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Status of group B streptococcal vaccine development

Streptococcus agalactiae (group B streptococcus, GBS) is a leading causal organism of neonatal invasive diseases and severe infections in the elderly. Despite significant advances in the diagnosis and treatment of GBS infections and improvement in personal hygiene standards, this pathogen is still a global health concern. Thus, an effective vaccine against GBS would augment existing strategies to substantially decrease GBS infection. In 2014, World Health Organization convened the first meeting for consultation on GBS vaccine development, focusing on the GBS maternal immunization program, which was aimed at reducing infections in neonates and young infants worldwide. Here, we review the history of GBS infections, the current vaccine candidates, and the current status of immunogenicity assays used to evaluate the clinical efficacy of GBS vaccines.

Keywords: Vaccines, Group B streptococcus, Streptococcus agalactiae, Conjugate vaccines, Polysaccharides

Lancefield group B streptococci (GBS), also referred to as Streptococcus agalactiae, is a gram-positive, opportunistic pathogen that colonizes the gastrointestinal and genitourinary tracts of up to 50% of healthy adults [1-3]. In 1938, it was first identified as a human pathogen, causing human fatal puerperal sepsis [4], but remained relatively unknown as sporadic asymptomatic cases were reported until the 1960s. By the 1970s, GBS had emerged as the predominant pathogen causing septicemia and meningitis in neonates and infants living in diverse regions [5-10]. GBS infection in newborns is usually classified as an early-onset disease (EOD) and late-onset disease (LOD), respectively depending on the age of the infant at the time of disease manifestation [11]. Recent advances in the diagnosis and treatment of GBS infections and global hygiene standards have significantly reduced the development of neonatal infections and mortality, particularly due to EOD. However, recent estimates also show 0.5–2 cases of neonatal GBS infections per 1,000 births with a mortality rate of 9.6%–22% [12,13]. In addition, recent reports have revealed that an increasing number of those infections occurred in pregnant women and non-pregnant adults who typically had an underlying medical condition. The incidence of GBS infection among those adults increased from 3.6 cases/100,000 persons in 1990 to 7.3 cases/100,000 persons in 2007, with significantly higher case fatality rate at 15% [14]. Although vaccination is the most promising strategy for preventing GBS infection, currently no licensed GBS vaccine is avail-
able in the market. Thus, the development of a GBS vaccine is the need of the hour, considering the risk involved in presently used prenatal strategies and the prevalence of GBS infections in the elderly.

GBS are covered with a sialic acid-rich capsular polysaccharide (CPS) and belong to one of the ten serotypes (Ia, Ib, and II–IX). Each CPS consists of variously arranged monosaccharides and a sialic acid residue on the branching terminus of the repeating unit (Fig. 1) [15,16]. Similar to that of pneumococci, GBS CPSs also show potential immune evasion mechanism for GBS by inhibiting complement deposition and phagocytosis [17,18]. Recent systemic meta-analyses indicate that five serotypes (Ia, Ib, II, III, and V) account for 97% of invasive isolates in all geographical regions [19]. Owing to its importance in GBS pathogenesis, CPS is considered to be the prime vaccine candidate for GBS.

In 2014, World Health Organization convened the first meeting of the Product Development for Vaccines Advisory Committee for consultation regarding the development of GBS vaccines [20]. In this meeting, they agreed that native CPS vaccine is ineffective due to its poor immunogenicity, but the immunogenicity of the GBS polysaccharide conjugate vac-

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**Fig. 1.** Chemical-repeating unit structures of group B streptococcus (GBS) capsular polysaccharides (CPSs). CPSs are classified in three classes depending on similarity of chemical structures and the enzymes involved in the assembly of the repeating units. (A) Class 1: the repeating unit consists of two sugars containing a β (1→3) linked side chain whose terminus possesses a sialic acid residue. (B) Class 2: the repeating unit consists of three sugars containing a β (1→6) linked side chain whose terminus possesses a sialic acid residue. (C) Class 3: CPS has no similarity with any other GBS CPSs.
cine (PCV) may be able to induce a stronger and higher functional CPS-specific IgG response [21,22]. Novartis has developed a trivalent (Ia, Ib, and III) CRM197 conjugate vaccine, and conducted a phase 1b/2 clinical trial (NCT01193920) in infants born to women who were vaccinated with a trivalent GBS PCV [23]. In infants born to the GBS PCV recipients, the level of CPS-specific antibody was higher at birth than at 43 and 91 days, indicating that maternal GBS PCV immunization is intended to protect infants by sufficient CPS-specific antibody transfer across the placenta. In 2017, Pfizer started to evaluate a pentavalent GBS PCV targeting Ia, Ib, II, III, and V in a phase 1 trial on healthy volunteer (NCT03170609). Despite the promising result from the clinical trial, recent change of serotype distribution worldwide requires the replacement of old serotypes, or the addition of new serotypes in the GBS PCV.

Although multivalent CPS PCVs are well established in terms of their production, safety, and immunogenicity, it has several limitations and various concerns have been raised regarding its use. The limitations are immune interference with similar type of conjugate vaccines, including *Haemophilus influenzae* type b, meningococcal, and pneumococcal conjugate vaccines, potential problems of serotype replacement and switching, and diverse serotype distribution across and within geographical regions [24-27]. In addition, an increasing number of reports show that nonencapsulated GBS strains are a concern for the implementation of an anti-CPS vaccine [28-31]. Structurally conserved protein antigens that are essential for GBS virulence and can induce a strong immune response against most of the GBS strains, are emerging as the most attractive and cost-effective vaccine candidates [12,32-34]. MinervaX Inc. recently announced that their protein-only vaccine based on the fusion of highly immunogenic N-terminal domains of Alpha C and Rib (GBS-NN) showed positive results from a phase I trial in 240 healthy adult women [35]. All subjects immunized with one or two doses of GBS-NN showed an increase of over 30-fold in GBS-NN specific antibodies compared to pre-immune level [36]. GlaxoSmithKline also identified a conserved sequence encoding components of GBS pili proteins, which induced the immune response against different GBS serotypes in preclinical studies [32,33]. We investigated that the C-terminal end of a serine-rich repeat surface glycoprotein named latch domain could provide serotype-independent protection in mouse meningitis model [37]. In addition, many surface proteins of GBS are being investigated, at the pre-clinical stage, as broad spectrum vaccines [35,38-47].

Because of the possibly low baseline incidence of the primary endpoint of invasive disease, there is an urgent need for a standardized clinical efficacy assay for GBS vaccines in order to support and accelerate the clinical studies. In pneumococcal PCV, two standard immunological methods, enzyme-linked immunosorbent assay (ELISA) and multiplex opsonophagocytosis assay (MOPA) for measuring CPS-specific antibody and functional antibody titers, are well established and accepted as standard vaccine efficacy assays. The radio-antigen binding assay (RABA) has been the gold standard for the quantification of anti-GBS antibody as it measures antibody in its native state [48]. However, the RABA has several limitations, such as low detection sensitivity, limited ability to quantify Ig isotypes, and the difficulties of procuring and using radioisotopes. Several ELISA protocols that are more sensitive and isotype-specific have subsequently been developed based on pneumococcal CPS ELISA. However, the sensitivity and non-specific binding remain a concern for these methods as well [49-51]. It is important to note that the results of RABA and ELISA might not always reflect functional antibody level for encapsulated bacteria, as experienced in pneumococcal vaccines [52].

In *vitro* opsonophagocytic assay (OPA) is believed to have a reliable correlation with the functional efficacy of pneumococcal PCVs, because host protection against pneumococcus is mainly mediated by opsonin-dependent phagocytosis [53, 54]. As pregnant women are mainly immunized with the GBS vaccine, and as the maternal transfer of anti-polysaccharide (PS) specific IgG should be tested using extremely small amounts of serum from the newborns, our group developed, standardized, and validated three-fold multiplexed GBS-OPA (GBS-MOPA) to enable practical, large-scale assessment of GBS vaccine immunogenicity [55]. Therefore, the standardized functional efficacy assay would be essentially used to evaluate the clinical efficacy in the process of GBS PCV approval and licensure.

Application of a GBS vaccine is the most promising strategy for the prevention of GBS infections in both newborns and adults with underlying diseases. However, numerous questions arise during the designing and evaluation of GBS vaccines. First, recent epidemiology studies have introduced the phenomenon of serotype switching and replacement occurring worldwide [25,56-58]. Thus, a streamlined effort is needed to update the global disease burden estimate and serotype distribution. Second, a standardized immunological assay is
urgently needed. Although we developed and standardized GBS-MOPA, it needs to be further optimized to suit the specific needs of different countries. In addition, standard ELISA to quantify CPS-specific antibody should be characterized by multi-national based assessment. This review will support the development of a new strategy for GBS vaccine development, evaluation to substantially reduce the global burden of GBS infections, achieve substantial reduction in premature and still births worldwide.

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References

1. Schuchat A. Epidemiology of group B streptococcal disease in the United States: shifting paradigms. Clin Microbiol Rev 1998;11:497-513.
2. Doran KS, Nizet V. Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy. Mol Microbiol 2004;54:23-31.
3. Hansen SM, Uldbjerg N, Kilian M, Sorensen UB. Dynamics of *Streptococcus agalactiae* colonization in women during and after pregnancy and in their infants. J Clin Microbiol 2004;42:83-9.
4. Fry RM. Prevention and control of puerperal sepsis: bacteriological aspects. Br Med J 1938;2:340-2.
5. Franciosi RA, Knostman JD, Zimmerman RA. Group B streptococcal neonatal and infant infections. J Pediatr 1973;82:707-18.
6. Baker CJ, Barrett FF, Gordon RC, Yow MD. Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. J Pediatr 1973;82:724-9.
7. Barton LL, Feigin RD, Lins R. Group B beta hemolytic streptococcal meningitis in infants. J Pediatr 1973;82:719-23.
8. Fluegge K, Siedler A, Heinrich B, et al. Incidence and clinical presentation of invasive neonatal group B streptococcal infections in Germany. Pediatrics 2006;117:e1139-45.
9. Kalliola S, Vuoipio-Varkila J, Takala AK, Eskola J. Neonatal group B streptococcal disease in Finland: a ten-year nationwide study. Pediatr Infect Dis J 1999;18:806-10.
10. Neto MT. Group B streptococcal disease in Portuguese infants younger than 90 days. Arch Dis Child Fetal Neonatal Ed 2008;93:F90-3.
11. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. MMWR Recomm Rep 2010;59:1-36.
12. Heath PT. Status of vaccine research and development of vaccines for GBS. Vaccine 2016;34:2876-9.
13. Schuchat A. Group B streptococcus. Lancet 1999;353:51-6.
14. Skoff TH, Farley MM, Petit S, et al. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990-2007. Clin Infect Dis 2009;49:85-92.
15. Paololetti LC, Kasper DL. Glycoconjugate vaccines to prevent group B streptococcal infections. Expert Opin Biol Ther 2003;3:975-84.
16. Chaffin DO, Mentele LM, Rubens CE. Sialylation of group B streptococcal capsular polysaccharide is mediated by cpsK and is required for optimal capsule polymerization and expression. J Bacteriol 2005;187:4615-26.
17. Aoyagi Y, Adderson EE, Min JG, et al. Role of L-ficolin/mannnose-binding lectin-associated serine protease complexes in the opsonophagocytosis of type III group B streptococci. J Immunol 2005;174:418-25.
18. Macauley MS, Crocker PR, Paulson JC. Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol 2014;14:653-66.
19. Madrid L, Seale AC, Kohli-Lynch M, et al. Infant group B streptococcal disease incidence and serotypes worldwide: systematic review and meta-analyses. Clin Infect Dis 2017;65(Suppl 2):S160-72.
20. Giersing BK, Modjarrad K, Kaslow DC, Moorthy VS; WHO Product Development for Vaccines Advisory Committee; WHO Product Development for Vaccines Product Development committee. Report from the World Health Organization’s Product Development for Vaccines Advisory Committee (PDVAC) meeting, Geneva, 7-9th Sep 2015. Vaccine 2016;34:2865-9.
21. Chen VL, Avci FY, Kasper DL. A maternal vaccine against group B streptococcus: past, present, and future. Vaccine 2013;31 Suppl 4:D13-9.
22. Leroux-Roels G, Maes C, Willekens J, et al. A randomized, observer-blind Phase Ib study to identify formulations and vaccine schedules of a trivalent group B streptococcus vac-
cine for use in non-pregnant and pregnant women. Vaccine 2016;34:1786-91.

23. Madhi SA, Koen A, Cutland CL, et al. Antibody kinetics and response to routine vaccinations in infants born to women who received an investigational trivalent group B streptococcus polysaccharide CRM197-conjugate vaccine during pregnancy. Clin Infect Dis 2017;65:1897-904.

24. Muller-Graf CD, Whatmore AM, King SJ, et al. Population biology of Streptococcus pneumoniae isolated from oropharyngeal carriage and invasive disease. Microbiology 1999;145(Pt 11):3283-93.

25. Teatero S, Ferrieri P, Martin I, Demczuk W, McGeer A, Fit-tipaldi N. Serotype distribution, population structure, and antimicrobial resistance of group B streptococcus strains recovered from colonized pregnant women. J Clin Microbiol 2017;55:412-22.

26. Flores AR, Galloway-Pena J, Sahasrabhojane P, et al. Sequence type 1 group B streptococcus, an emerging cause of invasive disease in adults, evolves by small genetic changes. Proc Natl Acad Sci U S A 2015;112:6431-6.

27. Bellais S, Six A, Fouet A, et al. Capsular switching in group B streptococcus CC17 hypervirulent clone: a future challenge for polysaccharide vaccine development. J Infect Dis 2012;206:1745-52.

28. Toyofuku M, Morozumi M, Hida M, et al. Effects of intrapartum antibiotic prophylaxis on neonatal acquisition of group B streptococci. J Pediatr 2017;190:169-73.e1.

29. Edwards MS, Rench MA, Rinaudo CD, et al. Immune responses to invasive group B streptococcal disease in adults, evolves by small genetic changes. Proc Natl Acad Sci U S A 2015;112:6431-6.

30. Alhhazmi A, Hurteau D, Tyrrell GJ. Epidemiology of invasive group B streptococcal disease in adults, evolves by small genetic changes. Proc Natl Acad Sci U S A 2015;112:6431-6.

31. Slotved HC, Kong F, Lambertsens L, Sauer S, Gilbert GL. Serotype IX, a proposed new Streptococcus agalactiae serotype. J Clin Microbiol 2007;45:2929-36.

32. Rioux S, Martin D, Ackermann HW, Dumont J, Hamel J, Brodeur BR. Localization of surface immunogenic protein on group B streptococcus. Infect Immun 2001;69:5162-5.

33. Nuccitelli A, Rinaudo CD, Maione D. Group B streptococcal vaccine: state of the art. Ther Adv Vaccines 2015;3:76-90.

34. Nuccitelli A, Cozzi R, Gourlay LJ, et al. Structure-based approach to rationally design a chimeric protein for an effective vaccine against group B streptococcus infections. Proc Natl Acad Sci U S A 2011;108:10278-83.

35. Areschoug T, Stalhammar-Carlemalm M, Larsson C, Lindahl G. Group B streptococcal surface proteins as targets for protective antibodies: identification of two novel proteins in strains of serotype V. Infect Immun 1999;67:6350-7.

36. Minervax [Internet]. Copenhagen: Minervax; 2017. Available from: http://minervax.com/news/2017/1/5/minervax-announces-positive-data-from-phase-i-clinical-trial.html.

37. Lin SM, Jang AU, Zhi Y, et al. Vaccination with a latch peptide provides serotype-independent protection against group B streptococcus infection in mice. J Infect Dis 2017;217:93-102.

38. Baker JA, Lewis EL, Byland LM, Bonakdar M, Randis TM, Ratner AJ. Mucosal vaccination promotes clearance of Streptococcus agalactiae vaginal colonization. Vaccine 2017;35:1273-80.

39. Gourlay LJ, Santi I, Pezzicoli A, Grandi G, Soriani M, Bolognesi M. Group B streptococcus pullulanase crystal structures in the context of a novel strategy for vaccine development. J Bacteriol 2009;191:3544-52.

40. Rioux S, Martin D, Ackermann HW, Dumont J, Hamel J, Brodeur BR. Localization of surface immunogenic protein on group B streptococcus. Infect Immun 2001;69:5162-5.

41. Manning SD, Ki M, Marrs CF, et al. The frequency of genes encoding three putative group B streptococcal virulence factors among invasive and colonizing isolates. BMC Infect Dis 2006;6:116.

42. Zhao Z, Kong F, Gilbert GL. Reverse line blot assay for direct identification of seven Streptococcus agalactiae major surface protein antigen genes. Clin Vaccine Immunol 2006;13:145-9.
Vaccine 1999;17:454-8.

47. Larsson C, Stalhammar-Carlemalm M, Lindahl G. Experimental vaccination against group B streptococcus, an encapsulated bacterium, with highly purified preparations of cell surface proteins Rib and alpha. Infect Immun 1996; 64:3518-23.

48. Baker CJ, Kasper DL. Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. N Engl J Med 1976;294:753-6.

49. Lin FY, Weisman LE, Azimi PH, et al. Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen. J Infect Dis 2004;190:928-34.

50. Baker CJ, Carey VJ, Rench MA, et al. Maternal antibody at delivery protects neonates from early onset group B streptococcal disease. J Infect Dis 2014;209:781-8.

51. Klegerman ME, Boyer KM, Papierniak CK, Gotoff SP. Estimation of the protective level of human IgG antibody to the type-specific polysaccharide of group B streptococcus type Ia. J Infect Dis 1983;148:648-55.

52. Guttormsen HK, Baker CJ, Edwards MS, Paoletti LC, Kasper DL. Quantitative determination of antibodies to type III group B streptococcal polysaccharide. J Infect Dis 1996; 173:142-50.

53. Burton RL, Nahm MH. Development and validation of a fourfold multiplexed opsonization assay (MOPA) for pneumococcal antibodies. Clin Vaccine Immunol 2006;13:1004-9.

54. Burton RL, Nahm MH. Development of a fourfold multiplexed opsonophagocytosis assay for pneumococcal antibodies against additional serotypes and discovery of serological subtypes in Streptococcus pneumoniae serotype 20. Clin Vaccine Immunol 2012;19:835-41.

55. Choi MJ, Noh JY, Cheong HJ, et al. Development of a multiplexed opsonophagocytic killing assay (MOPA) for group B streptococcus. Hum Vaccin Immunother 2018;14:67-73.

56. Seale AC, Koech AC, Sheppard AE, et al. Maternal colonization with Streptococcus agalactiae and associated stillbirth and neonatal disease in coastal Kenya. Nat Microbiol 2016;1:16067.

57. Meehan M, Cunney R, Cafferkey M. Molecular epidemiology of group B streptococci in Ireland reveals a diverse population with evidence of capsular switching. Eur J Clin Microbiol Infect Dis 2014;33:1155-62.

58. Ramaswamy SV, Ferrieri P, Flores AE, Paoletti LC. Molecular characterization of nontypeable group B streptococcus. J Clin Microbiol 2006;44:2398-403.