Serotype-Specific Cell-Mediated Immunity Associated With Clearance of Homotypic Group B Streptococcus Rectovaginal Colonization in Pregnant Women

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We investigated the association between group B Streptococcus (GBS) serotype-specific capsular polysaccharide cellular immunity, measured with enzyme-linked immunospot (ELISPOT) interferon γ release assay at 20 weeks gestation in pregnant women, and its effect on rectovaginal serotype-specific GBS colonization up to 37 weeks gestation. Among women colonized by serotype III at enrollment, interferon γ ELISPOT positivity was more common in those in whom colonization was cleared (44.4%) than in those in whom colonization persisted (7.4%; P = .008), with a similar trend observed for serotype Ia. Presence of serotype-specific capsular polysaccharide cell-mediated immunity contributes to the clearance of GBS rectovaginal colonization.

Keywords. group B Streptococcus; cellular immunity; colonization.

Maternal rectovaginal colonization with group B Streptococcus (GBS) and its vertical transmission to newborns is the primary source of early-onset (<7 days age) invasive GBS disease [1] and has also been associated with stillbirths and premature deliveries [2]. Spontaneous clearance and acquisition of GBS has been reported in pregnant women [3, 4]; however, the host immune mediators influencing the dynamics of GBS colonization remain to be fully elucidated. We established in an earlier study that capsular-specific antibody and opsonophagocytic activity reduced the risk of new homotypic serotype acquisition between 20 and 37+ weeks of pregnancy, but neither was associated with clearance of GBS colonization [5]. The aim of this study was to determine the association between the presence of GBS cellular immunity, as assessed by enzyme-linked immunospot (ELISPOT) interferon (IFN) γ release assays, and the dynamics of GBS colonization in women during the latter half of pregnancy.

MATERIAL AND METHODS

Study Participants and Design

Detailed study methods have been published elsewhere [6]. Briefly, pregnant women aged 18–45 years, who were without human immunodeficiency virus infection and at 20–25 weeks gestation, were enrolled at antenatal community clinics in Soweto, Johannesburg, from August 2010 to August 2011. Exclusion criteria included antibiotic treatment in the previous 2 weeks, any acute illness, symptomatic vaginal discharge, and a known or suspected condition in which clinical vaginal examination was contraindicated.

Study procedures included lower vaginal and rectal swab sample collection for GBS culture starting at 20–25 weeks (visit 1), followed by 3 subsequent visits (visits 2–4) at 5–6 weekly intervals, until 37–40 weeks gestation (visit 4). For GBS isolation, swab samples were inoculated onto CHROMagar StrepB agar (CA; MediaMage), as described elsewhere [7]. Serotyping was performed using the latex agglutination method, as described elsewhere [8]. Isolates that tested negative at latex agglutination for all serotypes were further typed with a polymerase chain reaction method, using primer sequences described by Poyart et al [9].

Measure of Cellular Immune Response

Cellular immune response was determined at visit 1 (enrollment) by IFN-γ release assays, with an ELISPOT assay kit for human IFN-γ according to the manufacturer’s instructions (catalog No. 3420-2A; Mabtech). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized blood using Ficoll gradient centrifugation and resuspended in AIM-V medium (Gibco, Invitrogen). About 0.25–0.3 × 10^6 PBMCs per well were added to ELISPOT plates (MAIP S4510; Millipore) coated with IFN-γ capture antibody (Mabtech). PBMCs were stimulated with GBS serotype-specific capsular polysaccharide (CPS) antigens separately (CPS Ia, III, and V) for 18–24 hours at 37°C in 5% carbon dioxide, with a final concentration of 40 µg/mL. (GBS CPS antigens were provided by Novartis Vaccines).

After incubation, ELISPOT plates were developed using biotinylated anti–IFN-γ detection antibody (Mabtech), detected with streptavidin–alkaline phosphatase (Mabtech) and visualized with an alkaline phosphatase conjugate substrate kit.
(Bio-Rad). Checkerboard titrations were performed to optimize PBMCs and antigen concentrations per well. AIM-V medium was used as a negative control to assess background levels, and phytohaemagglutinin (Sigma) was used as a positive control. Cellular immune responses against each antigen were detected in single wells only. Spot-forming units (SFUs) were counted with a magnifying lens. Background (SFUs in negative control) was subtracted, and results were expressed as SFUs per $10^6$ PBMCs. An ELISPOT response was considered positive if the number of antigen-specific spots was $\geq 7$ SFUs/$10^6$ PBMCs and at least double the number in the negative control well. In addition, serotype-specific serum CPS immunoglobulin (Ig) G antibody concentrations were measured by means multiplex Lumimex (serotypes Ia, III, and V) and opsonophagocytic activity (OPA) (serotypes Ia and III) assay, as described elsewhere [5].

**Study Definition**

A participant was considered colonized at a visit if GBS was cultured on either the vaginal or rectal swab sample and noncolonized if no growth was detected from either site. The association of cellular immunity with clearance of serotype-specific GBS colonization was evaluated in women who were colonized by the specific serotype at visit 1 and who completed all 4 study visits. The women were stratified into those in whom colonization of the specific serotypes was cleared by visit 4 (the "cleared-colonization" group) and those who remained colonized throughout the study (the "persistently colonized" group). Because of limitations associated with detecting multiple serotype carriage using standard culture methods, we excluded from analysis participants colonized with any GBS serotype at visit 1 who acquired a different GBS serotype at a subsequent visit and remained colonized with that serotype thereafter. We also evaluated the association of cellular immunity and acquisition of serotype-specific GBS colonization in women not colonized by a specific GBS serotype at visit 1, who were subsequently colonized by that specific serotype (irrespective of the number of study visits completed) ("new-acquisition" group), compared with those in women not colonized with that specific serotype at any study visit ("noncolonized" group).

**Statistical Analysis**

For categorical variables, groups were compared with either $\chi^2$ or Fisher 2-tailed exact tests, as appropriate. The Mann–Whitney test was used to detect differences in SFU counts between groups (persistently colonized vs cleared colonization). The correlations between serotype-specific serum IgG and serotype-specific OPA titers and the ELISPOT SFU count (per $10^6$ PBMCs) at visit 1 were calculated using Spearman correlation coefficients. To explore the association between SFU counts and clearance of GBS colonization, we used a Bayesian model to estimate the posterior distribution of the probability that a woman with a GBS homotypic SFU count (per $10^6$ PBMCs) greater or equal to $c$ would clear GBS colonization (D), denoted by Probability ($P$) (clearance of GBS colonization ($D = 1|SFU \geq c$) [10]. For each value $c$, we graphically display the 25%, 50%, and 75% quantiles of the posterior distribution of $P(D = 1|SFU \geq c)$. Data were analyzed using GraphPad (version 5.0; GraphPad Software) and R (version 2.15) software [11]. Differences were considered significant at $P < .05$.

**Ethics Statement**

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (institutional review board protocol M090937), and informed written consent was obtained from all participants.

**RESULTS**

Of 661 participants, 76.7% (507 of 661) completed all 4 study visits and 95.1% (629 of 661) completed at least 2 study visits. A detailed profile of participant demographics (Supplementary Table 1) and follow-up has been reported elsewhere [6]. Of the 507 women who completed all scheduled visits, 71 (14.0%), 56 (11.1%), and 15 (2.9%) were colonized by serotypes Ia, III, and V, respectively at enrollment. Of the women already colonized at enrollment, 21 of 71 (29.6%), 27 of 56 (48.2%), and 2 of 15 (13.3%) were categorized as being persistently colonized; and 43 (60.6%), 18 (32.1%), and 9 (60.0%) were in the cleared-colonization groups for serotypes Ia, III, and V, respectively. This excluded 22 participants colonized at enrollment who cleared the initial serotype (Ia (7 of 71; 9.8%), III (11 of 56; 19.6%), and V (4 of 15; 26.7%) but who acquired a new serotype at a subsequent visit and remained colonized with the subsequent serotype thereafter. There were no differences in demographic characteristics between the persistently colonized and cleared-colonization groups (data not shown).

Of those not colonized by specific GBS serotype Ia, III, or V at enrollment and who had $\geq 1$ subsequent study visit, 9.6% (52 of 541), 7.0% (39 of 560), and 2.8% (17 of 603) subsequently become colonized by the respective serotypes (ie, new-acquisition group). Of participants who completed all scheduled visits, 387 (76.3%), 414 (81.6%), and 471 (92.9%) for serotypes Ia, III, and V, respectively (ie, noncolonized group), were never colonized by that specific serotype. There were no differences in demographic characteristics between the new-acquisition and noncolonized group [5].

Of the 661 enrolled participants in whom cellular immune response was determined at the first visit, 6 participants were excluded from the analysis owing to contamination detected in the assay. Of the remaining 655 participants, serotype-specific ELISPOT responses were prevalent in 191 (29.2%), 154 (23.5%), and 143 (21.8%) for serotypes Ia, III, and V, respectively, at visit 1. There was no correlation between serotype-specific serum IgG concentrations and SFU counts at ELISPOT assay for serotypes Ia ($\rho = 0.006; P = .89$), III ($\rho = 0.023; P = .55$), or V ($\rho = 0.021; P = .59$) (Supplementary Figure 1A–C). Similarly,
no correlation was observed between serotype-specific OPA titers and SFU count for either serotype Ia (\(p = 0.017; P = .66\)) or III (\(p = 0.042; P = .28\)) (Supplementary Figure 1D and 1E).

Among women colonized with serotype III at enrollment, ELISPOT positivity was found in higher percentage of those who cleared colonization (44.4%) than of those who remained persistently colonized (7.4%; \(P = .008\)), with a similar trend for serotype Ia (44.2% vs 19.0%, respectively; \(P = .06\)) and serotype V (22.9% vs 0.0%, respectively; Figure 1). Serotype-specific median SFU counts tended to be higher among those who cleared colonization than among those persistently colonized for serotypes III (\(P = .055\)) and Ia (\(P = .14\)), albeit not significant (Supplementary Figure 2).

In a Bayesian framework, the probability of losing colonization increased with higher SFU counts (Supplementary Figure 3). For serotype III, we observed 50% and 70% probability of losing colonization with SFU counts \(\geq 7\times10^6\) and \(\geq 22\times10^6\) PBMCs, respectively. For serotype Ia, we observed 82% probability of losing colonization with SFU counts \(\geq 7\times10^6\) PBMCs. There was no correlation between serotype-specific ELISPOT positivity and clearance of nonhomotypic serotype. The lowest threshold associated with clearance of serotype III colonization was \(\geq 7\) SFUs/10^6, with prevalences of 44.4% (8 of 18) in the cleared-colonization versus 7.4% (2 of 27) in the persistently colonized group (odds ratio, 10.00; 95% confidence interval, 1.80–55.55; \(P = .008\)) and 48.8% (21 of 43) and 19% (4 of 21) in the respective groups for serotype Ia (4.06; 1.17–14.06; \(P = .03\)) (Table 1).

Furthermore, whereas we previously reported significant associations between serotype-specific antibody concentration and OPA in relation to subsequent new acquisition of GBS during pregnancy [5], there was no significant difference in ELISPOT positivity between women who became colonized (new-acquisition group) and those who remained uncolonized (noncolonized group) for serotypes Ia (21.6% [11 of 51] vs 29.4% [113 of 384]; \(P = .24\)), III (17.9% [7 of 39] vs 23.9% [98 of 410]; \(P = .40\)) or V (11.8% [2 of 17] vs 21.8% [102 of 467]; \(P = .55\)). Excluded from analysis owing to contamination were 3 participants for serotype Ia and 4 each for serotypes III and V from the noncolonized group and 1 participant for serotype Ia from the new-acquisition group.

**DISCUSSION**

We have shown that clearance of serotype-specific GBS rectovaginal colonization during pregnancy was associated with presence of homotypic capsular ELISPOT IFN-\(\gamma\) positivity, whereas no such association was observed for serotype-specific capsular antibody or OPA, as reported for the same cohort [5]. Conversely, ELISPOT positivity was not associated with a significantly reduced risk of GBS acquisition, which was, however, positively associated with serotype-specific capsular antibody and OPA for serotypes Ia and III, as reported. These data suggest that presence of cell-mediated immune response contributes to the clearance of rectovaginal GBS colonization, whereas we have reported elsewhere that humoral immunity is required to reduce the risk of rectovaginal GBS acquisition during pregnancy [5].

The results of the current study are in accordance with findings of studies on other capsular bacteria, such as *Streptococcus pneumoniae*, for which cell-mediated immune response is important for mucosal clearance in animal models [12, 13]. A serotype-specific capsular-based GBS vaccine able to elicit
both humoral and cell-mediated capsular immune responses could therefore confer protection against early-onset disease by reducing the exposure of newborns to GBS colonization during the peripartum period. This could offer an additional mode of protection against invasive GBS disease, especially for preterm newborns, who might not fully benefit from transplacental acquisition of maternal serotype-specific capsular antibody, which mainly occurs beyond 34 weeks of gestation. Moreover, clearance of GBS rectovaginal colonization during pregnancy, coupled with prevention of new acquisition mediated by the presence of humoral immunity, could theoretically mitigate against other GBS-associated illness, such as chorioamnionitis, and adverse birth outcomes, such as stillbirths and premature labor. However, to affect the full spectrum of GBS-associated morbidity and mortality in women, fetuses, and newborns, vaccination would need to occur early in the second trimester of pregnancy and induce serotype-specific functional antibody and cellular immunity.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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