS with brain endothelium. This report represents, to our knowledge, the first identification of a GBS surface-expressed protein that directly interacts with the BBB to promote CNS invasion and the development of GBS meningitis. Our data also suggest that Srr proteins may represent a target for pharmacologic or vaccine strategies to prevent the development of meningitis in the vulnerable neonate.

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