Persistence of IgG Antibody Following Routine Infant Immunization with the 7-Valent Pneumococcal Conjugate Vaccine

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Background: Pneumococcal conjugate vaccine (PCV) induces protective anticapsular IgG, which mediates disease immunity. IgG persistence may influence long-term protection.

Methods: An observational, prospective, longitudinal study of nasopharyngeal carriage among American Indian households from 2006 to 2008 evaluated long-term immunogenicity of 7-valent PCV (PCV7). Children unimmunized with PCV were age-matched to those PCV7 immunized at least 4 years prior (ratio 1:3 or 1:4). Blood collected at the final study visit was analyzed for PCV7 serotype IgG (enzyme-linked immunosorbent assay) and for functional activity (multiplex-opsonophagocytic assay) for serotypes 4, 6B, 14 and 23F. Geometric mean concentrations (GMCs), titers (GMTs) and the odds of serotype-specific IgG ≥0.35 μg/mL were compared according to immunization status using a matched regression approach.

Results: Eight unimmunized and 28 immunized children age-matched at the time of serum collection (mean age: 7.9 years) were included. Serotype-specific GMCs, GMTs and proportions above the correlate of protection did not differ between the groups except for serotypes 14 and 23F. Serotype 14 GMCs (immunized 0.7 vs. unimmunized 0.2; $P = 0.02$) and serotype 23F GMTs (immunized 388.3 vs. unimmunized 47.8; $P = 0.03$) were significantly higher among immunized children. IgG concentrations and functional titers among immunized children were strongly correlated for serotypes 4 ($r = 0.78$; $P \leq 0.001$) and 14 ($r = 0.52$; $P \leq 0.01$).

Conclusions: PCV serotype-specific IgG concentrations 4 years following PCV vaccination do not persist above natural levels for most serotypes. Exposure to pneumococcus may be critical in maintaining persistent serotype-specific IgG; the elimination of circulating vaccine type pneumococci by PCV may have effects on long-term immunity.

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The 7-valent pneumococcal conjugate vaccine (PCV7, Pfizer, Pearl River, NY; serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) was licensed and introduced into the US infant immunization schedule in 2000 with the primary series given at 2, 4 and 6 months and a booster dose at 12–15 months of age (ie, 3 + 1 schedule). Direct and indirect protection against vaccine serotype invasive disease and colonization was documented, including among high-risk populations.[1,2] In 2010, PCV7 was replaced with a 13-valent product (PCV13, Pfizer) adding serotypes 1, 3, 5, 6A, 7F and 19A. Although studies have demonstrated that PCVs generate a substantial immune response, limited data exist on the longevity of the response during routine PCV use when vaccine serotype colonization is virtually eliminated and replacement colonization with non-vaccine serotypes has occurred.[3]

Using pooled data from 3 PCV efficacy trials,[4–6] 0.35 μg/mL was established as the IgG concentration that predicted vaccine efficacy against invasive pneumococcal disease and is accepted by regulatory authorities as the evaluation threshold for licensure of new PCVs.[7] Most studies measure the IgG response following completion of the primary series or immediately following the booster dose; several have evaluated the long-term IgG levels in the context of an efficacy trial,[8–10] but none to our knowledge in the context of routine PCV use. Translating the 0.35 μg/mL correlate to contextualize the likelihood of IgG yielding protection years following the initial immunization series is poorly understood. The objective of this analysis was to measure serotype-specific pneumococcal IgG concentrations in children who were immunized with PCV7 at least 4 years before determining IgG persistence in a setting where vaccine serotype strains are no longer circulating.

MATERIALS AND METHODS

From 2006 to 2008, an observational, longitudinal study of 1072 individuals from 300 Navajo and White Mountain Apache families was conducted to evaluate the effects of long-term PCV7 use on nasopharyngeal (NP) carriage at the community level.[3] Study participants were followed monthly for 6 months (ie, 7 visits). An NP specimen was collected at each visit; serum was collected at the final visit. In a nested analysis, unimmunized children who had been age-eligible as infants to receive PCV7 but had no record of receipt were age-matched at a ratio of 1:3 or 1:4 to immunized children who had received at least 1 dose of PCV7 at least 4 years prior.

NP specimens were collected as previously described.[3] Pneumococci were isolated and serotyped by Neufeld Quellung reaction at the Centers for Disease Control and Prevention. Serotypes 6A and 6C isolates were distinguished by polymerase chain reaction.

TABLE 1. Characteristics of PCV7-Immunized and PCV7-Unimmunized Children

Characteristics	Unimmunized (N = 8)	Immunized* (N = 28)
Categorical variables	Number (%)	
Male	2 (25)	18 (64)
Smoker in house	4 (50)	5 (18)
Stove in house	6 (75)	19 (68)
Daycare, last 6 months	0 (0)	1 (4)
Vaccine-type colonization	0 (0)	2 (7)
Vaccine-type household colonization	0 (0)	5 (18)
Continuous variables	Mean (range)	
Number of siblings <6 years	1.5 (0–3)	1.8 (0–4)
Number of household members	6.5 (4–8)	5.8 (5–12)
Years from immunization to blood collection	()	6.4 (4–7.5)

* Immunized defined as children who received at least 1 dose of PCV7, at least 4 years before serum collection.

Venous blood was collected; sera were separated, stored at −80°C and tested for antibodies to the 7 vaccine-type capsular polysaccharides at the Institute of Child Health, University College London. IgG levels were measured by the World Health Organization reference enzyme-linked immunosorbent assay after adsorption with cell wall and 22F polysaccharide (http://www.vaccine.uab.edu/ELISA%20Protocol.pdf). Sera were analyzed for IgG function by a multiplex-opsonophagocytic assay for serotypes 4, 6B, 14 and 23F.[11] A multiplex-opsonophagocytic assay titer of ≥1:8 was considered positive.

The odds of achieving the serotype-specific IgG threshold of 0.35 and 4.0 μg/mL for NP colonization[12] were calculated and compared by conditional logistic regression. For small sample analyses, the odds ratio and 95% confidence intervals were estimated by a bootstrap with 2000 replications. Geometric mean concentrations (GMCs) and titers (GMTs) were calculated by linear regression using log-transformed IgG concentrations (or titers) and compared by immunization status. Correlations in IgG concentrations and functional titers were assessed by Pearson coefficient (r). A P value ≤0.05 was considered statistically significant. Statistical analyses were completed using Stata 12.0 (StataCorp, College Station, TX).

Ethical approvals were obtained from the review boards of Johns Hopkins Bloomberg School of Public Health, the Navajo Nation and the Phoenix Area Indian Health Service as well as from the tribes. Children were enrolled into the study following parental informed consent for participation.

RESULTS

Eight PCV7-unimmunized children were age-matched to 28 immunized children. Four of the unimmunized children were each matched to 4 immunized children, and the remaining 4 unimmunized children were matched to only 3 immunized children. Study participant characteristics are presented in Table 1. Immunized children received on average 3 PCV7 doses (min, max: 1, 5 doses) at least 4 years before the study (Table 2). Two immunized subjects, each with 4 doses of PCV7, acquired 19F NP colonization in the 6 months before serum collection. None of the unimmunized subjects acquired a vaccine serotype during the 6-month study period.

TABLE 2. Dosing Characteristics of PCV7-Immunized Children

Number of PCV7 Doses Received	Number of Immunized Children with PCV7 Doses Received	Age at Last Dose (Months)	
		Mean	Min, Max
1	1	62.0	62.0, 62.0
2	6	48.3	39.3, 61.3
3	4	52.7	38.8, 88.3
4	16	40.6	30.8, 94.9
5	1	61.0	61.0, 61.0

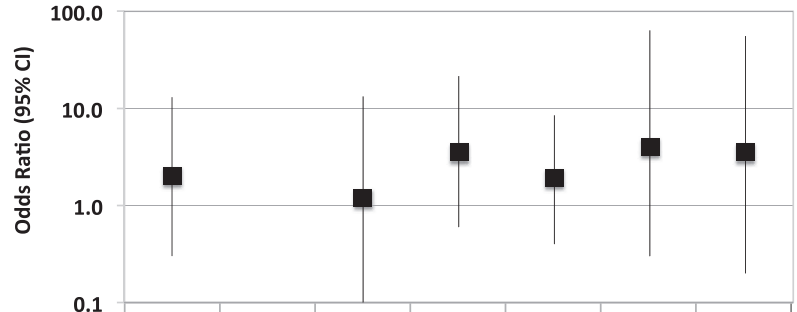
All immunized and unimmunized children had detectable levels of circulating serotype-specific IgG. Half or more immunized and unimmunized children had IgG concentrations ≥0.35 μg/mL. Few unimmunized and immunized children (<40%) had 6B, 18C and 23F serotype-specific IgG concentrations ≥4.0 μg/mL, and no unimmunized children had serotypes 4, 9V, 14 or 19F IgG concentrations ≥4.0 μg/mL. The odds of IgG concentrations ≥0.35 μg/mL were similar between unimmunized and immunized children for all serotypes (Fig. 1).

In general, GMCs were lower among unimmunized children (range: 0.2–2.4 μg/mL) than immunized children (range: 0.5–2.7 μg/mL; Fig. 2). GMCs for 6B and 19F were noticeably higher compared with other serotypes for both immunized and unimmunized. There was no significant difference in GMCs for immunized or unimmunized for any serotype except serotype 14 where the GMC for unimmunized was 3.5 times lower than among immunized children (0.2 vs. 0.7 μg/mL).

Both immunized and unimmunized children had functional IgG; however, GMTs were higher for immunized children and significantly higher for serotype 23F [47.8 (unimmunized) vs. 388.3 (immunized), P = 0.03; Fig. 3]. For immunized children, IgG concentrations and functional titers were highly correlated for serotypes 4 and 14 and only weakly correlated for serotypes 6B and 23F (Fig. 4). Correlations in IgG concentrations and functional titers among unimmunized children were found for serotype 14, but weak or absent for serotypes 4, 6B and 23F.

DISCUSSION

PCV7-immunized children have comparable amounts of circulating vaccine serotype-specific pneumococcal IgG 4 years following PCV7 immunization as unimmunized children. Childhood PCV7 immunization may have little to no long-term serological effects. The stimulus for the production of capsule-specific IgG in unimmunized children is unclear as introduction of PCV7 in 2000 led to community-wide reductions in serotypes 6B and 19F with very low-level colonization prevalence (0.1% and 0.5%, respectively).[3] Despite this, serotypes 6B and 19F had the highest GMCs in the unimmunized subjects with levels similar to those in the PCV-immunized children in our study. Interestingly, 2 immunized children acquired serotype 19F during the study period, but no 6B was detected at all. Both serotypes have been characterized as having



	4	6B	9V	14	18C	19F	23F
# (%) Imm ≥0.35 μg/mL	4 (50)	8 (100)	7 (88)	4 (50)	4 (50)	7 (88)	7 (88)
# (%) Unimm ≥0.35 μg/mL	18 (64)	28 (100)	25 (89)	21 (75)	19 (68)	27 (96)	27 (96)
Odds Ratio (95% CI)	2.00 (0.31, 13.14)	--	1.17 (0.10,13.31)	3.51 (0.57, 21.51)	1.86 (0.41, 8.55)	4.00 (0.25, 63.95)	3.46 (0.22, 55.78)

FIGURE 1. Comparing the odds of having serotype-specific pneumococcal IgG concentration ≥0.35 μg/mL for immunized versus unimmunized children.

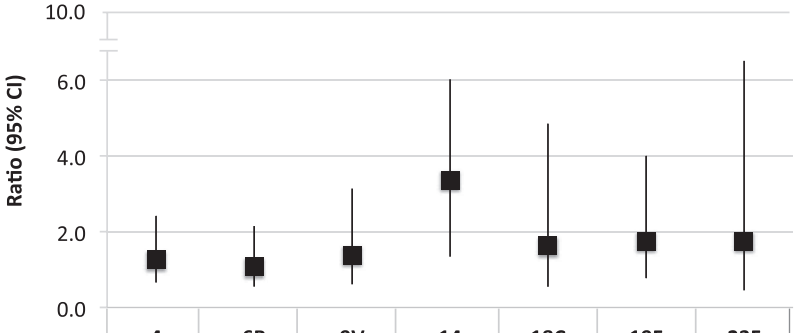
biochemical and physical properties associated with resistance to immunologic effector mechanisms, aiding in carriage duration and persistence and hindering elimination through vaccine use.[13]

Even though substantial reductions in circulating vaccine serotype strains have occurred with routine PCV use, it is possible that natural exposure to pneumococcus occurred among the unimmunized children producing IgG concentrations that resemble those of immunized children. A study of adults who generated capsule-specific antibodies in response to natural exposure by pneumococcal carriage supports this hypothesis.[14] This scenario is plausible if low-density colonization was occurring and contributing to persistent priming of the immune system. Low-density colonization may not be detected with standard culture-based methods used for isolation of pneumococcus in this study. Future evaluations should include an analysis of low-density colonization to understand its role in developing and maintaining serotype specific IgG concentrations.

It is also possible that cross-reactive serotype antigens may have stimulated or contributed to the persistence of PCV-induced IgG. Serotypes 6C and 19A, the 2 most prevalent colonizing serotypes across all ages in this population before introduction of PCV13,[3] could have induced antibodies to 6B and 19F, respectively. We have recently shown that adsorbing sera collected after PCV immunization with 6C or 19A reduces the opsonophagocytic activity against 6B and 19F, respectively.[15] This suggests that these cross-reactive, non-PCV7 strains could be inducing ongoing immunity to 19F and 6B.

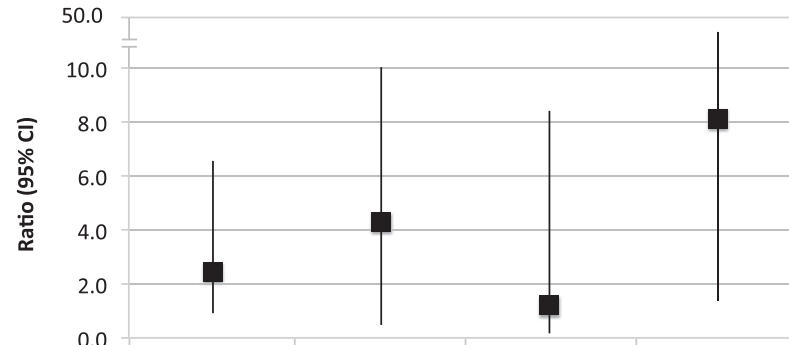
For most serotypes, comparable levels of circulating IgG and functional titers were observed in immunized and unimmunized children; however, the relationship between these concentrations and titers lacks uniformity across serotypes. High circulating IgG concentrations do not necessarily imply highly functional antibody titers. For example, even though the serotype 14 GMC is higher for immunized children, antibodies of immunized and unimmunized children have similar functional capacity. Some of the functional serotype 14 antibodies could represent a different immunoglobulin isotype (such as IgM) explaining these observed differences in concentrations and function for immunized children.[16]

Although this study has focused on circulating IgG concentrations, this may not be the effector mechanism by which PCVs protect against invasive disease years after vaccination. In particular, the 9-valent PCV demonstrated long-term efficacy (6.16 years) against vaccine-type invasive pneumococcal disease among HIV-negative children from South Africa.[8] PCVs generate immunological memory, which is likely required to mount a rapid immune response following colonization, and the cells mounting such a response may circulate systemically and then migrate to the NP mucosae in response to acquisition.[17] At this point, it is unclear whether circulating serotype-specific IgG measured 1 month after



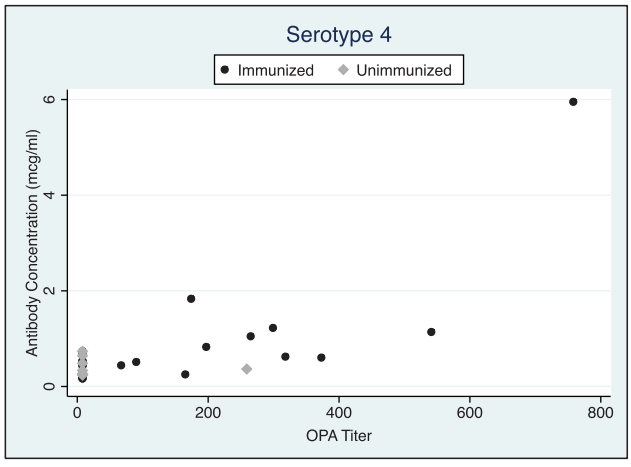
		4	6B	9V	14	18C	19F	23F
GMC ratio (95% CI)		1.26 (0.66, 2.42)	1.09 (0.55, 2.15)	1.38 (0.61, 3.14)	3.37 (1.34, 8.47)	1.62 (0.54, 4.86)	1.76 (0.77, 4.00)	1.77 (0.45, 6.93)
GMC	imm	0.48	2.62	0.90	0.67	0.64	2.72	1.43
	unimm	0.38	2.42	0.65	0.20	0.39	1.54	0.81

FIGURE 2. Comparison of serotype-specific pneumococcal GMCs between PCV7 immunized and unimmunized children.* Immunized defined as children who received at least one dose of PCV7 at least four years prior to the collection of sera.

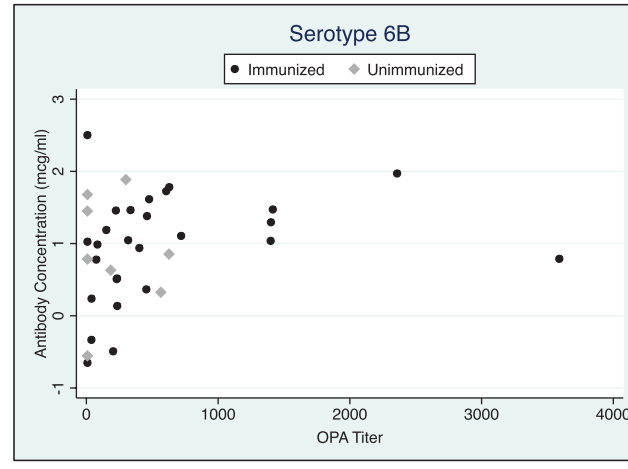


		4	6B	14	23F
GMT ratio (95% CI)		2.45 (0.92, 6.56)	4.31 (0.48, 38.78)	1.20 (0.17, 8.41)	8.12 (1.37, 48.09)
GMT	imm	30.28	235.66	560.36	388.34
	unimm	12.36	54.73	468.21	47.80

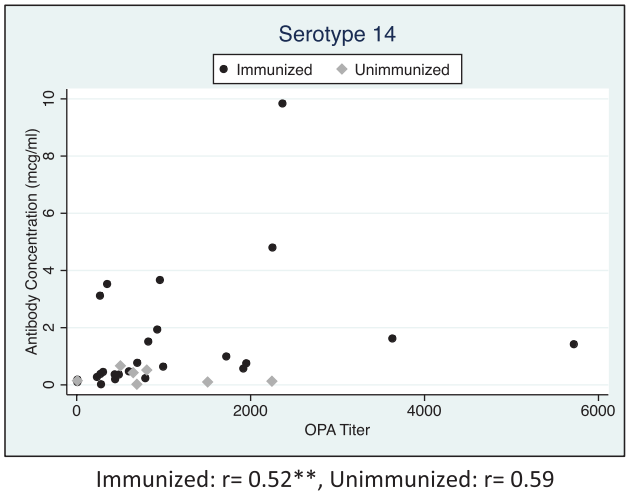
FIGURE 3. Comparison of serotype-specific pneumococcal GMTs between PCV7 immunized and unimmunized children.* Immunized defined as children who received at least one dose of PCV7 at least four years prior to the collection of sera.



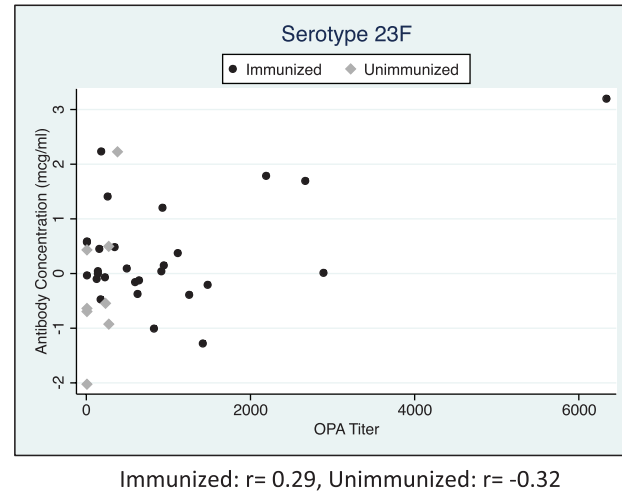
Immunized: r= 0.78*, Unimmunized: r= -0.11



Immunized: r= 0.25, Unimmunized: r= -0.05



Immunized: r= 0.52**, Unimmunized: r= 0.59



Immunized: r= 0.29, Unimmunized: r= -0.32

*p≤0.001, **p≤0.01

FIGURE 4. Correlation between serotype-specific pneumococcal IgG concentrations and functional titers by immunization status.

primary immunization (or post booster) or persistent IgG in a previously immunized child correlates with the presence or absence of memory B cells. Although circulating IgG concentrations in unimmunized children may reflect the presence of natural memory, it is not clear whether the same amount of IgG in serum of immunized and unimmunized children is a marker of equivalent protection.

Immunization with PCV7 and the development of naturally occurring antibody result in similar serotype-specific pneumococcal IgG concentrations over the long term, although the small sample size of this dataset limits interpretation of the odds ratios and comparison of GMCs. These IgG levels remain at or above the threshold used to evaluate vaccine efficacy against disease but may not persist at these concentrations as a national vaccine program enters into a fully mature phase when circulation of the strains is virtually eliminated. The relationship between community-level pneumococcal ecology and protection against disease through vaccination is intertwined especially in settings where vaccine coverage is suboptimal. A clear understanding of the relationships between colonization, immunity and disease will allow for anticipation and optimization of vaccine strategies.

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