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Impaired serotype-specific immune function following pneumococcal vaccination in infants with prior carriage

Paul V. Licciardi[a,b,*], Fiona M. Russell[a,c], Anne Balloch[a,b], Robert L. Burton[d,e], Moon H. Nahm[d,e], Gwendolyn Gilbert[f], Mimi L.K. Tang[a,g,h], Edward K. Mulholland[a,i,j]

[a] Pneumococcal Research Group, Australia
[b] Allergy and Immune Disorders Group, Murdoch Childrens Research Institute, Australia
[c] Centre for International Child Health, Department of Paediatrics, The University of Melbourne, Melbourne, Australia
[d] Department of Pathology, University of Alabama at Birmingham, USA
[e] Department of Microbiology, University of Alabama at Birmingham, USA
[f] Westmead Millennium Institute for Medical Research, University of Sydney, Australia
[g] The Royal Children's Hospital, Melbourne, Australia
[h] Department of Paediatrics, University of Melbourne, Melbourne, Australia
[i] Menzies School of Health Research, Darwin, Australia
[j] London School of Hygiene and Tropical Medicine, London, United Kingdom

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ABSTRACT

The impact of prior nasopharyngeal carriage on serotype-specific IgG responses following immunization with pneumococcal conjugate vaccines (PCV) has recently been described. This report extends these findings to describe the attenuation of functional immune responses following 23-valent pneumococcal polysaccharide vaccination (PPS). We report the attenuation of immune responses following booster with the 23-valent pneumococcal polysaccharide vaccination (PPS) in infants with prior nasopharyngeal carriage of *Streptococcus pneumoniae*. Fijian infants who were part of a phase II randomized, controlled trial of reduced dose PCV7 schedules were the basis of this study. Pneumococcal carriage was determined at 6, 9 and 12 months of age, prior to PPS immunization. Serum samples collected at 18 weeks (post-PCV7), 12 months (pre-PPS), 12.5 months and 17 months (post-PPS) of age were assessed for serotype-specific IgG and opsonophagocytic responses.

The most frequently carried serotypes were 6B ($N=11$), 19F ($N=14$) and 23F ($N=23$). Significantly lower serotype-specific IgG for 19F, 23F but not 6B post-PPS were detected in infants with homologous serotype carriage prior to PPS compared with non-carriers ($N=230$). However, OPA levels for 6B and 23F were lower in infants that carried these serotypes.

Pneumococcal carriage with 19F or 23F at any time prior to PPS immunization in infants at 12 months of age who were previously primed with PCV resulted in serotype-specific hyporesponsiveness that persisted until 17 months of age. These results may have implications for the timing of infant vaccine schedules, particularly in high disease burden settings.

1. Introduction

Infections with the bacterium *Streptococcus pneumoniae* (pneumococcus) are a significant cause of global morbidity and mortality, responsible for the deaths of at least 800,000 children under 5 years of age annually [1]. Invasive pneumococcal diseases (IPD) such as pneumonia, meningitis and sepsis are common conditions in young children, particularly in developing countries [2]. A major risk factor for the development of IPD is the acquisition of pneumococcal serotypes in the nasopharynx which may occur very early in infancy [3,4]. Colonization with *S. pneumoniae* is also immunogenic, leading to enhanced systemic and mucosal immune responses in the host [5,6].

Immunization with pneumococcal conjugate vaccines (PCVs) is highly efficacious against vaccine-type IPD in a number of geographical settings [7,8]. While some capsular polysaccharides are poorly immunogenic in children less than 2 years of age, their conjugation to a protein carrier increases immunogenicity in this age group by stimulation and recruitment of T-cell help, allowing

* Corresponding author at: Pneumococcal Research, Murdoch Childrens Research Institute, Melbourne, Australia. Tel.: +61 3 9345 5554.
E-mail address: paul.licciardi@mcri.edu.au (P.V. Licciardi).

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the successful implementation of PCV immunization schedules in children, particularly those living in high-risk settings [9]. However, emerging evidence has revealed that nasopharyngeal carriage can influence the response to PCV vaccination. The carriage of a particular serotype prior to or at the time of vaccination has been shown to impair antibody responses to the homologous serotype post-immunization, with the immune response for other serotypes being unaffected [10–12]. Moreover, serotype-specific hyporesponsiveness has also occurred following PCV immunization in infants with prior IPD, suggesting down-regulation of strain-specific host immunity during infection [13].

In contrast, the effect of recent pneumococcal colonization on the response to pneumococcal polysaccharide vaccination (PPS) has not been well described. The PPS vaccine induces good antibody responses in infants previously primed with 1, 2, or 3 PCV7 [14]. It has the potential to provide wider serotype coverage but studies have found that it has limited effectiveness against IPD and/or nasopharyngeal carriage (NP) [15,16,14]. Furthermore, the use of PPS has been met with controversy in terms of its reported induction of antibody hyporesponsiveness following repeated immunization [17]. Studies to date have focused on serotype-specific antibody responses but not function (opsonophagocytosis), although the mechanisms and clinical significance of these observations have yet to be fully elucidated.

In this study, we investigated the effect of prior serotype-specific pneumococcal NP carriage on the immune response to PPS in infants at 12 months of age following receipt of 1, 2 or 3 doses of PCV7 in infancy. We describe, for the first time, the effect of prior serotype-specific pneumococcal colonization at either 6, 9 or 12 months of age on both serotype-specific IgG and opsonophagocytic responses in Fijian children.

2. Methods

2.1. Study samples

The samples used in this study were collected as part of a phase II single-blind, open label, randomized controlled vaccine trial in Suva, Fiji aimed at examining the impact of reduced dose PCV7 (Prevenar, Pfizer Inc., USA) schedules on the safety, immunogenicity and nasopharyngeal (NP) carriage in 552 infants [18]. For this study, serum samples and NP swabs were analyzed from all infants who received one ($n = 66$), 2 ($n = 80$) or 3 ($n = 65$) PCV7 doses during infancy (at 14 weeks; 6 and 14 weeks, or 6, 10 and 14 weeks, respectively) and all were given PPS vaccine (Pneumovax, Merck, USA) at 12 months of age. For some analyses, several samples were unavailable for assay due to insufficient volume. The study was approved by the approved by the Fiji National Research Ethics Review Committee and the University of Melbourne Human Research Ethics Committee. All laboratory staff members were blinded to the group allocation for each of the study measurements.

2.2. Identification of pneumococcal isolates

Collection of NP swabs in this study has been previously described [14]. Briefly, buffered cotton NP swabs (Sarstedt, Australia) were taken at 6, 9 and 12 months of age and were processed according to the consensus guidelines of a World Health Organization working group [19]. Pneumococcal isolates were initially identified by alpha hemolysis, colony morphology, and optochin (Difco) sensitivity. Isolates with intermediate optochin sensitivity were confirmed as pneumococci by bile solubility testing. Single colonies were subcultured, and pure colonies were sent to the Pneumococcal Reference Laboratory, Centre for Infectious Diseases and Microbiology, ICPMR, Westmead, NSW, Australia, where they were serotyped by multiplex PCR and a reverse line blot assay [20]. Ten percent were also serotyped by a Quellung reaction using specific antisera (Statens Serum Institute, Copenhagen, Denmark). Any discrepancy in serotype between the 2 methods was resolved by a Quellung reaction.

2.3. Measurement of serotype-specific IgG

Serum samples were collected four weeks post-primary PCV7 series (at 18 weeks of age), at 2 weeks post-PPS (at 12.5 months of age) and at 17 months of age. The level of serotype-specific IgG was assayed using a modified WHO ELISA using a CPS/22F double absorption method as previously described [21]. Results were calculated from an 89-SF standard curve (FDA, USA) and reported in μg/mL.

2.4. Measurement of opsonophagocytosis

Opsonophagocytic assays were performed at each time point using a four-fold multiplexed OPA (MOPA) as previously described [22]. The MOPA results were expressed as opsonization indices (OIs) where an OI is defined as the interpolated dilution of serum that killed 50% of bacteria. The lowest dilution of serum tested in the assay is 4 and the OIs of samples that did not kill 50% of bacteria were reported as "2" for analysis purposes. The threshold of the assay was set at OI = 8. MOPA was not performed for serotype 19F in the original study.

2.5. Statistical analysis

Examination of the impact of NP carriage on pneumococcal immune responses post-PPS immunization was performed on serum samples that were pooled from infants who received 1, 2 or 3 doses of PCV7 during infancy and who received a PPS booster at 12 months of age. Serotype-specific IgG and OI levels were expressed as geometric mean concentration (GMC) and geometric mean opsonophagocytic index (GMOI) with 95% confidence intervals (CI). Comparison of GMC and GMOI between infants who were 'carriers' (defined as those infants colonized with a specified pneumococcal serotype at any time prior to PPS immunization) or 'non-carriers' (those infants that were not colonized with any pneumococci prior to PPS immunization) were analyzed using an unpaired *t*-test on log-transformed data. Differences in the proportions of infants with serotype-specific IgG levels $\geq$0.35 μg/mL and $\geq$1.0 μg/mL thresholds were assessed using the Fisher's exact test. *p*-Values less than 0.05 were considered statistically significant in all cases. Analyses were performed using GraphPad Prism Version 5 software package.

3. Results

Post-PPS immunization serum samples at 12.5 months of age (post-PPS) were available from a total of 230 infants for this study.

3.1. Pneumococcal carriage

The cumulative proportion of children who were positive for pneumococcal carriage at either 6, 9, or 12 months of age was 78% (179 infants) (Table 1). Serotypes 6B (5%), 19F (6%) and 23F (10%) were the most frequently carried serotypes and common causes of IPD and were therefore the serotypes that were analyzed in this study. There were 51 non-carriers (22%). Similar demographic characteristics were observed between carriers and non-carriers (data not shown).