Reduction of Nasopharyngeal Carriage of *Streptococcus pneumoniae* after Administration of a 9-Valent Pneumococcal Conjugate Vaccine to Toddlers Attending Day Care Centers

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A double-blind, randomized study involving 264 toddlers attending day care centers was conducted to document the effect of a 9-valent pneumococcal conjugate vaccine on the carriage rate of pneumococci. Of 3750 cultures done on nasopharyngeal samples obtained from subjects during a 2-year follow-up period after vaccination, 65% were positive for *Streptococcus pneumoniae*. In all age windows, the rate of carriage of vaccine-type pneumococci was lower among subjects who received the pneumococcal vaccine than among control subjects, because the acquisition rate was lower in the former group. The effect was most pronounced among subjects aged ≤36 months. The sample size enabled us to study protection against carriage of *S. pneumoniae* serotypes 6B, 9V, 14, 19F, and 23F; significant protection against all serotypes except 19F was seen in the pneumococcal-vaccine group. The rate of carriage of serotype 6A (not included in the vaccine) was also reduced significantly, but the rate of carriage of serotype 19A (not included in the vaccine) was not. The rate of carriage of non–vaccine-type pneumococci (excluding serotype 6A) was higher in the pneumococcal-vaccine group than in the control group.

Streptococcus pneumoniae resides in the nasopharynx of humans, most commonly without adverse effects to the host, but it may spread and cause upper or lower respiratory tract infection. In some circumstances, the organism is able to enter the bloodstream, leading to bacteremia and, occasionally, to infection of other organ systems. Asymptomatic nasopharyngeal carriage of pneumococci is widely prevalent among young children in both developed and developing countries [1–8]. Pneumococcal nasopharyngeal carriage is important because it is involved both in development of disease and in spread of the pathogen to other susceptible individuals [3, 9–15].

Most of the reported outbreaks of pneumococcal disease in the United States have involved child care facilities, usually day care centers (DCCs) [2]. Day care attendance is associated with an increased incidence of pneumococcal carriage and infections in infants and children [16–21]. Conditions that favor the development and transmission of pneumococci include presence of a large number of susceptible children, frequent and close person-to-person contact, and frequent occurrence of viral respiratory tract infections, which have been shown to increase both the frequency of pneumococcal carriage and the density of organisms during carriage [22].

In a prospective study published elsewhere [23] that was conducted in a DCC in southern Israel, we demonstrated extensive person-to-person spread of pneumococci. This finding suggests that the DCC may serve as a reservoir of pneumococci and, thus, augment the carriage of these organisms in the community. In preparation for the present study, we established that carriage rates of *S. pneumoniae* among attendees of the 8 DCCs located in the city of Beer-Sheva in southern Israel were high and ranged from 35% to 93% [24].

The ability of pneumococcal conjugate vaccines to reduce the rate of carriage of serotypes included in the vaccine has been demonstrated in several studies [25–33]. However, because pneumococcal conjugate vaccines are multivalent and each reported study of carriage has included only a limited number of subjects, the effect of such vaccines on carriage of individual serotypes has not been clearly demonstrated.

The primary objective of the present prospective, double-blind, randomized study was to investigate whether a 9-valent pneumococcal conjugate vaccine could protect immunized toddlers against carriage of *S. pneumoniae* of serotypes included in the vaccine (vaccine-type [VT] *S. pneumoniae*). Additional objectives were (1) to calculate protection against individual serotypes; (2) to investigate whether the inclusion of serotypes 6B

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This study was approved by the Soroka Medical Center Institutional Review Board and the Israel Ministry of Health National Review Board, and written, signed informed consent was obtained from the parents of all the children before immunization.

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and 19F in the vaccine induces protection against carriage of the cross-reactive serotypes 6A and 19A, respectively; and (3) to investigate whether the reduction in the rate of carriage of VT *S. pneumoniae* is associated with a parallel increase in the rate of carriage of non-VT *S. pneumoniae*.

Subjects, Materials, and Methods

Population. Healthy boys and girls aged 12–35 months attending 8 DCCs in the city of Beer-Sheva in southern Israel were recruited. The 8 DCCs were within a 5-km radius and were in 6 different neighborhoods. Subjects were excluded if they (1) had received or were expected to receive any vaccine or immunoglobulin within 8 weeks of study vaccination (within the 4-week period before or during the 4-week period after the administration of the study vaccines), (2) had any known or suspected impairment of immunologic functions, (3) had major congenital malformation or serious chronic disease, (4) had a known hypersensitivity to any component of the study vaccines or had experienced any previous severe vaccine-associated adverse reaction, or (5) had been previously vaccinated with any pneumococcal or meningococcal vaccine. Subjects were also temporarily excluded if they had experienced any febrile illness (rectal temperature, $\geqslant 38^{\circ}C$) within 72 h before vaccination.

Vaccines. Children received 1 of the 2 following vaccines: (1) a 9-valent pneumococcal conjugate vaccine in which 2 μg each of serotype 1, 4, 5, 9V, 14, 18C, 19F, and 23F carbohydrates and 4 μg of serotype 6B carbohydrate were coupled to diphtheria toxin CRM_{197} and were presented as a lyophilized preparation provided by Wyeth-Lederle Vaccines (WLV) or (2) the control vaccine, a meningococcus group C conjugate vaccine in which 10 μg of carbohydrate was coupled to CRM_{197} (WLV).

Study design. This was a double-blind study with 1:1 individual randomization within DCCs to groups receiving the pneumococcal or the control vaccine. For randomization, subjects were stratified by age group (12–17, 18–23, 24–29, and 30–35 months). Subjects aged 12–17 months at the time of enrollment ($n = 35$) received 2 intramuscular injections 2–3 months apart, and those aged 18–35 months at the time of enrollment ($n = 229$) received a single intramuscular injection. For each child, 18 encounters were planned that would take place during a 2-year follow-up period that started >1 month after complete immunization; encounters were to occur monthly during the first year and every 2 months during the second year of follow-up. At each visit, a nasopharyngeal sample was obtained for culture for *S. pneumoniae*.

Cultures of nasopharyngeal samples. Nasopharyngeal samples were obtained by use of a flexible cotton-tipped swab, which was introduced into the nostrils and advanced until resistance was found. These swabs were inoculated into modified Stewart transport medium (Medical Wire & Equipment) and processed within 1 h at the Clinical Microbiology Laboratory of the Soroka University Medical Center (Beer-Sheva, Israel). Swabs were plated on Columbia agar with 5% sheep blood and 5.0 μg/mL gentamicin and incubated aerobically at 35°C in a CO_2-enriched atmosphere for 48 h. This method was used in our other studies and yielded a high rate of positive cultures [20, 23, 24, 26, 27].

Presumptive identification of *S. pneumoniae* was based on the presence of α-hemolysis and inhibition by optochin and confirmed by a positive slide agglutination test result (Phadebact; Pharmacia Diagnostics). One *S. pneumoniae* colony per plate was then subcultured, harvested, and kept frozen at $-70^{\circ}C$ for further testing.

Serogrouping and serotyping. Serogrouping and serotyping of *S. pneumoniae* were performed by the quellung reaction, using sera produced by the Statens Seruminstitut [34]. Isolates that had negative reactions to all pooled sera and to omni serum were considered to be nontypeable. Serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F were classified as VT pneumococci.

Data management and statistical analysis. The primary end point for the study was the carriage of pneumococci of all serotypes included in the vaccine. Secondary end points were carriage of individual VT pneumococci; carriage of antibiotic-resistant pneumococci; carriage of *S. pneumoniae* of serotypes not belonging to the vaccine (not 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F), as a group and as individual serotypes; and acquisition of new VT and non-VT pneumococci.

Statistical analyses were performed by use of SPSS statistical software package version 10.0 and Egret version 2.0.3 software (CYTEL Software Corporation). Contingency table analysis was performed by use of the χ^2 test or Fisher's exact test, as appropriate. Odds ratios (ORs), as measures of relative rates in pneumococcal-vaccine recipients compared with control subjects, were calculated and 95% confidence intervals computed. Because data from sequential nasopharyngeal swabs from the same child were sometimes correlated, the Egret software was used to calculate the OR by use of logistic regression random effects, matching on the child.

"New acquisition of pneumococci" was defined as the detection of a serotype, once the child was fully vaccinated, that had not been detected previously. Only 1 new acquisition could therefore be counted per serotype for each child.

For duration of carriage of newly acquired *S. pneumoniae,* each positive culture was considered to indicate carriage of 1 child-month's duration. The mean number of new acquisitions and the mean duration of carriage of newly acquired *S. pneumoniae* were compared using Student's *t* test.

Results

A total of 264 subjects were enrolled in our study between 28 August 1996 and 6 February 1997. Of those, 2 subjects who belonged to the control group were not included in the analysis—one patient who, despite being <18 months of age, received only 1 instead of 2 injections, and another patient who received 2 doses of the vaccine but whose parents then refused any further nasopharyngeal follow-up. Consequently, the final analysis was performed on a study group that included 262 subjects, 132 subjects who received the pneumococcal conjugate vaccine and 130 who received the control vaccine (table 1). An analysis that included data from the child aged <18 months who received only 1 vaccine did not differ significantly from the analysis in which the child was excluded. The number of children followed up for <6, 6–11, 12–17, and 18–24 months after complete immunization was 4, 9, 6, and 113, respectively, in the pneumococcal-vaccine group, and 5, 8, 4, and 113, respectively, in the control group. The distribution of sex, mean age at enrollment, follow-up time,

Table 1. Demographic and clinical characteristics of children included in a study of the effect of a 9-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *Streptococcus pneumoniae*.

Variable	Pneumococcal-vaccine recipients (n = 132)	Control subjects (n = 130)
Sex, male/female	76/56	68/62
Median age at enrollment, months (25th–75th percentile)	27.89 (21.55–31.75)	27.75 (21.76–32.11)
No. (%) of children aged <18 months at enrollment	19 (14)	16 (12)
Total child-months of follow-up	2682	2666
No. (%) of children followed up for <12 months	13 (10)	13 (10)
Total no. of samples cultured	1887	1863
No. of samples obtained in each age window		
15–23 months	137	121
24–35 months	679	669
36–47 months	792	785
⩾48 months	279	288
Mean no. (± SD) of cultures per child	14.3 ± 2.95	14.3 ± 2.70

and number of cultures obtained for each age window were similar in the 2 groups.

At enrollment (before immunization), the total number of children carrying pneumococci was 105 (80%) among the 132 children who received the pneumococcal vaccine and 105 (81%) among the 130 children who received the control vaccine. Carriage of VT *S. pneumoniae* was 51 (39%) versus 53 (41%); of serotype 6A, 22 (17%) versus 19 (15%); and of non-VT *S. pneumoniae* (excluding serotype 6A), 32 (24%) versus 33 (25%), among pneumococcal-vaccine and control groups, respectively.

Beginning 1 month after complete immunization, 3750 nasopharyngeal samples were obtained for culture (1887 from the pneumococcal-vaccine group and 1863 from the control group). Of those, cultures of 2450 samples (65%) were positive for *S. pneumoniae:* 642 isolates (17%) were VT, 348 (9%) were serotype 6A, 113 (3%) were serotype 19A, and 1346 (36%) were other non-VT pneumococci (4% of which were untypeable; 1 isolate was not typed).

When the pneumococcal carriage rate in general and that of VT *S. pneumoniae* in particular were examined, it became clear that the carriage rate decreased in the control group as mean age increased. This finding prompted us to analyze the carriage rate by age window (the age interval within which the culture was obtained) rather than by the length of time since vaccination, because time since vaccination is related to age. Thus, our results are shown by age window. Four age windows were chosen: <24 months, 24–35 months, 36–47 months, and ⩾48 months.

In the control group, the rate of carriage of VT *S. pneumoniae* decreased as age increased, from 40% among subjects aged 15–23 months to 12% among those aged ⩾48 months (table 2). For any given age window, the rate of carriage of VT pneumococci was lower among recipients of the pneumococcal conjugate vaccine than among control subjects. However, because the carriage rate decreased in older age windows, even among control subjects, the relative risk was closest to 1.0 among children aged ⩾36 months. Therefore, the protective effect was greater in the younger age groups than in the older age groups (68%, 60%, 43%, and 29% for 15–23, 24–35, 36–47, and ⩾48 months of age, respectively; $P < .001$ for trend).

We calculated the protection against carriage conferred by the pneumococcal conjugate vaccine for *S. pneumoniae* serotypes 6B, 9V, 14, 19F, and 23F (all serotypes for which the number of positive cultures was >30). For 4 of 5 serotypes, we could demonstrate a significant protection that was associated with use of the pneumococcal conjugate vaccine (table 3 and figure 1). The lowest relative risk (representing the strongest effect) was seen for serotype 6B, followed by that for 9V, 14, and 23F. A lesser protective effect against serotype 19F was demonstrated that was not statistically significant. We did not have a sufficient number of subjects who carried serotypes 1, 4, 5, and 18C (2, 2, 7, and 23 isolates, respectively) to examine serotype-specific protective efficacy for these serotypes.

To assess whether cross-protection from carriage occurs between different serotypes belonging to the same group, we chose to analyze the effect for serotype 6A (after receipt of serotype 6B antigen) and 19A (after receipt of serotype 19F antigen) (figure 2). These 2 pairs were chosen because they were sufficiently prevalent to provide a sizeable sample. A significantly lower rate of carriage of serotype 6A was observed in the pneumococcal-vaccine recipients, who received the serotype 6B antigen (overall relative risk, 0.56; $P = .0032$). This effect was seen in particular among subjects aged <36 months. No effect of

Table 2. Carriage of vaccine-type *Streptococcus pneumoniae* among 262 toddlers attending day care centers who received a 9-valent pneumococcal conjugate vaccine (132 children) or the control vaccine (130 children).

Age window in which sample was obtained, in months	Pneumococcal-vaccine recipients, *n/N* (%)	Control subjects, *n/N* (%)	OR_{RE} (95% CI)	*P*
15–23	33/137 (24.1)	48/121 (39.7)	0.32 (0.09–1.02)	.062
24–35	101/679 (14.9)	186/669 (27.8)	0.40 (0.26–0.60)	<.001
36–47	87/792 (11.0)	127/785 (16.2)	0.57 (0.38–0.87)	.009
⩾48	25/279 (9.0)	35/288 (12.2)	0.71 (0.41–1.25)	.240
Total	246/1887 (13.0)	396/1863 (21.3)	0.50 (0.38–0.66)[a]	<.001

NOTE. Samples were obtained beginning 1 month after the administration of a single dose for children aged ⩾18 months at enrollment and 1 month after the second dose for children aged <18 months at enrollment. CI, confidence interval; *n/N,* no. of cultures yielding vaccine-type isolates/total no. of cultures; OR_{RE}, odds ratio from logistic regression with random effects, matching on child.

[a] Controlled for age.

the vaccine could be demonstrated for serotype 19A among recipients of the pneumococcal conjugate vaccine (which contained serotype 19F). The overall relative risk was 1.15 ($P = .68$).

We examined the rate of carriage of *S. pneumoniae* with non-VT serotypes among pneumococcal-vaccine recipients and control subjects. For this analysis, we excluded serotype 6A, because the rate of carriage of that serotype was shown to be reduced by use of the 9-valent pneumococcal conjugate vaccine that contains 6B polysaccharide antigen. The overall positive-culture rate for non-VT pneumococci (excluding serotype 6A) was 1459 (39%) of 3750. The carriage rate increased as age increased in both groups, but it was significantly higher among subjects who received the pneumococcal conjugate vaccine than among control subjects (table 4).

We also examined whether the rate of carriage of pneumococci of the same serogroup but not of the same serotype as the VT pneumococci was reduced. For this analysis, we excluded serotype 6A, for which a reduction in carriage rate had been demonstrated among pneumococcal-vaccine recipients. The total number of isolates in the serogroup (including serogroups 9 non-V, 18 non-C, 19 non-F, and 23 non-F) was 268 (7%) of 3750 cultures. Contrary to our expectation, a trend toward a higher carriage rate was seen in the pneumococcal-vaccine group, compared with the control group: 155 (8%) of 1887 versus 113 (6%) of 1863 cultures, respectively ($P = .16$). This trend was mainly the result of the difference in the carriage rate among children <36 months of age and children ⩾36 months of age, which was 45 (6%) of 816 among pneumococcal-vaccine recipients versus 42 (5%) of 790 among control subjects ($P = .86$) and 110 (10%) of 1071 among pneumococcal-vaccine recipients versus 71 (7%) of 1073 among control subjects ($P = .009$), respectively.

Because ⩾2 positive cultures from the same child were often included in the analysis (if carriage lasted >1 month), we examined the effect of the pneumococcal conjugate vaccine by asking 2 additional questions: (1) Does the pneumococcal conjugate vaccine protect against new acquisition of VT pneumococci and, at the same time, promote new acquisition of non-VT pneumococci? (2) Once a new VT strain has been acquired, are pneumococcal-vaccine recipients likely to carry it for a shorter period of time than the control subjects and, if pneumococcal-vaccine recipients acquire non-VT pneumococci, will the duration of carriage be longer?

Table 5 shows that significantly fewer children who received the pneumococcal conjugate vaccine than children in the control group acquired VT pneumococci (mean carriage per child ± SD, 0.8 ± 0.87 vs. 1.48 ± 1.02, respectively), and significantly more pneumococcal-vaccine recipients acquired non-VT pneumococci (3.63 ± 1.80 vs. 3.10 ± 1.62). The rate of acquisition of serotype 6A differed between the 2 groups only among children <24 months of age (it was lower in the pneumococcal-vaccine group).Therefore, a significant difference in acquisition of VT, non-VT (excluding serotype 6A), and serotype 6A pneumococci was seen between the pneumococcal-vaccine and control groups when duration of follow-up was equal (mean follow-up, 14.3 months in both groups).

The mean duration of carriage after acquisition of an *S. pneumoniae* strain in the pneumococcal-vaccine and control groups did not differ. This was true for VT pneumococci, serotype 6A, and non-VT pneumococci (excluding serotype 6A): the mean duration of carriage ± SD was 1.64 ± 1.14 months in the pneumococcal-vaccine group and 1.60 ± 1.03 months in the control group, for VT pneumococci; 2.14 ± 1.52 and 2.57 ± 1.69, for serotype 6A; and 1.56 ± 0.95 and 1.49 ± 0.88, for non-VT pneumococci.

Discussion

DCCs are the most common form of out-of-home care for preschool children in the Western world [35, 36], and the number of children attending such facilities is increasing. In the United States, >13 million children aged <5 years are enrolled in some form of out-of-home child care [36]. DCC attendees constitute a special population with unique behavioral traits, incompletely matured immune systems, and susceptibility to both viral and bacterial upper respiratory infections. They spend

Table 3. Carriage of *Streptococcus pneumoniae* among toddlers who received a 9-valent pneumococcal conjugate vaccine or the control vaccine, according to age at which the first culture positive for serotype 6B, 9V, 14, 19F, or 23F was obtained.

	Age <36 months				Age ≥ 36 months				All ages			
Serotype	Pneumococcal-vaccine recipients, *n/N* (%)	Control subjects, *n/N* (%)	OR_{RE1} (95% CI)	*P*	Pneumococcal-vaccine recipients, *n/N* (%)	Control subjects, *n/N* (%)	OR_{RE1} (95% CI)	*P*	Pneumococcal-vaccine recipients, *n/N* (%)	Control subjects, *n/N* (%)	OR_{RE2} (95% CI)	*P*
6B	7/816 (1)	22/790 (3)	0.25 (0.08–0.81)	.021	4/1071 (0)	21/1073 (2)	0.18 (0.05–0.63)	.007	11/1887 (1)	43/1863 (2)	0.22 (0.09–0.54)	$<.001$
9V	5/816 (1)	15/790 (2)	0.31 (0.11–0.93)	.037	6/1071 (1)	12/1073 (1)	0.50 (0.18–1.41)	.191	11/1887 (1)	27/1863 (1)	0.39 (0.18–0.83)	.015
14	18/816 (2)	29/790 (4)	0.40 (0.16–1.02)	.056	13/1071 (1)	23/1073 (2)	0.52 (0.23–1.16)	.111	31/1887 (2)	52/1863 (3)	0.51 (0.27–0.96)	.037
19F	41/816 (5)	62/790 (8)	0.58 (0.31–1.07)	.079	57/1071 (5)	52/1073 (5)	1.11 (0.68–1.81)	.689	98/1887 (5)	114/1863 (6)	0.79 (0.54–1.17)	.246
23F	60/816 (7)	91/790 (12)	0.55 (0.31–1.01)	.052	27/1071 (3)	43/1073 (4)	0.48 (0.22–1.02)	.058	87/1887 (5)	134/1863 (7)	0.53 (0.33–0.88)	.013

NOTE. CI, confidence interval; *n/N,* no. of positive cultures/total no. of cultures; OR_{RE1}, odds ratio from logistic regression with random effects, matching on child; OR_{RE2}, odds ratio from logistic regression with random effects, matching on child and controlled for age.

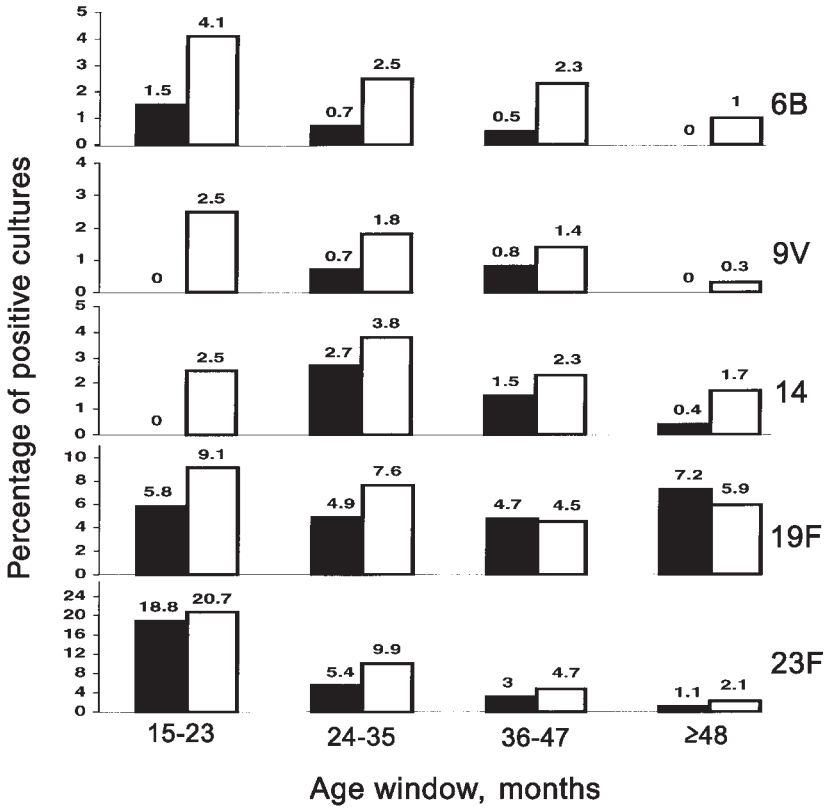

Figure 1. Carriage of *Streptococcus pneumoniae* serotypes 6B ($n = 54$), 9V ($n = 38$), 14 ($n = 83$), 19F ($n = 212$), and 23F ($n = 221$) among toddlers attending day care centers who received a 9-valent pneumococcal conjugate vaccine or the control vaccine, by age window in which the first positive culture was obtained. Results are given as the percentage of positive cultures in each age window in the pneumococcal-vaccine *(filled bars)* and control *(open bars)* groups.

many hours each day together in crowded conditions that facilitate colonization and spread of *S. pneumoniae* [23, 24, 37, 38]. As a result, DCC attendees are at a high risk of developing pneumococcal infections, often with antibiotic-resistant strains [2, 16–19]. It is therefore essential to find ways of decreasing the rate of carriage and spread of *S. pneumoniae,* to reduce morbidity associated with pneumococcal infection in DCC attendees.

Furthermore, DCCs have been shown to be responsible for the spread of *S. pneumoniae* to the community, particularly to younger siblings of attendees at home [21]. Therefore, reduction of the carriage rate among DCC attendees could result in a reduction in the spread of these organisms in the community as a whole. The importance of pneumococcal carriage in the development of pneumococcal morbidity, in conjunction with the key role played by DCCs in the spread of *S. pneumoniae* in the community, makes DCC attendees a particularly attractive target population for intervention aimed at reducing carriage rates by vaccination.

Several studies have demonstrated that conjugate vaccines decrease the rate of nasopharyngeal carriage of pneumococci of serotypes included in the vaccine. These studies were performed in different sites (Israel [26, 27, 32], The Gambia [28, 33], South Africa [29], Iceland [30], and the United States [31]). In these studies, different vaccines were used (from 4- to 11-valent vaccines, conjugated to diphtheria or tetanus toxoids, CRM_{197} protein, or meningococcus outer protein complex), and the age at administration of vaccines ranged from early infancy to older toddlers. However, in all studies, it was shown that the administration of pneumococcal conjugate vaccines resulted in reduction in the rate of carriage of VT *S. pneumoniae,* which is consistent with our findings. Replacement of VT *S. pneumoniae* with non-VT pneumococci also was seen in most of these studies [28–33] but not in all of them [26, 27, 32]. It has been suggested that replacement occurs when extensive contact with children carrying non-VT pneumococci occurs or, alternatively, when multiple serotypes are carried and an "unmasking" phenomenon occurs after reduction of carriage of VT pneumococci in response to vaccination. The extensive reduction of VT pneumococci colonization after vaccination that was seen in the present study, coupled with the extensive replacement by non-VT pneumococci that was observed, suggests

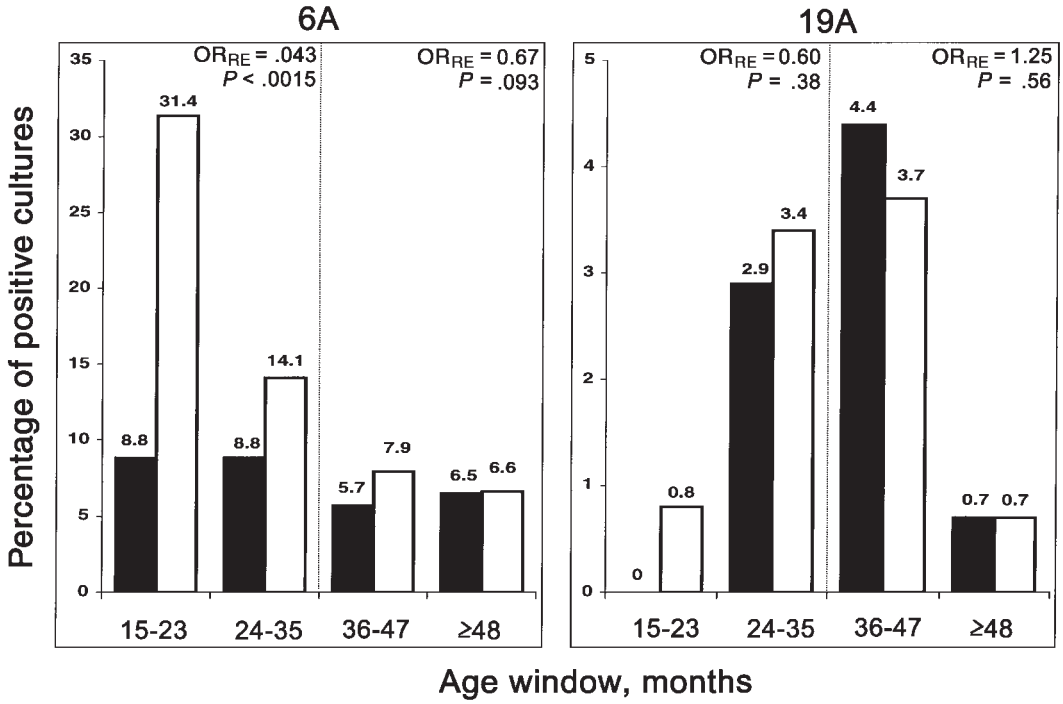

Figure 2. Carriage of *Streptococcus pneumoniae* serotypes 6A ($n = 238$) and 19A ($n = 113$) among toddlers attending day care centers who received a 9-valent pneumococcal conjugate vaccine or the control vaccine, by age window in which the first positive culture was obtained. Results are given as a percentage of positive cultures in each age window in pneumococcal-vaccine *(filled bars)* and control *(open bars)* groups. OR_{RE}, odds ratio from logistic regression with distinguishable binomial random effects, matching on child and day care center.

that, in a crowded population with a high rate of pneumococcal colonization and spread, such as in a DCC, widespread vaccination will result in a rapid replacement of pneumococci by new serotypes not included in the vaccine. The clinical significance of such a replacement is not yet clear.

In theory, the observed reduction in the rate of carriage of VT pneumococci after administration of a conjugate vaccine could be the result of reduction in acquisition of new VT pneumococci, reduction of the duration of carriage once pneumococci have been acquired, or both. The present study shows clearly that the most important mechanism is the prevention of new acquisition and that, once pneumococci have been acquired, the duration of carriage in pneumococcal-vaccine recipients is not different from that in children who have not received a pneumococcal conjugate vaccine. This observation is similar to that made elsewhere with regard to *Haemophilus influenzae* type b conjugate vaccines [39].

Although protection against carriage of VT *S. pneumoniae* has been demonstrated in various studies [26–33], this effect so far has been demonstrated for all VT pneumococci included in a

Table 4. Carriage of non–vaccine-type *Streptococcus pneumoniae* (excluding 6A) in 262 toddlers attending day care centers who received a 9-valent pneumococcal conjugate vaccine (132 children) or the control vaccine (130 children).

Age window in which sample was obtained, in months	Pneumococcal-vaccine recipients, *n/N* (%)	Control subjects, *n/N* (%)	OR_{RE} (95% CI)	*P*
15–23	33/137 (24.1)	11/121 (9.1)	3.72 (1.40–9.83)	.008
24–35	260/679 (38.3)	206/669 (30.8)	1.46 (1.04–2.04)	.029
36–47	405/792 (52.2)	314/785 (40.1)	1.65 (1.24–2.19)	<.001
≥48	126/279 (45.2)	104/288 (36.1)	1.66 (1.00–2.77)	.054
Total	824/1887 (43.7)	635/1863 (34.1)	1.59 (1.28–1.96)[a]	<.001

NOTE. Samples were obtained beginning 1 month after the administration of a single dose of vaccine in children aged ≥18 months at enrollment and 1 month after the second dose in children aged <18 months at enrollment. CI, confidence interval; *n/N,* no. of cultures yielding non–vaccine-type isolates (excluding serotype 6A)/total no. of cultures; OR_{RE}, odds ratio from logistic regression with random effects, matching on child.

[a] Controlled for age.

Table 5. New acquisition of vaccine-type (VT), serotype 6A, and non-VT *S. pneumoniae* among 262 toddlers attending day care centers who received a 9-valent pneumococcal conjugate vaccine (132 children) or the control vaccine (130 children).

Isolate type, age window in which sample was obtained, in months	Pneumococcal-vaccine recipients		Control subjects		
	n/N	Mean (SD)	*n/N*	Mean (SD)	*P*
VT					
15–23	6/35	0.17 (0.45)	18/35	0.51 (0.70)	.018
24–35	44/109	0.40 (0.63)	92/110	0.84 (0.78)	<.001
36–47	41/126	0.33 (0.59)	63/122	0.52 (0.66)	.017
⩾48	15/75	0.20 (0.40)	20/78	0.26 (0.47)	.426
Total	106/132	0.80 (0.87)	192/130	1.48 (1.02)	<.001
6A					
15–23	2/35	0.06 (0.24)	8/35	0.23 (0.43)	.041
24–35	27/109	0.25 (0.43)	27/110	0.25 (0.43)	.969
36–47	16/26	0.13 (0.33)	18/122	0.15 (0.36)	.640
⩾48	6/75	0.08 (0.27)	3/78	0.04 (0.19)	.278
Total	51/132	0.39 (0.49)	56/130	0.43 (0.50)	.467
Non-VT (excluding 6A)					
15–23	24/35	0.69 (0.63)	10/35	0.29 (0.52)	.005
24–35	172/109	1.58 (1.24)	147/110	1.34 (1.06)	.123
36–47	210/126	1.67 (1.19)	187/122	1.53 (1.17)	.374
⩾48	73/75	0.97 (1.05)	59/78	0.76 (0.93)	.178
Total	479/132	3.63 (1.80)	403/130	3.10 (1.62)	.013

NOTE. More than 1 serotype was isolated from some children. *n/N,* no. of acquisitions of new serotypes/no. of children tested.

particular vaccine as a group (serotypes 4–11). Because sample sizes have been small, serotype-specific analyses have not been possible. Only in a single study from South Africa [29] was the sample size for 2 serotypes (6B and 19F) large enough to demonstrate protection against individual serotypes. The South African study, which was conducted on infants, used a vaccine identical to that used in the present study (9-valent vaccine conjugated to CRM_{197}). A trend toward protection against 9V, 14, and 23F was seen but was not statistically significant, probably because the sample size was too small. To the best of our knowledge, the present study is the first study with a sample size large enough to demonstrate individual protection against 4 serotypes. Importantly, in both this and the South African study, there was no evidence that the vaccine failed to protect against any of the vaccine serotypes; the inability to show protection against some of the serotypes resulted from the paucity of these serotypes.

We observed a reduction in the rate of carriage of serotype 6A after vaccination with serotype 6B conjugate, demonstrating cross-protection between serotypes 6B and 6A. However, such cross-protection was not demonstrated for other pneumococcal strains that were of the same serogroups as strains represented in the vaccine but did not have serotypes identical to the VT pneumococci. The differences in cross-protection between serotypes 6B and 6A and serotypes 19F and 19A match previous observations in animals [40, 41] and in the laboratory [42, 43], which suggested that cross-protection exists between serotypes 6B and 6A but not between 19F and 19A. On the other hand, protection against serotype 19F was weak, compared with protection against other VT pneumococci, and therefore we cannot state with complete certainty that no cross-protection exists until further studies in humans are performed. For the other serotypes (9 non-V, 18 non-C, and 23 non-F), little information exists in the literature to suggest whether cross-protection should be expected. Because we had only a small number of those isolates, we could not reliably demonstrate the presence of cross-protection for each individual serotype. However, the fact that these groups behaved in the same manner as non-VT pneumococci, with higher carriage rates among pneumococcal-vaccine recipients than among control subjects (representing a replacement phenomenon), strongly suggests that no protective effect exists.

An important question is whether the effect of the conjugate vaccines on carriage of VT pneumococci in general, and on individual serotypes in particular, can serve as a surrogate for protection against respiratory or invasive infections. This is relevant because efficacy studies with bacteriologic results, especially for end points such as acute otitis media or pneumonia, are difficult to perform, and such studies will no longer be conducted after widespread vaccination is introduced. Data obtained from clinical studies presented or published thus far are scarce. However, 2 points can be generally made.

First, serotypes such as 1, 3, 4, and 5 are associated with disease but are underrepresented among the pneumococci asymptomatically carried by children. Furthermore, in developed countries, serotypes 1, 4, and 5 are not common in childhood disease. Even in populations in which disease caused by these serotypes is common, carriage is rare, as has been shown for serotypes 1 and 5

[44, 45]. Therefore, it is unlikely that we can draw any conclusion about these serotypes from the effect of vaccination on carriage and disease.

Second, the commonly carried VT pneumococci—namely, 6B, 9V, 14, 19F, and 23F—are also those most commonly found in patients experiencing episodes of acute otitis media. Information available from efficacy studies conducted in California [46] and Finland [47] permits us to make some comparisons between the effect of vaccine on carriage and the efficacy of vaccine against invasive infections and otitis media. In California [46], only a few culture-proven cases were recorded, but it was clear that serotype 19F was responsible for most cases of invasive and infectious otitis media that occurred in individuals who had been vaccinated. In Finland, the efficacy of two 7-valent conjugate vaccines (1 was conjugated to CRM_{197} [47] and the other to meningococcus B outer membrane protein complex [48]) was examined, with acute otitis media as the end point. For both vaccines, the most protection was seen against serotype 6B and the least against 19F. Our results for the effect of the 9-valent vaccine (which also showed the greatest efficacy against serotypes 6B and 9V and the least against serotype 19F) on nasopharyngeal carriage suggest that the effect of conjugate vaccines on carriage of VT *S. pneumoniae* can serve as a surrogate, at least to some extent, for efficacy against pneumococcal disease.

In conclusion, we have demonstrated a marked protection against carriage of VT pneumococci in DCC attendees who received a 9-valent pneumococcal conjugate vaccine. This protection was demonstrated individually for each of the 4 serotypes for which such calculation was possible. Because DCCs are associated with extensive spread of pneumococci, our findings suggest that widespread vaccination may result in marked herd immunity. The similarity between our findings with regard to reduction of carriage rates and findings from other studies with regard to disease reduction suggest that reduction in carriage rates can serve, at least to some extent, as a surrogate for vaccine efficacy against disease. The clinical significance of the observed increase in the rate of carriage of non-VT pneumococci is not yet clear, but this phenomenon points to a need to conduct further surveillance after the initiation of extensive vaccination programs.

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