

Placental transfer of anti-group B *Streptococcus* immunoglobulin G antibody subclasses from HIV-infected and uninfected women to their uninfected infants

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Objectives: Placental antibody transfer is impaired in the context of HIV infection, which may render HIV-exposed, uninfected infants vulnerable to group B *Streptococcus* (GBS) disease. The GBS antibody response predominately consists of immunoglobulin G2 (IgG2) antibody. Thus we determined whether concentration and placental transfer of anti-GBS antibody subclasses was altered in HIV-infected compared with HIV-uninfected mothers.

Design: A retrospective analysis of anti-GBS antibody subclasses in 38 HIV-infected and 33 HIV-uninfected mothers and their uninfected infants.

Methods: Sera were analysed using a novel flow cytometric assay that quantified binding of IgG1, IgG2, IgG3 and IgG4 to serotype (ST)Ia, STIII and STV GBS bacteria.

Results: IgG2 binding to GBS STIa and V was lower in HIV-infected women compared with HIV-uninfected women. Moreover, IgG2 binding to GBS STIa was also lower in HIV-exposed, uninfected infants compared with unexposed infants. However, there were no statistically significant differences in the transplacental transfer ratio of IgG2 for any GBS serotype. The transplacental transfer of total IgG was reduced for GBS STIII and V and IgG1 subclass for STIII; placental transfer of all other subclasses was comparable in HIV-affected and HIV-unaffected pregnancies.

Conclusion: Anti-GBS IgG2 placental transfer is not affected by HIV infection. This is important for functional antibody against the capsular polysaccharide of GBS and provides confidence that maternal GBS vaccination may result in functional activity in HIV-infected and uninfected women.

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Introduction

Opsonophagocytosis of group B *Streptococcus* (GBS) is mediated predominately by the immunoglobulin G2 (IgG2) subclass [1], which is poorly transported across the placenta compared with IgG1 [2,3]. This may reflect the lower affinity of IgG2 for the FcRn receptors on syncytiotrophoblasts of placental cells as compared with the other subclasses of IgG [4,5]. Studies comparing maternal and infant antibody concentrations demonstrate that anti-GBS capsular antibody is transferred across the placenta with a high degree of efficiency [6]. However, the proportionally higher concentration of antibody in infant compared with maternal serum at birth is thought to be mainly due to excess foetal IgG1 [2]. It has been demonstrated for GBS serotype (ST) Ia and STIII that not only is total anti-GBS antibody concentration lower in GBS-infected infants than in infants without infection born to colonized mothers [7,8], but that IgG2 is also lower in infants with GBS infection [9]. Recent studies have shown that maternal and placental transfer of total anti-GBS antibody is reduced in the context of maternal HIV-infection [10,11]. This might explain the greater reported incidence of early, and especially late onset, GBS disease observed amongst HIV-exposed, uninfected infants compared with unexposed infants [12]. A West African study identified hypergammaglobulinaemia as a risk factor for reduced placental transfer of IgG subclasses and this might be a further contributing factor to the observed excess of GBS morbidity amongst HIV-exposed infants [13]. Thus, we compared total and subclass anti-GBS antibody placental transfer in HIV-infected and HIV-uninfected South African women.

Methods

Samples were collected from mothers and infants enrolled in a cohort study investigating the influence of maternal HIV and mycobacterial sensitization on infant immune responses to *Bacillus Calmette-Guérin* (BCG) vaccination carried out between 2009 and 2011 [14]. The demographic details, CD4⁺ cell count, viral load and antiretroviral medication have been previously described [14]. Informed consent was obtained from all mothers participating in the study. The study was approved by the Universities of Cape Town (382/2008) and Stellenbosch (N08/10/278), South Africa, and the National Health Service Research Ethics Committee, England (07/H0720/178).

Paired sera from 38 HIV-infected and 33 HIV-uninfected mothers and their uninfected infants were available to analyse immunoglobulin subclasses. Deposition of total IgG and IgG1, IgG2, IgG3 and IgG4 anti-GBS antibody subclasses onto the surface of formaldehyde-fixed GBS bacteria was measured using a flow cytometric assay

performed in 96-well microtitre plates as previously described [10]. Mouse monoclonal antibodies: 4E3 antihuman IgG1 H&L, HP6014 antihuman IgG2 fd gamma, HP6050 antihuman IgG3 hinge heavy chain and mouse HP6025 antihuman IgG4 Fc (FITC) (Life Technologies, Carlsbad, California, USA) (1:500) in blocking buffer were added and samples incubated for 20 min at 4°C. Assays were analysed using a Beckman Coulter Cyan flow cytometer equipped with a Cytek 96-well microtitre plate loader. A fluorescence index (FI) was calculated for each sample, which involved the multiplication of the percentage of bacteria in the horizontal gate (%-gated), by the mean fluorescence of that population (X-mean). The final result for each test was expressed as the average FI of duplicate test samples minus the average FI of the bacteria and conjugate-only control. A standard unit (SU) measurement for each serum sample was then calculated by comparing to the serum FI response obtained with the positive control serum for each serotype which was given an arbitrary value of 1000 (kind gift from Professor Carol Baker, Baylor College of Medicine, Texas, USA) to give a result in SU/ml.

Statistical analyses were completed using STATA version 12 (StataCorp 2013, College Station, Texas, USA) and GraphPad Prism version 6.0 (GraphPad Software Inc., La Jolla, California, USA). The sample size was calculated to demonstrate a 50% difference in IgG subclass concentrations between HIV-exposed and HIV-unexposed infants with the predefined assumption that antibody subclass concentrations would be lower in HIV-exposed infants. Using an α error of 5%, 80% test power and a 95% confidence interval, the sample size ranged from 10 to 30 in each group. Placental transfer was defined as the ratio of infant-to-mother GBS IgG concentration at birth. Missing data were excluded from analysis. We considered *P* values less than 0.05 as statistically significant.

Results

The paired sera from 71 mother–infant dyads (38 HIV-infected mothers and their uninfected infants and 33 HIV-uninfected mothers and their infants) at birth were available for analysis of total IgG, IgG1 and IgG2 for all serotypes. For serotypes ST1a and STV, sufficient sera was available from 60 mother–infant pairs to analyse subclasses IgG3 and IgG4. Sufficient sera were available from 40 mother–infant pairs for analysis of IgG3 and IgG4 for STIII.

Antibody subclass concentrations

Concentrations of anti-GBS IgG1 in mothers and infants were higher than concentrations of IgG2 for all GBS serotypes, independent of HIV status (Fig. 1).

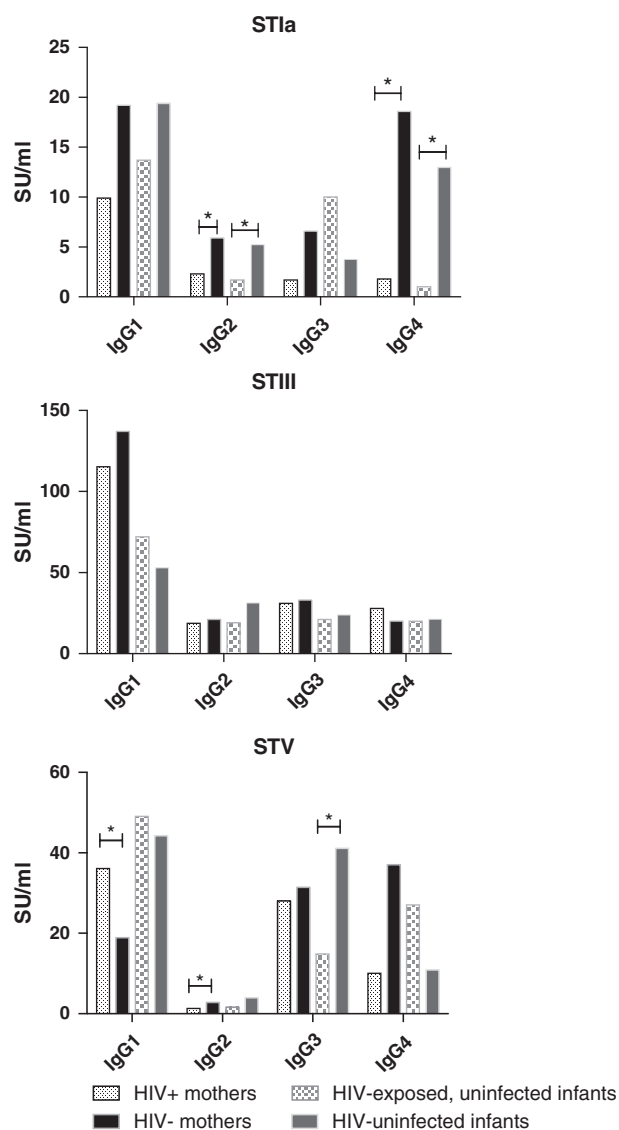


Fig. 1. Anti-group B *Streptococcus* subclass distribution in HIV-infected and HIV-uninfected mothers and their uninfected infants at birth. HIV-, HIV uninfected; HIV+, HIV infected; IgG, immunoglobulin G; ST, serotype; SU/ml, standard units per millilitre. Horizontal bars compare groups, * $P < 0.05$.

For STIa, HIV-uninfected women had significantly higher geometric mean concentration (GMC) of subclass IgG2 and IgG4 than HIV-infected women (IgG2 2.3 [95% confidence interval (CI) 1.6–3.3] vs. 5.9 [4.0–8.9] SU/ml, $P = 0.02$; IgG4 1.8 [0.9–3.6] vs. 18.6 [6.0–57.8] SU/ml, $P = 0.03$) but comparable concentrations of IgG1 and IgG3 (Fig. 1). In HIV-unexposed compared with HIV-exposed, uninfected infants, there was a significantly higher concentration of IgG2 ($P = 0.03$) and IgG4 ($P = 0.03$). In addition, we noted a trend towards higher concentrations of anti-STIa IgG1 and lower concentration of IgG3 but this did not reach statistical significance (Fig. 1).

For STV, HIV-infected women had a trend towards higher concentrations of IgG1 than HIV-uninfected women ($P = 0.049$) and HIV-uninfected women had significantly higher concentrations of IgG2 than HIV-infected women ($P = 0.047$), although the concentration of IgG2 was low in both groups. HIV-unexposed infants had higher concentrations of anti-STV IgG3 antibody compared with HIV-exposed infants ($P = 0.039$). There was no statistical difference between HIV-exposed, uninfected infants and HIV-unexposed infants for any of the other IgG subclasses (Fig. 1).

There was no significant difference between anti-STIII IgG subclasses in HIV-infected and HIV-uninfected women and HIV-exposed, uninfected and HIV-unexposed infants.

Placental transport of total group B *Streptococcus* antibody

Total IgG transplacental antibody transfer ratio (TPR) was reduced in HIV-infected compared with HIV-uninfected women for GBS STIII [0.6 (0.3–1.1) vs. 1.3 (0.8–2.3) SU/ml, $P = 0.04$] and STV [1.2 (0.7–1.6) vs. 2.0 (1.1–3.0) SU/ml, $P = 0.04$] but not for STIa. (Table 1). There was no association between CD4⁺ cell count or viral load and TPR.

Placental transport of subclasses group B *Streptococcus* antibody

TPR of IgG1 was decreased to HIV-exposed infants compared with HIV-unexposed infants [0.4 (0.1–1.4) vs.

Table 1. Geometric mean concentration (GMC) transplacental transfer ratios of total and subclass IgG1, IgG2, IgG3 and IgG4 against GBS STIa, III and V in HIV-infected and HIV-uninfected mothers to their infants [95% confidence interval (CI)].

	STIa			STIII			STV		
	HIV+	HIV-	<i>P</i>	HIV+	HIV-	<i>P</i>	HIV+	HIV-	<i>P</i>
Total	1.1 (0.8–1.6)	1.0 (0.6–1.6)	0.75	0.6 (0.3–1.1)	1.3 (0.8–2.3)	0.04	1.2 (0.7–1.6)	2.0 (1.1–3.0)	0.04
IgG1	1.0 (0.7–1.5)	1.4 (1.0–2.0)	0.44	0.4 (0.1–1.4)	1.3 (0.7–2.5)	0.04	1.0 (0.7–1.4)	1.4 (0.9–2.1)	0.14
IgG2	0.9 (0.5–1.5)	0.7 (0.5–1.2)	0.43	1.1 (0.4–3.2)	0.8 (0.4–1.4)	0.38	0.7 (0.5–1.1)	0.8 (0.6–1.1)	0.59
IgG3	0.3 (0–9)	1.0 (0.3–3.3)	0.5				0.9 (0.6–1.4)	1.3 (0.8–2.1)	0.23
IgG4	0.5 (0.2–1.1)	1.6 (0–4)	0.08				1.2 (0.5–2.9)	0.6 (0.3–1.4)	0.31

GBS, group B *Streptococcus*; HIV-, HIV-uninfected; HIV+, HIV infected; IgG, immunoglobulin G; ST, serotype.

1.3 (0.7–2.5) SU/ml; $P=0.04$] for STIII but not for STIa or STV (Table 1). The TPR for IgG2 was unaffected by maternal HIV infection status for any serotype. There were no statistical differences between antibody TPR for any other subclass or any other serotype of GBS between groups.

Discussion

Our findings demonstrate that whilst total transplacental transfer ratios are reduced in HIV-infected mothers for GBS STIII and V, the proportion of individual subclasses transferred from mother to infant is unaffected by maternal HIV infection, with the exception of IgG1 to GBS STIII. Importantly, there was no difference in the placental transfer of IgG2 that binds to any of the GBS serotypes.

HIV-exposed, uninfected infants may be at increased risk of GBS infection, in particular late onset disease [10,12]. The observation that there is no difference in the proportion of IgG subclass placental transfer (except for IgG1 for STIII) may mean that HIV-exposed, uninfected infants are at increased risk of disease as a result of their mother's reduced total antibodies that bind to GBS bacteria, rather than a selective deficit in IgG2 transfer. It may alternatively be due to impaired B cell function in the context of HIV infection that results from poor function of T helper cellular response and function of antibody in the context of HIV-infection [15].

The fact that IgG2 surface deposition was lower in HIV-exposed infants to STIa and in women to STV in HIV-infected women may indicate reduced antibody function due to HIV-infection. In this study we were unable to assess the opsonophagocytic ability of the antibody which would provide additional information on the functional ability of antibody to protect from GBS infection. In studies of pneumococcal vaccination, opsonophagocytosis was impaired in HIV-infected compared with HIV-uninfected individuals and the same may be true of GBS [16].

The serotypes we have selected in this study represent 77% of colonizing strains in South African pregnant women (STIII, 33%; STIa, 39%; STV, 12% [17]). They also represent 86.1% of early onset GBS disease and 100% of late onset disease (STIII 57.7% of early onset, 84.4% of late onset disease; STIa 22.6%/ 13.9%; STV 5.8%/1.9% [18]). Thus, the serotype-specific antibody observed corresponds to the predominant colonizing and disease-causing serotypes in South Africa.

Although patients with HIV disease develop high levels of total IgG in serum soon after infection [19], the IgG1 subclass is preferentially increased, whereas levels of total IgG2 may be normal or decreased [20]. We confirmed the

relatively similar concentration of IgG2 antibody that binds to GBS STIII in the sera of HIV-infected and HIV-uninfected women. IgG2 responses to the bacterial capsular polysaccharide in humans may be driven in part by IFN- γ production [21], which may be impaired during HIV-infection [22]. However, HIV-infected patients in our cohort showed no selective deficit in IgG2 production. The differences in subclass distribution by GBS serotype may be as a result of different reactivity of the different serotypes in our assay. However, comparison with the larger study demonstrated good correlation between total antibody concentrations for all serotypes tested [10].

Maternal HIV infection has been associated with reduced placental transfer of antibodies against several common viral and bacterial antigens [23,24]. Additionally, the finding that HIV-exposed, uninfected infants are more likely to have sub-protective antibody levels to vaccine preventable diseases means that these infants are likely to be more prone to these diseases in early childhood. Our finding that the placental transfer of IgG2 subclass, important for the opsonization and killing of GBS, was not impaired indicates potential for a maternal vaccine to boost both maternal and infant immunity in areas of high HIV-prevalence.

Our sample size was limited and we are not able to draw conclusions on other possible effects of HIV-infection on placental transfer. Additionally, we cannot comment on the colonizing serotypes of the mothers. However, the fact that the antibody against the major GBS serotypes was detected in all women suggests prior colonization with GBS. Finally, we cannot comment on protective levels of antibody in this population, as there were no cases of GBS disease in our cohort.

In summary, the decreased concentration of serum IgG that binds to the surface of GBS in HIV-infected women and their infants compared with non-HIV-infected dyads is not due to a selective defect in the generation of GBS-specific IgG2 antibody. Future efforts should address enhancing protective antibody concentrations through maternal vaccination against GBS during pregnancy to optimize IgG2 concentration in the neonate.

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substantially contributed to the development of the manuscript.

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Conflicts of interest

The authors do not have a commercial or other association that might pose a conflict of interest.

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