Serum antibody responses to pneumococcal colonization in the first 2 years of life: results from an SE Asian longitudinal cohort study

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Abstract

Assessment of antibody responses to pneumococcal colonization in early childhood may aid our understanding of protection and inform vaccine antigen selection. Serum samples were collected from mother-infant pairs during a longitudinal pneumococcal colonization study in Burmese refugees. Maternal and cord sera were collected at birth and infants were bled monthly (1–24 months of age). Nasopharyngeal swabs were taken monthly to detect colonization. Serum IgG titres to 27 pneumococcal protein antigens were measured in 2624 sera and IgG to dominant serotypes (6B, 14, 19F, 19A and 23F) were quantified in 864 infant sera. Antibodies to all protein antigens were detectable in maternal sera. Titres to four proteins (LytB, PcpA, PhtD and PhtE) were significantly higher in mothers colonized by pneumococci at delivery. Maternally-derived antibodies to PiuA and Spr0096 were associated with delayed pneumococcal acquisition in infants in univariate, but not multivariate models. Controlling for infant age and previous homologous serotype exposure, nasopharyngeal acquisition of serotypes 19A, 23F, 14 or 19F was associated significantly with a ≥2-fold antibody response to the homologous capsule (OR 12.84, 7.52, 6.52, 5.33; $p < 0.05$). Acquisition of pneumococcal serotypes in the nasopharynx of infants was not significantly associated with a ≥2-fold rise in antibodies to any of the protein antigens studied. In conclusion, nasopharyngeal colonization in young children resulted in demonstrable serum IgG responses to pneumococcal capsules and surface/virulence proteins. However, the relationship between serum IgG and the prevention of, or response to, pneumococcal nasopharyngeal colonization remains complex. Mechanisms other than serum IgG are likely to have a role but are currently poorly understood.

Keywords: Antibody, capsule, colonization, polysaccharide, protein, *Streptococcus pneumoniae*, vaccine
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Background

Nasopharyngeal colonization is thought to always precede pneumococcal infection [1]. Immune responses to colonization are complex: experimental murine models suggest a limited role for antibody and that an IL-17A-mediated CD-4$^+$ T-cell pathway is the dominant mechanism involved in immunity to pneumococcal colonization [2,3]. However, colonization-related serum immunoglobulin G (IgG) antibody responses to capsular polysaccharide and surface/virulence proteins have been described in adult humans [4,5], although these antibodies may not protect against colonization [6]. In children aged <2 years, studies have demonstrated limited serum anti-capsular antibody development in the absence of pneumococcal immunization [7]. Several pneumococcal proteins are immunogenic in young children and antibody development is correlated with pneumococcal exposure [8]. Most studies in the literature use relatively infrequent sampling of blood to assess development of natural immunity, few combine with nasopharyngeal carriage data, and none to our knowledge have ever sampled monthly for 24 months and

then performed combined analyses of anti-capsular and anti-protein antibody responses.

Vaccines containing pneumococcal proteins have provided protection against invasive infections in mice [9]. Assessments of the relationship between nasopharyngeal colonization and antigen-specific immune responses in young children are critical for prediction of potential carriage-mediated protein vaccine hyporesponsiveness. This is important because serotype-specific hyporesponsiveness has been previously demonstrated for the currently available conjugate vaccines [10].

This manuscript describes the temporal relationships between nasopharyngeal colonization and serum IgG to pneumococcal capsular polysaccharides and surface/virulence proteins over the first 2 years of life in a population where there is early/sustained pneumococcal carriage. Effects of transplacentally-transferred IgG antibodies on infant pneumococcal colonization are explored.

Methods

Study population

Mother-infant pairs were followed from birth for 24 months during a pneumococcal colonization and pneumonia study in a camp for Burmese refugees [11,12]. At delivery, nasopharyngeal swab (NPS) and serum specimens were collected from the mother and a serum specimen from the umbilical cord. NPS were taken from mother to infant, plus serum from infants, at monthly intervals (1–24 months of age). Pneumococcal vaccines were not available to the study population.

Pneumococcal nasopharyngeal colonization

Nasopharyngeal swabs were cultured using the WHO standard protocol to detect pneumococcal colonization [13]. Briefly, a Dacron-tipped nasopharyngeal swab was used to sample the nasopharynx and the tip immediately excised into a cryotube containing 1 mL STGG (skim milk, tryptone, glucose, glycerol) transport medium. NPS-STGG specimens were transferred back to the laboratory in a cool box before being promptly frozen at −80°C. Ten microlitres of the thawed and vortexed NPS-STGG specimen were cultured overnight on sheep blood-CNA agar (bioMerieux, Marcy L'Etoile, France) at 36°C and 5% CO_2. All morphologically distinct alpha-haemolytic colonies were subcultured onto plain sheep blood agar (Clinical Diagnostics, Bangkok, Thailand) and *S. pneumoniae* was confirmed by colonial morphology and susceptibility to optochin (Oxoid, Basingstoke, UK). The bile solubility test was used to confirm isolates with equivocal optochin disc susceptibility and those non-typeable by Omniserum (SSI Diagnostica, Hillerod, Denmark). Pneumococcal isolates were serotyped by latex agglutination using a full panel of pneumococcal antiserum (SSI Diagnostica), with Quellung confirmation of equivocal results [14].

Antigens and serological methods

Serum IgG antibodies to 27 pneumococcal protein antigens were measured using a direct binding electrochemiluminescence-based multiplex assay (Table 1). The assay was based on that described for pneumococcal polysaccharide antigens utilizing MesoScale Discovery (MSD, Rockville, MD, USA) technology [15]. Pneumococcal reference serum 007 was used

TABLE 1. Protein antigens assessed in the study

Name	Internal ID	Protein details	Provided by
CbpA	PP01	Choline binding protein A, without choline binding domain (CbpA NR1XR2P)	GSK
LytB	PP11	Endo-beta-N-acetylglucosaminidase (LytB-T-PO1)	Sanofi Pasteur
LytC	PP02	Lysozyme (LytC C-ter)	GSK
NanA	PP33	Neuraminidase (NanA)	UAB
PcpA	PP13	Choline binding protein (PcpA DC6842)	Sanofi Pasteur
PcsB-1	PP06	Secreted 45 kDa protein (PcsB, SP2216-1)	Intercell
PcsB-2	PP32	Secreted 45 kDa protein (PcsB, Spr2021)	Novartis
PhtD-1	PP03	Pneumococcal histidine triad D (PhtD)	GSK
PhtD-2	PP14	Pneumococcal histidine triad protein (PhtD DC6857)	Sanofi Pasteur
PhtE	PP10	Truncated histidine triad protein (PhtE-T1 DC6286)	Sanofi Pasteur
PiaA	PP09	Part of iron uptake ABC transporter (PiaA)	PATH
PiuA	PP08	Part of iron uptake ABC transporter (PiuA)	PATH
Ply-1	PP12	Pneumolysin (WtPly DC6968)	Sanofi Pasteur
Ply-2	PP17	Pneumolysin (P10V12/13 Ply)	UAB
PsaA	PP04	Pneumococcal surface adhesin A (PsaA, SP1650)	Intercell
PspA-Fam1	PP16	Pneumococcal surface protein A, family 1 (PspA, P18-01/P18-02)	UAB
PspA-Fam2	PP15	Pneumococcal surface protein A, family 2 (PspA, UAB099 P9V63)	UAB
RrgA-T4	PP22	RrgA pilus subunit, adhesin (RrgA, T4)	Novartis
RrgB-T4	PP18	RrgB pilus subunit, backbone (RrgB, T4)	Novartis
RrgB-6B	PP19	RrgB pilus subunit, backbone (RrgB, 6B)	Novartis
RrgB-23F	PP20	RrgB pilus subunit, backbone (RrgB, 23F)	Novartis
StkP	PP05	Serine threonine kinase protein (StkP, SP1732-3)	Intercell
StrH	PP29	Beta-N-acetylhexosaminidase (StrH, Spr0057)	Novartis
SP0609	PP31	Amino acid ABC transporter, amino acid-binding protein	Intercell
SP2027	PP07	Conserved hypothetical protein (Spr1/SP2027)	Intercell
SP2194	PP30	ATP-dependent Clp protease, ATP-binding subunit	Intercell
Spr0096	PP24	LysM domain-containing protein	Novartis