Antibodies to Pneumococcal Proteins PhtD, CbpA, and LytC in Filipino Pregnant Women and Their Infants in Relation to Pneumococcal Carriage

Emma Holmlund,1* Beatriz Quiambao,2 Jukka Ollgren,1 Teija Jaakkola,1 Cécile Neyt,3 Jan Poolman,3 Hanna Nohynek,1 and Helena Käyhty1

National Institute for Health and Welfare, Helsinki, Finland; Research Institute for Tropical Medicine, Manila, Philippines2; and GlaxoSmithKline Biologicals, Rixensart, Belgium3

Received 4 February 2009/Returned for modification 26 February 2009/Accepted 21 April 2009

This study focuses on the immunogenicity of the following three pneumococcal vaccine candidate proteins in Filipino infants, all inducing protection in animal models: pneumococcal histidine triad protein D (PhtD), choline binding protein A (CbpA), and the lysozyme LytC. The immunoglobulin G antibody concentrations to PhtD, its putative, protective, and exposed C-terminal fragment (PhtD C), CbpA, and LytC were measured by enzyme immunoassay in 52 serum samples from pregnant women, 39 cord blood samples, and consecutive serum samples (n = 263) from 52 newborns between 6 weeks and 10 months of age scheduled to be taken at six time points. A nasopharyngeal swab to detect pneumococcal carriage was taken parallel to the serum samples. The antibody concentrations in the cord blood samples were similar to those in the samples from the mothers. In infant sera, the geometric mean antibody concentrations (GMCS) for all three proteins decreased until the age of 18 weeks and started to increase after that age, suggesting that the infants’ own antibody production started close to the age of 4 to 5 months. The increase in GMCS by age, most clear-cut for CbpA, was associated with pneumococcal carriage. Anti-PhtD concentrations were higher than anti-PhtD C concentrations but correlated well (r of 0.89 at 10.5 months), suggesting that antibodies are directed to the supposedly exposed and protective C-terminal part of PhtD. Our results show that young children are able to develop an antibody response to PhtD, CbpA, and LytC and encourage the development of pneumococcal protein vaccines for this age group.

Several pneumococcal proteins participate in the development of pneumococcal infection and progression into disease (18). Certain pneumococcal proteins are common to all pneumococcal types, and novel vaccines containing these proteins could provide broad protection. This study focuses on three such proteins, as follows: pneumococcal histidine triad protein D (PhtD), choline binding protein A (CbpA), and the lysozyme LytC. In addition, we have included in our analyses a putative, protective, and exposed C-terminal fragment of the PhtD protein (PhtD C).

PhtD belongs to the family of surface-exposed pneumococcal proteins that has a histidine triad motif in the amino acid sequence (1). In the literature, different names for the members of this protein family have been used, as follows: PhtA, also called Sp36 and BVH-11-3; PhtB, also called PhpA and BVH-11; PhtD, also called BVH-11-2; and PhtE, also called BVH-3 (1, 10, 39, 44). The PhtD protein is highly conserved among various strains (1) and has been suggested to be involved in the invasion process of pneumococcus (27). Recent data suggest that the Pht proteins are also involved in the inhibition of complement deposition through binding to factor H (24). In a mouse model, PhtD has been shown to elicit protection against pneumococcal systemic infection caused by pneumococci of serotypes 3 (WU2), 4 (EF5668), 6A (EF6796), and 6B (SJ2) (1, 24). In humans, anti-PhtD antibodies have been detected in the convalescent-phase sera of three out of five infants and children with pneumococcal bacteremia, indicating that this protein is exposed and recognized by the immune system during pneumococcal disease (1). In addition, a fragment of the PhtD protein reacted with anti-PhtD in 83% of 30 serum samples from healthy adults (3).

CbpA belongs to the family of choline binding proteins. Sequence analyses have shown that there are many allelic variants of the CbpA protein, and different biological functions have given these variants different names, as follows: PspC, SpsA, PbcA, and Hic (6, 7, 11, 15, 16, 33). This polymorphic protein has strong molecular and serologic similarities with PspA, another choline binding protein (6). CbpA has been suggested to contribute to the pneumococcal colonization of the nasopharynx and also to contribute to the transition of pneumococcus to the lower respiratory tract (26, 33). By adhering to the human polymeric immunoglobulin receptor, CbpA is suggested to translocate across the mucosal barrier (40). Further, the Hic protein has been suggested to protect pneumococcal cells from opsonization with the components of the alternative complement pathway, since Hic binds to factor H, which accelerates the degradation of C3b by factor I (16, 17).

In a mouse model, PspC is able to elicit protection against nasopharyngeal colonization (2), and CbpA offers protection against death when challenged with the highly virulent pneu-
Pneumococcal strain D39 (25). Quin et al. have shown that mice infected intranasally with strain D39 preincubated with factor H (supposedly bound to PspC) increased lung invasion and bacteremia (29). An antibody response to CbpA in an experimental human pneumococcal colonization model indicates that the protein is exposed and immunogenic in adults (22). Culturing adenoidal lymphocytes from 20 children in a concentrated pneumococcal culture supernatant, including pneumococcal proteins, stimulated specific anti-CbpA antibody production, suggesting that CbpA may be a good upper respiratory mucosal antigen in children (43).

LytC is a lysozyme that degrades the cell walls of pneumococci and is located on the surface of the bacterium (8). In a rat model, LytC has been shown to promote pneumococcal colonization of the nasopharynx (9), and mice immunized with LytC were protected against a lethal challenge with serotype 6B pneumococci (39). In adherence experiments with human nasopharyngeal Detroit cells, a LytC deletion mutant showed 70% loss of adherence (9). The existence of antibodies in the majority of the 17 convalescent-phase serum samples from patients recovering from bacteremic pneumococcal pneumonia suggests that LytC is expressed in vivo and is immunogenic during disease in humans. Additionally, LytC has also been shown to be serologically cross-reactive among pneumococcal strains of different capsular serotypes (39).

To our knowledge, the development of antibodies to these vaccine candidate proteins in infants and children has not been reported before, except for anti-CbpA (42). In this study, we measured the antibody concentrations in Filipino adults and infants and followed the development of antibodies in consecutive samples from infants in relation to pneumococcal carriage. We show that the three proteins are immunogenic in infancy, that the antibody concentrations start to increase at 18 to 22 weeks (4 to 5 months) of age, and that the increases in antibody concentrations are related to pneumococcal upper respiratory tract carriage.

### MATERIALS AND METHODS

**Study cohort and samples.** The study cohort consisted of 54 Filipino mothers and their infants enrolled into the MATER study as a control group (13, 28). The mothers were not vaccinated with the pneumococcal polysaccharide (PS) vaccine, but the infants received the pneumococcal PS vaccine at an average age of 7 weeks. The vaccination of the infants with the 23-valent PS vaccine had a negligible effect on anti-PS antibody concentrations (12) and no effect on the pneumococcal colonization (13) and, thus, did not affect this study.

The group assignment of one mother-infant pair was unclear and thus was left out of this study. For one mother-infant pair, no infant sera were obtained, and for another mother-infant pair, the serum from the mother was finished. Thus, there were 52 mothers and 52 infants in this analysis.

Serum samples were taken from the mothers during their second to third trimester of pregnancy, and 39 umbilical cord blood samples were available. From the infants, serum samples were scheduled to be taken at 6, 10, 14, and 18 weeks and at 9 and 10 months of age, but the range of ages at each visit was wide, and the average ages at the time of samplings were 7 (range, 6.0 to 8.7), 12 (10.0 to 18.7), 17 (14.3 to 22.7), and 22 (18.4 to 28.7) weeks and 9 and 10.5 months of age (40 weeks, with a range of 37.9 to 49.0, and 46 weeks, with a range of 42.7 to 55.0 weeks, respectively).

Because of the wide range of ages at each sampling point and since the actual age is important when measuring natural antibodies, the samples were regrouped into eight new groups according to age, instead of using the original six groups based on visits, as described in Table 1. A total of 263 infant samples were available for this study. There were some variations in the number of samples used for each antigen, because some sera were finished during the determinations for this study. The serum samples had been thawed several times before entering into this study, but our experience is that this does not affect the immunoglobulin G (IgG) antibody concentrations (our unpublished data).

**Serological methods.** An enzyme immunoassay was used for measuring the anti-PhtD-, -PhtD C-, -CbpA, and -LytC antibody concentrations. The wells of the microtiter plate (Microlon 655061; Greiner Bio-One, Frickenhausen, Germany) were coated with the antigens at a concentration of 1 μg/ml in phosphate-buffered saline (PBS) (100 μl/well; overnight at 4°C). The wells were blocked with 1% bovine serum albumin (BSA) (PBS) for 1 h at 37°C. The samples were serially diluted (starting at 1:100) in the milk/PBS buffer, 100 μl/well in duplicate, and incubated at 37°C for 2 h. Alkaline phosphatase-conjugated anti-human IgG (Sigma Chemicals, St. Louis, MO) was diluted in the milk/PBS buffer, pipetted at 100 μl/well, and incubated for 2 h at 37°C. Finally, the substrate solution containing 1 mg/ml of p-nitrophenyl phosphate disodium (Sigma Chemicals; St. Louis, MO) diluted in carbonate buffer (pH 9.8) was added and incubated at 37°C. After 60 min, the optical density at 405 nm of the wells was measured by a photometer (Multiskan MCC/340; Labsystems, Helsinki, Finland).

The plates were washed four times between each step with PBS containing 0.05% Tween 20, except before addition of the substrate, when they were washed three times with PBS containing 0.05% Tween 20 and two times with distilled water. As a reference serum, we used a commercial human serum pool (Ludion H006W11; Ludion Bioproducts, De Pinte, Belgium) for which the IgG anti-PhtD-, -PhtD C-, -CbpA, and -LytC concentrations had been determined previously (12.3 μg/ml, 2.99 μg/ml, 23.49 μg/ml, and 3.89 μg/ml, respectively). Samples with antibody concentrations below the quantitation limit (0.01 μg/ml for PhtD, 0.07 μg/ml for PhtD C, 0.16 μg/ml for CbpA, and 0.41 μg/ml for LytC) were assigned values equivalent to half the detection limit.

**Definitions and subsets of children.** When the antibody concentrations were related to the pneumococcal carriage of the infants, the sera were grouped into two categories in each of the eight age groups, carrier (Pnc+) and noncarrier (Pnc−) subjects, according to the pneumococcal carriage state of the infant (13). Once the infant had become a carrier, he/she stayed in the Pnc+ group, which means that the proportion of carriers was cumulatively increasing while the proportion of noncarriers was cumulatively decreasing. The group assignment of one mother-infant pair was unclear and thus was left out of this study. For one mother-infant pair, no infant sera were obtained, and for another mother-infant pair, the serum from the mother was finished. Thus, there were 52 mothers and 52 infants in this analysis.

### TABLE 1. Infant samples were grouped into eight new age groups according to the real age of the infant at the time when the sample was taken

| Age group (wk) | Average age within group (wk) | No. of infant samples (n = 263) |
|---------------|-----------------------------|---------------------------------|
| <8            | 7                           | 42                              |
| 8–11          | 11                          | 34                              |
| 12–15         | 14                          | 40                              |
| 16–19         | 18                          | 32                              |
| 20–28         | 22                          | 33                              |
| 37–41         | 40                          | 41                              |
| 42–45         | 44                          | 28                              |
| ≥46           | 49                          | 13                              |

The average age (in weeks) and the number of samples within each age group are shown.

From July 20, 2018 by guest
http://cvi.asm.org/ on July 20, 2018 by guest
was primarily due to the increase in the concentrations of the antibodies in the carrier \((\text{Pnc}^+)\) group (Fig. 2) after the age of 18 to 22 weeks, while the concentrations in the noncarrier \((\text{Pnc}^-)\) group continued to decrease or stayed at the same level. The difference between the \(\text{Pnc}^+\) and \(\text{Pnc}^-\) groups was most clear-cut for anti-CbpA, while the differences were smaller for anti-PhtD, -PhtD C, and -LytC but showed the same trend. The small number of samples in the \(\text{Pnc}^-\) group, closer to the end of the observation period, made the 95% CI wide.

Rises in antibody levels after nasopharyngeal pneumococcal acquisitions were also examined individually (Fig. 3). The infants who were found to be carriers for the first time at an average age of 7 weeks (Fig. 3, left column) showed in general, in concordance with Fig. 1 and 2, a decrease in antibodies, and the responses were seen only at an older age in association with persisting colonization or new acquisition. In addition, the kinetic curves of the two infants who were pneumococcal carriers at 7 weeks of age and remained pneumaticcoccus negative after that continued to fall like the curves of the noncarrier infants, since no new pneumococcal contact stimulated antibody production. At an average age of 18 weeks (Fig. 3, middle column), those who became carriers for the first time \((n = 5)\) showed a clear increase in antibodies to LytC \((4/5)\), but lower and fewer responses to the other antigens were noted. In the noncarrier group (Fig. 3, right column), the antibody concentrations decrease in general, but there are some rises in antibody, probably due to undetected carriage during the long gap (about 5 months) in sampling between the fourth and the fifth samples. One noncarrier infant had increases in antibody concentration by >9-fold to LytC, PhtD, and PhtD C between the fourth (infant aged 21 weeks) or the fifth (infant aged 39 weeks) and the sixth (infant aged 43 weeks) samples. The increase in antibody concentration to CbpA was 1.5-fold between the fifth (infant aged 39 weeks) and the sixth (infant aged 43 weeks) samples.

Correlation between antigens. In general, the concentrations of antibodies to the different proteins correlated modestly in the infant sera taken at an average age of 10.5 months and poorly in the adult sera (Table 2). The only exceptions were the anti-PhtD and -PhtD C concentrations. The concentrations of anti-PhtD were higher than those of anti-PhtD C, but the correlation between the antigens was good for both infant and adult sera (Fig. 4).

**RESULTS**

The kinetics of anti-PhtD, -PhtD C, -CbpA, and -LytC in early infancy. The anti-PhtD, -PhtD C, -CbpA, and -LytC antibody concentrations in the cord blood samples were similar to those in the samples from the mothers but notably higher than those in the subsequent samples of the infants (Fig. 1). During the first months of life, the GMCs decreased, which is consistent with the decline of the maternal antibodies. The synthesis of the infants’ own anti-PhtD, -PhtD C, -CbpA, and -LytC antibodies started to be detected after 18 to 22 weeks of age (4 to 5 months). At the end of our follow-up period, at the mean age of 49 weeks (11 months), the concentrations in the infant samples were still lower than those in the mother samples, with the exception of the anti-LytC concentrations, which were similar in the samples from the mothers and the infants (Fig. 1).

Development of anti-PhtD, -PhtD C, -CbpA, and -LytC antibodies in relation to pneumococcal carriage. The frequency of pneumococcal upper respiratory tract carriage in this study cohort has been described previously (see group 1 of Fig. 1 in reference 13). The increase in GMCs described above (Fig. 1) was primarily due to the increase in the concentrations of the antibodies in the carrier \((\text{Pnc}^+)\) group (Fig. 2) after the age of 18 to 22 weeks, while the concentrations in the noncarrier \((\text{Pnc}^-)\) group continued to decrease or stayed at the same level. The difference between the \(\text{Pnc}^+\) and \(\text{Pnc}^-\) groups was most clear-cut for anti-CbpA, while the differences were smaller for anti-PhtD, -PhtD C, and -LytC but showed the same trend. The small number of samples in the \(\text{Pnc}^-\) group, closer to the end of the observation period, made the 95% CI wide.

Rises in antibody levels after nasopharyngeal pneumococcal acquisitions were also examined individually (Fig. 3). The infants who were found to be carriers for the first time at an average age of 7 weeks (Fig. 3, left column) showed in general, in concordance with Fig. 1 and 2, a decrease in antibodies, and the responses were seen only at an older age in association with persisting colonization or new acquisition. In addition, the kinetic curves of the two infants who were pneumococcal carriers at 7 weeks of age and remained pneumococcus negative after that continued to fall like the curves of the noncarrier infants, since no new pneumococcal contact stimulated antibody production. At an average age of 18 weeks (Fig. 3, middle column), those who became carriers for the first time \((n = 5)\) showed a clear increase in antibodies to LytC \((4/5)\), but lower and fewer responses to the other antigens were noted. In the noncarrier group (Fig. 3, right column), the antibody concentrations decrease in general, but there are some rises in antibody, probably due to undetected carriage during the long gap (about 5 months) in sampling between the fourth and the fifth samples. One noncarrier infant had increases in antibody concentration by >9-fold to LytC, PhtD, and PhtD C between the fourth (infant aged 21 weeks) or the fifth (infant aged 39 weeks) and the sixth (infant aged 43 weeks) samples. The increase in antibody concentration to CbpA was 1.5-fold between the fifth (infant aged 39 weeks) and the sixth (infant aged 43 weeks) samples.

**Correlation between antigens.** In general, the concentrations of antibodies to the different proteins correlated modestly in the infant sera taken at an average age of 10.5 months and poorly in the adult sera (Table 2). The only exceptions were the anti-PhtD and -PhtD C concentrations. The concentrations of anti-PhtD were higher than those of anti-PhtD C, but the correlation between the antigens was good for both infant and adult sera (Fig. 4).

**DISCUSSION**

We show here that the development of antibodies to the pneumococcal virulence and vaccine candidate proteins PhtD, CbpA, and LytC have very similar kinetics in early infancy (Fig. 1). At the age of 4 to 5 months, the infants start to have increases in the antibody concentrations, and the increases are associated with culture-proven pneumococcal carriage (Fig. 2). From the individual kinetic curves, we can see that for the LytC antigen, the detectable responses to carriage seem to take place earlier than for the others (Fig. 3).

The kinetics of anti-PhtD, -PhtD C, -CbpA, and -LytC go along with the kinetics found in our previous studies for antibodies to PspA, Ply \((13, 30)\), PhtB, PhtE \((14)\), PpmA \((4)\), and NanA \((34)\). The LytC and NanA antigens differ from those of

![FIG. 1. Kinetics of antibodies to pneumococcal proteins PhtD, PhtD C, CbpA, and LytC. The GMCs (µg/ml) and the 95% CI of antibody concentrations in serum samples from mothers (M) taken during the second or third trimester of pregnancy, in the cord blood (CB) samples, and in consecutive serum samples from infants (Table 1). The number of samples at each time point is as follows: M, 52; CB, 38 or 39; and infants, 42, 33 or 34, 40, 29 to 32, 31 to 33, 40 or 41, 27 or 28, and 12 or 13 for symbols from left to right, respectively.](image-url)
the other proteins; infants have already reached the adult anti-LytC (this study) and anti-NanA (34) concentrations by about 11 months and 2 years of age, respectively, while the antibody concentrations for the other proteins remain lower in children than in adults (4, 13, 14, 30). The anti-PsaA concentrations are clearly increased in the second month of life in pneumococcal carriers (Pnc+) and noncarriers (Pnc−). Once the infant had become a carrier, he/she stayed in the Pnc+ group, which means that the carrier group was cumulatively increasing and that the Pnc− group was decreasing. For the Pnc+ group (filled squares), the number of samples/indicated time point was as follows: 13, 15 or 16, 26, 21 to 24, 26 to 28, 36 or 37, 25 or 26, and 11 or 12 (for symbols from left to right). For the Pnc− group (open squares), the numbers of samples/indicated time point were as follows: 29, 18, 14, 8, 5, 4, 2, 1 (for symbols from left to right). Number of cord blood (CB) samples, 38 or 39.

Zhang et al. (42) have studied the kinetics of the anti-CbpA antibody concentrations in healthy British children. From 2 months to 5 months of age, the anti-CbpA concentrations decreased (like in this study), but in contrast to this study, the concentrations started to increase only after 13 months of age. In addition, Simell et al. (B. Simell, P. Ahokas, M. Lahdenkari, J. Poolman, I. Henckaerts, T. Kilpi, and H. Käyhty, submitted for publication) have shown that the anti-CbpA antibody concentrations in general start to increase after the age of 12 months in Finnish children. Thus, the different antibody kinetics can be due to different exposures to pneumococcus in the Philippines (13) compared to those of the industrialized world (36).}

Zhang et al. (42) have further shown that British children (2 to 12 years) colonized with pneumococcus have lower serum anti-CbpA and -Ply concentrations at the time of swabbing than noncolonized children. In accordance, Obaro et al. (23) reported that Gambian infants (3 to 5 months) colonized with pneumococcus have lower anti-PsaA concentrations than infants with cultures negative for pneumococcus. In contrast to this, we have shown in our previous studies (4, 13, 14, 30, 34) and in this study that colonized children (<1 to 2 years of age) have higher antibody concentrations to PspA, PsaA, Ply, PhTB, PhTE, PpmA, NanA, PhTD, PhtD C, CbpA, and LytC than children who are culture negative for pneumococcus. Clearly, this study and our previous studies suggest that pneumococcal colonization alone is immunogenic to the proteins studied, but the studies of Zhang et al. and Obaro et al. indicate that the lack of specific antibody-to-protein determinants may correlate with a risk for colonization (23, 42). The different results may
be due to differences in study design, different aims of the studies, and differences in the study subjects. The Finnish and Filipino studies use previous and current colonization, while the studies of Zhang et al. and Obaro et al. have data only on current colonization. To clarify this, the children of this study and our previous study (13) were regrouped according to current colonization only, like in the studies of Zhang et al. and Obaro et al. Also, in this analysis, colonized infants have concentrations of anti-CbpA, -LytC, -PhdT, -PhdT C, -PspA, -PsaA, and -Ply antibodies similar to or higher than those of...
TABLE 2. Correlation coefficients and 95% CI for the anti-PhtD, -PhtD C, -CbpA, and -LytC antibody concentrations in serum samples from the mothers and infants

| Antigens          | Correlation coefficient (95% CI) | Mothers | Infants |
|-------------------|----------------------------------|---------|---------|
| PhtD vs PhtD C    | 0.79 (0.66 vs 0.88)              | 0.89 (0.79 vs 0.94) |
| PhtD vs CbpA      | 0.21 (–0.07 vs 0.45)             | 0.34 (0.02 vs 0.59) |
| PhtD vs LytC      | 0.30 (0.03 vs 0.53)              | 0.36 (0.06 vs 0.61) |
| PhtD C vs CbpA    | 0.11 (–0.17 vs 0.37)             | 0.35 (0.04 vs 0.60) |
| PhtD C vs LytC    | 0.26 (–0.02 vs 0.50)             | 0.38 (0.08 vs 0.62) |
| CbpA vs LytC      | 0.18 (–0.10 vs 0.43)             | 0.61 (0.36 vs 0.77) |

*Statistically significant correlation at 5% level.

Culture-negative children (data not shown). The children of the study of Zhang et al. were all undergoing adenoidectomies, while the children of this study are healthy. However, the children of the study of Obaro et al. were also healthy. Clearly, further studies are needed to clarify the reason for the differences in the results.

There were five infants in this study and in our previous study (13) whose nasopharyngeal swabs remained negative for pneumococcus. Of these five infants, none had increases of >2-fold in anti-PspA or -CbpA antibody concentration, but one infant had an increase of >2-fold in concentrations of antibodies to all the other antigens, as follows: PsaA, Ply (13), PhtD, PhtD C, and LytC (this study). The increases in antibody concentrations occurred during the long gap in sampling (about 5 months) between the fourth and the fifth sample. It is quite likely that the infant had been colonized with pneumococcus between the samplings. The increases in concentrations of antibodies to common protein antigens of children whose swabs remained negative for pneumococcus found in this study and the previous study highlight the need for an interval shorter than 5 months between sampling for longitudinal carriage acquisition studies.

We expected good correlations among the antiprotein antibodies in the infant sera, since we anticipated a simultaneous increase in antibodies of several specificities after recent or current pneumococcal contacts. However, we found only modest correlation among the different antibody concentrations (Table 2), which suggests that infants respond selectively and with different strengths to different antigens. The correlation of the antiprotein antibody concentrations of infants was slightly better than that of adults. This could be due to fewer recent pneumococcal carriage episodes with antigenic stimuli among adults than with those among infants (32). As expected, the anti-PhtD and -PhtD C concentrations showed a good correlation (Table 2 and Fig. 4), and this was true for both infant and adult samples. The concentrations of anti-PhtD were higher than those of anti-PhtD C. This could be due to different folding of the recombinant proteins PhtD and PhtD C or just due to PhtD C being shorter, with fewer epitopes than PhtD. Our data suggest that a marked proportion of the antiphtD antibodies of both adults and infants are directed against the exposed and possibly protective epitopes.

This study and others (4, 13, 14, 19, 23, 30, 34, 35) confirm that serum antibodies to proteins are also being produced after pneumococcal carriage or disease in young infants, although the simultaneous responses to different antigens can vary (indicated by modest correlation of the antibody concentrations). The role of serum antiprotein antibodies in infants for protection against pneumococcal infection needs to be investigated; this study was not designed for that. Animal passive protection models suggest that serum antiprotein antibodies can have a role in protection against pneumococcal infection (5, 37), and studies using an adult human colonization model have shown an association between antibodies to CbpA and PspA and acquisition of pneumococcal colonization (21). In addition, Rapola et al. have shown an association between antibodies to PsaA and the development of pneumococcal acute otitis media (31). However, T-cell-mediated immunity as well as mucosal immunity might have a role in protection. Simell et al. have shown that the existence of salivary anti-PspA is associated with a lowered risk of pneumococcal acute otitis media but not of carriage (35). Zhang et al. (41) have shown that T-cell-derived gamma interferon and interleukin 10 may be regulators of the production of mucosal antibodies to pneumolysin and CbpA in the nasopharynx, and they suggest that cytokines may play an important role in local protection against pneumococcal infection in children. In a mouse model, protection against pneumococcal carriage is dependent on CD4+ T cells and independent of pneumococcal antibodies, although the anti-PspA and -PsaA antibody concentrations correlated with protection against colonization (38). A recent study suggests that immunity to pneumococcus is mediated by interleukin 17A (20).

The role of pneumococcal protein antibodies after the immunization of infants still needs to be clarified by future vaccine studies. Immunizing animals with the three proteins in this study has been shown to induce protection (1, 25, 39), and the data on the natural antibody development of these antigens

FIG. 4. Correlation between individual anti-PhtD and -PhtD C antibody concentrations in sera from mothers and infants (10.5 months). The filled boxes are samples from the mothers, and the open boxes are samples from the infants.
provided by the present study are encouraging. The finding relevant to vaccine development is that young infants are able to produce serum anti-PhtD, -PhtD C, -CbpA, and -LytC antibodies in response to pneumococcal carriage.

ACKNOWLEDGMENTS

We thank the mothers and infants enrolled in this study. In particular, we thank the MATER study personnel in the Philippines: Conchita Gepanayao, Marife Amarante, Lea Saturno, and Remedios Borja for patient recruitment and follow-up; and Melissa Munday, Crezulando Cuenca, and Jenny Argana for laboratory work. Ana Tamundong is acknowledged for data entry and validation. We thank P. Helena Makela for critical comments.

This work was supported by the Finnish Academy (SA-ARIVAC) Officials grant no. 5569 and 206283; the European Union, DG XII, INCO-DC Programme (EU-ARIVAC-1) contract IC18CT950025; the European Union, DG XII, INCO-DC Programme (EU-ARIVAC-2) contract IC18-C79-0219; the European Union, DG XII, INCO-DC Programme (EU-ARIVAC-3) contract ICA4-CT-1999-10008; the European Union, DG Research INCO Programme (EU-ARIVAC-4) contract ICA4-CT-2002-10062; and GlaxoSmithKline Biologicals.

REFERENCES

1. Adamou, J. E., J. H. Heinrichs, A. L. Erwin, W. Walsh, T. Gayle, M. Dor- mitzer, R. Dagan, Y. A. Brewah, P. Barren, R. Latham, S. Langermann, S. Konig, and S. Jnowick. 2001. Identification and characterization of a novel family of pneumococcal proteins that are protective against sepsis. Infect. Immun. 69:949–958.

2. Balachandran, P., A. Brooks-Walter, A. Virolina-Julkenen, S. K. Holling- shaw, and D. L. Braun. 2002. Role of pneumococcal surface protein C in nasopharyngeal carriage and pneumonia and its ability to elicit protection against carriage of Streptococcus pneumoniae. Infect. Immun. 70:2526–2534.

3. Beghetto, N., G. Gargano, S. Ricci, G. Garusi, S. Peppoloni, F. Montagnani, M. Oggioni, G. Pozzi, and F. Felici. 2006. Discovery of novel Streptococcus pneumoniae antigens by screening a whole-genome lambda-display library. FEMS Microbiol. Lett. 262:14–21.

4. Bogaert, D., E. Holmlund, M. Lahdenkari, R. de Groot, T. Kilpi, P. W. Scott. 2007. Factor H binding to PspC of Streptococcus pneumoniae reveals evolutionary mobile domains. Mol. Microbiol. 65:3724–3732.

5. Briles, D. E., S. K. Hollingshead, J. King, A. Swift, P. A. Braun, M. K. Park, and D. E. Briles, J. C. Paton, A. K. Takala, T. M. Kilpi, and H. Ka ¨yhty. 2001. Do antibodies to pneumococcal surface adhesin A prevent complement deposition through the recruitment of complement factor H. FASEB J. 15:731–738.

6. Briles, D. E., M. C. Woodrow, J. T. Poolman, and J. C. Paton. 2001. Protection against Streptococcus pneumoniae elicits by immunization with antibodies to pneumolysin and the pneumococcal surface adhesin A. Vaccine 19:1661–1669.

7. Jarva, H., R. Janulczyk, J. Hellwage, P. F. Zipfel, L. Bjorck, and S. Meri. 2000. Novel purification scheme for pneumococcal surface adhesin A and pneumolysin in relation to pneumococcal carriage and acute otitis media. J. Infect. Dis. 182:1166–1172.

8. Qiambao, B. P., H. Nohynek, H. Kayhty, J. Ollgren, L. Gozum, C. P. Gepanayao, V. Soriano, and P. H. Makela. 2003. Maternal immunization with pneumococcal polysaccharide vaccine in the Philippines. Vaccine 21:3451–3454.

9. Qiambao, B. P., H. Nohynek, H. Kayhty, J. Ollgren, L. Gozum, C. P. Gepanayao, V. Soriano, and P. H. Makela. 2003. Maternal immunization with pneumococcal polysaccharide vaccine in the Philippines. Vaccine 21:3451–3454.

10. Regev-Yochay, G., M. Raz, R. Dagan, N. Porat, B. Shainberg, E. Pinco, N. Keller, and E. Rubinstein. 2004. Nasopharyngeal carriage of Streptococcus pneumoniae by adults and children in community and family settings. Clin. Exp. Immunol. 138:632–639.

11. Rosenov, C., P. Ryan, J. N. Weiser, S. Johnson, P. Fontan, A. Orquiste, and H. R. Masure. 2001. Congenital nasopharyngeal carriage of Streptococcus pneumoniae in young African infants. Vaccine 19:2457–2467.

12. Holmlund, E., B. Qiambao, S. Grönholt, V. C. Soriano, H. Nohynek, and H. Käyhty. 2004. Abstr. 4th Int. Symp. Pneumococci Pneumococcal Dis. (ISPDP-4), abstr. P25v-26. Helsinki, Finland, 9 to 13 May 2004.

13. Hammerschmidt, S., S. R. Talay, P. Brandtzæg, and G. S. Chhatwal. 1997. SpsA, a novel pneumococcal surface protein with specific binding to secretory immunoglobulin A and secretory component. Mol. Microbiol. 25:1113–1124.

14. Holmlund, E., B. Qiambao, S. Grönholt, V. C. Soriano, H. Nohynek, and H. Käyhty. 2004. Abstr. 4th Int. Symp. Pneumococci Pneumococcal Dis. (ISPDP-4), abstr. P25v-26. Helsinki, Finland, 9 to 13 May 2004.

15. Holmlund, E., B. Qiambao, S. Grönholt, V. C. Soriano, H. Nohynek, and H. Käyhty. 2004. Abstr. 4th Int. Symp. Pneumococci Pneumococcal Dis. (ISPDP-4), abstr. P25v-26. Helsinki, Finland, 9 to 13 May 2004.

16. Holmlund, E., B. Qiambao, S. Grönholt, V. C. Soriano, H. Nohynek, and H. Käyhty. 2004. Abstr. 4th Int. Symp. Pneumococci Pneumococcal Dis. (ISPDP-4), abstr. P25v-26. Helsinki, Finland, 9 to 13 May 2004.
38. Trzcinski, K., C. Thompson, R. Malley, and M. Lipsitch. 2005. Antibodies to conserved pneumococcal antigens correlate with, but are not required for, protection against pneumococcal colonization induced by prior exposure in a mouse model. Infect. Immun. 73:7043–7046.

39. Wizemann, T. M., J. H. Heinrichs, J. E. Adamou, A. L. Erwin, C. Kunsch, E. Choi, S. C. Barash, C. A. Rosen, H. R. Masure, E. Tuomanen, T. Gayle, Y. A. Brewah, W. Walsh, P. Barren, R. Lathigra, M. Hanson, S. Langermann, S. Johnson, and S. Koenig. 2001. Use of a whole genome approach to identify vaccine molecules affording protection against Streptococcus pneumoniae infection. Infect. Immun. 69:1593–1598.

40. Zhang, J. R., K. E. Mostov, M. E. Lamm, M. Nanno, S. Shimida, M. Ohwaki, and E. Tuomanen. 2000. The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. Cell 102:827–837.

41. Zhang, Q., J. Bernatoniene, L. Bagrade, J. C. Paton, T. J. Mitchell, S. Hammerschmidt, D. A. Nunez, and A. Finn. 2006. Regulation of production of mucosal antibody to pneumococcal protein antigens by T-cell-derived gamma interferon and interleukin-10 in children. Infect. Immun. 74:4735–4743.

42. Zhang, Q., J. Bernatoniene, L. Bagrade, A. J. Pollard, T. J. Mitchell, J. C. Paton, and A. Finn. 2006. Serum and mucosal antibody responses to pneumococcal protein antigens in children: relationships with carriage status. Eur. J. Immunol. 36:46–57.

43. Zhang, Q., S. Choo, and A. Finn. 2002. Immune responses to novel pneumococcal proteins pneumolysin, PsaA, PspA, and CbpA in adenoidal B cells from children. Infect. Immun. 70:5363–5369.

44. Zhang, Y., A. W. Masi, V. Barniak, K. Moutzouros, M. K. Hostetter, and B. A. Green. 2001. Recombinant PhpA protein, a unique histidine motif-containing protein from Streptococcus pneumoniae, protects mice against intranasal pneumococcal challenge. Infect. Immun. 69:3827–3836.