Pneumococcal polysaccharide vaccine at 12 months of age produces functional immune responses

Paul V. Licciardi, PhD,[a]* Anne Balloch, MSc,[a]* Fiona M. Russell, MBBS, PhD,[b] Robert L. Burton, MS,[c] Jisheng Lin, MD,[c] Moon H. Nahm, MD,[c] Edward K. Mulholland, MBBS,[d] and Mimi L. K. Tang, MBBS, PhD[a,e,f] *Melbourne, Australia, Birmingham, Ala, and London, United Kingdom*

Background: Infections with *Streptococcus pneumoniae* (pneumococcus) are a cause of significant child mortality. Pneumococcal glycoconjugate vaccines are expensive and provide limited serotype coverage. The 23-valent pneumococcal polysaccharide vaccine (Pneumovax) might provide wider serotype coverage but is reported to be weakly immunogenic in children less than 2 years of age. We have previously reported that Pneumovax administered to healthy 12-month-old Fijian infants elicits significant serotype-specific IgG responses. However, the functional capacity of these responses in 12-month-old infants is not known.

Objective: We sought to assess the functional, serotype-specific immune response of 12-month-old infants after immunization with Pneumovax.

Methods: Functional responses of 12-month-old infants were assessed by using the opsonophagocytic and antibody avidity assay against 8 serotypes and 23 serotypes, respectively.

Results: Seventy-one percent of infants produced strong opsonophagocytic activity against 4 of 8 serotypes, and 30% produced high-avidity serotype-specific IgG antibodies to 10 of 23 serotypes at 2 weeks after Pneumovax. Responses were protective for most serotypes that cause disease in Western countries, whereas responses to most of the epidemiologically relevant serotypes for developing countries were low.

Conclusion: This is the first comprehensive study evaluating the functional antibody response to Pneumovax in 12-month-old infants. Pneumovax induced functional antibody responses to several serotypes causing disease in Western countries but induced poorer responses to serotypes that are responsible for the majority of disease in developing countries. Pneumovax might be of benefit in some populations, but further studies are required before this can be recommended in developing countries. (J Allergy Clin Immunol 2012;129:794-800.)

Key words: *Pneumococcal polysaccharide vaccine, antibody, opsonophagocytosis, avidity, function, serotype, Pneumovax, 23-valent pneumococcal polysaccharide vaccine (Pneumovax)*

Streptococcus pneumoniae is the most common cause of bacterial pneumonia, nonepidemic meningitis, bacteremia, and otitis media in children. In developing countries an estimated one million deaths per year in children less than 5 years of age are attributable to pneumococcal disease.[1] More than 90 serotypes have been described based on the capsular polysaccharide structure of pneumococcus.[2]

Pneumovax (Merck & Co, Inc, Whitehouse Station, NJ), a 23-valent pneumococcal polysaccharide vaccine (23vPPV), contains the 23 pneumococcal serotypes that cause up to 90% of the invasive pneumococcal disease (IPD) in unvaccinated children less than 5 years of age in the United States[3] and 83% of the IPD in children less than 5 years of age in Fiji.[4] Immunization with 23vPPV induces production of anticapsular IgG antibodies through T cell–independent mechanisms. Protection against *S pneumoniae* is mediated by opsonophagocytosis of the organism in the presence of complement and serotype-specific antibody.[5,6] Current opinion suggests that although 23vPPV provides immune protection in children greater than 2 years of age, the polysaccharide-specific T-independent response is poorly developed in younger children because of the immaturity of the infant immune system characterized by the lack of a functional splenic marginal zone.[7] Consequently, the World Health Organization (WHO) and national health authorities do not advocate the use of 23vPPV in children less than 2 years of age. Nevertheless, Indigenous Australian children receive 23vPPV at 18 months after a 3-dose 7-valent pneumococcal conjugate vaccine (Prevenar, Wyeth-Pfizer Inc, New York, NY; PCV7) primary series, and the use of 23vPPV after a primary series with PCV7 has been shown to boost the response to Prevenar serotypes and be immunogenic for non-PCV7 serotypes.[8-10] However, the capacity of infants less than 2 years of age to produce functional antibody responses to purified polysaccharide antigens remains uncertain.

The serotype-specific IgG response to 23vPPV is one of the main tests used to investigate children with suspected immune

From [a]the Pneumococcal Laboratory and [b]the Centre for International Child Health, Murdoch Childrens Research Institute, Melbourne; [c]the Departments of Pathology and Microbiology, University of Alabama at Birmingham; [d]the London School of Hygiene and Tropical Medicine; [e]the Department of Paediatrics, University of Melbourne; and [f]the Department of Allergy and Immunology, Royal Children's Hospital, Melbourne.

*These authors contributed equally to this work.

Supported by grants from the National Institute of Allergy and Infectious Diseases (NIAID; 2 U01 AI052337-05), the Australian National Health and Medical Research Council (NHMRC grant no. 251648), and the Victorian Government's Operational Infrastructure Support Program. Pneumovax was kindly donated by CSL Biotherapies, Australia. M.H.N. is supported by National Institutes of Health contract AI-30021.

Disclosure of potential conflict of interest: M. H. Nahm has consultant arrangements with Merck and receives research support from the National Institutes of Health. E. K. Mulholland receives research support from the National Institutes of Health and the Gates Foundation. M. L. K. Tang is a member of the Asia Pacific Medical Advisory Board for Nestlé Nutrition Institute, is a member of the Medical Advisory Board for Wyeth, is a speaker for Danone, and receives research support from the Nestlé Research Center. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication July 1, 2011; revised November 10, 2011; accepted for publication November 15, 2011.

Available online February 6, 2012.

Corresponding author: Mimi L. K. Tang, MBBS, PhD, Department of Allergy and Immunology, Royal Children's Hospital, Melbourne, Australia. E-mail: mimi.tang@rch.org.au.

0091-6749/$36.00

doi:10.1016/j.jaci.2011.11.043

Abbreviations used

AI: Avidity index
GAVI: Global Alliance for Vaccines and Immunisation
GMOI: Geometric mean opsonization index
GMP: Geometric mean potency
IPD: Invasive pneumococcal disease
MAI: Mean avidity index
NaSCN: Sodium thiocyanate
OI: Opsonization index
OPA: Opsonophagocytic assay
PCV7: 7-Valent pneumococcal conjugate vaccine (Prevenar)
23vPPV: 23-Valent pneumococcal polysaccharide vaccine (Pneumovax)
WHO: World Health Organization

deficiency. However, data on the normal immune response to 23vPPV are limited.[11,12] Expert guidelines for the interpretation of an adequate response to 23vPPV in the context of evaluation of immunocompetence and specific antibody deficiency[13] are available. In children aged 2 to 5 years, it has been suggested that an adequate response to 23vPPV be defined as a postimmunization titer of 1.3 μg/mL or greater and/or a 4-fold or greater increase from the preimmunization titer for ≥50% of serotypes tested.[13,14] Importantly, however, these criteria were derived from small study cohorts, selected patient populations, or studies examining IgG responses to a limited number of serotypes by using an older-generation ELISA. We have recently characterized the serotype-specific IgG response after 23vPPV in 12-month-old children.[15] We found that 95% of infants generated an "adequate antibody response" to polysaccharide antigens (as defined by current expert guidelines[13]), although some serotypes were poorly immunogenic in the majority of infants, with less than 30% mounting adequate IgG responses to serotypes 6B, 14, and 23F after immunization.[15] This was the first detailed evaluation of serotype-specific IgG responses to polysaccharide antigens in infants less than 2 years of age and, moreover, was the only study to assess infant antibody responses using the third-generation WHO ELISA,[16] which is known to offer higher specificity and correlate more closely with the functional opsonophagocytic assay (OPA).[17,18]

Nevertheless, the functional activity of serotype-specific IgG produced by infants less than 2 years of age has not been fully established, and confirmation of an adequate immune response to polysaccharide antigens in infants requires more detailed characterization of the functional capacity of serotype-specific IgG.

In this study we evaluated the functional antibody response to a single dose of 23vPPV in the absence of prior pneumococcal immunization in 12-month-old infants by means of measurement of serotype-specific antibody avidity to all 23 serotypes in Pneumovax and serotype-specific opsonophagocytic activity against 8 serotypes in preimmunization and postimmunization sera. This is the first comprehensive evaluation of functional antibody responses to all 23 serotypes in Pneumovax in a cohort of healthy infants.

METHODS

Study population

The Fiji Pneumococcal Project was a single-blind, open-label, randomized phase II study assessing the safety, immunogenicity, and effect on *S pneumoniae* nasopharyngeal carriage of 0, 1, 2, or 3 doses of PCV7 followed by a booster of 23vPPV. Fijian infants were recruited at 6 weeks of age and stratified by ethnicity at randomization. Fijians are Pacific Islanders comprising 57% Indigenous Fijians and 38% Indo-Fijians (of Indian ethnicity).[4] The ethnic composition of this study reflected the composition of Fiji (see Table E1 in this article's Online Repository at www.jacionline.org). Sixty-three infants were randomized to receive a full dose of 23vPPV at 12 months of age without prior PCV7. This study was approved by the Fiji National Research Ethics Review Committee and the University of Melbourne Human Research Ethics Committee, and written informed consent was obtained from the study participants' parents. Blood samples were collected immediately before 23vPPV and at 2 weeks and 5 months after 23vPPV.

OPA

OPAs to serotypes 1, 4, 5, 6B, 9V, 14, 18C, and 23F were performed at the University of Alabama at Birmingham (Birmingham, Ala) by using standard multiplexed methods measuring the killing of pneumococci by differentiated HL-60 cells.[19] Results were expressed as an opsonization index (OI) representing the serum interpolated dilution that kills 50% of bacteria. The lower limit of detection was an OI of 4, and samples with an OI reading of less than the limit of detection were assigned an OI value of 2. An adequate functional response for each serotype was defined as a serotype-specific OI of 8 or greater.[19] Antibody potency for each serotype was calculated by dividing the OI by the IgG antibody concentration for each serotype. Concentrations of serotype-specific IgG to the 23 serotypes in 23vPPV were measured by using a modified third-generation WHO ELISA[15,20] and have been reported previously.[15]

Antibody avidity

The avidity of serotype-specific IgG for each serotype in 23vPPV was determined by using a previously published method.[21] The assay is based on the dissociation of low-avidity antigen-antibody complexes by the chaotropic agent sodium thiocyanate (NaSCN) in the modified third-generation WHO ELISA validated in our laboratory.[22,23] A 0.5 mol/L NaSCN value was used for all serotypes except serotype 19F (0.65 mol/L) and serotype 14 (0.8 mol/L). Avidity indices (AIs) were calculated as the percentage of antibody that remained bound after NaSCN elution by using an interpolation OD value of 0.5 U as follows:

$$\left(\text{Interpolation value}_{\text{NaSCN}}/\text{Interpolation value}_{\text{PBS/FCS}}\right)\times 100.$$

The avidity of serotype-specific IgG was arbitrarily assigned as low (AI, <40%), intermediate (AI, 40% to 49%), or high (AI, ≥50%).

Statistical analysis

Analysis was performed by using GraphPad Prism version 4.03 software (GraphPad Software, Inc, La Jolla, Calif). For avidity analyses, the median avidity index (MAI) and interquartile range were calculated, and statistical significance was assessed by using the Wilcoxon matched-pairs test. The geometric mean opsonic indices (GMOIs) and antibody geometric mean potencies (GMPs) with 95% CIs were compared by using the Student *t* test. The proportions of infants with OIs of 8 or greater and AIs of 50 or greater were analyzed by using the McNemar test. A *P* value of less than .01 was considered statistically significant to account for the multiple comparisons.

RESULTS

Sixty-three infants were randomized at 6 weeks of age to receive 23vPPV as their primary pneumococcal immunization at 12 months of age. Fifty-six infants received the 12-month 23vPPV vaccination. Information on infants' characteristics and withdrawals from the study are available in the online repository (see Tables E1 and E2 in this article's Online Repository at www.jacionline.org) and has been reported previously.[20]