Published in final edited form as:

Vaccine. 2012 June 13; 30(28): 4123–4126. doi:10.1016/j.vaccine.2012.04.048.

PERSISTENCE OF FUNCTIONAL ANTIBODIES TO GROUP B STREPTOCOCCAL CAPSULAR POLYSACCHARIDES FOLLOWING IMMUNIZATION WITH GLYCOCONJUGATE VACCINES

Morven S. Edwards, M.D.^a, Heidi J. Lane, B.S.^a, Sharon L. Hillier, Ph.D.^b, Marcia A. Rench, R.N.^a, and Carol J. Baker, M.D.^{a,c}

^aDepartment of Pediatrics, Baylor College of Medicine, Houston, TX

^bDepartments of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine and Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh PA

^cDepartment of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

Abstract

The duration of functional activity of group B streptococcal (GBS) glycoconjugate vaccine-induced capsular polysaccharide-specific (CPS) IgG was evaluated among healthy adult responders. Opsonophagocytic activity declined significantly from a 4-week post-immunization peak, but substantial functional activity, exceeding 1 log₁₀ reduction in GBS cfu/mL, was retained at 18 months to 2 years post-immunization for each GBS type assessed. The persistence of functional antibody activity when GBS CPS-specific IgG concentrations decline, although remaining significantly higher than pre-immunization levels, suggests that long-term protection may be expected from candidate GBS glycoconjugates administered to this population.

Keywords

Group B *Streptococcus*; vaccine; antibody; glycoconjugate; polysaccharide; durability

1. INTRODUCTION

Infections caused by group B *Streptococcus* (GBS) continue to comprise a substantial disease burden among neonates, young infants, pregnant women, and adults [1]. The Centers for Disease Control and Prevention's Active Bacterial Core network estimated that invasive GBS infections afflicted 21,500 people in 2010 with an estimated incidence of 7 cases per 100,000 population [2]. Infants accounted for approximately 2,050 annual cases.

© 2012 Elsevier Ltd. All rights reserved.

Corresponding Author: Morven S. Edwards, M.D., 1102 Bates Street, Suite 1120, Houston, Texas 77030 USA, Telephone: 011 (832) 824-1780 Fax: 011 (832) 825-1048 morvene@bcm.edu.

Financial Disclosure and Conflict of Interest: Dr. Edwards is a consultant to and receives research funding from Novartis Vaccines & Diagnostics. The other authors have no financial relationships relevant to this article to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Approximately 90% of cases and almost 85% of the estimated 1,400 annual deaths from GBS occurred among adults 50 years of age and older.

Prevention of GBS infection in infants and adults through immunization is a theoretically attainable goal. Maternal transfer of antibodies could prevent newborn GBS disease and GBS vaccines offer the potential to prevent disease in low-income settings where prenatal screening and intrapartum antibiotics are generally not feasible. Susceptibility to invasive GBS infection correlates with low concentrations of GBS capsular polysaccharide (CPS)-specific antibodies in serum [3, 4]. GBS CPS-protein glycoconjugate vaccines are well-tolerated and immunogenic in healthy adults, including pregnant women and those 65 years of age and older [5–10]. Recently, commercial interest in vaccine development has increased and strategies capable of inducing in women strong, durable protective immunity against GBS are being assessed [11]. Data regarding the persistence of antibodies elicited in response to GBS glycoconjugate vaccines and their function in vitro would provide insight into their protective potential in different populations.

Our objective was to determine the persistence of functional activity of GBS CPS-specific IgG in sera after immunization with candidate CPS type Ia, III or V GBS glycoconjugate vaccines. The antibody data and functional activity at 4–8 weeks after immunization have already been presented [5, 9, 12]. We employed opsonophagocytosis assays to assess in vitro function of these antibodies [5–6, 8–10]. We hypothesized that robust functional activity would be sustained for at least 18 to 24 months post-immunization.

2. MATERIALS AND METHODS

2.1. Sera

Previously collected sera from healthy adults participating in prior phase 1 and 2 evaluations of GBS type Ia-capsular polysaccharide (CPS)-tetanus toxoid (TT) conjugate vaccine (n = 10), GBS type III CPS-TT vaccine (n = 13), and GBS type V CPS-TT or V CPS-cross-reactive material (CRM₁₉₇) vaccines (n = 10) [5, 9, 12] were evaluated. Sera from all 33 subjects selected were from individuals who had low pre-immunization concentrations of GBS CPS-specific IgG (<0.5 µg/mL for types III and V and <1.8 µg/mL for type Ia). Each of these subjects responded to a single dose of GBS glycoconjugate vaccine containing 50 µg (types III and V) or 60 µg (type Ia) GBS CPS with at least a 1 µg/mL increase in GBS CPS-specific antibody at 4-weeks post-immunization. Four-week, 6 or 12 month, and 18 or 24 month post-immunization samples were evaluated. Sera, selected based upon availability, included 10 of 12 subjects among 15 immunized who met the stated criteria for GBS type Ia-TT and 10 of 21 subjects among 30 immunized who met criteria for type V CPS-conjugates [5, 9]. The first available sera meeting study criteria were selected from among a cohort of 333 recipients of GBS III-TT vaccine [12]. All sera had been stored at –80°C since collection.

2.2 Bacterial Strains

The GBS strains used in these studies included type Ia strain 515, type III strain COH1 and type V strain 117. All are clinical isolates from neonates having GBS invasive infection. The strains had been maintained with minimal laboratory passage.

2.3 Opsonophagocytosis assay

Pre-immunization and post-immunization sera were tested on the same day for their ability to promote opsonization, phagocytosis and killing of the GBS CPS type contained in the glycoconjugate by adult polymorphonuclear leukocytes (PMN). The assay for CPS types Ia, III and V was performed as described for type V [13]. The assay for types Ia and III was

modified to include a pre-opsonization step because an exogenous complement source rather than endogenous complement was employed. Each assay contained well-characterized positive control sera known to promote $>1 \log_{10}$ reduction in cfu and well-characterized negative control sera. Each assay also included control tubes lacking complement, serum or PMN which, in all cases, permitted growth of GBS. Reaction mixtures for opsonization consisted of 50 μl of GBS containing $\sim 1\text{--}2 \times 10^6$ cfu and heat-inactivated serum (53°C for 90 min) at 10% (types Ia and III) or 33 % (type V) in 0.3 mL total volume in PBS. The reaction mixtures were incubated for 30 min at 4°C and then centrifuged at 4°C. Supernatants were decanted and 30 μl (10%), 6 μl (2%), and 15 μl (5%) of infant rabbit complement (Serotec, MorphoSys U.K., Ltd., Oxford) was added to reaction mixtures for types Ia, III and V GBS assays, respectively. PMNs from healthy adults ($\sim 1 \times 10^6$ in 50 μl) were added and the volume brought to 0.3 mL with PBS. Results were expressed as the $\log_{10}$ reduction in cfu of GBS after incubation for 40 min at 37°C and represented a mean of 2 or 3 experiments.

2.4 Statistics

Student's paired *t*-test and the Wilcoxon signed ranks test on log-transformed data was used to compare the geometric mean concentrations (GMC) of CPS-specific antibodies before and after immunization. Differences in $\log_{10}$ reduction in cfu of GBS in sera at intervals after immunization were assessed by the Wilcoxon signed ranks test.

3. RESULTS

A robust immune response in CPS-specific IgG was observed 4-weeks after immunization of healthy adults with each of the GBS conjugate vaccines as summarized in Table 1. By 52 weeks post-immunization, a significant decline to approximately one-half of the 4-week peak serum concentrations was observed for types Ia and III CPS-specific IgG, but no further decline was observed 78 weeks to 104 weeks post-immunization. The GMC of GBS CPS-specific IgG also declined for recipients of type V GBS conjugate vaccine after immunization and, by 104 weeks post-immunization, was approximately 40% of the 4-week peak concentration. For each GBS type, CPS-specific IgG remained significantly greater than pre-immunization concentrations at the latest interval after immunization.

Opsonophagocytic activity in sera from vaccine recipients prior to and at intervals ranging from 4 weeks to 2 years after immunization is shown in Figure 1. Functional activity was significantly greater at each post-immunization interval tested when compared to pre-immunization samples ($P < 0.01$). The mean functional activity 4 weeks after immunization ranged from 1.2 $\log_{10}$ (type V) to 1.5 $\log_{10}$ reduction in cfu/mL (types Ia and III) and, for each GBS type, this represented an increase of at least 1.0 $\log_{10}$ cfu/mL in killing compared to the pre-immunization specimens. Among type Ia-TT recipients, a significant decline occurred between 4 weeks and 2 years after immunization ($P < 0.01$). Among type III-TT recipients, functional activity declined when 4 week sera were compared to those collected 78 weeks after immunization ($P < 0.01$) and, for type V-TT and V-CRM₁₉₇ recipients, functional activity declined significantly between the 1 and 2 year intervals ($P = 0.047$) post-immunization.

Notably, the mean $\pm$ SEM $\log_{10}$ reduction in cfu/mL of GBS exceeded 1.0 $\log_{10}$ at the latest time point, indicating that substantial functional activity persisted despite the decay in CPS-specific IgG (Figure 1). However, several individual sera dropped below this 1.0 $\log_{10}$ threshold reduction in cfu/mL at each time point, as depicted in Figure 2. With one exception, each of these sera retained opsonophagocytic activity ranging from 0.34–0.94 $\log_{10}$ reduction in cfu/mL.

4. DISCUSSION

Our findings demonstrate that healthy adults who develop an immune response to GBS CPS type Ia, III or V glycoconjugate vaccine retain robust *in vitro* functional activity at least 18 to 24 months after immunization. The CPS-specific IgG levels at these time points have already been presented for these vaccines [5, 9, 12]. The results of functional assays at intervals beyond 8 weeks post-immunization [5–6, 8–10] represent new data. Although the GMCs of CPS-specific IgG declined to 40 to 60% of peak concentrations at the latest interval, significant opsonophagocytic killing persisted, and suggests a possible correlate of protection.

Antibody to the capsular polysaccharide of GBS in a sufficient concentration was recognized as an immunologic correlate of protection against invasive GBS infection in the 1970's [3–4]. The specific concentration of maternal IgG required to protect neonates against invasive early-onset disease has been evaluated [14–15]. Neonates whose mothers had levels of IgG to the CPS of type Ia GBS $5 \mu\text{g/mL}$ had an 88% lower risk of developing early-onset disease caused by type Ia GBS compared with those whose mothers had levels $<0.5 \mu\text{g/mL}$ [14]. Neonates whose mothers had $10 \mu\text{g/mL}$ of IgG to the CPS of type III GBS had a 91% lower risk for early-onset disease compared with those whose mothers had levels of $<2 \mu\text{g/mL}$ [15]. Use of an opsonophagocytic assay as a functional correlate has been employed in prior studies of GBS vaccines [5–6, 8–10] but the assay has not previously been used to examine persistence of functional activity after immunization.

The importance of our observations is three-fold. First, it is probable that long-term protection after immunization against encapsulated bacteria is dependent upon persistence of functional antibodies and maintenance of B-cell memory [16]. A functional assay that correlates with vaccine effectiveness is a particularly important surrogate of protection against a rapidly invasive pathogen such as GBS because immunological memory takes several days to activate after challenge by a pathogen, a response which may be too slow to prevent invasive infection. Second, a functional assay can be used as a correlate of protection over time after immunization. For example, serum bactericidal titers of at least 1:8, defined as a correlate of seroprotection for group C *Neisseria meningitidis*, persisted for 5 years in 84.1% (95% confidence interval, 81.6% to 86.3%) of 987 participants immunized with a serogroup C meningococcal glycoconjugate vaccine at 6 to 15 years of age [17]. In that cohort, geometric mean titers were significantly higher in those immunized at 10 years of age or older than among the younger children, suggesting that an adolescent immunization program could generate sustained protection against serogroup C meningococcal infection. Both opsonophagocytic activity and avidity have been shown to correlate better than antibody levels with *in vivo* passive protection after immunization with pneumococcal polysaccharide vaccine, highlighting the importance of measuring functional activity [18]. Finally, the finding that the functionality of CPS-specific IgG persists for at least 18 months to 2 years after GBS glycoconjugate immunization has implications for timing of immunization of women of childbearing age. Our results affirm the notion that women responding to GBS glycoconjugate vaccines administered early in pregnancy or prior to conception should have functionally active antibodies throughout pregnancy that theoretically could prevent early- or late-onset invasive infant infection.

Our findings are subject to several limitations. First, we studied vaccine recipients with very low pre-immunization concentrations of CPS-specific IgG to the immunizing GBS CPS type who responded with least a $1 \mu\text{g/mL}$ increase in CPS-specific IgG 4 weeks after immunization. It is likely that the kinetics of antibody function would differ, for example, among the 20% to 30% of individuals with moderate or high pre-immunization serum concentrations of CPS-specific IgG for whom immunization would be expected to boost

existing immunity and potentially sustain functionality without decline for an even more prolonged interval after immunization [6, 19]. Colonization at the time of vaccination may also impact immune response. Studies of pneumococcal conjugate vaccines have found reduced response, using both qualitative and functional assays, among children who are colonized with vaccine serotypes at vaccine receipt [20]. This potential correlation has not been assessed for recipients of GBS conjugate vaccines. Next, our data are limited to 2 years after immunization and evaluation of durability of function over a more extended interval of time would be required to direct vaccine platform constructs. Vaccination during adolescence would provide ample time for development of CPS-specific IgG before placental antibody transfer if pregnancy ensued quickly, but there are no data to date regarding durability of vaccine-induced antibodies or their function beyond 2 years. Finally, we evaluated persistence of function among adults 18–45 years of age. Aging reduces the functionality of anti-pneumococcal antibodies produced after natural exposure to the pathogen and in response to pneumococcal vaccine [21–22]. A reduction in function in healthy elderly adults correlates with low IgG antibody avidity [18]. Our evaluation of function of IgG elicited in response to a GBS conjugate in younger adults cannot be applied to durability in adults age 65 years or older who bear the highest attack rate and case-fatality ratio from invasive GBS infection among non-neonatal populations.

In conclusion, substantial functional activity, exceeding a 1 log₁₀ reduction in GBS cfu/mL, was retained at 18 months to 2 years post-immunization among recipients of GBS Ia, III or V glycoconjugate vaccines. While additional long term assessment of vaccine-induced immunity should be undertaken, functional correlates should be considered in ongoing clinical trials of candidate GBS glycoconjugates to inform decisions regarding vaccine platforms and expected durability of vaccine-induced protection.

Acknowledgments

We gratefully acknowledge the assistance of Robin D. Schroeder in preparation of the manuscript.

ABBREVIATIONS

GBS	group B <i>Streptococcus</i>
CPS	capsular polysaccharide
TT	tetanus toxoid
PMN	polymorphonuclear leukocyte
GMC	geometric mean concentration

References

1. Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison L, Petit S, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005. *JAMA*. 2008; 299:2056–65. [PubMed: 18460666]
2. Centers for Disease Control and Prevention. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, Group B *Streptococcus*, provisional-2010. 2010. Available via the internet: <http://cdc.gov/abcs/reports-findings/survreports/gbs10.pdf>
3. 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. [PubMed: 768760]
4. Baker CJ, Kasper DL, Tager IB, Paredes A, Alpert S, McCormack WM, et al. Quantitative determination of antibody to capsular polysaccharide in infection with type III strains of group B *Streptococcus*. *J Clin Invest*. 1977; 59:810–8. [PubMed: 856869]

5. Baker CJ, Paoletti LC, Wessels MR, Guttormsen H-K, Rench MA, Hickman ME, et al. Safety and immunogenicity of capsular polysaccharide-tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib. *J Infect Dis.* 1999; 179:142–50. [PubMed: 9841833]
6. Baker CJ, Rench MA, Fernandez M, Paoletti LC, Kasper DL, Edwards MS. Safety and immunogenicity of a bivalent group B streptococcal conjugate vaccine for serotypes II and III. *J Infect Dis.* 2003; 188:66–73. [PubMed: 12825173]
7. Baker CJ, Rench MA, McInnes P. Immunization of pregnant women with group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine. *Vaccine.* 2003; 21:3468–72. [PubMed: 12850362]
8. Baker CJ, Rench MA, Paoletti LC, Edwards MS. Dose-response to type V group B streptococcal polysaccharide-tetanus toxoid conjugate vaccine in healthy adults. *Vaccine.* 2007; 25:55–63. [PubMed: 16919857]
9. Baker CJ, Paoletti LC, Rench MA, Guttormsen H-K, Edwards MS, Kasper DL. Immune response of healthy women to 2 different group B streptococcal type V capsular polysaccharide-protein conjugate vaccines. *J Infect Dis.* 2004; 189:1103–12. [PubMed: 14999615]
10. Palazzi DL, Rench MA, Edwards MS, Baker CJ. Use of type V group B streptococcal conjugate vaccine in adults 65–85 years old. *J Infect Dis.* 2004; 190:558–64. [PubMed: 15243932]
11. DEVANI. [Accessed 4/20/2011] Vaccine against neonatal infections: Design of a vaccine to immunize neonates against GBS infections through a durable maternal response. at <http://www.devaniproject.org/>
12. Hillier, S.; Ferris, D.; Fine, P.; Ferrieri, P.; Edwards, M.; Carey, V., et al. Women receiving group B *Streptococcus* serotype III tetanus toxoid (GBS III-TT) vaccine have reduced vaginal and rectal acquisition of GBS type III. Abstracts of the 47th Infectious Diseases Society of America Annual Meeting; Philadelphia, PA. 2009. p. abstract #186
13. Hall MA, Edwards MS, Baker CJ. Complement and antibody participation in opsonophagocytosis of type IV and V group B streptococci. *Infect Immun.* 1992; 60:5030–5. [PubMed: 1452334]
14. Lin F-YC, Philips JB III, Azimi PH, Weisman LE, Clark P, Rhoads GG, et al. Level of maternal antibody required to protect neonates against early-onset disease caused by group B *Streptococcus* type Ia: A multicenter, seroepidemiology study. *J Infect Dis.* 2001; 184:1022–8. [PubMed: 11574917]
15. Lin F-YC, Weisman LE, Azimi PH, Philips JB III, Clark P, Regan J, 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. [PubMed: 15295698]
16. Pollard AJ, Perrett KP, Beverley PC. Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. *Nat Rev Immunol.* 2009; 9:213–20. [PubMed: 19214194]
17. Snape MD, Kelly DF, Lewis S, Banner C, Kibwana L, Moore CE, et al. Seroprotection against serogroup C meningococcal disease in adolescents in the United Kingdom: observational study. *BMJ.* 2008; 336:1487–91. [PubMed: 18535032]
18. Artz AS, Ershler WB, Longo DL. Pneumococcal vaccination and revaccination of older adults. *Clin Microbiol Rev.* 2003; 16:308–18. [PubMed: 12692100]
19. Campbell JR, Hillier SL, Krohn MA, Ferrieri P, Zaleznik DF, Baker CJ. Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery. *Obstet Gynecol.* 2000; 96:498–503. [PubMed: 11004347]
20. Madhi SA, et al. Inferior quantitative and qualitative immune responses to pneumococcal conjugate vaccine in infants with nasopharyngeal colonization by *Streptococcus pneumoniae* during the primary series of immunization. *Vaccine.* 2011
21. Simell B, Vuorela A, Ekström N, Palmu A, Reunanen A, Meri S, et al. Aging reduces the functionality of anti-pneumococcal antibodies and the killing of *Streptococcus pneumoniae* by neutrophil phagocytosis. *Vaccine.* 2011; 29:1929–34. [PubMed: 21236231]
22. Romero-Steiner S, Musher DM, Cetron MS, Pais LB, Groover JE, Fiore AE, et al. Reduction in functional antibody activity against *Streptococcus pneumoniae* in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity. *Clin Infect Dis.* 1999; 29:281–8. [PubMed: 10476727]

HIGHLIGHTS

- Antibody responses persist 18–24 months after receipt of GBS glycoconjugate vaccines
- Substantial functional activity against GBS persisted 18–24 months after immunization
- Robust killing of GBS persisted despite a decline in antibody concentrations

Figure 1.

Opsonophagocytosis of the immunizing type of group B *Streptococcus* (GBS) before and at specified post-immunization intervals for 10 recipients of Ia-TT (finely hatched bars), 13 recipients of III-TT (open bars) and 10 recipients of V-CRM₁₉₇ (n = 7) or V-TT (n = 3 subjects) (coarsely hatched bars) who were selected to have low pre-immunization concentrations of type-specific CPS IgG (vaccine naïve). The mean ± S.E.M. log₁₀ reduction in cfu of GBS in post-immunization sera from each of the three groups of GBS glycoconjugate vaccine recipients exhibited significantly greater functional activity (P 0.01) when compared to pre-immunization specimens at each interval tested. Declines in functional activity were observed comparing 4 week to 2 years (Ia-TT) or 78 weeks (III-TT) (* P < 0.01), 52 to 78 weeks (III-TT) (** P = 0.04) and 1 to 2 years (V-TT) (*** P = 0.047) post-immunization.

Figure 2.

Percent of sera with at least 1 log₁₀ reduction in cfu by opsonophagocytic assay at the intervals specified after immunization with Ia-TT (finely hatched bars), III-TT (open bars) or V-CRM₁₉₇ or V-TT (coarsely hatched bars) group B streptococcal (GBS) glycoconjugate vaccines.

TABLE Durability of immune responses to group B streptococcal (GBS) tetanus toxoid (TT) or CRM₁₉₇ glycoconjugate vaccines.

Vaccine (number of recipients)	Weeks after immunization, GMC (μg/mL) of CPS-specific IgG (range) [95% CI]			
	0	4	52 ^a	104 ^b
Ia-TT (10)	0.2 (0.03–1.8) [0.05–0.6]	8.1 ^c (0.88–99.6) [2.6–25.8]	4.7 ^{c,e} (1.4–41.5) [2.0–11.1]	4.8 ^{c,e,g} (1.0–32.8) [1.7–13.2]
III-TT (13)	0.06 (0.03–0.3) [0.04–0.8]	5.7 ^c (1.5–14.1) [3.8–8.5]	2.3 ^{c,f} (0.7–6.2) [1.5–3.5]	2.1 ^{c,f,g} (0.6–9.5) ^a [1.3–3.3]
V-TT or V-CRM ₁₉₇ (10)	0.05 (0.02–0.2) [0.03–0.09]	6.9 ^d (1.1–44.0) [2.8–16.9]	4.3 ^{d,e} (0.6–24.8) ^b [1.7–10.9]	2.7 ^{d,f,h} (0.4–12.4) [1.2–5.8]

^aSera were obtained at 26 weeks post-immunization for V-TT recipients.

^bSera were obtained at 78 weeks post-immunization for III-TT recipients.

^c $P < 0.001$ when compared to pre-immunization values by paired *t*-test on log-transformed data.

^d $P = 0.02$ when compared to pre-immunization values by paired *t*-test on log-transformed data.

^e $P < 0.04$ when compared to 4-week values by Wilcoxon ranked sum test on log-transformed data.

^f $P < 0.01$ when compared to 4-weeks values by Wilcoxon ranked sum test on log-transformed data.

^g $P > 0.05$ when compared to 52-week values by Wilcoxon ranked sum test on log-transformed data.

^h $P = 0.02$ when compared to 26-week values by Wilcoxon ranked sum test on log-transformed data.