Association of Serotype-Specific Antibody Concentrations and Functional Antibody Titers with Subsequent Pneumococcal Carriage in Toddlers Immunized with a 9-Valent Pneumococcal Conjugate Vaccine

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Association of pneumococcal nasopharyngeal carriage with the concentration and opsonophagocytic activity (OPA) of serum serotype-specific antibodies was determined for toddlers 1 month after immunization with a 9-valent pneumococcal conjugate vaccine. Higher anti-serotype 14 and anti-serotype 19F IgG and anti-serotype 14 IgM correlated with a lowered probability of pneumococcal acquisition. Postvaccination OPA did not correlate with pneumococcal carriage.

Streptococcus pneumoniae is a leading cause of morbidity and mortality worldwide. The first essential step in all pneumococcal diseases is the symptomless colonization of the nasopharynx (carriage). Use of pneumococcal conjugate vaccines (PCVs) effectively prevents pneumococcal carriage of vaccine serotypes and serotype 6A (4–6, 9, 13, 14). In our previous study, an increasing serum antipolysaccharide IgG concentration after vaccination with a 9-valent PCV (PCV9) significantly decreased the probability of new acquisitions for vaccine serotypes 14 and 19F and for the vaccine-related serotype 6A (3). The objective of the present study was to update and supplement these findings by analyzing the same samples with additional, up-to-date assays. More precisely, we compared the association of postvaccination serum serotype-specific IgG and IgM and opsonophagocytic activity (OPA) with serotype 6A, 9V, 14, 19F, and 23F were measured by a 4-fold multiplexed opsonophagocytic activity (MOPA4) assay (1, 18). The opsonophagocytic activities are given as geometric mean opsonic titers (GMOPTs) with 95% CIs.

We first compared the GMCs and GMOPTs of the serotype-specific antibodies in toddlers who carried S. pneumoniae of the same serotype in their nasopharynx (carriers) and those who did not (noncarriers) 1 month after PCV9 immunization (Table 1). The noncarriers had significantly higher GMCs of anti-serotype 14 and anti-serotype 19F IgG (P = 0.002 and 0.04, respectively) and anti-serotype 14 IgM (P = 0.04) than the carriers. For the other serotypes, noncarriers had slightly higher GMCs of anti-serotype 23F IgG as well as anti-serotype 6A IgM, but these differences did not reach statistical significance. The GMOPT of anti-serotype 6A tended to be slightly higher in the noncarriers than in the carriers (P = 0.05).

To evaluate whether the postvaccination serological variables were associated with new acquisitions of pneumococcal carriage, we used a logistic regression model reporting the odds ratio (OR) for the association between a serological variable and pneumococcal acquisition (with logarithmic IgG, IgM, or MOPA as a covariate). In this model, higher postvaccination IgG and IgM concentrations against serotype 14 and higher IgG concentrations against serotype 19F significantly reduced the probability of having a new acquisition of these serotypes (Table 2 and Fig. 1A and B). A similar but not statistically significant trend was detected for new
TABLE 1 GMCs of serotype-specific anti-pneumococcal polysaccharide (anti-PPS) IgG and IgM and GMOPTs of anti-PPS antibodies 1 month after PCV9 immunization in toddlers who carried pneumococci of the same serotype in their nasopharynx (carriers) and those who did not carry the serotype (noncarriers)

| Variable and serotype | Carriers | Noncarriers | P value* |
|-----------------------|----------|-------------|----------|
|  | n | GMC or GMOPT (95% CI) | n | GMC or GMOPT (95% CI) |  |
| IgG  |  |  |  |  |  |
| 6A | 33 | 0.34 (0.23–0.51) | 38 | 0.40 (0.26–0.62) | 0.61 |
| 9V | 6 | 2.90 (1.25–6.72) | 65 | 1.72 (1.35–2.20) | 0.22 |
| 14 | 12 | 0.51 (0.28–0.93) | 55 | 1.65 (1.20–2.26) | 0.002 |
| 19F | 31 | 1.10 (0.75–1.63) | 40 | 1.91 (1.32–2.75) | 0.043 |
| 23F | 18 | 0.63 (0.28–1.41) | 53 | 0.93 (0.60–1.45) | 0.37 |
| IgM  |  |  |  |  |  |
| 6A | 33 | 6.26 (5.00–7.84) | 38 | 7.71 (6.01–9.88) | 0.22 |
| 9V | 6 | 1.54 (0.87–2.75) | 66 | 1.54 (1.26–1.88) | 1 |
| 14 | 12 | 2.62 (1.61–4.27) | 60 | 4.47 (3.62–5.52) | 0.041 |
| 19F | 31 | 1.91 (1.38–2.66) | 40 | 2.07 (1.70–2.53) | 0.66 |
| 23F | 18 | 0.85 (0.43–1.68) | 53 | 0.93 (0.69–1.26) | 0.77 |
| MOPA  |  |  |  |  |  |
| 6A | 37 | 621 (229–1687) | 42 | 1939 (1023–3674) | 0.06 |
| 9V | 7 | 9545 (3403–26771) | 72 | 10276 (7183–14702) | 0.90 |
| 14 | 13 | 4995 (2385–10458) | 67 | 5458 (3720–8008) | 0.85 |
| 19F | 33 | 533 (250–1136) | 48 | 729 (404–1317) | 0.51 |
| 23F | 25 | 687 (185–2551) | 56 | 1811 (976–3358) | 0.12 |

* Student’s t test was used for statistical comparisons.

acquisitions of serotype 6A in relation to higher anti-serotype 6A IgM concentrations (Table 2 and Fig. 1B). Higher postvaccination IgM concentrations against the other three serotypes (9V, 19F, and 23F) were not associated with the subsequent acquisition of these serotypes (Table 2 and Fig. 1B). No significant associations were found for any serotype between the postvaccination MOPA and subsequent acquisition (Table 2 and Fig. 1C).

While the approach of this study was similar to that reported earlier for the same sample material (3), we now used an improved EIA method with serotype 22F inhibition and also measured IgM and MOPA in addition to IgG. To our knowledge, there are no earlier reports that associate serum IgM concentration with pneumococcal carriage. A dose of PCV is able to induce a significant IgM response that is measurable 1 month after immunization in Israeli (unpublished data) and Finnish (12) toddlers, which according to our recent findings may contribute to the opsonophagocytosis of S. pneumoniae (B. Simell, A. Nurkka, K. Jousimies, S. Grönholm, N. Givon-Lavi, H. Käyhty, and R. Dagan, presented at the 7th International Symposium on Pneumococci and Pneumococcal Diseases, Tel Aviv, Israel, 14 to 18 March 2010). Small amounts of IgM are usually detected on mucosal surfaces, but this IgM is in a secretory form and thus originates from local production by mucosal plasma cells (2).

In an earlier report (3), higher IgG concentrations led to a decreasing probability of having a new acquisition, which achieved statistical significance for serotypes 14 and 19F. This observation was now confirmed in the present study. In the earlier report (3), cross-protection against serotype 6A was observed for anti-serotype 6B IgG, whereas in the present study setting anti-serotype 6A IgG was not associated with new acquisitions of serotype 6A. Besides anti-serotype 14 IgG, a higher anti-serotype 14 IgM concentration was inversely correlated with new acquisitions of this serotype. Comparison of the IgG and IgM concentrations in carrier and noncarrier children (Table 1) gave concordant results with the logistic regression model (Table 2).

We did not find any significant associations between pneumococcal carriage and postvaccination MOPA. The primary mechanism for eliminating S. pneumoniae from the host during invasive infection is opsonophagocytosis, where bacteria are opsonized with anticapsular polysaccharide antibodies followed by activation of the complement system and receptor-mediated uptake and killing of S. pneumoniae by phagocytic cells (7, 16, 20). While MOPA has been shown to be predictive for the serotype-specific efficacy of PCVs against invasive pneumococcal disease (8), the finding of MOPA not being associated with pneumococcal carriage—an event taking place on mucosal surfaces, where complement and phagocytes may be less abundant—is not surprising.

We conclude that no association could be demonstrated with antipneumococcal antibody MOPA in toddlers 1 month after immunization with PCV9 and the probability of new acquisition of

TABLE 2 Prediction of acquisition of postimmunization pneumococcal carriage 1 month after immunization with a 9-valent pneumococcal conjugate vaccine, with serum serotype-specific IgG and IgM antibodies and MOPA of antipneumococcal antibodies as covariates, in a logistic regression model

| Serotype | IgG (OR [95% CI]) | IgM (OR [95% CI]) | MOPA (OR [95% CI]) |
|----------|------------------|------------------|------------------|
| 6A | 0.90 (0.62–1.32) | 0.65 (0.33–1.29) | 0.83 (0.69–1.01) |
| 9V | 1.88 (0.69–5.09) | 1.00 (0.34–2.91) | 0.97 (0.59–1.60) |
| 14 | 0.39 (0.20–0.76) | 0.43 (0.19–0.99) | 0.96 (0.66–1.41) |
| 19F | 0.63 (0.40–0.996) | 0.87 (0.46–1.63) | 0.93 (0.75–1.15) |
| 23F | 0.85 (0.60–1.20) | 0.93 (0.58–1.49) | 0.87 (0.73–1.04) |

* Statistically significant results are shown in bold.
pneumococcal nasopharyngeal carriage, whereas higher IgG concentrations specific to serotypes 14 and 19F and higher IgM concentrations specific to serotype 14 inversely correlated with new acquisitions of these serotypes. It was speculated recently that serum antibodies may represent merely markers of the immune response against pneumococcal colonization, while the actual effectors lie elsewhere (10, 11, 19). Optimal strategies for prevention of pneumococcal carriage and generation of herd immunity by vaccination may require the induction of both antipolysaccharide and antiprotein antibodies, the stimulation of both antibody-dependent and cell-mediated arms of the acquired immune system, and mucosal immunity.

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FIG 1 Probability (OR) of acquisition of pneumococcal carriage 1 month after immunization with one dose of 9-valent pneumococcal conjugate vaccine in association with postimmunization IgG (A), IgM (B), or MOPA (C). Pnc, S. pneumoniae.
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