

Progress toward a group B streptococcal vaccine

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ABSTRACT

Streptococcus agalactiae (group B *Streptococcus*, GBS) is a leading cause of severe invasive disease in neonate, elderly, and immunocompromised patients worldwide. Despite recent advances in the diagnosis and intrapartum antibiotic prophylaxis (IAP) of GBS infections, it remains one of the most common causes of neonatal morbidity and mortality, causing serious infections. Furthermore, recent studies reported an increasing number of GBS infections in pregnant women and elderly. Although IAP is effective, it has several limitations, including increasing antimicrobial resistance and late GBS infection after negative antenatal screening. Maternal immunization is the most promising and effective countermeasure against GBS infection in neonates. However, no vaccine is available to date, but two types of vaccines, protein subunit and capsular polysaccharide conjugate vaccines, were investigated in clinical trials. Here, we provide an overview of the GBS vaccine development status and recent advances in the development of immunoassays to evaluate the GBS vaccine clinical efficacy.

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Introduction



Group B streptococcus (*Streptococcus agalactiae*; GBS) is an opportunistic gram-positive pathogen and one of the most common causes of life-threatening bacterial infections worldwide. In the human neonates, GBS infection commonly results in the development of pneumonia, sepsis, and meningitis.^{1,2} Despite considerable advances in the diagnosis, prevention, and treatment of neonatal GBS infections, it remains an important public concern globally. Additionally, an increasing number of GBS infections in pregnant women and non-pregnant adults typically with underlying medical conditions, has been reported. In the first meeting of the Product Development for Vaccines Advisory Committee (PDVAC) convened by the World Health Organization (WHO) in 2014, GBS was identified as an important pathogen leading to a large burden of disease worldwide and a high priority for the development of a vaccine.² Although vaccination is the most promising strategy for the prevention of GBS infection, currently no licensed GBS vaccine is available in the market. Here, we reviewed and described the studies investigating this pathogen and potential future directions of GBS vaccine development and assay methods for the evaluation of the clinical efficacy of GBS vaccines.

Disease burden and clinical spectrum of GBS infections

GBS has been identified as a major cause of invasive infections during the first three months of life since the 1970s. The incidence of invasive GBS infections varies geographically, ranging

from 0.02 per 1,000 live births in Southeast Asia to 1.21 per 1,000 live births in Africa.³ GBS infection cases in the neonates and infants can be divided into two categories: an early-onset disease (EOD), which occurs within 0–6 days after birth, and a late-onset disease (LOD), which occurs within 7–90 days after birth. GBS is a common colonizer of digestive and female genital tract in approximately one-third of human population. Maternal colonization and subsequent neonatal acquisition of GBS is an established risk factor for GBS sepsis during the early periods of life.⁴ Therefore, intrapartum antibiotic prophylaxis (IAP) strategy to reduce the neonatal acquisition of GBS has been applied, and the incidence of EOD declined from 1.8 cases per 1,000 live births in 1990 to 0.23 cases per 1,000 live births in 2015. However, IAP has had no impact on the incidence of LOD and only a limited impact on disease development in pregnant women. The incidence of LOD in the United States has remained stable since 1990 at approximately 0.3 to 0.4 per 1,000 live births.^{5–9} Moreover, approximately 60–80% of LOD occurred in infants whose mothers had negative results in the GBS screening at 35–37 weeks' gestation.^{10,11} This may be due to the recolonization of GBS shortly before the delivery in these individuals, and their infants may acquire GBS from breast milk or diverse community/nosocomial sources after birth.¹²

In neonates and infants younger than 3 months, GBS causes invasive bacterial diseases including sepsis, meningitis, and pneumonia (Table 1). An unexpected, rapidly progressing sepsis is the dominant manifestation of a GBS infection (80–85%) in EOD, while both primary bacteremia (65%) and meningitis (25–30%) are common in LOD.^{8,13–15} Localized GBS infections,

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Table 1. Disease spectrum and serotype distribution of group B streptococcal (GBS) infection according to the age of the patient.

	Neonates and infants			
	Early-onset disease	Late-onset disease	Pregnant women	Non-pregnant adults (old adults)
Common manifestations ^{5,12,13,16-18}	Sepsis with unknown source (80-85%) Pneumonia (10%) Meningitis (7%)	Primary bacteremia (65%) Meningitis (25-30%) Bone and joint infection (5%) Cellulitis and/or adenitis (4%)	Genitourinary tract infection (50%) Chorioamnionitis (4%) Endometritis (8%) Primary bacteremia (31%) Pneumonia (2%) Puerperal sepsis (2%) Genital GBS colonization	Primary bacteremia (24%) Skin and soft tissue infection (20%) Respiratory tract infection (12%) Urinary tract infection (10%) Bone and joint infection (8%) Intra-abdominal infection (5%) Endocarditis (4%) Central nervous system infection (4%) Old age (≥ 65 years), diabetes mellitus, liver cirrhosis, stroke, cancer, neurogenic bladder, decubitus ulcer
Risk factors ¹¹⁻¹³	Maternal obstetric complications* Maternal genital GBS colonization GBS bacteriuria	Prematurity	Not available	
Case-fatality rate ^{11,16}	5-10%	2-6%	Not available	3.7% (up to 15% in old adults)
Serotype distribution ^{11,20}	Ia (27.7%) Ib (7.3%) II (4.2%) III (46.2%) IV (1.5%) V (12.9%)	Ia (11.3%) Ib (5.4%) II (0.7%) III (76.0%) IV (0%) V (6.6%)	Infection Ia (32.7%) Ib (4.0%) II (14.9%) III (28.7%) IV (0%) V (19.8%) Others (0.2%)	Colonization Ia/Ib (36.0%) II (14.5%) III (24.0%) IV (1.2%) V (20.2%) Others (4.1%)

*Premature rupture of membrane, preterm delivery, prolonged rupture of membrane

such as skin and soft tissue infections, bone and joint infections, and urinary tract infections, occasionally occur in LOD cases as well.¹³ GBS is vertically transmitted to neonates during labor and delivery, so maternal GBS carriage is an important risk factor for EOD, particularly with obstetric complications, such as preterm rupture of membrane, preterm delivery, and prolonged rupture of membrane. GBS carriage rates in pregnant women vary geographically between 6.5% and 43.6%, with approximately 20-30% of pregnant women carrying GBS in the developed countries.^{12,16,17} In about 50% of the cases, the infection is transmitted to their babies, leading to invasive diseases in 1% of neonatal carriers.¹⁸

Although GBS infection has been primarily recognized as a pediatric disease, it has also emerged as an important pathogen colonizing pregnant and non-pregnant adults, particularly older ones or adults with underlying medical conditions. A two-to-four-fold increase in the incidence of invasive GBS diseases in adults has been observed over the past two decades, reaching 25.4 cases per 100,000 adults.¹⁹ Approximately 5% of adults with these disease experienced relapse with an average of 13-week intervals.²⁰ Moreover, more than 50% of fatal GBS infections occur in elderly people.¹⁹ Common clinical manifestations of adult GBS diseases includes skin and soft tissue infections, primary bacteremia, bone and joint infections, pneumonia, etc. (Table 1).²⁰ Contrary to the pregnancy-related cases, which occur in the otherwise healthy women, most of the non-pregnant adult patients with GBS infections have underlying medical conditions.^{19,20} Old age (≥ 65 years), diabetes mellitus, liver cirrhosis, stroke, and cancer are considered the common risk factors increasing the invasiveness of GBS infections among non-pregnant adults.^{19,20} GBS colonization rate at the genital and gastrointestinal tract ranges from 20% to 35% in adults irrespective of age.²⁰ However, in bed-ridden elderly people, GBS has been shown to colonize the dental plaque and pharynx as well,²⁰ increasing the probability of GBS pneumonia development in these patients.

Serotype distribution and antimicrobial resistance

Capsular polysaccharide (CPS) is an important virulence factor of encapsulated bacteria, including GBS, and has been related to the bacterial disease clinical manifestations and invasiveness.²¹ Among 10 distinct serotypes, more than 90% of EOD are caused by serotypes Ia, Ib, II, III, and V, while LODs are caused predominantly by serotype III (Table 1).¹⁷ Similar to the neonates and infants, maternal carriage has been associated with the GBS disease development in the pregnant women, with five-fold increase in the risk of disease development, including genitourinary tract infection, chorioamnionitis, and endometritis, compared with that in the non-pregnant women.¹⁸ Pregnancy-associated GBS infections may lead to poor pregnancy outcomes, including spontaneous abortion, stillbirth, and preterm birth. Serotype distribution of clinical and colonized isolates was shown to be well correlated in pregnant women, and they were determined to be similar to those leading to the development of EOD (Table 1).^{17,18} In adults, serotype V (27.5%) was shown to be a predominant serotype, followed by Ia (24.3%) and III (16.5%).¹⁹

Similar to pneumococci, some GBS capsular serotypes commonly colonize the gastrointestinal/genital tract, but rarely cause the development of invasive diseases. Other serotypes, however, are more likely to cause invasive diseases with each episode of colonization.²²⁻²⁶ Previously, the invasive disease-causing potential of each GBS capsular serotype was investigated, based on the invasive odds ratio (OR) (Table 2).²²⁻²⁶ Invasive OR is calculated by referring to all other serotypes as follows: $OR = (ad)/(bc)$, where a is the number of invasive A serotypes, b is the number of carriage A serotypes, c is the number of invasive non-A serotypes, and d is the number of carriage non-A serotypes.²¹ An OR of 1 indicates that the serotype is equally likely to cause invasive disease or be recovered from carriage, an $OR > 1$ indicates an increased probability for a

Table 2. Serotype (ST)-specific invasive diseases potential of group B streptococci.^{14,22–25}

ST	Odds ratio (95% confidence interval)				
	Madzivhandila et al.	Berg et al.	Martins et al.	Bisharat et al.	Ip et al.
Ia	0.8 (0.6-1.0)	0.9 (0.5-1.9)	2.1 (1.1-4.0)	1.9 (0.8-4.8)	0.6 (0.4-0.8)
Ib	0.9 (0.5-1.5)	1.7 (0.9-3.4)	0.6 (0.1-2.3)	0.5 (0.1-2.1)	1.0 (0.7-1.3)
II	0.5 (0.2-0.8)	0.9 (0.4-2.0)	0.7 (0.3-1.4)	0.3 (0.1-0.9)	0.9 (0.6-1.3)
III	4.2 (3.5-5.0)	2.0 (1.3-3.0)	2.4 (1.4-4.3)	1.8 (0.9-3.8)	III-1: 1.1 (0.8-1.5) III-2: 1.5 (1.1-2.1) III-3: 1.1 (0.4-2.9) III-4: 19.4 (9.1-41.2)
IV	0.5 (0.2-1.2)	0.8 (0.2-3.9)	1.4 (0.2-6.7)	—	0.1 (0.0-2.2)
V	0.4 (0.2-0.7)	0.6 (0.3-1.1)	0.4 (0.2-0.9)	1.0 (0.4-2.5)	0.6 (0.5-0.9)

serotype to cause invasive disease, and an OR<1 indicates a reduced probability for a serotype to cause invasive disease. These studies demonstrated that serotype III is the predominant invasive serotype with high invasive OR (1.8-4.2).^{22–26} In a study conducted in Hong Kong, the virulence of serotype III was further assessed at subtype level, and serotype III-subtype 4 GBS was shown to be more highly invasive compared with other subtypes (invasive OR, 19.4).²⁶

As recommended by the United States Centers for Disease Control and Prevention (CDC) guidelines for the IAP for the prevention of neonatal GBS infections, penicillin or ampicillin is administered to the pregnant women before delivery.²⁷ For the patients allergic to β -lactam agents, either erythromycin or clindamycin can be used as an alternative agent. However, an increasing rate of resistance to erythromycin and clindamycin has been observed among clinical GBS isolates (Table 3).^{28–33} High resistance rate against macrolide antibiotics was reported in South Korea (51.8%) and China (74.1%).^{28,29} In South Korea, serotype V was predominantly shown to have a high macrolide resistance rate, reflecting clonal spread with the selective advantage of antimicrobial resistance.²⁸ However, in China, more than 70% of GBS were resistant to macrolide antibiotics, irrespective of serotype.²⁹ In South Korea, 42.9% of the GBS isolates were shown to carry *ermB*, while in China, 52.3% of the isolates carried this gene, which provided a high level of resistance to both erythromycin and clindamycin.^{28,29}

Virulence factors for a GBS vaccine

GBS expresses numerous virulence factors that are involved in its colonization, adherence, invasion, and immune evasion,^{34–36}

and these may be used as potential vaccine candidates. Biochemical and molecular analyses of these factors can provide a better understanding of the infectious process, further assisting the development of new diagnostic techniques, specific antimicrobial compounds, and effective vaccines.

Capsular Polysaccharide (CPS)

GBS expresses a unique CPS that is the most well-studied virulence factor contributing to the evasion of host immune defense mechanisms by protecting the bacteria from opsonophagocytosis by immune cells.^{12,37,38} CPS can also increase the invasiveness of GBS by enhancing biofilm formation, inhibiting the binding of antimicrobial peptides and neutrophil extracellular traps (NET), and affecting bacterial adherence to the epithelium and mucus.^{39–41} Moreover, a correlation between the presence of CPS-specific antibodies in serum and the increased risk of GBS EOD and LOD development was reported,^{42–44} and CPS is considered the best target for the development of GBS vaccine.

Structure and biosynthesis

GBS expresses at least 10 structurally and antigenically different types of CPS (Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX) (Fig. 1).^{45,46} All identified CPSs of GBS are high-molecular weight polymers with the short side-chain capped terminally with a sialic acid (N-acetylneuraminic acid) residue. Pneumococcal type 14 polysaccharide (Pn14) is structurally related to GBS type III polysaccharide (GBS-III), except for the presence of a terminal sialic acid residue in the side chain.^{47–50} However, immunoglobulin G (IgG), induced by the presence of Pn14,

Table 3. Resistance rates (%) to macrolide antibiotics in group B streptococcus isolates.

	Yoon et al. ²⁷	Lu et al. ²⁸	Morozumi et al. ²⁹	Von Both et al. ³⁰	Lin et al. ³¹	Dutra et al. ³²
Country	South Korea	China	Japan	Germany	US	Brazil
No. of isolates	56	193	443	146	346	185
Overall	51.8%	74.1%	19.2%	19.9%	20.2%	9.7%
Serotype Ia	12.5%	69.4%	13.2%	7.7%	16.3%	5.0%
Serotype Ib	50.0%	76.7%	14.3%	63.6%	8.1%	2.4%
Serotype II	—	100%	18.2%	18.2%	8.7%	4.8%
Serotype III	40.0%	78.4%	49.2%	16.3%	26.0%	10.3%
Serotype IV	—	—	—	0%	—	—
Serotype V	93.8%	74.1%	22.5%	52.6%	34.9%	1.7%
Serotype VI	0%	33.3%	4.8%	—	—	—
<i>ermB</i>	42.9%	52.3%	11.7%	8.9%	—	2.7%
<i>ermA</i>	5.4%	10.9%	5.4%	5.5%	—	3.8%
<i>mefA</i>	1.8%	32.1%	2.0%	5.5%	—	0%

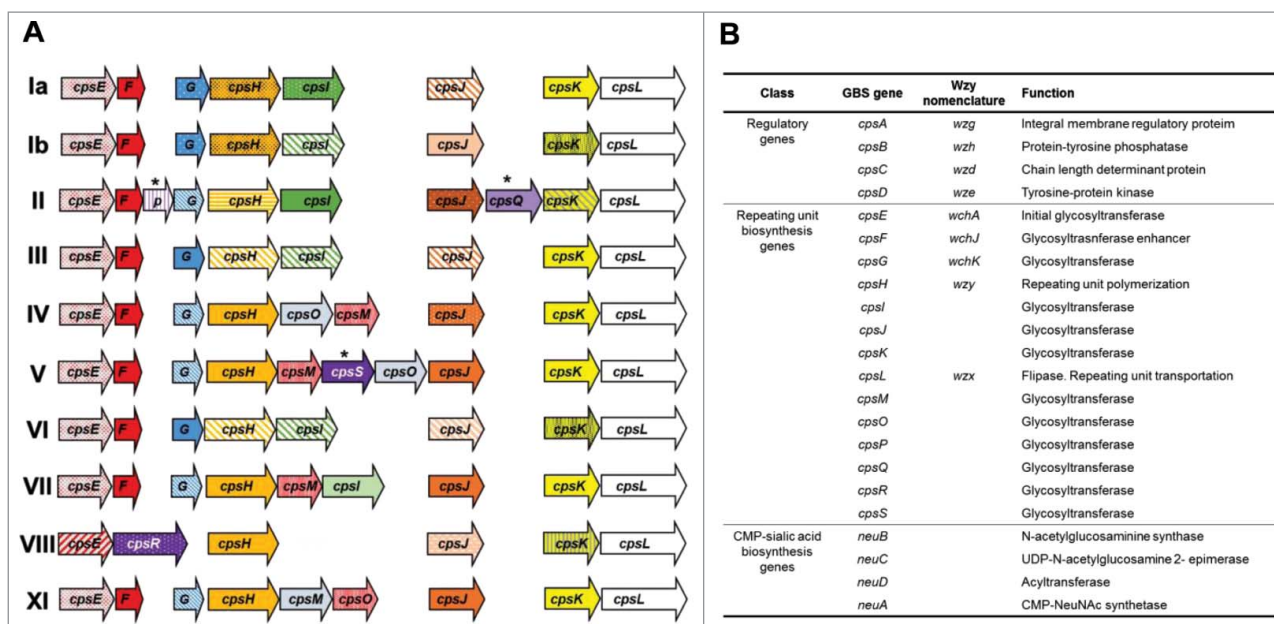


Figure 1. Genetic organization of the *cps* locus in *Streptococcus agalactiae*. (A) Comparative *cps* gene organization in nine serotypes: Ia (ABO28896.2), Ib (AAJS01000021.1), II (ALQD01000015.1), III (AF163833.1), IV (AF355776.1), V (AF349539.1), VI (AF337958.1), VII (LT671990.1), VIII (ALST01000010.1), and IX (LT671992.1). Gene designations are indicated on each arrow. Similarity between the genes is indicated by the same or similar colors. Gene names are the same as those used in a previous study,⁴⁵ except for *cpsP*, *cpsS*, and *cpsQ*. (B) Predicted CPS functions based on the results of previous studies and sequence comparisons.

poorly cross-reacts with GBS-III polysaccharide, suggesting that the sialic acid-dependent functional epitope may provide the protective immunity.^{47,51} Moreover, sialylated CPS of GBS is recognized as a critical structural moiety for the attachment to human brain microvascular endothelium and evasion of human immune system.^{52,53}

Based on the genetic structure of the GBS-CPS synthesis loci, the genes involved in CPS synthesis are located at the same chromosomal locus (*cps*) and are generally synthesized through the Wzx/Wzy-dependent pathway, similar to the pneumococcal CPS and *Salmonella* O-antigen synthesis (Fig. 2, Fig. 3A, and B).^{37,54} The locus contains the conserved genes (*cpsA*-*D*), whose products are involved in the regulation of capsule synthesis and the determination of the length of the repeating unit.^{37,55,56} *CpsA* is a membrane anchoring protein and functions as a key regulator of *CpsD* phosphorylation.⁵⁶ *CpsB*, -C, and -D compose a phosphoregulatory system, where the *CpsD* autokinase phosphorylates its C-terminal tyrosine residues in a *CpsC*-dependent manner.⁵⁵ Capsule synthesis is initiated by the transfer of monosaccharide phosphate to a membrane-associated undecaprenyl-phosphate by *CpsE*,⁵⁷⁻⁶⁰ and the additional sugars are sequentially added to form a repeat unit through the activity of different transferases, such as *CpsF*-*G*, *CpsI*-*K*, and *CpsM*-*S*.^{37,60} The repeat unit is transferred to the outside of the cytoplasmic membrane by flippase (*CpsL*) and polymerized to form the mature CPS by polymerase (*CpsH*). High molecular weight CPS is covalently linked to GlcNAc C-6 of peptidoglycan, but the genetic and enzymatic mechanisms involved in polysaccharide-PGN ligation remain unclear.⁶¹

CPS vaccines

Around 1920, Avery and Heidelberger performed a series of studies establishing the bacterial capsule as a critical virulence

factor of the encapsulated bacteria.⁶²⁻⁶⁹ Clinical trials investigating a multivalent polysaccharide vaccine demonstrated its high efficacy in humans against pneumococcal disease. Hexavalent, 14-valent, and 23-valent pneumococcal polysaccharide vaccines (PPVs) were licensed in 1947, 1977, and 1983, respectively, for the vaccination of adults and children.⁷⁰ GBS-CPS vaccine underwent clinical trials in healthy adults including pregnant women in the 1980s.⁷¹⁻⁷⁵ Although tetravalent GBS polysaccharide vaccine with serotypes Ia, Ib, and III was shown to be well-tolerated, the proportion of subjects with more than four-fold increase in the serotype-specific Ig titers compared with those in the unvaccinated group was only 33% for serotype Ia, 0% for serotype Ib, 17% for serotype II, and 70% for serotype III.⁷³ During the PDVAC meeting in 2014, it was concluded that the native CPS vaccine was ineffective due to its poor immunogenicity.⁷⁶

Around 1980, CPS coupling to protein carriers, which transforms T-cell-independent CPS antigens to T-cell-dependent antigenic vaccines that can elicit a larger IgG response than the native CPS vaccine, was developed by Robbins and Schneerson.^{77,78} Monovalent or multivalent GBS CPS-protein conjugated vaccines (GBS-PCV) were designed and evaluated in clinical trials.⁷⁷⁻⁹³ In healthy adults, trivalent conjugate vaccines with serotypes Ia, Ib, and III were well tolerated, significantly more immunogenic than the uncoupled CPSs, and induced predominantly the generation of the IgG antibodies, which promote opsonophagocytic killing and are effectively transferred across the placenta.^{81,94} Currently, two large companies are undertaking efforts to develop GBS conjugate vaccines. GlaxoSmithKline (GSK) has conducted phase I/II trials with a trivalent CPS-CRM197 GBS conjugate vaccine (serotypes Ia, Ib, and III), and this company plans to conduct a clinical trial of the pentavalent GBS vaccine with serotypes Ia, Ib, II, III, and V.¹ Pfizer has also announced a phase 1/2 trial of

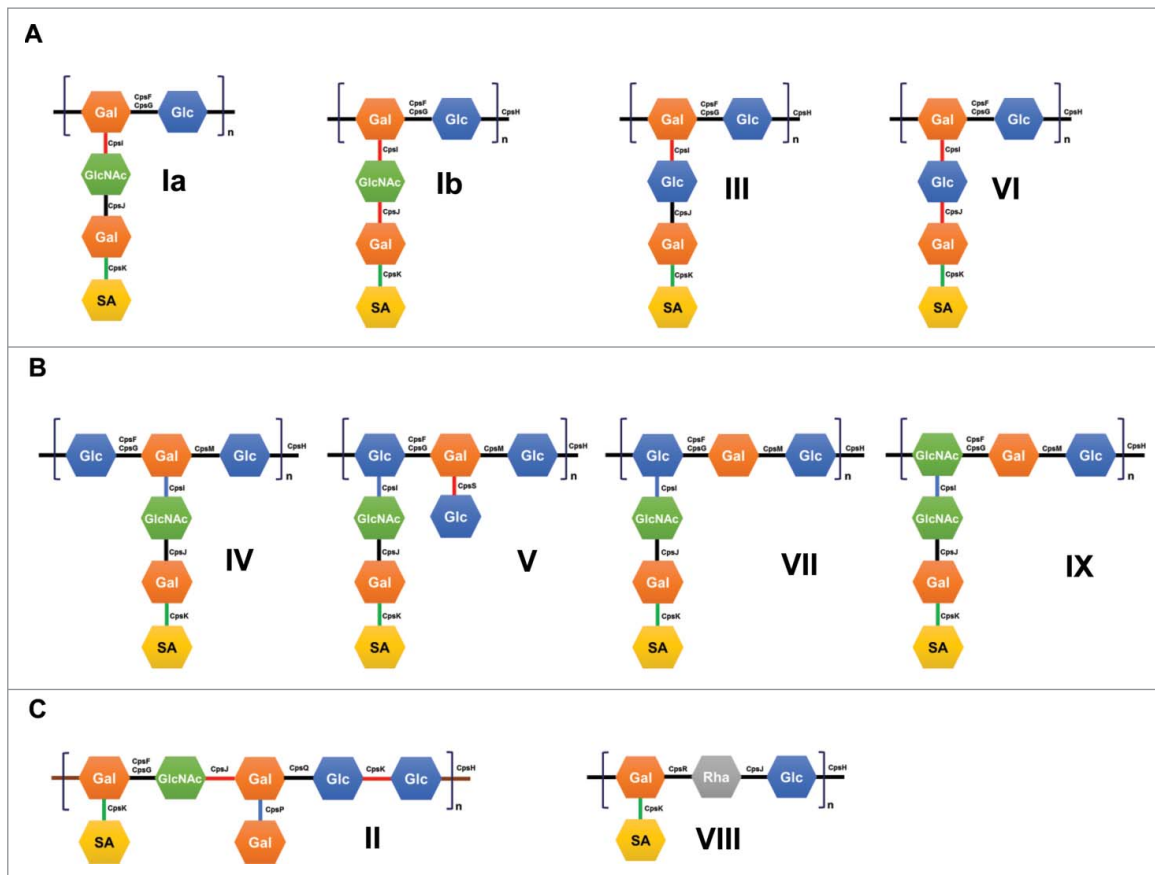


Figure 2. Biochemical capsular polysaccharide (CPS) structure of *Streptococcus agalactiae*. Association of the encoded sugar transferases and polymerases (cpsH) with each corresponding CPS structure. Links between two sugars are represented as black lines ($\beta 1 \rightarrow 4$), red lines ($\beta 1 \rightarrow 4$), blue lines ($\beta 1 \rightarrow 6$), and green lines ($\alpha 2 \rightarrow 3$).

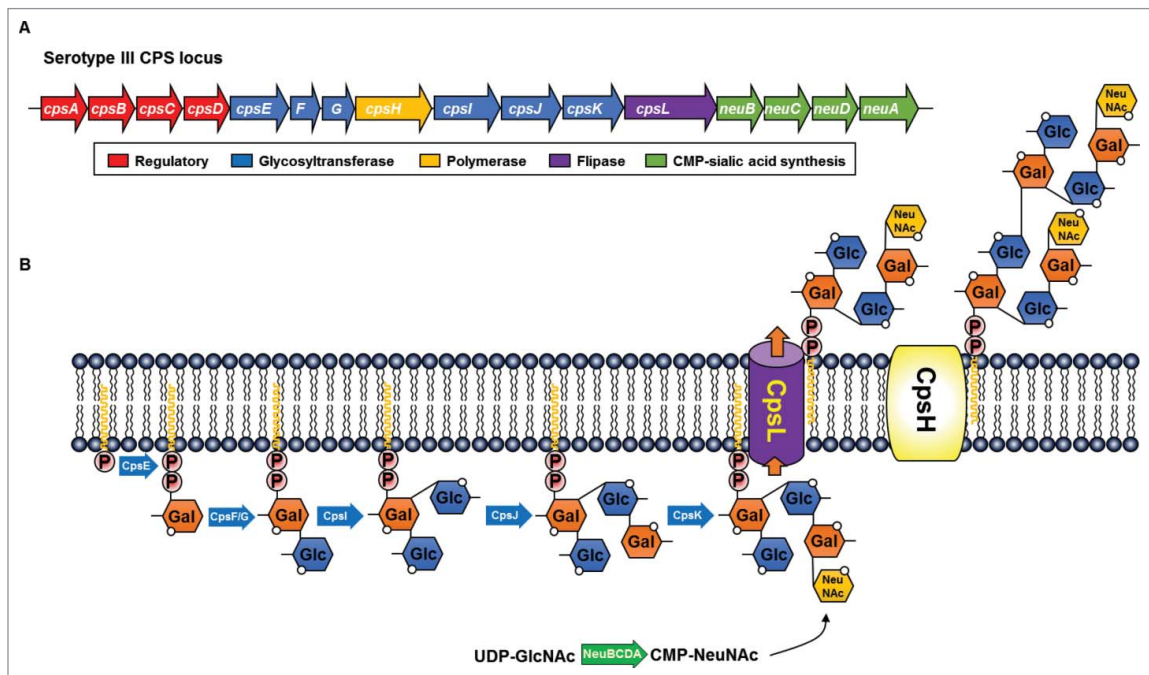


Figure 3. Representative Wzx/Wzy-dependent capsular polysaccharide (CPS) biosynthesis pathway in the group B streptococcal serotype III. (A) Serotype III cps gene organization and putative functions of the gene products.³⁷ (B) Biochemical steps during the CPS synthesis. Galactose-1-phosphate is initially transferred to an undecaprenyl-phosphate by CpsE and the repeat unit is rapidly assembled by glycosyltransferases. Individual repeat units are translocated across the cytoplasmic membrane by flippase (cpsL) and linked to form lipid-linked CPS by polymerase (cpsH).

pentavalent conjugate vaccine with serotypes Ia, Ib, II, III, and V to evaluate its safety, tolerability, and immunogenicity (NCT03170609).

Surface anchoring adhesins

Although the production, coverage, safety and immunogenicity of CPS conjugate vaccines have been well established, they show several limitations, including^{95–98} (1) their limited usability in the low-income countries due to the high cost,⁹⁶ (2) potential immune interference with other type of conjugate vaccines,⁹⁹ (3) the possibility of serotype replacement and switching following the vaccination,^{37,100–102} and (4) an increase in the occurrence of the unencapsulated GBS.^{103–105}

Therefore, a structurally conserved protein antigen-based vaccine against GBS has been investigated as alternative vaccines.^{106,107} Conserved bacterial surface proteins play important roles during different stages of infection and likely represent the promising universal vaccine candidates.^{106,107,108}

To initiate infection and invasion of a specific organs, bacterial pathogens must first be able to attach to an appropriate target tissue by specific multiple tropisms between bacterial surface ligands and host receptors. Surface-anchoring adhesion molecules of GBS may therefore represent good candidates for vaccine development.

Alp protein family

Most well-known gram-positive bacterial surface proteins are cell wall proteins covalently anchored to the peptidoglycan layer through an LPXTG motif by the activity of enzyme sortase A (SrtA).¹⁰⁹ The genome of GBS encodes at least 20–25 LPXTG-linked surface proteins.^{110–112} Proteomic analysis of GBS was conducted to identify the major surface proteins of GBS, and Rip protein, one of Alp family protein, was found to be the most abundant surface protein.¹¹⁰ Furthermore, over 90% of GBS clinical isolates were found to express or encode at least one of the Alp protein family genes.¹¹³ Seven members of the Alp family, including AlphaC, BetaC, Alp1, Alp2, Alp3 (R28), Alp4, and Rib, have been identified to date. They contain an N-terminal secretion signal sequence (S), N-terminal conserved domain (N), a variable number of tandemly arranged repeats of 70–80 amino acids (R), 8 to 10 repeats, and a C-terminal LPXTG cell-wall anchoring motif (Fig. 4A).¹¹¹ Certain domains of Alp proteins may display high sequence similarity, which provides a structural basis for their interactions with the same host receptor and cross-protective immunity.¹¹⁴ The roles of AlphaC and BetaC proteins have been extensively studied, compared with those of the other members of the Alp family. AlphaC protein was shown to be an important ligand involved in the GBS binding to human cervical epithelial cells through its interaction with glycosaminoglycan (GAG).¹¹⁵ MLKKIE sequence motif of BetaC protein binds to the Fc region of human IgA, predominantly found on the mucus surface (Fig. 4B).^{116,117} Additionally, it was also shown to bind to human factor H (FH) to protect GBS from opsonophagocytosis.¹¹⁸ Rib protein shares several biochemical features with AlphaC protein, but no immunological cross-reaction with either AlphaC or BetaC proteins has been found.¹¹⁹ The

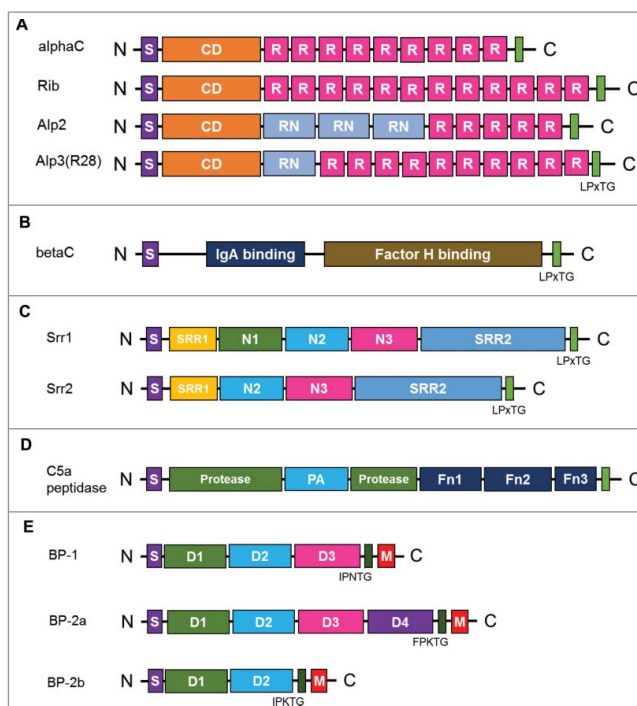


Figure 4. Schematic representation of the group B streptococcal surface proteins. (A) Alp protein family. Signal peptide (S), conserved domain (CD), repeating domain (R), LPXTG cell-wall anchoring domain (green). (B) BetaC protein, containing IgA binding domain (blue) and factor H binding domain (brown). (C) Serine-rich repeat protein family. Ig-like domain (N1, N2, and N3), serine-rich repeat glycosylation domain (SRR1 and SRR2). (D) C5a peptidase. Protease activity domain (Protease), protease-associated domain (PA), fibronectin type III domain (Fn1, Fn2, Fn3). (E) Pilin proteins. Ig-like domain (D1–D3), pilin sorting motif (IPNTG, FPKTG), and a membrane-spanning hydrophobic domain (M).

potential invasive properties of other Alp proteins have not been studied.

Preclinical vaccine investigations of the AlphaC, Alp3, and Rib proteins have been conducted, but the use of Alp proteins as universal vaccines has been limited due to the heterogeneity of the Alp sequence.^{114,120–123} Nevertheless, MinervaX Inc. recently reported that the fusion protein of the highly immunogenic N-terminal domains of AlphaC and Rib (GBS-NN) led to over 30-fold increase of GBS-NN-specific antibody in their phase I clinical trial with 240 healthy adult women (NCT02459262).¹²⁴

Serine-rich repeat proteins

Doro *et al.*¹²⁵ performed surfome analysis to identify GBS proteins with domains protruding from the bacterial surface. Among 43 surface-associated proteins identified using GBS COH1 strain (serotype III), serine-rich repeat 2 (Srr2) protein was shown to be the most abundant surface protein, which can be used to generate a protective immune response against GBS serotype III in mice. Serine-rich repeat (SRR) glycoproteins are a large and diverse family of adhesins found in most gram-positive bacteria.^{126–129} GBS expresses either one of two-allelic SRR proteins, Srr1 and Srr2,¹³⁰ with a highly conserved domain organization that includes a secretion signal sequence, two SRR domains that are glycosylated, a specialized fibrinogen binding domain between two SRR domains, and an LPXTG cell wall-anchoring motif (Fig. 4C). Both Srr1 and Srr2 identified in GBS can bind fibrinogen α chain through the “dock, lock, and

latch” mechanism, and these interactions contributes to the pathogenesis of GBS meningitis and GBS colonization of the vaginal surface.^{131–133} An antigenic domain with 13 amino acids in Srr1 and Srr2, latch domain, was shown to be crucial for the pathogenesis of GBS diseases, and latch-peptide vaccination was demonstrated to provide serotype-independent protection against GBS infection in mice.¹³⁴

C5a peptidase (ScpB)

C5a peptidase is a highly conserved surface protein that is expressed on the surface of most GBS serotypes and can specifically inactivate a human phagocyte chemotaxin, C5a.^{135–138} C5a peptidase is also involved in GBS invasion, as it interacts with the human fibronectin through its RGD motif.^{139,140} This cell wall-anchoring protease contains N-terminal subtilisin-like protease domain, two RGD motifs targeting integrin, and three C-terminal fibronectin type III (Fn) domains (Fig. 4D).¹³⁷ C5a peptidase-deficient GBS pathogens were shown to be more rapidly cleared from mice supplemented with human C5a, suggesting that this peptidase is an important GBS virulence factor.¹⁴¹ Recombinant C5a peptidase has been investigated as a universal protein vaccine or a carrier protein of GBS-CPS instead of the tetanus toxoid.^{142,143} In a murine model, antibodies raised against recombinant C5a peptidase were opsonic and enhanced phagocytic killing of various GBS serotypes. The immunization with C5a peptidase-conjugated GBS type III CPS led to an increase in the IgG immune response against both CPS and C5a peptidase. To enhance the immunogenicity of the recombinant C5a peptidase further, the researchers encapsulated it within microspheres composed of a lactic and glycolic acid copolymer, which enabled this molecule to induce systemic and mucosal immune responses, offering protection against multiple GBS serotypes.^{144,145}

Pilus

The genome sequences of five GBS serotypes were analyzed to identify pan-genome genes that encode putative surface-associated proteins and possible antigens suitable for the development of a universal GBS vaccine.^{95,146,147} Among 396 core genes, pilin proteins were shown to induce a protective, serotype-independent immune response against GBS infection. Pili are long filamentous structures protruding from the bacterial surface, which are important for the bacterial virulence and disease pathogenesis.¹⁴⁸ Extensive genomic analyses of a large panel of GBS isolates revealed the presence of three pilus islands, PI-1, PI-2a, and PI-2b, which are further classified as pilus type 1, 2a, and 2b, respectively.^{147,149,150} Each island encodes a pilus composed of three structural proteins, the major pilus subunit (backbone protein, BP) that forms the pilus shaft and two ancillary proteins that appear to be located at the pilus tip (AP1) and at the base (AP2) as anchor protein of the pilus to bacterial cell-wall.^{151–156} Although vaccination using either BP or AP1 induced protective immune responses against GBS, it was pilus type-specific and better in immunization with BP. Furthermore, at least six immunologically different variants were found in BP-2a, which limited BP for the use of vaccine development.^{95,149} Nuccitelli *et al.*¹⁵⁷ found that BP-2a variants share similar four Ig like domain (D1 to D4) and a D3 domain of BP-2a is a major epitope for a protective immune response.

They further developed a six D3 fused chimeric protein from six BP-2a variants by using structural vaccine technology and showed strong protective immune responses against all six BP-2a variant carrying GBS strains. If this structural vaccine technology is further expanded to successfully include BP1 and BP-2b in a six D3 chimeric protein vaccine, a pilus is going to be a good vaccine candidate for a universal GBS protein vaccine.

Vaccine evaluation assays

Several vaccine candidates are under clinical and preclinical investigations, but the low baseline incidence of the primary endpoint of GBS invasive disease requires phase III clinical efficacy trials to be very large.² Based on a good correlation between immune response and clinical protection, some experts suggested that GBS vaccine can be approved based on the immunogenicity assay.^{42,158,159} Similarly, *Neisseria meningitidis* group C conjugate vaccine was successfully introduced in the UK on the basis of the immunogenicity assay results.¹⁶⁰ Therefore, the standardization of the clinical immunogenicity assays is urgently required for the development of GBS vaccines. The basic approach to the determination of vaccine immunogenicity is the measuring of antigen-specific antibody levels in the patient sera before and after vaccination, to determine whether an appropriate response has been induced.

For the PCV vaccine, two standard immunological methods, enzyme-linked immunosorbent assay (ELISA) and opsonophagocytic killing assay (OPKA) for measuring the quantity and quality of CPS-specific antibodies, are well established and accepted as the standard vaccine efficacy assays.^{51,161,162} Although an immunogenicity assay for the analysis of GBS vaccine has been developed using certain modifications of the existing PCV vaccine assay, the modified protocol has not been standardized and validated in different laboratories to date, and consequently, the standardization of the GBS vaccine immunogenicity assays is necessary.

Antibody quantification

Standard ELISA can be used for the quantification of antibodies generated due to the immunization by protein-based vaccine.^{163,164} However, the capacity of this test to determine the levels of the antibodies against serotype-specific CPS antigens largely depends on the ability of the CPS immobilization on an ELISA plate, which can be accompanied by considerable technical difficulties, such as an inconsistent binding of immobilized CPS to the solid phase or a nonspecific serotype-independent binding with lower avidity.^{85,89,91,165} Despite the high degree of similarity between the repeat unit structure of CPS in different serotypes, their immunogenicity may quite differ. Therefore, methods used for the quantification of capsular serotype-specific antibody in serum must be not only sensitive, but also serotype-specific.⁸⁹ Baker and Kasper reported that the use of horse serum albumin-conjugated CPS obtained from different GBS serotypes as coating antigens results in at least 13- to 215-fold higher binding of antigens to the ELISA plates than when CPS alone is used and an improved sensitivity of the ELISA compared with that of the unconjugated CPS.^{51,81,87,89,166} However, another study demonstrated that the chemical conjugation of CPSs and proteins as ELISA antigens

can alter the antigenic structure of CPS, resulting in the reduction of antigenic specificity.¹⁶⁷ These results demonstrate that the design of specific ELISA protocol for the determination of serotype-specific GBS antibodies should be further optimized.

Assessment of functional antibodies

The quantity of antibodies generated against CPS or protein antigens highly correlates with the level of protection against GBS infections, but the functional quality of the antibodies induced by vaccines represents a critical determinant for the protection against GBS infections as well. Since the application of ELISA cannot differentiate between poorly functional antibodies with low avidity and the high-avidity antibodies, ELISAs may not be sufficient to determine the functional quality of antibodies.^{168–171} OPKA has been useful for the direct measurement of the protective capacity of antibodies, which function by opsonizing GBS for phagocytosis.¹⁶¹ The classical OPKA is a tedious procedure for the examination of several serotype-specific OPKA in a large number of samples.^{172,173} For the clinical testing of pneumococcal vaccine efficacy, OPKA for pneumococcus has been modified to use a granulocytic cell line (HL60) that allows more convenient use of it with specificity and reproducibility.¹⁷⁴ Additionally, this assay has been further simplified from a single OPKA to a multiplexed OPKA to reduce the assay time and the amount of serum required for the test.^{175–177} Evaluation of the functional efficacy of vaccine after immunization of pregnant women with GBS vaccine is performed in the newborns, in which extremely small amounts of serum can be obtained. With the technical advantages of the multiplexed OPKA, three-fold multiplexed OPKA for GBS (GBS-MOPA) has been developed, standardized, and validated to be used in newborns.¹⁷⁸ This standardized GBS-MOPA protocol enabled a practical, large-scale assessment of GBS vaccine immunogenicity against serotypes Ia, III, and V. An additional set of GBS-MOPA, covering all possible vaccine serotypes, is required to be developed.

Concluding remarks and perspectives

Despite the remarkable advances in the prevention and treatment of GBS infections over the recent decades, invasive GBS infections are still important public health problems, particularly in the neonates and infants. Although several vaccine candidates are under clinical development, a key issue of the phase III trials is the low baseline incidence of the primary clinical endpoints of GBS infections in both neonates and elderly. Additionally, the optimization of the number, concentrations, and timing of maternal vaccination conferring protection against GBS infections in both pregnant women and neonates is complicated. Therefore, it is critical to develop a standardized immunogenicity assay and establish GBS serotype-specific protective cut-off values to succeed in the development of effective vaccines. Several efforts were made to modify the standard immunogenicity assay for pneumococcal PCV for the application in the GBS vaccine development, however, several concerns were highlighted here. First, no reference serum for the standardization of GBS ELISA is available, furthermore, the immobilization of CPS on ELISA plate has to be optimized, and finally, the low affinity of natural and non-specific binding

antibodies. Moreover, standard immunogenicity assays should be further optimized and validated in multiple laboratories across different counties, together with the worldwide epidemiological studies of the GBS serotype and genotype distribution. After the introduction of pneumococcal PCV, new serotypes and serotype replacement were identified in the countries where PCV has been used nationwide. Due to this, the vaccine effectiveness and changes in the disease incidence should be constantly assessed and monitored before and after the licensing and implementation of GBS vaccine.

Disclosure of potential conflicts of interest

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