Lower Immunoglobulin G Antibody Responses to Pneumococcal Conjugate Vaccination at the Age of 2 Years after Previous Nasopharyngeal Carriage of *Streptococcus pneumoniae*

Gerwin D. Rodenburg, MD, PhD, Elske J. M. van Gils, MD, Reinier H. Veenhoven, MD, PhD, Debby Bogaert, MD, PhD, Germie P. J. M. van den Dobbelsteen, PhD, Guy A. M. Berbers, PhD, and Elisabeth A. M. Sanders, MD, PhD

Objective To determine whether nasopharyngeal pneumococcal carriage with serotypes 6B, 19F, or 23F interferes with immunoglobulin G (IgG) antibody responses to vaccination with 7-valent pneumococcal conjugate vaccine (PCV7) at the age 24 months.

Study design Blood samples were collected before and after a PCV7 challenge vaccination at age 24 months from subsets of children participating in a randomized controlled trial. Children previously had received two doses of PCV7 at 2 and 4 months, two plus one doses of PCV7 at 2, 4, and 11 months, or no dosage until 24 months. Nasopharyngeal swabs were cultured at for *Streptococcus pneumoniae* at age 6 weeks and at 6, 12, 18, and 24 months. IgG responses were determined with enzyme immunoassay.

Results Lower IgG responses against serotypes 6B, 19F, and 23F were observed on PCV7 challenge vaccination at 24 months in children who had received earlier PCV7 vaccinations and had been found positive for homologous carriage compared with non-carriers of these serotypes. Lower non-homologous IgG responses were observed after the PCV7 challenge at 24 months against serotype 6B after earlier 19F carriage and against serotype 19F after earlier 23F carriage compared with children who had not been positive for carriage of these serotypes.

Conclusions Pneumococcal colonization with serotypes 6B, 19F, and 23F is associated with diminished immune responses against these serotypes on PCV7 vaccination at 2 years of age. Underlying mechanisms deserve further investigation. *(J Pediatr 2011;159:965-70)*.

Streptococcus pneumoniae is a major cause of pneumonia, bacteremia, and meningitis, especially in children in the first years of life.[1,2] Pneumococcus is a polysaccharide encapsulated bacterium and commonly but transiently colonizes the human nasopharynx. In young children, asymptomatic nasopharyngeal carriage rates may be as high as 70% to 80%.[3]

Some pneumococcal capsular polysaccharides have been described as poorly immunogenic, especially in infants as old as 2 years,[4-6] and the multivalent pneumococcal polysaccharide vaccines failed to show efficacy against invasive diseases in most clinical trials in children.[7] By recruitment of CD4+ T-helper cells, pneumococcal polysaccharide-protein-conjugate vaccines proved to be immunogenic in infants as young as a few weeks of age.[8] Clinically, the 7-valent pneumococcal conjugate vaccine (PCV7) showed high efficacy against invasive pneumococcal disease (IPD) caused by vaccine serotypes and, to a lesser extent, protection against pneumococcal respiratory disease and nasopharyngeal acquisition by vaccine serotypes.[3,9]

IPD in infants has been reported to cause serotype-specific hyporesponsiveness to later vaccination with PCV7.[10] Also, repeated immunization with pneumococcal polysaccharide vaccines has been associated with immune hyporesponsiveness to polysaccharide antigens and with blunted responses to later PCV7 administration.[6,7,11] Recently, nasopharyngeal carriage of the most commonly carried pneumococcal serotypes shortly before administration of the primary series in infants also was reported to result in serotype-specific hyporesponsiveness toward different pneumococcal conjugate vaccines.[12,13]

In this study, we investigated the effect of nasopharyngeal carriage of pneumococci after the primary series of PCV7 vaccination during the first 2 years of life on serum immunoglobulin G (IgG) immune responses to a PCV7 challenge vaccination at 24 months of age.

GMC	Geometric mean concentration
IgG	Immunoglobulin G
IPD	Invasive pneumococcal disease
PCV7	7-valent pneumococcal conjugate vaccine

From the Department of Pediatric Immunology and Infectious Diseases, Wilhelmina Children's Hospital, University Medical Center Utrecht, The Netherlands (G.R., E.vG., D.B., E.S.); Linnaeus Institute, Spaarne Hospital, Hoofddorp, The Netherlands (E.vG., R.V.); National Institute for Public Health and the Environment, Bilthoven, The Netherlands (G.B.); and Unit research and development, Netherlands Vaccine Institute, Bilthoven, The Netherlands (G.vdD.)

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Trial registered at clinicaltrials.gov: NCT00189020.

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Methods

Between July 2005 and February 2006, 1005 infants were enrolled in a randomized controlled trial investigating the effects of reduced-dose PCV7 schedules on pneumococcal carriage during the first 2 years of life (NCT00189020).[3] Healthy infants were randomly allocated to receive various vaccination schedules: (1) PCV7 at 2 and 4 months of age (2-dose group; n = 333); (2) PCV7 at 2 and 4 months followed by a booster dose at 11 months of age (2+1-dose group; n = 336); or (3) no PCV7 (control group; n = 336). At 24 months of age, participants of all 3 study groups were offered a PCV7 immunization on voluntary basis. Exclusion criteria for the study were known immunodeficiency, craniofacial or chromosomal abnormalities, language barrier, or expected relocation within the follow-up period. The study vaccine was the 7-valent non-toxic derivative of diphtheria toxin 197-conjugated pneumococcal vaccine (Prevenar, Wyeth, Maidenhead, United Kingdom). The study was approved by a national medical ethics committee. Informed consent was obtained from the parents or guardians of all study participants. The trial was registered at clinicaltrials.gov (NCT00189020).

Nasopharyngeal swabs were taken at the age of 6 weeks before the first PCV7 vaccination and at 6, 12, 18, and 24 months of age. The last swab was collected immediately before the PCV7 challenge vaccination at 24 months. Identification of *S pneumoniae* nasopharyngeal carriage was based on single colony morphology and conventional methods of determination, as described earlier.[3] Blood samples were obtained on voluntary basis at 24 months of age from approximately 80 children per study group before the challenge PCV7 vaccination and from approximately 80 children per group 28 to 42 days after PCV7 challenge vaccination. Serum IgG antibody levels to the 7 vaccine serotypes were determined with double adsorption enzyme immunoassay with cell wall polysaccharide and 22F polysaccharide, as described earlier.[14] In all measurements, the operator was blinded to the study group of samples being processed.

Statistical Analysis

Pre- and post-challenge antibody levels are expressed as geometric mean concentration (GMC) with 95% CI. For analyses of potential correlations between carriage and consecutive antibody responses to PCV7, we focused on the 3 most frequently carried vaccine serotypes in the study population: 6B, 19F, and 23F. Infants were defined as prior carriers when found positive for carriage of that serotype at one of the nasopharyngeal sampling moments at 6 weeks or at 6, 12, 18, and 24 months of age. The children with nasopharyngeal samples who were negative for the given serotype 6B, 19F, or 23F as old as 24 months of age were defined as non-carriers. Statistical differences in IgG GMC values for the serotypes 6B, 19F, and 23F were assessed with log-transformed unpaired *t* test when >5 blood samples were available per group. With all infants who were vaccinated with PCV7 at 2 and 4 months pooled together, a multivariable linear regression model was used to assess the relationship between the different moments of carriage (6 weeks, 6, 12, 18, or 24 months) and log-transformed IgG antibody levels 28 to 42 days after PCV7 vaccination at 24 months of age. These were adjusted for vaccination group (2-dose and 2+1-dose schedule) and earlier carriage of non-homologous serotypes. Serotype-specific antibody levels ≥0.35 μg/mL against vaccine serotypes were defined as adequate response to the PCV7 challenge vaccination at 24 months.[10] This threshold is used as correlate of protection against IPD and is used for assessing non-inferiority of novel PCVs.[15] Differences in proportions of participants reaching serotype-specific antibody levels ≥0.35 μg/mL with vaccination at 24 months were calculated by using the Fisher exact test. All reported *P* values are two-sided; *P* values <.05 were considered significant. Analyses were performed with SPSS software version 15.0 (SPSS Inc, Chicago, Illinois).

Results

Blood samples were collected from 231 infants before (pre-challenge sample group) and from 230 children 28 to 42 days after the PCV7 challenge vaccination (post-challenge sample group) at 24 months of age. Paired samples (pre- and post-PCV7 challenge) were available for 137 participants (60%). The cumulative proportion of children who were found to be positive for pneumococcal carriage during the total study period until 2 years of age was found to be 90% and 92% of the children in the pre- and post- challenge group, respectively. Serotype 6B carriage was found in 47 (20%) and 62 (27%) children in one or more consecutive nasopharyngeal samples; serotype 19F carriage was found in 50 (22%) and 46 (20%) subjects; and serotype 23F carriage was found in 37 (16%) and 41 (18%) children. At 6 weeks of age, however, only 5 and 7 children (pre- and post-challenge sample groups) were found to be positive for carriage of serotype 6B, 19F, or 23F. No major differences in demographic characteristics and risk factors for pneumococcal carriage were found between children in the original main trial and the immunogenicity subsets described in this report.[3]

Immunoglobulin G Serum Antibody Levels before 7-valent Pneumococcal Conjugate Vaccine Challenge at 24 Months

Before the PCV7 challenge vaccination at 24 months of age, no differences in homologous IgG GMC values could be observed between non-carriers of serotypes 6B, 19F, and 23F and earlier carriers of these serotypes in both the 2-dose and 2+1-dose group (**Table I**). In unvaccinated control subjects, earlier carriage of the serotypes 19F and 23F was associated with higher homologous IgG serum antibody levels at 24 months of age before the challenge vaccination compared with non-carriers (**Table I**).