MAJOR ARTICLE

Differential B-Cell Memory Around the 11-Month Booster in Children Vaccinated With a 10- or 13-Valent Pneumococcal Conjugate Vaccine

Els van Westen,[1] Alienke J. Wijmenga-Monsuur,[1] Harry H. van Dijken,[1] Jacqueline A. M. van Gaans-van den Brink,[1] Betsy Kuipers,[1] Mirjam J. Knol,[1] Guy A. M. Berbers,[1] Elisabeth A. M. Sanders,[1,2] Nynke Y. Rots,[1] and Cécile A. C. M. van Els[1]

[1]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, and [2]Department of Pediatric Immunology and Infectious Diseases, Wilhelmina Children's Hospital, University Medical Center, Utrecht, The Netherlands

Background. Both the 10- and 13-valent pneumococcal conjugate vaccines (PCV10 and PCV13) induce immunological memory against *Streptococcus pneumoniae* infections caused by vaccine serotypes. In addition to comparing serum antibody levels, we investigated frequencies of serotype-specific plasma cells (PCs) and memory B-cells (Bmems) as potential predictors of long-term immunity around the booster vaccination at 11 months of age.

Methods. Infants were immunized with PCV10 or PCV13 at 2, 3, 4, and 11 months of age. Blood was collected before the 11-month booster or 7–9 days afterward. Serotype-specific immunoglobulin G (IgG) levels were determined in serum samples by multiplex immunoassay. Circulating specific PCs and Bmems against shared serotypes 1, 6B, 7F, and 19F and against PCV13 serotypes 6A and 19A were measured in peripheral blood mononuclear cells by enzyme-linked immunospot assay.

Results. No major differences in IgG levels and PC frequencies between groups were found for the 4 shared serotypes. Notably, PCV13 vaccination resulted in higher frequencies of Bmems than PCV10 vaccination, both before and after the booster dose, for all 4 shared serotypes except for serotype 1 postbooster. For PCV13-specific serotypes 6A and 19A, the IgG levels and frequencies of PCs and Bmems were higher in the PCV13 group, pre- and postbooster, except for PC frequencies prebooster.

Conclusions. Both PCVs are immunogenic and induce measurable IgG, PC, and Bmem booster responses at 11 months. Compared to PCV10, vaccination with PCV13 was associated with overall similar IgG levels and PC frequencies but with higher Bmem frequencies before and after the 11-month booster. The clinical implications of these results need further follow-up.

Clinical Trials Registration. NTR3069.

Keywords. pneumococcal conjugate vaccine; PCV10; PHiD-CV; PCV13; memory B cells.

Streptococcus pneumoniae can cause acute and life-threatening invasive diseases including meningitis and sepsis and is the most common pathogen in respiratory infections such as pneumonia and otitis media. The heptavalent CRM_{197}-conjugated pneumococcal conjugate vaccine (PCV7; Prevenar) was licensed for children in 2000 in the United States and introduced in the National Immunization Program in the Netherlands in 2006. Afterward, a steep decline in vaccine serotype invasive pneumococcal disease (IPD) and nasopharyngeal carriage was observed [1–5]. Nonvaccinated groups benefited due to indirect herd effects through strong reduction of vaccine serotype carriage in vaccinated children and subsequent reduced host-to-host transmission to individuals of all ages [3]. In 2009, broader-coverage

Received 29 January 2015; accepted 26 March 2015; electronically published 1 April 2015.

Correspondence: Els van Westen, MSc, Centre for Infectious Disease Control, National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands (els.van.westen@rivm.nl).

Clinical Infectious Diseases® 2015;61(3):342–9

DOI: 10.1093/cid/civ274

PCVs became available with 3 (PCV10) and 6 (PCV13) additional serotypes. Besides differing in number of serotypes, PVC10 and PCV13 differ in concentration of the capsular polysaccharides, the conjugation process, and carrier proteins, possibly leading to different immunogenicity and memory induction between these 2 vaccines [6].

Serum immunoglobulin G (IgG) concentrations against vaccine serotypes are now being evaluated as a predictor for clinical protection against IPD. Apart from circulating antibodies, the induction of differentiated B cells, such as plasma cells (PCs) and memory B-cells (Bmems), may determine immediate and long-term protection against disease. PCs are the source of antibodies, but the short-lived type has a half-life of only 1–10 days [7]. Hence, long-lived PCs and Bmems are important for induction of long-term protection by providing a continuous antibody response and a rapid booster response, respectively. These 2 cell types are both generated in germinal centers and preferentially home in the bone marrow, from which they perform their function [8]. Assessment of the presence of these cell types might refine the prediction of long-term vaccine-induced immunological memory and protection against IPD [9].

Several immunogenicity studies comparing PCV10 or PCV13 with PCV7 have been performed, showing them to be similar to PCV7 in immunogenicity and safety [10–13]. However, to our knowledge, no direct comparison of the induction of PCs and Bmems by PCV10 and PCV13 has been published.

In this clinical study, we directly compared the immunogenicity profiles of PCV10- and PCV13-vaccinated children. Their IgG levels and frequencies of circulating PCs and Bmems were described before and after a booster dose at 11 months of age, with a focus on shared serotypes 1, 6B, 7F, and 19F, and on the PCV13-specific serotypes 6A and 19A.

MATERIALS AND METHODS

Study Design

Infants born in the Netherlands during September–December 2011 were enrolled in a controlled parallel group intervention study comparing immunogenicity before and after a booster dose with PCV10 or PCV13 (NTR3069; www.trialregister.nl) (Figure 1). In accordance with the Dutch National Immunization Program, the children were vaccinated at 2, 3, 4, and 11 months of age. All children received the same vaccine for all primary series doses and for the booster dose. Children were randomly assigned to groups in which an intravenous 8 mL blood sample was collected just before the booster or 7–9 days afterward for analyses of PC and Bmem frequencies.

From the parents and/or guardians of all study participants, informed consent was obtained before enrollment. The study was approved by a national medical ethics committee and undertaken in accordance with Good Clinical Practice, which includes the provisions of the Declaration of Helsinki. The study

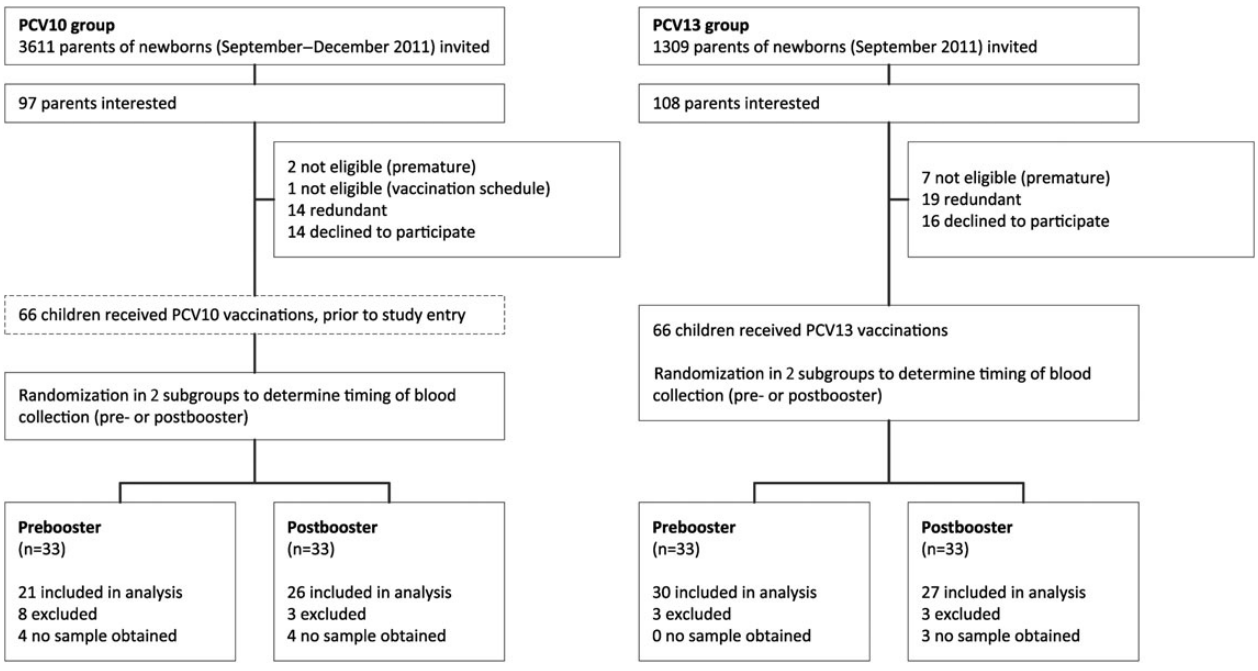

Figure 1. Enrollment diagram. Abbreviations: PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine.

staff members and parents were aware of the intervention, but laboratory staff was blinded.

Vaccines

Children in the PCV10 group were vaccinated with Synflorix (GSK, Belgium) during regular visits to well-baby clinics. PCV10 contains 1 μg of serotypes 1, 5, 6B, 7F, 9V, 14, and 23F and 3 μg of serotypes 4, 18C, and 19F. The polysaccharides are conjugated to protein D, except for 18C (tetanus toxoid) and 19F (diphtheria toxoid). Children in the PCV13 group received Prevenar13 (Pfizer, UK) during home visits by the study team. This vaccine contains 2.2 μg of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, all conjugated to diphtheria toxoid CRM_{197}. Both groups concomitantly received Infanrix-hexa (GSK, Belgium) against diphtheria, tetanus, acellular pertussis, hepatitis B, poliovirus, and *Haemophilus influenzae* type B (conjugated to tetanus toxoid), at 2, 3, 4, and 11 months of age.

Blood Collection and Storage

The 8 mL blood volume was collected in two 4 mL cell preparation tubes (BD). Plasma and peripheral blood mononuclear cells (PBMCs) were separated within 24 hours by density gradient centrifugation according to the manufacturers' instructions. PBMCs were used fresh, and plasma samples were stored at −20°C until use. For serum isolation, 300 μL blood was collected and stored at −20°C until use.

Serotype Selection

Due to the limited available blood sample volume, 6 serotypes were selected as antigens for qualitative serological and B-cell analysis. These were serotypes 6B and 19F, included in all PCV vaccines (PCV7, PCV10, and PCV13); serotypes 1 and 7F, shared by PCV10 and PCV13; and serotypes 6A and 19A, which are unique to PCV13.

Multiplex Immunoassay

The multiplex immunoassay was used to determine serotype-specific IgG antibody levels, here shown for 6 serotypes (1, 6A, 6B, 7F, 19A, and 19F) as described by Elberse et al [14]. In brief, beads were coated with individual pneumococcal polysaccharides. Serum samples were diluted and incubated together with the beads. IgG concentrations (μg/mL) against the individual serotypes were calculated from the mean fluorescence intensity by interpolation of the plot from the reference serum, 89-SF, and expressed per group as geometric mean concentration with 95% confidence intervals.

PBMC Stimulation

After isolation, PBMCs were washed and resuspended in AIM-V medium (Gibco) containing 10% fetal bovine serum (FBS; HyClone) and penicillin-streptomycin-glutamine (Gibco). Measurement of PC frequencies was performed using freshly isolated cells, whereas Bmems were measured after 5 days of PBMC stimulation in vitro. At 37°C and 5% CO_2 in a 24-well culture plate, the PBMCs were stimulated at a concentration of 2×10^6 cells/mL with 3 μg/mL CpG (with a phosphorothioate backbone; Isogen Life Science), 10 ng/mL interleukin 2 (Miltenyi Biotec), 10 ng/mL interleukin 10 (Merck), and a pool of polysaccharides including serotypes 1, 6A, 6B, 7F, and 19F (Netherlands Vaccine Institute) and 19A (American Type Culture Collection) at 2 ng/mL per serotype.

Enzyme-Linked Immunospot Assay

Frequencies of serotype-specific antibody-secreting cells (ASCs), using unstimulated freshly isolated PBMCs (for PCs) or the stimulated PBMCs (for Bmems), were determined by B cell enzyme-linked immunospot assay (ELISpot). Multiscreen Filter Plates (MSIPS4510, Millipore) were pretreated with 35% ethanol for 1 minute, washed with phosphate-buffered saline (PBS), and coated with 25 μg/mL of a single polysaccharide (1, 6A, 6B, 7F, 19A, or 19F) or PBS as negative control. Plates were incubated overnight at 4°C, washed, and blocked for at least 30 minutes with PBS containing 5% FBS. Eight 3-fold dilutions of PBMC suspensions, with a starting concentration of 3×10^5 cells/well, were added to the plates and incubated overnight at 37°C and 5% CO_2. After washing with PBS containing 0.05% Tween-20 (PBS-Twn20), plates were incubated with goat-anti-human IgG conjugated to alkaline phosphatase (1:5000; SouthernBiotech) for 2–4 hours at 37°C and 5% CO_2. Plates were washed with PBS-Twn20 and PBS. Finally, 50 μL substrate (5-bromo-4-chloro-3-indolyl phosphate in nitro blue tetrazolium phosphatase; Sigma) was incubated for 10–30 minutes, and afterward plates were washed with tap water and dried in the dark. Blue/purple spots, representing ASCs, were measured using an ELISpot reader and software (CTL Europe). Spots from at least 2 dilutions were used to calculate the number of ASCs per 10^5 PBMCs per child. Results are displayed as frequencies per individual, with medians per group. The lower detection limit for PCs and for Bmems is 0.10 ASCs per 10^5 PBMCs. Percentages of children per group below this detection limit were calculated.

Statistical Analyses

Samples were included in the analyses only if serum IgG levels and PC and Bmem frequencies could be determined for all 6 serotypes. Excluding samples with limited cell numbers and serum volume, final group sizes are shown in Figure 1. Differences between groups were tested using a Mann–Whitney test for PC and Bmem frequencies and a *t* test on log-transformed IgG levels. Correlations between Bmem frequencies and IgG levels were assessed using Spearman correlation. A P value <.05 was considered statistically significant. Analyses were performed using SPSS 19.0 and GraphPad Prism 6 software. Regression analyses of log-transformed IgG levels were

Table 1. Characteristics of the Participants

Characteristic	PCV10		PCV13	
	Prebooster (n = 21)	Postbooster (n = 26)	Prebooster (n = 30)	Postbooster (n = 27)
Male sex, %	66.7	42.3	60.0	66.7
Month of birth, median (min–max)	November (October–December)	November (October–December)	September (September–September)	September (September–September)
Daycare attendance age 11 mo, %	90.5	69.2	73.3	88.9
Half-days at daycare age 11 mo, mean (SD)	4.9 (1.9)	4.7 (1.4)	4.3 (1.3)	4.7 (1.9)
Birth weight, g, mean (SD)	3684 (556)	3699 (410)	3643 (499)	3536 (415)
Gestational age, wk, mean (SD)	39.8 (1.4)	39.8 (1.1)	39.5 (1.3)	39.8 (1.4)
Presence of siblings aged <5 y, %	42.9	61.5	56.7	25.9**
Breastfeeding, %	85.7	84.6	80.0	85.2
Duration of breastfeeding, mo, mean (SD)	5.8 (4.1)	6.8 (3.1)	5.6 (3.3)	5.9 (2.9)
Passive smoking, %	0.0	0.0	0.0	0.0
Age, mo, mean (SD)				
At 2-mo vaccination	2.1 (0.2)	2.0 (0.1)	1.9 (0.1)*	1.9 (0.1)*
At 3-mo vaccination	3.3 (0.3)	3.1 (0.2)	2.9 (0.1)***	2.9 (0.2)***
At 4-mo vaccination	4.4 (0.4)	4.3 (0.3)	3.9 (0.1)***	3.9 (0.2)***
At 11-mo vaccination	11.2 (0.3)	11.2 (0.2)	11.3 (0.1)	11.3 (0.2)*
At prebooster blood sampling	11.0 (0.3)	. . .	11.3 (0.1)	. . .
Time of sampling, days postbooster, mean (SD)	. . .	7.7 (0.8)	. . .	7.3 (0.7)*

An unpaired *t* test is used to compare PCV13 to the PCV10-counterparts.

Abbreviations: PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; SD, standard deviation.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

performed to assess the influence of age at vaccination and blood sampling.

RESULTS

Study Population

A total of 132 children were enrolled, with final group sizes varying from 21 to 30 children per group (Figure 1). All groups had comparable baseline characteristics, with the most noticeable difference being age at vaccination, which was lower in the PCV13 group than in the PCV10 group for most time-points (Table 1). Shared serotypes (1, 6B, 7F, and 19F), included in both PCV10 and PCV13, were compared. PCV13 serotypes 6A and 19A, not included in PCV10, were analyzed separately.

Serum IgG Responses for Shared Serotypes 1, 6B, 7F, and 19F

Seven months after the primary vaccination series and just before the 11-month booster dose, the pneumococcal serotype-specific serum IgG concentrations had declined to low levels in both groups. Seven to 9 days after the 11-month booster dose, IgG levels against all 4 serotypes increased in both groups (Figure 2; Supplementary Table 1).

PCV13-vaccinated children showed lower prebooster IgG levels for serotype 6B and higher pre- and postbooster IgG levels for serotype 19F. For serotypes 1 and 7F, IgG responses did not differ between groups, before or after the booster dose (Figure 2; Supplementary Table 1).

PC Frequencies for Shared Serotypes 1, 6B, 7F, and 19F

Prebooster, the PCV13 group showed lower PC frequencies for serotype 6B and higher PC frequencies for serotype 1, compared with the PCV10 group. The PCV13 booster dose increased serotype-specific PCs for 3 of 4 shared serotypes (serotypes 6B, 7F, and 19F), whereas the PCV10 booster increased frequencies of PCs for 2 of 4 shared serotypes (serotypes 1 and 7F) (Figure 3; Supplementary Table 2). After the booster, no differences were detectable between PCV10 and PCV13 for these 4 serotypes (Figure 3; Supplementary Table 2). The percentages of children with PC frequencies below the detection limit, variable between groups and serotypes, declined after the booster (Supplementary Table 3).

Bmem Frequencies for Shared Serotypes 1, 6B, 7F, and 19F

In both groups, Bmems were detectable for all serotypes prebooster, approximately 7 months after the primary series. Both vaccines

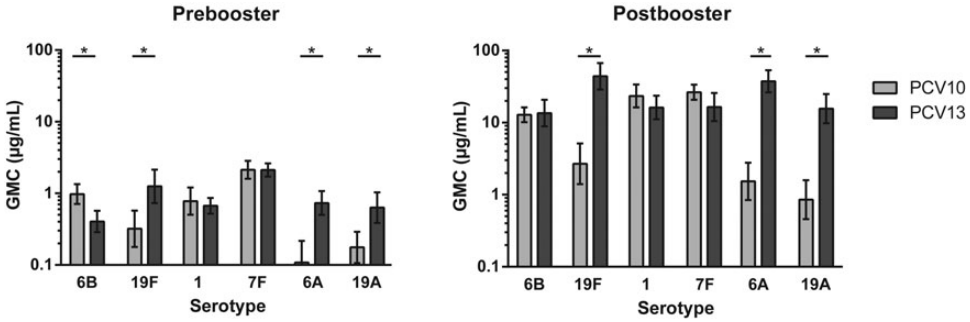

Figure 2. Comparative analysis of serotype-specific immunoglobulin G (IgG) responses in 10- and 13-valent pneumococcal conjugate vaccine (PCV10 and PCV13) recipients. Geometric mean IgG concentrations (GMC) with 95% confidence intervals from sera of PCV10- or PCV13-vaccinated children, before (left panel) and after (right panel) the 11-month booster. Differences were tested with an unpaired *t* test on the log-transformed IgG levels. **P* < .05 was considered significant.

induced a booster response, consisting of an increase in Bmem frequencies after the 11-month vaccination, compared with the prebooster frequencies (Figure 4; Supplementary Table 4).

Notably, children vaccinated with PCV13 showed higher pre- and postbooster frequencies of Bmems compared to children vaccinated with PCV10 for 3 of the 4 shared serotypes. The exception was serotype 1, for which the PCV10 group had similar postbooster frequencies compared with the PCV13 group (Figure 4; Supplementary Table 4).

Several infants in the PCV10 group had prebooster only Bmem frequencies below the detection limit, for all serotypes (Supplementary Table 3).

Responses to Serotypes Unique to PCV13 (6A and 19A)

Seven months after the primary series with PCV13, PC and Bmem frequencies and IgG levels were still detectable for serotypes 6A and 19A and increased again after the booster dose, except for Bmem frequencies for serotype 19A. Notably, prebooster PC and Bmem frequencies for 6A and 19A were likewise detectable in children primed with PCV10, but they did not increase after the 11-month booster dose (Figures 3 and 4; Supplementary Tables 2 and 3). In contrast, prebooster IgG levels for serotypes 6A and 19A were boosted by the 11-month PCV10 dose (Figure 2; Supplementary Table 1). The PCV13 group had higher PC and Bmem frequencies and IgG levels for the PCV13 serotypes than in PCV10-vaccinated children, pre- as well as postbooster. The one exception was that PC frequencies for both serotypes did not differ between both vaccines at the prebooster time-point.

DISCUSSION

To our knowledge, our report is the first direct comparison of B-cell responses induced by PCV10 and PCV13 in Dutch children vaccinated at 2, 3, 4, and 11 months of age. Both vaccines were observed to induce adequate booster responses against the vaccine serotypes. Differences in IgG levels were observed between groups for shared serotype 6B postbooster and for

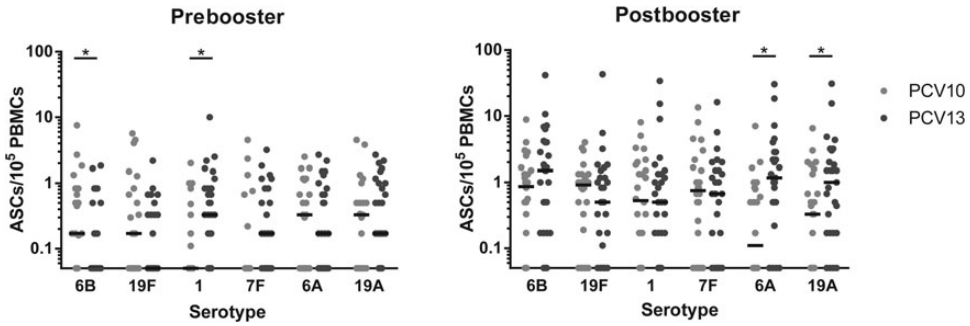

Figure 3. Comparative analysis of serotype-specific plasma cell responses in 10- and 13-valent pneumococcal conjugate vaccine (PCV10 and PCV13) recipients. Frequencies of plasma cells are expressed as number of antibody-secreting cells (ASCs) per 10^5 peripheral blood mononuclear cells (PBMCs), before (left panel) and after (right panel) the 11-month booster dose. Individual frequencies with medians per group are displayed. **P* < .05 was considered significant, using Mann–Whitney test.

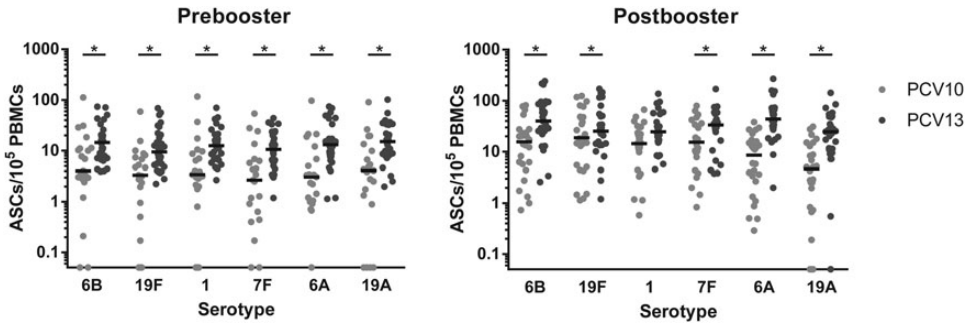

Figure 4. Comparative analysis of serotype-specific memory B-cell (Bmem) responses in 10- and 13-valent pneumococcal conjugate vaccine (PCV10 and PCV13) recipients. Frequencies of Bmems are expressed as number of antibody-secreting cells (ASCs) per 10^5 stimulated peripheral blood mononuclear cells (PBMCs), before (left panel) and after (right panel) the 11-month booster dose. Individual frequencies with medians per group are displayed. *$P < .05$ was considered significant, using Mann–Whitney test.

serotype 19F pre- and postbooster. PC frequencies appeared to be comparable between PCV10 and PCV13 before and after the 11-month booster for the 4 evaluated shared serotypes, but were lower for the PCV13-specific serotypes 6A and 19A in the PCV10 group. We found higher serotype-specific Bmem responses in PCV13-vaccinated children compared with PCV10-vaccinated children both before and after the 11-month booster for the shared serotypes, and postbooster for serotypes 6A and 19A.

Our data depict a significant booster-induced increase in PC frequencies for 2 and 3 of 4 shared serotypes (for PCV10 and PCV13, respectively), and in Bmems for all 4 shared serotypes. These findings are similar to booster dose responses in antigen-specific B-cell subsets known from other studies of protein–polysaccharide conjugate vaccine in infants and young children, such as for *H. influenzae* type B [15], meningococcal serogroups A, C, W, and Y [7, 16–18], and *S. pneumoniae* serotypes in PCV7 [19]. Moreover, we observed higher frequencies of serotype-specific Bmems 7 months after the primary PCV13 series compared with the PCV10 series for all 4 of the shared serotypes evaluated. Higher Bmem frequencies were likewise found in the PCV13 group 1 week after the 11-month booster dose for 3 of the 4 shared serotypes, with the exception being serotype 1. These differences in Bmem immunogenicity might result from the higher concentration of polysaccharides in PCV13 compared with PCV10 (except for serotype 19F) or from the variability of conjugate protein or conjugation process. Immunogenicity and functionality of polysaccharide antibodies depends not only on which carrier protein is used, but also on the method of conjugation [20]. Long-term follow-up of antibody levels and clinical surveillance should be used to learn whether the observed differences have clinical consequences.

Only serotype 1 postbooster did not differ in the induction of serotype-specific Bmem frequencies between the 2 vaccines. This polysaccharide is zwitterionic, containing negative as well as positive charges, whereas the other serotypes are negatively charged [21, 22]. A zwitterionic antigen might induce a T-cell–dependent response, through major histocompatibility complex class II, initiating a memory response without help from a conjugate protein [21, 23]. Even though the behavior of zwitterionic polysaccharides in immunological responses is not completely clear, they are known to induce different responses than do nonzwitterionic polysaccharides [21]. This may in part explain why serotype 1 showed no difference in Bmem frequencies in the comparison between PCV10 and PCV13.

Several studies have compared the serotype-specific IgG antibody levels in PCV10 or PCV13 to levels in PCV7, showing that they are noninferior to those of PCV7 [10–13]. So far, however, no direct comparisons of immunogenicity induced by PCV10 and PCV13 have been published. Interestingly, our data indicated an IgG booster effect for all 6 evaluated serotypes in both vaccine groups, even for serotype 6A and 19A, which are not included in PCV10. Induction of IgG for these 2 serotypes can be explained by cross-protection by serotypes 6B and 19F, respectively. Shown in PCV7 studies for serotype 6A, cross-protection led to carriage reduction and reduction of invasive 6A pneumococcal disease [11, 24]. Cross-reactivity between serotypes 19F and 19A might contribute to the high 19F responses in the PCV13 group [11]. Responses to serotype 19F can likewise be cross-reactive to serotype 19A [11, 20], but there are conflicting data as to whether these cross-reactive antibodies are functional and protect against 19A disease [11]. Our findings are noteworthy in that although IgG levels of serotypes 6A and 19A increased after vaccination with PCV10, PC and especially Bmem frequencies against these serotypes did not increase. This finding suggests a suboptimal recall for cellular expansion among the cross-reactive vaccine serotypes. Baseline

PC and Bmem frequencies specific to serotypes 6A and 19A in PCV10-vaccinated children could also have been induced by natural circulation. In studies of nasopharyngeal carriage in infants, serotype 19A was the dominant serotype then circulating in the Netherlands (Bosch et al, manuscript submitted). In other countries before introduction of PCV13, serotype 19A was likewise one of the most predominantly carried serotypes, as was serotype 6C [25, 26].

Our study clearly affirms that B-cell memory is measurable for all serotypes tested after 3 primary doses of either vaccine, as a fourth dose rapidly boosted the respective PC and IgG pools. When testing for relationships between the various immune compartments assessed, we only found significant correlations postbooster between the size of the newly expanded Bmem pools and the increased IgG titers, for serotypes 6B and 1 in the PCV10 group, and for serotypes 6B, 19F, 6A, and 19A in the PCV13 group (Supplementary Figure 1). Such correlations highly depend on the phase in the immune response studied and are not consistently seen throughout vaccine studies [17, 27–29]. It is possible that the capacity of the primary B-cell memory to drive a renewed Bmem response as well as an IgG response when boosted may differ for serotypes between PCV10 and PCV13. Whether the Bmems induced by these 2 vaccines might intrinsically differ in their differentiation capacities needs further investigation.

The strength of this study is the direct comparison of PCV10- and PCV13-vaccinated children, which overcame problems such as differences in immunization schedule, age at vaccination, and environmental exposure (eg, differences in the natural circulation of serotypes across geographic regions) [30, 31]. Limitations are that the PCV13 children were younger during the primary series and on average postbooster blood samples were collected 0.4 days earlier, compared with the PCV10 group. However, similar findings were obtained after we corrected for these differences by regression analyses for IgG titers and by selection of more restricted age groups for the frequency analysis of PCs and Bmems. Furthermore, the age difference is unlikely to have contributed to the higher Bmem responsiveness of the younger PCV13 infants, as an older and more matured immune system is more likely to show a higher immunological response [7, 30]. Therefore, the observed effect was probably induced by the vaccine itself and is not due to a difference in age at vaccination.

Last, this study shows that differences in immunogenicity between 2 conjugate vaccines can be serotype specific, as for some serotypes PCV13 induced similar serum IgG levels compared with PCV10, whereas for others the IgG levels were higher. Serotype-specific immunogenicity of pneumococcal conjugate vaccines was also addressed in an indirect cohort study by Andrews et al [32]. These results indicate that serotype-specific results cannot be extrapolated to other serotypes.

In summary, our study showed that both PCV10 and PCV13 are immunogenic, evoking mostly comparable PC frequencies and IgG booster responses for the serotypes they share, but that PCV13 induces higher frequencies of serotype-specific Bmems. The clinical implications of the observed differences in Bmem frequencies and booster profiles of the PCV10 and PCV13 vaccines are unknown. Further studies to include taking additional blood samples from these children when they are older may elucidate the impact of this difference on, for example, maintenance levels and the functionality of specific IgG in the longer term.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank the study team for their dedication to this study and also all the participating children and their families for their time and commitment. We also thank Lucy D. Phillips for editing the final manuscript.

Financial support. The clinical study was supported by the Dutch Ministry of Health.

Potential conflicts of interest. E. A. M. S. has received unrestricted research support from Pfizer, grant support for vaccine studies from Pfizer and GlaxoSmithKline (GSK), and fees paid to her institution for advisory boards or participation in independent data monitoring committees for Pfizer and GSK. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Spijkerman J, Prevaes SM, van Gils EJ, et al. Long-term effects of pneumococcal conjugate vaccine on nasopharyngeal carriage of *S. pneumoniae*, *S. aureus*, *H. influenzae* and *M. catarrhalis*. PLoS One **2012**; 7:e39730.
2. Spijkerman J, van Gils EJ, Veenhoven RH, et al. Carriage of *Streptococcus pneumoniae* 3 years after start of vaccination program, the Netherlands. Emerg Infect Dis **2011**; 17:584–91.
3. Elberse KE, van der Heide HG, Witteveen S, et al. Changes in the composition of the pneumococcal population and in IPD incidence in the Netherlands after the implementation of the 7-valent pneumococcal conjugate vaccine. Vaccine **2012**; 30:7644–51.
4. Rodenburg GD, de Greeff SC, Jansen AG, et al. Effects of pneumococcal conjugate vaccine 2 years after its introduction, the Netherlands. Emerg Infect Dis **2010**; 16:816–23.
5. van Deursen AM, van Mens SP, Sanders EA, et al. Invasive pneumococcal disease and 7-valent pneumococcal conjugate vaccine, the Netherlands. Emerg Infect Dis **2012**; 18:1729–37.
6. Poolman JT, Peeters CC, van den Dobbelsteen GP. The history of pneumococcal conjugate vaccine development: dose selection. Expert Rev Vaccines **2013**; 12:1379–94.
7. Blanchard-Rohner G, Pollard AJ. Long-term protection after immunization with protein-polysaccharide conjugate vaccines in infancy. Expert Rev Vaccines **2011**; 10:673–84.

8. McHeyzer-Williams LJ, Malherbe LP, McHeyzer-Williams MG. Checkpoints in memory B-cell evolution. Immunol Rev **2006**; 211:255–68.
9. Lanzavecchia A, Bernasconi N, Traggiai E, Ruprecht CR, Corti D, Sallusto F. Understanding and making use of human memory B cells. Immunol Rev **2006**; 211:303–9.
10. Dagan R, Patterson S, Juergens C, et al. Comparative immunogenicity and efficacy of 13-valent and 7-valent pneumococcal conjugate vaccines in reducing nasopharyngeal colonization: a randomized double-blind trial. Clin Infect Dis **2013**; 57:952–62.
11. Grant LR, O'Brien SE, Burbidge P, et al. Comparative immunogenicity of 7 and 13-valent pneumococcal conjugate vaccines and the development of functional antibodies to cross-reactive serotypes. PLoS One **2013**; 8:e74906.
12. van den Bergh MR, Spijkerman J, Francois N, et al. Immunogenicity, safety, and reactogenicity of the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine and DTPa-IPV-Hib when coadministered as a 3-dose primary vaccination schedule in the Netherlands: a randomized controlled trial. Pediatr Infect Dis J **2011**; 30:e170–8.
13. Vesikari T, Wysocki J, Chevallier B, et al. Immunogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) compared to the licensed 7vCRM vaccine. Pediatr Infect Dis J **2009**; 28(4 suppl):S66–76.
14. Elberse KE, Tcherniaeva I, Berbers GA, Schouls LM. Optimization and application of a multiplex bead-based assay to quantify serotype-specific IgG against *Streptococcus pneumoniae* polysaccharides: response to the booster vaccine after immunization with the pneumococcal 7-valent conjugate vaccine. Clin Vaccine Immunol **2010**; 17:674–82.
15. Perrett KP, John TM, Jin C, et al. Long-term persistence of immunity and B-cell memory following *Haemophilus influenzae* type b conjugate vaccine in early childhood and response to booster. Clin Infect Dis **2014**; 58:949–59.
16. Kelly DF, Snape MD, Perrett KP, et al. Plasma and memory B-cell kinetics in infants following a primary schedule of CRM 197-conjugated serogroup C meningococcal polysaccharide vaccine. Immunology **2009**; 127:134–43.
17. Perrett KP, Jin C, Clutterbuck E, et al. B cell memory to a serogroup C meningococcal conjugate vaccine in childhood and response to booster: little association with serum IgG antibody. J Immunol **2012**; 189: 2673–81.
18. Blanchard-Rohner G, Snape MD, Kelly DF, et al. The B-cell response to a primary and booster course of MenACWY-CRM(1)(9)(7) vaccine administered at 2, 4 and 12 months of age. Vaccine **2013**; 31:2441–8.
19. Clutterbuck EA, Oh S, Hamaluba M, Westcar S, Beverley PC, Pollard AJ. Serotype-specific and age-dependent generation of pneumococcal polysaccharide-specific memory B-cell and antibody responses to immunization with a pneumococcal conjugate vaccine. Clin Vaccine Immunol **2008**; 15:182–93.
20. Poolman J, Frasch C, Nurkka A, Kayhty H, Biemans R, Schuerman L. Impact of the conjugation method on the immunogenicity of *Streptococcus pneumoniae* serotype 19F polysaccharide in conjugate vaccines. Clin Vaccine Immunol **2011**; 18:327–36.
21. Cobb BA, Kasper DL. Zwitterionic capsular polysaccharides: the new MHCII-dependent antigens. Cell Microbiol **2005**; 7:1398–403.
22. Truck J, Lazarus R, Clutterbuck EA, et al. The zwitterionic type I *Streptococcus pneumoniae* polysaccharide does not induce memory B cell formation in humans. Immunobiology **2013**; 218:368–72.
23. Mazmanian SK, Kasper DL. The love-hate relationship between bacterial polysaccharides and the host immune system. Nat Rev Immunol **2006**; 6:849–58.
24. Park IH, Moore MR, Treanor JJ, et al. Differential effects of pneumococcal vaccines against serotypes 6A and 6C. J Infect Dis **2008**; 198: 1818–22.
25. Scott JR, Millar EV, Lipsitch M, et al. Impact of more than a decade of pneumococcal conjugate vaccine use on carriage and invasive potential in Native American communities. J Infect Dis **2012**; 205:280–8.
26. Tocheva AS, Jefferies JM, Rubery H, et al. Declining serotype coverage of new pneumococcal conjugate vaccines relating to the carriage of *Streptococcus pneumoniae* in young children. Vaccine **2011**; 29:4400–4.
27. Hendrikx LH, de Rond LG, Ozturk K, et al. Impact of infant and preschool pertussis vaccinations on memory B-cell responses in children at 4 years of age. Vaccine **2011**; 29:5725–30.
28. Hendrikx LH, Felderhof MK, Ozturk K, et al. Enhanced memory B-cell immune responses after a second acellular pertussis booster vaccination in children 9 years of age. Vaccine **2011**; 30:51–8.
29. Kakoulidou M, Ingelman-Sundberg H, Johansson E, et al. Kinetics of antibody and memory B cell responses after MMR immunization in children and young adults. Vaccine **2013**; 31:711–7.
30. Spijkerman J, Veenhoven RH, Wijmenga-Monsuur AJ, et al. Immunogenicity of 13-valent pneumococcal conjugate vaccine administered according to 4 different primary immunization schedules in infants: a randomized clinical trial. JAMA **2013**; 310:930–7.
31. Park DE, Johnson TS, Nonyane BA, et al. The differential impact of co-administered vaccines, geographic region, vaccine product and other covariates on pneumococcal conjugate vaccine immunogenicity. Pediatr Infect Dis J **2014**; 33(suppl 2):S130–9.
32. Andrews NJ, Waight PA, Burbidge P, et al. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. Lancet Infect Dis **2014**; 14:839–46.