MAJOR ARTICLE

Nasopharyngeal Carriage of *Streptococcus pneumoniae* Shortly before Vaccination with a Pneumococcal Conjugate Vaccine Causes Serotype-Specific Hyporesponsiveness in Early Infancy

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Background. The antibody response to pneumococcal conjugate vaccines (PCVs) in infants is variable. Factors responsible for this variability have not been fully elucidated. The objective of this study was to investigate whether pneumococcal carriage around the time of the first dose of 7-valent PCV (PCV7) affects serotype-specific immunologic response.

Methods. Healthy 2-month old infants were randomized to receive 2 (at the ages of 4 and 6 months) or 3 (at the ages of 2, 4, and 6 months) PCV7 doses and a booster dose (at the age of 12 months). Nasopharyngeal or oropharyngeal specimens were obtained for culture shortly before the first PCV7 dose. Serotype-specific immunoglobulin (Ig) G levels were measured at ages 2, 7, and 13 months.

Results. Of 545 children studied, 332 received a booster dose. The most common serotypes carried around the time of the first PCV7 dose were 6B ($n = 37$), 19F ($n = 22$), and 23F ($n = 14$). In carriers before the first dose, the IgG response to the carried serotype after 2 or 3 doses was significantly lower than in noncarriers. In contrast, response to the noncarried serotypes was not affected. Although all children responded to the booster dose, the response to the originally carried serotype was generally lower.

Conclusions. Serotype-specific hyporesponsiveness to PCV7 after pneumococcal carriage in infants is demonstrated for the first time. This phenomenon was common, lasted for at least several months, and was only partially overcome by the 12-month booster.

Trial registration. isrctn.org identifier: ISRCTN28445844.

Global prevention of pneumococcal disease and death through vaccination with pneumococcal conjugate vaccines (PCVs) is now possible. In many developing countries, early nasopharyngeal acquisition of *Streptococcus pneumoniae* is associated with both high carriage in young infants and a high rate of pneumococcal disease [1]. This is the main reason why the World Health Organization's Strategic Advisory Group of Experts (SAGE) recommended that the introduction of pneumococcal vaccination into developing countries be considered a high priority [2].

Despite multiple studies conducted during >30 years, the efficacy and effectiveness of 23-valent pneumococcal polysaccharide vaccine (PPSV) in children and adults remain poorly defined and the subject of controversy [3]. Furthermore, repeated doses of PPSV are associated with hyporesponsiveness, when antibody lev-

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els after a second antigenic challenge are lower than after the first, either in the same individual or in age-matched individuals receiving PPSV at the same time [4], a phenomenon previously described with *Neisseria meningitidis* polysaccharides, mainly the group C polysaccharides [5–7].

Protein polysaccharide conjugate vaccines, unlike unconjugated polysaccharide vaccines, induce T cell–dependent responses, making them already highly immunogenic during early infancy. In addition, after priming with conjugate vaccines, subsequent doses with either conjugate or polysaccharide vaccines evoke a booster response, rather than hyporesponsiveness [5]. Furthermore, conjugate vaccines can overcome hyporesponsiveness evoked by polysaccharide vaccines, although the response may be lower than in those not previously primed or those primed with PCVs. This was found in both adults and children immunized with meningococcus C vaccines [6–8] or pneumococcal vaccines [9–11].

A recent report suggested that invasive disease caused by a given serotype in children <18 months old can result in hyporesponsiveness to the infecting serotype, but not the other serotypes included in the PCV7, after subsequent administration of the 7-valent PCV conjugated to CRM_{197} (PCV7) [5]. The authors speculated that this phenomenon could reflect immune paralysis due to large pneumococcal polysaccharide antigen loads during invasive pneumococcal disease (IPD) and/or a potential genetic basis for nonresponse to individual serotypes.

Pneumococcal carriage is considerably more frequent than IPD [12] and may be associated with absorption and circulation of the carried polysaccharide, although probably with lower loads than during IPD. An indirect evidence of the polysaccharide absorption during carriage is the association of pneumococcal C polysaccharide detection in urine with pneumococcal carriage in healthy children [13–16]. Our objective was therefore to determine whether nasopharyngeal carriage of *S. pneumoniae* serotypes included in PCV7, at or immediately before the first PCV7 dose, affects the response to the primary immunization series and/or to the booster dose.

METHODS

Setting. In southern Israel (the Negev region), the Jewish and Bedouin populations, differing in their socioeconomic conditions and lifestyles, live side by side. However, both have access to the same medical services. The Jewish population is mainly urban, whereas the Bedouin population, formerly desert nomads, is in transition to a western lifestyle [17]. Contact between children of the 2 populations is rare. The Bedouin population lives in scattered clusters and Bedouin townships, whereas the Jewish population lives mostly in cities, with the rest living in Jewish townships and villages. Children of the 2 populations do not frequent the same daycare facilities or schools and do not have a common social life.

The Bedouin population is characterized by overcrowding, lower education level, larger family size, and lower income than the Jewish populations [17]. In 2004, the crude birth rate was 55.3 versus 21.0 births per 1000 population in the Bedouin versus Jewish populations [18], respectively; the mean family size ($\pm$ standard deviation) in the Bedouin population was 8.2 ± 0.9 persons versus only 3.2 ± 0.1 in the Jewish population [19]. The average monthly family income was 2-fold higher in the Jewish population [18]. Hospitalization rates for respiratory and other infectious diseases were higher among Bedouins [20]. Pneumococcal nasopharyngeal carriage is usually higher among Bedouin infants than among Jewish infants (data not shown), and maternally derived pneumococcal anti-polysaccharide antibodies are often higher in young Bedouin infants [21].

All children in the area are born in 1 hospital, where they also receive all emergency and inpatient services. Vaccines are given in public sector mother and child health centers for a token annual family membership fee. All children in Israel are entitled to medical insurance free of charge.

Participants. Healthy infants presenting to the mother and child health centers in southern Israel for vaccination were screened for eligibility. The study included male and female subjects aged 2 months $\pm$ 3 weeks, presenting for routine immunization, for whom the parents or legal guardians signed informed consent. Exclusion criteria were as follows: premature birth at <35 weeks; acute disease at enrollment; axillary temperature >38.0°C; any congenital abnormality judged to be clinically important or any inherited metabolic disorder, thrombocytopenia, or coagulation disorder; use of immune-modifying drugs for $\geq$14 days; known or suspected allergy or intolerance to constituents of products administered in the study; hypotonic-hyporesponsiveness or persistent inconsolable crying for $\geq$3 h within 48 h after a prior dose of any vaccine; and human immunodeficiency virus infection.

The study and all protocol revisions were approved by the Ethics Committees of the Soroka University Medical Center, the Maccabi Health Services Helsinki Committee, and the National Ministry of Health Ethics Committee.

Procedures. The study was initiated in August 2005, and enrollment lasted through March 2008. The study was initially designed to examine the effect of various immunization schedules on vaccine immunogenicity and pneumococcal nasopharyngeal carriage.

In this open-label, randomized trial, 2 intervention groups (randomized at a 2:1 ratio) were studied: a 3-dose primary immunization series (at the ages of 2, 4, and 6 months) and a 2-dose primary immunization series (at the ages of 4 and 6 months) (Figure 1). Randomization was performed using random number calculation, and the assigned randomization was

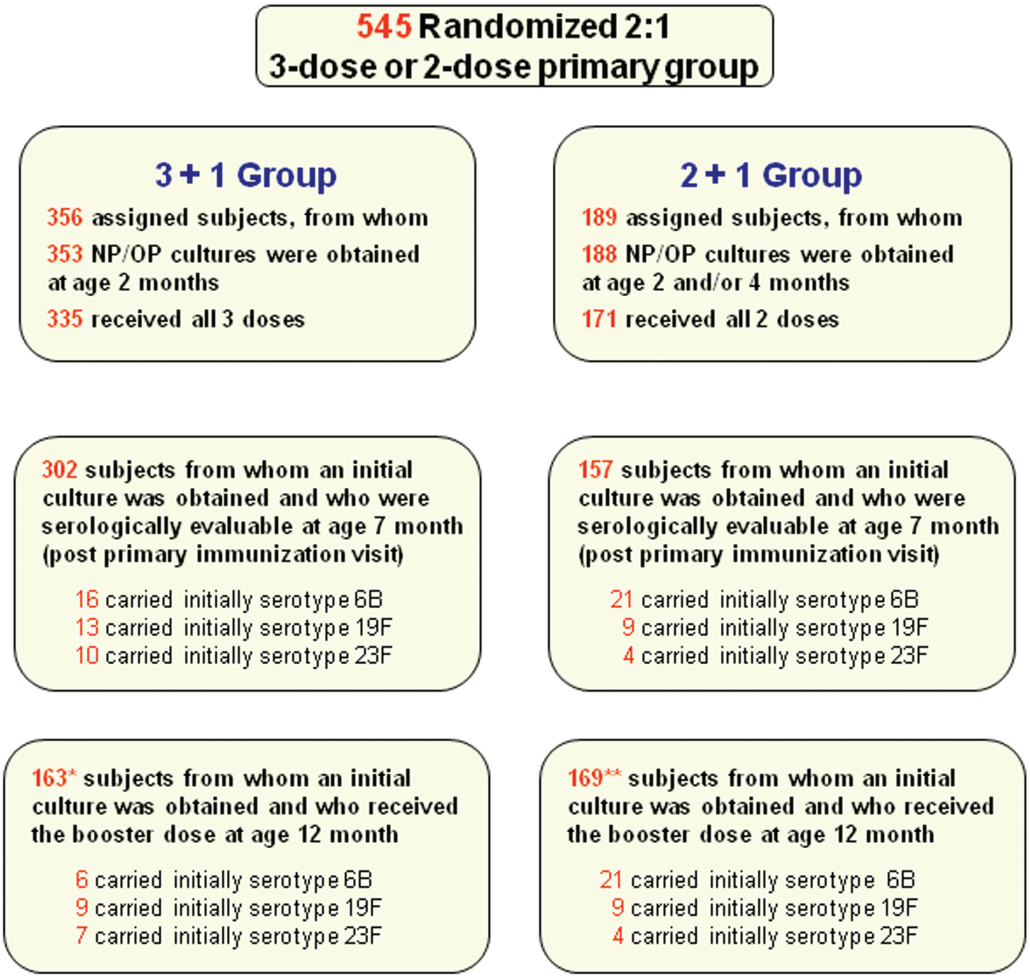

Figure 1. Flow diagrams for study groups. NP, nasopharyngeal; OP, oropharyngeal. *The 3-dose group was randomized 1:1 to receive either a 7-valent pneumococcal conjugate vaccine booster dose or no booster dose at 12 months of age. **In 4 children, serum for serologic tests was available after the booster doses but not after the primary immunization series.

presented in a sealed opaque envelope that was opened only after consent was obtained. The 3-dose primary immunization group was further randomized (1:1) to receive or not receive a booster PCV7 dose at 12 months of age. All children receiving the 2-dose primary vaccination series were assigned to receive a booster dose at 12 months.

The study vaccine was PCV7 (Wyeth Vaccines) containing 2 μg of serotypes 4, 9V, 14, 19F, and 23F polysaccharide, 4 μg of serotype 6B polysaccharide, and 2 μg of serotype 18C oligosaccharide, linked to the diphtheria toxoid protein CRM_{197} (lots A94431D, B08636C, 808672K, and C659732).

All infants received concomitant vaccines, including a combined diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae* type b, and inactivated poliovirus vaccine (GlaxoSmithKline Biologicals) at ages 2, 4, 6 and 12 months and a measles, mumps, and rubella vaccine (GlaxoSmithKline Biologicals) at age 12 months.

Nasopharyngeal and oropharyngeal swab samples were obtained at the time of first dose (age, 2 months) for the 3-dose primary group and at ages 2 and 4 months for the 2-dose primary group. The nasopharyngeal cultures were kept in transport swabs (MW173 Amies transport medium [Transwab, Medical Wire and Equipment] for nasopharyngeal samples; Culture Swab Transport System [Venturi Transystem, Copan Innovation] oropharyngeal samples) at room temperature until processed by the microbiology laboratory within 16 h. Results of the oropharyngeal swab samples were used only if *S. pneumoniae* did not grow from the nasopharyngeal swab samples. Identification and serotyping of *S. pneumoniae* were performed as described elsewhere [22].

A total of 2–4 mL of blood was drawn before vaccination (age, 2 months), 1 month after the primary dose (age, 7 months), and 1 month after the booster dose (age, 13 months). Blood samples were kept refrigerated and centrifuged in the laboratory within 24 h.

Serologic assays. Serum serotype-specific pneumococcal an-

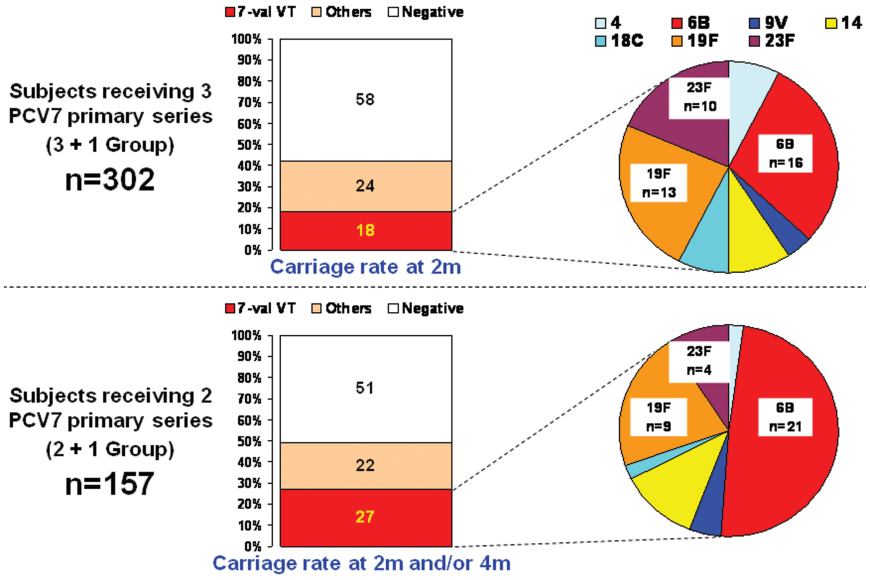

Figure 2. *Streptococcus pneumoniae* nasopharyngeal carriers at or shortly before the first 7-valent (val) pneumococcal conjugate vaccine booster (PCV7) dose in children receiving either 3 primary PCV7 doses (at ages 2, 4, and 6 months [m]) or 2 primary PCV7 doses (ages 4 and 6 months) who were serologically evaluable 1 month after the primary PCV7 series (age 7 months); 7-val VT indicates the serotypes included in PCV7. In 14 children of the 2+1 group (see Methods), from whom a VT serotype was isolated, a non-VT serotype was also isolated at a previous or subsequent visit.

ticapsular immunoglobulin (Ig) G concentrations were tested by enzyme-linked immunosorbent assay at the Department of Applied Immunology and Endocrinology, Kinder und Jugendklinik Universitätsklinikum Erlangen, after double absorption with C polysaccharide and serotype 22F polysaccharide [23–25].

Statistical analysis. Data were analyzed with SPSS software (version 14.0 for Windows). Contingency table analyses for comparing rates were performed using χ^2 or Fisher's exact test, as appropriate. Continuous variables including geometric mean concentration (GMC) comparisons were analyzed using *t* test or analysis of variance procedures. Because carriage rates often differ between Jewish and Bedouin infants, and because maternally derived pneumococcal anti-polysaccharide antibodies are often more common in Bedouin infants, ethnicity was corrected for using Mantel-Haenszel or linear regression analysis. Differences were considered significant at $P < .05$.

RESULTS

A total of 545 infants were randomized to receive either 3-dose primary vaccination (3+1 group; $n = 356$) or 2-dose primary vaccination (2+1 group; $n = 189$) (Figure 1). Of these, 302 (85%) of 356 subjects and 157 (83%) of 189 subjects in the 3 + 1 and 2+1 groups, respectively, had an initial nasopharyngeal culture and were evaluable for serologic response at the postprimary vaccination visit (age 7 months). The 3+1 and 2+1 groups were comparable in proportions of male subjects (45%–50% for all visits). The mean ages (± standard deviations) at the first, second, third, and booster doses for the 3+1 group were 2.1 ± 0.2, 4.0 ± 0.4, 5.8 ± 0.6, and 12.5 ± 0.7 months. The mean ages at the first, second, and booster doses for the 2+1 group were 3.9 ± 0.3, 5.7 ± 0.4, and 12.4 ± 0.5 months. At enrollment, 51% and 38% of the subjects were Bedouin in the 3+1 and 2+1 groups, respectively.

At the enrollment visit (age, 2 months), 127 (42%) of 302 infants in the 3+1 group carried *S. pneumoniae,* of whom 54 (18%) carried a serotype included in PCV7 (VT serotype) and 73 (24%) carried a non-VT serotype (Figure 2). For the 2+1 group, because the first dose was given at 4 months of age, carriage at both 2 and 4 months was investigated. Pneumococcal carriage at 2 and/or 4 months was detected in 77 of the 157 infants (49%) (9 at age 2 months, 25 at 4 months, and 43 at both 2 and 4 months). Of the 157 infants, 42 (27%) and 48 (31%) carried VT and non-VT serotypes, respectively.

The most commonly carried VT serotypes at or shortly before the first PCV7 dose were 6B, 19F, and 23F (Figure 2); these constituted the study serotypes, because carriage of the other 4 VT serotypes was not prevalent enough to be studied.

Carriage of serotypes 6B, 19F, and 23F at or shortly before the first PCV7 dose resulted in both a decreased GMC of the anticapsular IgG antibodies and a lower proportion of children with concentrations of ⩾0.35 μg/mL against the carried serotype in both the 3+1 and 2+1 groups (Tables 1 and 2 and Figures 3–5). This difference was significant in all comparisons, except for serotype 23F in the 2+1 group, an arm with only 4 children. In contrast, when IgG GMCs were measured for the serotypes not retrieved from the nasopharynx around the

Table 1. Serotype-Specific Anticapsular Immunoglobulin (Ig) G Levels in Children Who Carried *Streptococcus pneumoniae* Serotypes 6B, 19F, or 23F at or Shortly before the First Dose of 7-Valent Pneumococcal Conjugate Vaccine Booster (PCV7)

Serotype carriage groups and anticapsular IgG antibodies	Subjects receiving PCV7 at ages 2, 4, and 6 months (3 + 1 group)			Subjects receiving PCV7 at ages 4 and 6 months (2 + 1 group)		
	Positive before vaccination	Negative before vaccination	P^a	Positive before vaccination (*n*	Negative before vaccination	P^a
Serotype 6B carriers vs noncarriers						
No. of subjects	16	286		21	136	
IgG, GMC (95% CI), μg/mL						
Anti-6B IgG[b]	0.35 (0.12–1.00)	2.29 (1.92–2.72)	<.001	0.23 (0.11–0.46)	0.66 (0.53–0.82)	<.001
Anti-19F IgG	2.78 (1.63–4.74)	1.90 (1.66–2.18)	.175	3.31 (1.48–7.40)	1.99 (1.56–2.54)	.054
Anti-23F IgG	1.78 (1.10–2.88)	1.11 (0.96–1.28)	.124	0.78 (0.42–1.44)	0.61 (050–0.75)	.479
Serotype 19F carriers vs noncarriers						
No. of subjects	13	289		9	148	
IgG, GMC (95% CI), μg/mL						
Anti-6B IgG	1.42 (0.52–3.83)	2.11 (1.76–2.53)	.429	1.11 (0.48–2.54)	0.55 (0.44–0.68)	.154
Anti-19F IgG[b]	0.49 (0.13–1.87)	2.07 (1.82–2.34)	<.001	0.17 (0.05–0.66)	2.48 (2.00–3.08)	<.001
Anti-23F IgG	1.50 (0.68–3.29)	1.12 (0.98–1.30)	.324	0.50 (0.30–0.83)	0.64 (0.53–0.79)	.430
Serotype 23F carriers vs noncarriers						
No. of subjects	10	292		4	153	
IgG, GMC (95% CI), μg/mL						
Anti-6B IgG	3.45 (1.49–7.99)	2.03 (1.69–2.44)	.266	1.20 (0.15–9.78)	0.56 (0.45–0.69)	.298
Anti-19F IgG	2.31 (0.90–5.91)	1.93 (1.89–2.21)	.511	1.17 (0.12–11.33)	2.17 (1.71–2.75)	.612
Anti-23F IgG[b]	0.44 (0.10–1.93)	1.19 (1.04–1.36)	.014	0.45 (0.03–6.76)	0.64 (0.53–0.78)	.517

NOTE. Antibody concentrations were measured at age 7 months (1 month after completion of the primary series). CI, confidence interval; GMC, geometric mean concentration.

[a] *P* values adjusted for ethnicity (Jewish vs Bedouin infants).

[b] Antibodies against the carried serotype.

time of first PCV7 dose (namely, GMC for serotypes 19F and 23F for those carrying serotype 6B, serotypes 6B and 23F for those carrying 19F, and serotypes 6B and 19F for those carrying 23F), no significant differences in postprimary series IgG GMCs were observed between carriers and noncarriers. Thus, a clear hyporesponsiveness was observed for each of the 3 studied serotypes in subjects carrying these serotypes around the time of the first PCV7 dose. This phenomenon was not seen when these serotypes were not carried at this time.

We investigated whether this hyporesponsiveness persisted after booster dose administration at age 12 months (Figures 3–5). As can be clearly seen, although all groups showed a clear booster response, the serotype-specific GMCs were lower in children carrying the given serotypes around the administration of the first PCV7 dose than in those not carrying the serotypes. These differences reached statistical significance for serotype 6B in both 3 + 1 and 2 + 1 groups and for serotype 19F in the 2 + 1 group. In contrast to the carried serotypes, no significant differences in postbooster IgG GMC between groups were observed for serotypes not carried around the time of the first dose.

We determined whether carriage or its effect was related to serotype-specific IgG concentrations at the time of vaccination (representing maternal antibody) (Figure 3–5). No significant difference between carriers and noncarriers was found in serum anticapsular IgG GMCs at the time of first dose administration for any of the serotypes tested.

DISCUSSION

PCVs elicit a T cell–dependent response and thus are highly immunogenic in infancy [4, 7]. However, we demonstrated in the present study that pneumococcal nasopharyngeal carriage shortly before the first infant dose of PCV7 resulted in hyporesponsiveness to the capsular polysaccharide of the carried strain. This serotype-specific hyporesponsiveness was only partially overcome after 2 or 3 PCV7 doses. Furthermore, although an additional PCV7 dose administered at 12 months elicited a booster dose response, the response was blunted in children who had carried the specific serotype around the time of the first PCV7 dose, at age 2 or 4 months. Preliminary results from our study showed high correlation between the concentrations measured by enzyme-linked immunosorbent assay (as shown in the present article) and the more functional opsonophagocytosis assay (data not shown).

What could be the reasons for this hyporesponsiveness to PCV7 observed in pneumococcal carriers? Because the first PCV7 dose was administered at age 2 months, when the cir-

Table 2. Proportions of Subjects with Serotype-Specific Anticapsular Immunoglobulin (Ig) G Levels ⩾0.35 μg/mL among Infants Who Carried *Streptococcus pneumoniae* Serotypes 6B, 19F, or 23F at or Shortly before the First Dose of 7-Valent Pneumococcal Conjugate Vaccine Booster (PCV7)

Group	Subjects receiving PCV7 at ages 2, 4, and 6 months (3 + 1 group)			Subjects receiving PCV7 at ages 4 and 6 months (2 + 1 group)		
	Positive before vaccination	Negative before vaccination	*P*[a]	Positive before vaccination	Negative before vaccination	*P*
Serotype 6B carriers vs noncarriers						
No. of subjects	16	285		21	136	
No. (%) with IgG ⩾0.35 μg/mL						
Anti-6B IgG[b]	6 (38)	255 (89)	<.001	6 (29)	90 (66)	.002
Anti-19F IgG	16 (100)	265 (93)	.998	19 (90)	121 (89)	.586
Anti-23F IgG	16 (100)	241 (85)	.998	15 (71)	95 (70)	.924
Serotype 19F carriers vs noncarriers						
No. of subjects	13	288		9	148	
No. (%) with IgG ⩾0.35 μg/mL						
Anti-6B IgG	12 (92)	249 (86)	.520	7 (78)	89 (60)	.303
Anti-19F IgG[b]	7 (54)	274 (95)	<.001	4 (44)	136 (92)	.002
Anti-23F IgG	11 (85)	246 (85)	.856	7 (78)	103 (70)	.643
Serotype 23F carriers vs noncarriers						
No. of subjects	10	291		4	153	
No. (%) with IgG ⩾0.35 μg/mL						
Anti-6B IgG	10 (100)	251 (86)	.999	3 (75)	93 (61)	.576
Anti-19F IgG	9 (90)	272 (93)	.913	3 (75)	137 (90)	.583
Anti-23F IgG[b]	6 (60)	251 (86)	.053	2 (50)	108 (71)	.360

NOTE. Antibody concentrations were measured at age 7 months (1 month after completion of the primary series). PCV7, 7-valent pneumococcal conjugate vaccine booster.

[a] *P* values adjusted for ethnicity (Jewish vs Bedouin infants).

[b] Antibodies against the carried serotype.

culating anti-polysaccharide IgG is essentially maternally derived, can these results reflect differences in maternally derived antibody concentration? In principle, maternally derived antibodies can be associated with both differences in colonization rates and responses to PCV7. However, no differences in specific anti-polysaccharide IgG antibody concentrations between carriers and noncarriers for any of the serotypes tested were observed at 2 months of age. Thus, the role of maternal antibodies could be ruled out.

Could pneumococcal carriage affect innate immunity, antigen-presenting cells, or T cells required for B cell help, resulting in reduced antibody response to PCVs? Such a general mechanism can be excluded, because the hyporesponsiveness was serotype specific: immune responses were blunted only to the carried serotype, responses to other serotypes remaining unaffected. The same rationale rules out genetic factors, because the subjects responded appropriately to all tested serotypes other than the carried serotype.

Another potentially plausible explanation is that carriage resulted in the absorption and circulation of high loads of pneumococcal polysaccharide, which could compete with the respective polysaccharide antigen included in PCV7. Although such a competition could affect responses to the first vaccine dose, serotype-specific carriage-associated polysaccharide load is unlikely to persist throughout the primary and booster dose series (ie, ⩾10 months) [26–28].

Having excluded the previous potential mechanisms for the observed serotype-specific hyporesponsiveness, we are left with the most likely plausible explanation, namely, the B cell exhaustion or fatigue mechanism. B cell hyporesponsiveness caused by the injection of polysaccharide antigens has been largely described. Several randomized clinical trials in adults ⩾50 years old using 23-valent PPSV (PPSV23) showed that a second dose of PPSV23 administered within <5 years after the first dose was associated with clear hyporesponsiveness [9, 10, 29, 30]. Hyporesponsiveness observed after PPSV23 administration in the elderly was partially overcome by PCV7 administered as the second dose. However, a PCV7 dose administered 1 year after PPSV23 injection still elicited generally lower anticapsular IgG concentrations than these obtained in subjects not previously primed with PPSV23 [9, 31].

B cell hyporesponsiveness after the administration of PPSV was also observed in infants. This was first described in group C meningococcal polysaccharide vaccines [32, 33]. This hyporesponsiveness was partially overcome by meningococcal conjugate vaccine [7, 34]. Only a few studies have investigated mul-

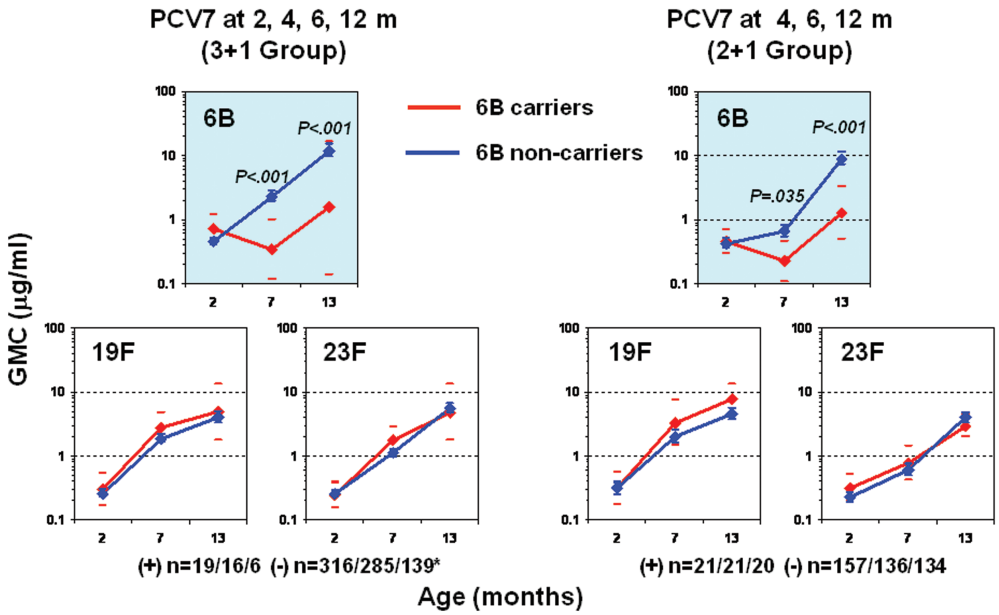

Figure 3. Anticapsular immunoglobulin G geometric mean concentrations (GMCs) and 95% confidence intervals at ages 2 months (m) (prevaccination), 7 months (1 month after primary 7-valent pneumococcal conjugate vaccine booster [PCV7] series), and 13 months (1 month after booster dose) in subjects who carried serotype 6B shortly before or at the first PCV7 dose versus noncarriers. Children belonged to either the 3+1 or the 2+1 group (see Methods). In the 3+1 group, only half of the children were assigned to receive a booster dose. *P* values were adjusted for ethnicity (Jewish vs Bedouin infants). *In the 3+1 group, only one-half of the children were assigned to receive a booster dose.

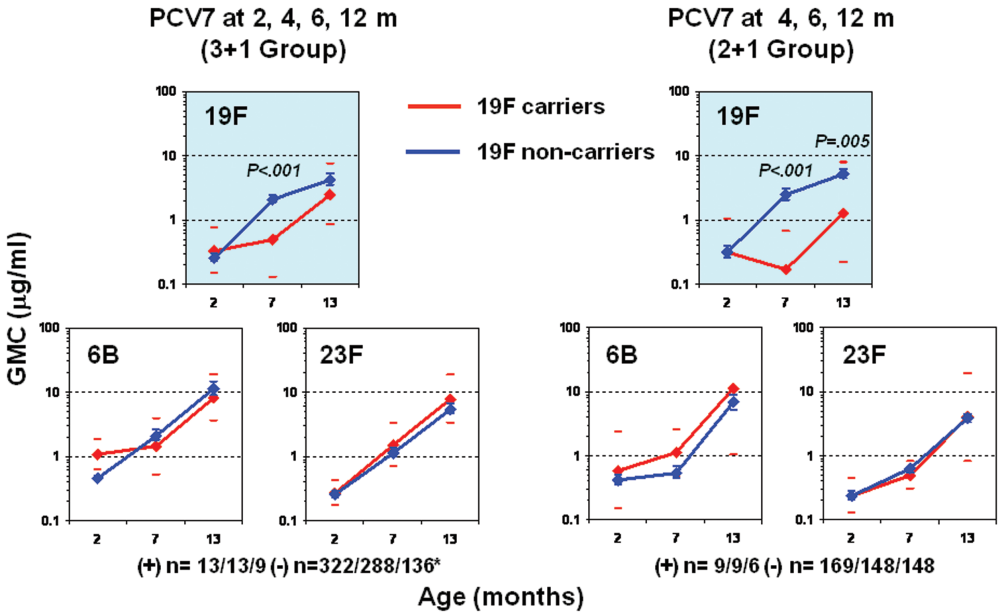

Figure 4. Anticapsular immunoglobulin G geometric mean concentrations (GMCs) and 95% confidence intervals at ages 2 months (m) (prevaccination), 7 months (1 month after primary 7-valent pneumococcal conjugate vaccine booster [PCV7] series), and 13 months (1 month after booster dose) in subjects who carried serotype 19F shortly before or at the first PCV7 dose versus noncarriers. Children belonged to either the 3+1 or the 2+1 group (see Methods). In the 3+1 group, only half of the children were assigned to receive a booster dose. *P* values were adjusted for ethnicity (Jewish vs Bedouin infants). *In the 3+1 group, only one-half of the children were assigned to receive a booster dose.

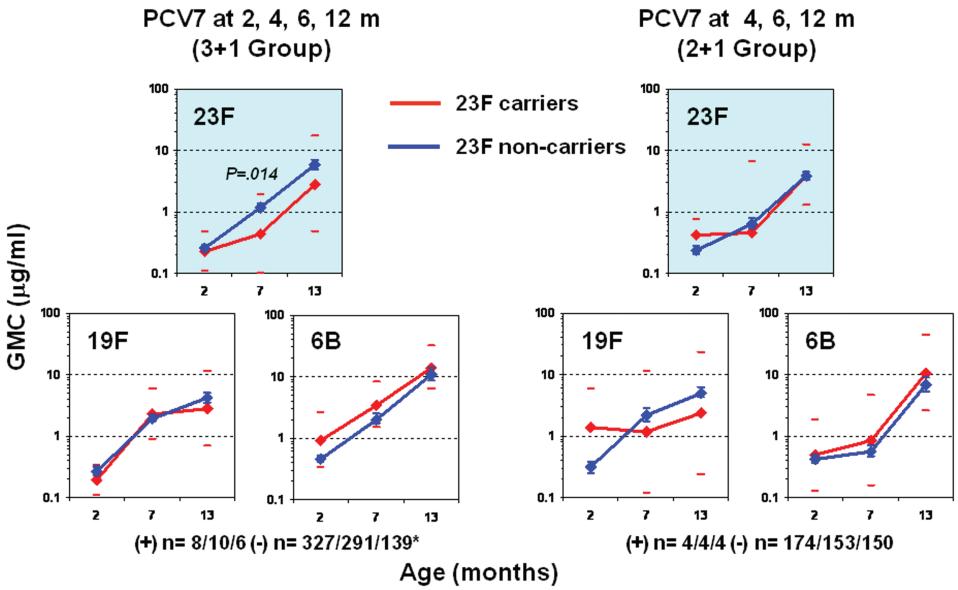

Figure 5. Anticapsular immunoglobulin G geometric mean concentrations (GMCs) and 95% confidence intervals at ages 2 months (prevaccination), 7 months (1 month after primary 7-valent pneumococcal conjugate vaccine booster [PCV7] series) and 13 months (1 month after booster dose) in subjects who carried serotype 23F shortly before or at the first PCV7 dose versus noncarriers. Children belonged to either the 3+1 or the 2+1 group (see Methods). In the 3+1 group, only half of the children were assigned to receive a booster dose. *P* values were adjusted for ethnicity (Jewish vs Bedouin infants). *In the 3+1 group, only one-half of the children were assigned to receive a booster dose.

tiple doses of PPSV in infants, and most showed that antibody concentrations after the second PPSV dose were lower than after the first [4]. However, no systematic studies were conducted to assess whether the use of PCVs can overcome PPSV-induced hyporesponsiveness in healthy infants or toddlers. In 1 study with a limited number of subjects, we showed that when a second PPSV23 dose was administered to healthy toddlers 1 year after the first dose, the response was lower than that after the first dose for several serotypes. This reduction was not seen if PCV was given instead of PPSV23 [11].

Anecdotal reports have suggested that serotype-specific hyporesponsiveness to polysaccharide antigens can be induced by pneumococcal invasive infections. In 2 case reports, 2 children who experienced IPD were unable to respond to the infecting serotype but could respond to other serotypes when given a PPSV (2 weeks and 27 months after the events for serotypes 14 and 18C, respectively) [35, 36]. In a recent report, 8 children aged 3.1–16.3 months who had experienced IPD failed to develop a response to the infecting serotype (despite receiving ⩾3 doses of PCV7). None of these children had underlying risk factors for IPD, and their responses to the other PCV7 serotypes were adequate [5].

The most likely explanation for our observation is that B cell fatigue or unresponsiveness may also be elicited in healthy infants by nasopharyngeal carriage. Carriage during early infancy may result in absorption and circulation of high polysaccharide antigen loads and may persist for several months [26–28]. These circulating polysaccharides are expected to bind to serotype-specific infant B cells, especially in the marginal zone of the spleen and nodes. In the absence of carrier-induced T cell help, in the poorly developed marginal zones, immature infant B cells do not effectively differentiate into antibody-producing plasma cells in response to polysaccharides [37, 38]. Our observation that children carrying a given vaccine serotype shortly before the first PCV7 immunization fail to respond to this specific serotype indicates that their B cells may not be activated even by an otherwise immunogenic conjugate vaccine. This implies that B cell fatigue or unresponsiveness may be driven by nasopharyngeal carriage in healthy young infants, whose pool of marginal zone B cells is limited [37, 38]. In our study, this phenomenon persisted at least through the first year of life. Whether and when it can be fully overcome is an open question.

The blunting of vaccine responses in carrier children may not be restricted to young infants. In 1 study, the administration of 1 dose of PCV7 to 1-year-old toddlers resulted in significantly lower anti-9V antibody concentration 9–11 days after immunization in 8 children carrying *S. pneumoniae* serotype 9V at the time of immunization than in 152 children not carrying this serotype [39]. However, no significant differences were demonstrated for the other serotypes.

Although we could study only the 3 serotypes carried at a sufficient frequency at 2–4 months of age, which is a limitation of our study, the importance of carriage-induced B cell ex-

haustion is indeed expected to decline with the age-associated maturation of the marginal zone B cell pool. Whether various serotypes differ in their vulnerability to carriage-induced B cell exhaustion, depending on their binding characteristics and the size of the serotype-specific B cell pool, is a plausible hypothesis that remains to be studied.

The present study clearly demonstrates, for the first time, that serotype-specific hyporesponsiveness can occur in infants even as a result of pneumococcal colonization, a much more common situation than IPD, and that PCV7 can only partially overcome this phenomenon. Nasopharyngeal pneumococcal colonization in infants is common, especially in crowded populations such as those in the developing world, where most severe and fatal pneumococcal diseases occur. In developing countries or among indigenous populations, pneumococcal carriage exceeds 50%–75% by the age of 2 months, about the time the first PCV7 dose is usually recommended [40]. In our study >40% of infants carried *S. pneumoniae* at the time of the first PCV7 dose, and 18%–27% carried a serotype included in PCV7. Thus, the potential of reduced response to PCV7 in populations with high pneumococcal carriage rates during early infancy may be substantial. The clinical significance of this phenomenon is still unclear. However, the fact that hyporesponsiveness persisted after 3 doses and often, though to a lesser extent, after a booster dose, suggests that these infants may be at higher risk than their noncarrier peers for disease caused by the carried serotypes.

Can carriage-induced hyporesponsiveness limit PCV7 vaccine efficacy? The exact relationship between reduced antibody response and disease is not entirely clear. A reduced proportion of children achieving antibody concentrations above a given threshold after the primary immunization series, as observed here for serotypes 6B, 19F, and 23F, is indeed expected to result in reduced PCV7 efficacy against IPD [41, 42]. Protection against the more common mucosal pneumococcal diseases, such as acute otitis media and pneumonia, or against carriage, is more likely to relate to higher IgG concentration, but the exact threshold is not known [43]. Accordingly, the finding that carriage at the time of the first PCV7 dose results in IgG GMCs that are several-fold lower for each of the carried serotypes suggests a lower vaccine efficacy against mucosal end points among carriers [44, 45].

This first report of the negative influence of previous exposure or carriage on PCV vaccine responses calls for a careful examination and selection of optimal immunization schedules. In areas of intense and early pneumococcal colonization, carriage may affect immune response in a large number of infants, posing a public health threat. In such areas, providing a booster dose may be important. In addition, immunization during or shortly after the 4-week neonatal period may result in improved protective efficacy, by reducing the chance of becoming a carrier shortly before immunization. However, this possibility needs still to be studied.

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