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Natural acquired group B *Streptococcus* capsular polysaccharide and surface protein antibodies in HIV-infected and HIV-uninfected children

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ABSTRACT

Group B *Streptococcus* (GBS) is a major cause of invasive disease in young infants and also in older immunocompromised individuals, including HIV-infected persons. We compared naturally acquired antibody titres to GBS polysaccharide and surface protein antigens in HIV-uninfected and HIV-infected children aged 4–7 years.

A multiplex Luminex immunoassay was used to measure IgG concentrations against GBS capsular polysaccharides (CPS) for serotypes Ia, Ib, III and V; and also extracellular localizing proteins which included cell-wall anchored proteins: Fibrinogen binding surface Antigen (FbsA), GBS Immunogenic Bacterial Adhesin (BibA), Surface immunogenic protein (Sip), gbs0393, gbs1356, gbs1539, gbs0392; and lipoproteins gbs0233, gbs2106 and Foldase PsrA.

HIV-infected children (n = 68) had significantly lower IgG GMT compared to HIV-uninfected (n = 77) children against CPS of serotype Ib ($p = 0.012$) and V ($p = 0.0045$), and surface proteins Sip ($p < 0.001$) and gbs2106 ($p = 0.0014$). IgG GMT against GBS surface proteins: FbsA, gbs1539, gbs1356, gbs0392, gbs0393 and Foldase PsrA were significantly higher in HIV-infected children ($p < 0.004$). Moreover, amongst HIV infected children, IgG GMT to GBS surface proteins were higher in those with $CD4^+$ lymphocyte counts <500 cell/μL compared to those who had $CD4^+$ lymphocyte count ⩾500 cell/μL with the exception of Sip.

The increased susceptibility to invasive GBS disease in HIV-infected individuals could be due to the lower serotype specific capsular antibody and possibly due to lower antibody to some of the GBS proteins such as Sip and gbs2106.

1. Introduction

Group B *Streptococcus* (GBS) is an important cause of invasive disease in neonates, but also causes disease in pregnant women, the elderly and immunocompromised individuals such as those with HIV-infection [1]. Prior to widespread use of intrapartum antibiotic prophylaxis in the USA, the age-group distribution of invasive GBS disease was 46% in neonates (⩽1 month age), 2% in children aged 1–17 years and 33% in those ⩾18 years [2]. Notably, the majority of invasive GBS disease in adults was associated with underlying conditions such as diabetes mellitus, cancer and HIV-infection [2]. HIV infection increases susceptibility to invasive bacterial infection in children, particularly encapsulated bacteria such as *Haemophilus influenzae* type b and *Streptococcus pneumoniae* [3]. There is, however, limited data on invasive GBS infection in HIV-infected children, with only few reports among severely immunocompromised children [3–7].

An inverse association has been observed for the risk of developing invasive GBS disease during early-infancy and the level of serotype-specific capsular polysaccharide antibodies derived from trans-placental transmission [8]. In healthy infants, serotype-specific capsular IgG concentration to the dominant invasive serotypes (Ia, II and III) gradually decline post-partum, reaching approximately 10% of cord-blood IgG concentrations by 6 months of age [8]. Nevertheless, invasive GBS disease is uncommon beyond three months of age in non-immunocompromised children. This could be due to immunological responses to other GBS virulence factors including surface proteins, which might protect against disease, as well as underlying immunological responses elicited by colonization during early life.

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GBS surface proteins bacterial adhesion protein A (BibA), fibrinogen binding protein (FbsA) and surface immunogenic protein (Sip) have been identified as protective immunogens in murine models [9], including influencing the risk of GBS acquisition and clearance of colonization. BibA, an anti-phagocytosis protein that inhibits a complement regulator (C4 bp) was shown to confer 69% protection against a lethal challenge dose of GBS serotype-III in mice models [10,11]. FbsA induces fibrinogen aggregation to mask GBS from opsonophagocytosis [12] and Sip, a surface exposed protein occurring in almost all GBS strains was reported to confer serotype-independent protection in 89% of mice challenged with a lethal dose of either GBS serotype Ia/c, Ib, II-II and V-VI [13].

The aim of this study was to determine whether differences in antibody titres to capsular polysaccharide (CPS) and surface protein epitopes may play a role in susceptibility to invasive GBS disease in HIV-infected children. To explore this, we compared natural acquired serum IgG antibody to serotype-specific CPS and selected GBS protein antigens between HIV-infected and HIV-uninfected children. The surface proteins evaluated, included known virulence proteins together with other novel proteins which were identified using a bioinformatics approach [14].

2. Materials and methods

2.1. Study population

We retrospectively tested available serum samples from a group of 77 HIV-uninfected and 68 HIV-infected children who had previously participated in a study on the durability of antibody responses to an investigational 9-valent pneumococcal conjugate vaccine (PCV) as detailed [15]. These children were enrolled between December 2004 to April 2005 and serum samples were collected and stored at −70 °C, before processing for this study. The sera collected were analysed for the prevalence of IgG antibody concentration for select GBS surface proteins and serotype specific CPS.

2.2. In silico selection of GBS immunogenic surface proteins

Using GBS NEM316 as the reference strain for serotype III, surface proteins were predicted using an adaptation of the Giombini et al. [16] protocol that performs automated identification of bacterial surface proteins from inserted genome and proteome sequences. A systemic process was adopted rather than a filtration method to analyse all proteins for potential cell surface features using available bioinformatics tools. SingalP was used to screen for proteins that contain a signal peptide at their N-terminal sequence required for protein export [17]. Proteins with signal peptides were cross-validated using TargetP to assess their final subcellular localization [18]. Transmembrane spanning proteins were identified using TMHMM and prodiv-HMM [19]. A cut-off $\leqslant$3 membrane helices per protein was permitted since proteins with more than 3 membrane spanning regions tend to be insoluble [20]. Extracellular localization of the proteins was further predicted using pSORTb and LipoP that screens for cell-wall anchor motifs (LPxTG or LxxC) [21,22]. Proteins that were identified as extracellular and/or cell-wall surface anchored proteins were screened for the presence of B-cell epitope regions using BepiPred and an average threshold score of 0.4 was considered significant for immunogenic proteins [23]. The selected proteins were varied according to subcellular localization and degree of heterogeneity to evaluate the association of these features to antibody response and the influence of HIV infection thereupon. GBS proteins with surface localization motifs; Sip, gbs0393, gbs0392, gbs1356, gbs2106, gbs1539 were selected to be cloned, expressed and for immunogenicity testing and Foldase PsrA was included as a representative lipoprotein.

2.3. Recombinant GBS surface proteins

GBS NEM316 serogroup III (CDC) was grown overnight on 5% sheep blood agar and subsequently cultured overnight in Todd Hewitt broth (Merck, South Africa) at 37 °C with shaking. Genomic DNA was extracted and purified with the QIAgenDNAamp kit (QIAgen, Netherlands). Genes encoding immunogenic proteins were amplified by PCR with manually-designed primers that truncated the signal peptides and cell-wall anchor motifs, Table 1. PCR mixture was inclusive of 1.0 μM of each primer (Inqaba Biotec, South Africa), 25–50 ng gDNA and Phusion Flash Enzyme mix (2.5 U DNA polymerase, DMSO, 50 μM MgCl, 400 μμM per dNTPs) (Thermo Scientific) and the reaction cycles were: an initial denaturation cycle at 98 °C for 2 min, 30 cycles of 98 °C for 5 s, 50–55 °C for 30 s, 72 °C for 15 s/kb and a final extension cycle at 72oC for 5 min. The gene amplicons were then cloned into pETite C-His vector (Lucigen, USA) and propagated in HI *E. Coli* competent 10G cells (Lucigen, USA) as per the manufacturer's recommendations. Gene sequencing of the cloned insert was performed to confirm the integrity and orientation of the cloned fragment, prior to protein expression and purification. His tagged proteins were purified with Ni^{2+} affinity chromatography, and visualized with SDS PAGE to confirm their purity and polypeptide molecular weight and were subsequently stored in PBS with 50% glycerol at −70 °C. FbsA-6313, gbs0233-NEM316, BibA-COH1 and BibA-NEM316 proteins of serotype II were obtained from Valneva Austria GmbH (Vienna, Austria). Sequence comparisons of the BibA protein of NEM316 and COH1 strains have highly identical flanking sequences and a variable internal region [24]. To represent both BibA variants in a multiplex assay with minimum cross reactivity the complete protein from GBS-COH1 strain was used and only the variable fragment of the polypeptide from the NEM316 strain was included.

2.4. Luminex assay for the quantification of antibodies to GBS antigens

We developed a multiplex Luminex assay to quantify the IgG titres against GBS CPS (Norvatis Vaccines, now part of GSK) and 11 surface proteins. CPS were coupled to carboxylated microbeads with a cross-linking reagent 4-(4,6-dimethoxy-[1,3,5]-triazi-2-yl)-4-methyl-morpholinium (DMTMM) as described previously [25]. Purified proteins were desalted by gel filtration with PD-10 desalting columns (GE healthcare, Germany). Desalted proteins were immobilized onto carboxylated beads using the 2-step carbodiimide reaction [26], at a protein concentration of 50 μg/ml per unique bead region. Conjugated beads were blocked with PBSFN buffer (PBS, 10% fetal bovine serum, 0.05% NaN_3) and stored at 4 °C.

Pooled purified IgG from adult humans (Polygam; National Bioproducts, South Africa) was used as a reference serum to measure IgG antibodies. Polygam calibrated with a standard homotypic GBS was used as reference serum to extrapolate IgG geometric mean concentration (GMC) to CPS reported as micrograms per ml (μg/ml). IgG geometric mean titres (GMT) for the protein antigens were reported as arbitrary units (U/ml) as extrapolated from Polygam sera which was assigned a value of 100 U/ml. In house high and low control sera were prepared from pooling sera from HIV-uninfected pregnant women that were either colonized or uncolonized with GBS respectively. These controls were included in the multiplex assay to assess inter-assay variation. All serum samples, controls and the reference serum were diluted 1:100 with PBSFN for immunological analysis. Samples with titres above the detection limits were further diluted. Competitive inhibition assays were conducted for all proteins in a multiplex assay with 5 μg of