Effects of Absorption with Pneumococcal Type 22F Polysaccharide on Maternal, Cord Blood, and Infant Immunoglobulin G Antipneumococcal Polysaccharide Antibodies

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The aim of this study was to evaluate the effect of absorption with pneumococcal type 22F polysaccharide on antipneumococcal antibody titers in unimmunized Chilean pregnant women and on antibodies in their offspring at birth and 3, 6, and 12 months of age. Sera from 10 healthy pregnant women and from their offspring at birth and at 3, 6, and 12 months of age were studied. Immunoglobulin G antibodies against serotypes 1, 3, 4, 5, 6B, 9V, 14, 18, 19F, and 23F were measured by a standardized enzyme-linked immunosorbent assay method. All sera were absorbed with polysaccharide C, and aliquots of each serum were absorbed with polysaccharide 22F. Individual results were expressed in μg/ml based on the standard serum pool 89-SF. Absorption with polysaccharide 22F reduced antibody concentrations in all samples and to all 10 serotypes studied. Reduction was highest in maternal sera and in cord blood, but it was also present at 3, 6, and 12 months of age. The percent reduction ranged from 24% for serotype 14 to 50% for serotype 1 in maternal samples and from 20% for serotype 18C to 49% for serotype 4 in cord blood samples. The percentages of transplacental transmission were similar for nonabsorbed and absorbed maternal fetal pairs. Absorption with serotype 22F had a significant impact on antipneumococcal antibody concentrations in unimmunized pregnant women and in their offspring. Our results suggest that absorption with 22F polysaccharide needs to be performed in studies of transplacental transmission of antipneumococcal antibodies.

Streptococcus pneumoniae is the leading cause of invasive bacterial infections in young children throughout the world, causing bacteremia, meningitis, pneumonia, and also otitis media, sinusitis, and other complications of respiratory tract infections. The rate of infection is greater for children under 2 years of age, reaching rates as high as 228 cases per 100,000 in those 6 to 12 months old (3, 4). Some protection against invasive infections in the first few months of life is afforded by the transplacental transfer of maternal antibodies. The concentration of antibodies present in unimmunized mothers (6, 1, 8), as well as in mothers immunized with the 23-valent pneumococcal polysaccharide vaccine during pregnancy (16, 14, 12).

Recently, it has been shown that even after absorption with pneumococcal cell wall polysaccharide, the enzyme-linked immunosorbent assay (ELISA) often measures some quantity of nonfunctional, nonspecific antibodies (5, 7, 17, 23). Absorption with 22F polysaccharide of sera from individuals above 8 years of age significantly improves the correlation of antibody concentrations with functional opsonophagocytic assays that predict protection against invasive pneumococcal disease. Pneumococcal type 22F polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide ELISA (7).

There is conflicting evidence about whether nonspecific antibodies are present in infants as well as in adults. One study showed that these antibodies were not present in infants (7), while another showed they were present in children studied at 18 and 24 months of age, although in a lower concentration than in adults (17).

We wished to determine the effect of absorption with 22F polysaccharide on maternal antibody concentrations and also on the antibody concentration in unimmunized infants in the first year of life since these antibodies are mostly of maternal origin. The effects of absorption with serotype 22F polysaccharide on maternal antibodies, on transplacental transmission of serotype-specific antibodies, and on antibodies present in infants during the first year of life were evaluated in 10 unim-

| Subject no. | Maternal age (yr) | Parity | Gestational age (wk) | Birth weight (g) | Length (cm) | Infant gender |
|-------------|------------------|--------|----------------------|-----------------|-------------|---------------|
| 1           | 20               | 0      | 39                   | 3,200           | 50.0        | Male          |
| 2           | 30               | 1      | 39                   | 3,850           | 51.5        | Female        |
| 3           | 34               | 3      | 39                   | 3,500           | 51.0        | Female        |
| 4           | 21               | 0      | 39                   | 3,160           | 49.0        | Female        |
| 5           | 25               | 1      | 37                   | 3,070           | 48.5        | Male          |
| 6           | 23               | 0      | 40                   | 3,000           | 48.5        | Female        |
| 7           | 40               | 0      | 40                   | 4,500           | 52.0        | Female        |
| 8           | 24               | 0      | 40                   | 4,070           | 52.0        | Male          |
| 9           | 17               | 0      | 40                   | 3,620           | 51.0        | Female        |
| 10          | 21               | 0      | 39                   | 3,830           | 52.0        | Male          |

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munized pregnant Chilean women and in their offspring at birth and at 3, 6, and 12 months of age.

**MATERIALS AND METHODS**

**Study population.** Ten healthy Chilean pregnant females and their term offspring were studied as part of a prospective study of breast milk and formula feeding (Table 1). None had received a pneumococcal vaccine. Samples from the mother were obtained in the third trimester of pregnancy. Cord blood was obtained by cutting the cord at one-third of the distance to the placenta, letting blood drip freely from the cut cord on the placental side into a sterile tube. Infant serum samples were obtained at 3, 6, and 12 months of age. All serum samples were frozen until the pneumococcal antibodies were determined.

**ELISA for antipneumococcal IgG.** Immunoglobulin G (IgG) antipneumococcal serotypes 1, 3, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F were determined by a modified ELISA protocol intended to detect serum antibodies to pneumococcal C polysaccharide in children by determining the response to acute pneumococcal otitis media or to vaccination (11, 15). Standard, control, and serum samples were preabsorbed with S. pneumoniae cell wall polysaccharide (500 μg/ml in undiluted serum; Statens Seruminstitut, Denmark) for 30 min at room temperature. The serotype-specific IgG antibody concentration (in micrograms per milliliter) was calculated by measuring the absorbance (optical density at 450 nm) against a standard curve obtained using a serum pool (standard). Serotype-specific IgG levels in this standard had previously been determined using the FDA 89-SF reference sample (Center for Biologies Evaluation and Research, U.S. Food and Drug Administration, Rockville, MD). The antibody concentration against serotype 3, which is not included in the conjugate vaccines, was determined by cross-standardization of our standard sample against the assigned IgG values for the other serotypes in the FDA 89-SF sample. In this method, six different standardized serotypes were used to generate standard curves which were used individually to evaluate the antibody concentration against serotype 3 (21).

**Absorption of sera with pneumococcal polysaccharide 22F.** Prior to IgG antibody concentration determinations, sera were absorbed with pneumococcal serotype 22F polysaccharide by incubating the serum sample diluted 1:10 in phosphate-buffered saline with 0.05% Tween 20 and 0.05% bovine serum albumin with 50 μg of 22F polysaccharide per ml for 30 min at room temperature. Absorbed and nonabsorbed samples from the mother-and-infant pairs were studied simultaneously.

**Statistical methods.** Results of specific antibody concentrations were analyzed following a logarithmic transformation in order to decrease the influence of a few extreme measurements. Results were expressed as geometric means and confidence intervals.

The effect of absorption with polysaccharide 22F was calculated as the percent change due to absorption (absorption ratio). Ratios were calculated for mother, cord, and all infant samples and for each serotype separately. Transplacental transmission coefficients of specific antipneumococcal antibodies were calculated dividing the cord blood concentrations by the concentration found in the corresponding maternal blood. Original antibody concentrations, and not the log-transformed values, were used in the calculations of both ratios and transplacental transmission coefficients.

Differences between the mother and cord absorbed and nonabsorbed samples were analyzed using the Wilcoxon signed-rank test, a procedure designed for small samples with nonindependent measures. All data management and analyses were done in SAS Statistical Software, version 9.

**Informed consent.** This study was approved by the Universidad de la Frontera, Temuco, Chile, Institutional Review Board. Written informed consent was obtained from each participating mother.

**RESULTS**

Absorption with polysaccharide 22F reduced antibody concentrations in all samples and to all 10 serotypes studied, with the exception of two instances where the specific antibody concentration in the absorbed serum was slightly higher than in the nonabsorbed sample. The mean specific antibody reduction ranged from 24% for serotype 14 to 50% for serotype 1 in maternal samples and from 20% for serotype 18C to 49% for serotype 4 in cord blood samples. The percentages of transplacental transmission

**TABLE 2. Mean antibody concentrations for absorbed and nonabsorbed mother and cord blood samples and transplacental transmission coefficients for absorbed and nonabsorbed sera based on 10 mother-infant pairs**

| Group and/or parameter | Transplacental transmission coefficient | Nonabsorbed antibody concn | Absorbed antibody concn | Absorbed GM/Non-absorbed GM (95% CI) | Absorption ratio (mean % SD) | Absorbed GM (99% CI) |
|------------------------|----------------------------------------|-----------------------------|-------------------------|-------------------------------------|-----------------------------|---------------------|
| 1                      |                                         | 0.40 ± 0.16                 | 0.46 ± 0.15             | 0.56 ± 0.42                         | 0.63 ± 0.26                 | 0.49 ± 0.22         |
| 3                      |                                         | 0.65 ± 0.26                 | 0.69 ± 0.26             | 0.71 ± 0.29                         | 0.73 ± 0.24                 | 0.59 ± 0.22         |
| 4                      |                                         | 0.41 ± 0.16                 | 0.47 ± 0.22             | 0.59 ± 0.42                         | 0.69 ± 0.25                 | 0.45 ± 0.22         |
| 5                      |                                         | 0.23 ± 0.17                 | 0.34 ± 0.22             | 0.36 ± 0.41                         | 0.46 ± 0.26                 | 0.28 ± 0.22         |
| 6B                     |                                         | 0.67 ± 0.21                 | 0.72 ± 0.21             | 0.78 ± 0.30                         | 0.87 ± 0.28                 | 0.67 ± 0.24         |
| 9V                     |                                         | 0.40 ± 0.12                 | 0.44 ± 0.13             | 0.44 ± 0.16                         | 0.47 ± 0.13                 | 0.38 ± 0.17         |
| 14                     |                                         | 0.78 ± 0.30                 | 0.86 ± 0.36             | 0.88 ± 0.41                         | 0.91 ± 0.30                 | 0.75 ± 0.31         |
| 18C                    |                                         | 0.34 ± 0.15                 | 0.34 ± 0.15             | 0.35 ± 0.20                         | 0.36 ± 0.21                 | 0.33 ± 0.21         |
| 19F                    |                                         | 0.42 ± 0.18                 | 0.45 ± 0.20             | 0.46 ± 0.22                         | 0.50 ± 0.25                 | 0.43 ± 0.23         |
| 23F                    |                                         | 0.58 ± 0.28                 | 0.63 ± 0.30             | 0.66 ± 0.35                         | 0.70 ± 0.33                 | 0.56 ± 0.33         |
ranged from 0.34 to 0.65 for nonabsorbed antibodies and from 0.30 to 0.63 for absorbed serum pairs. Transmission coefficients were identical or very similar in nonabsorbed and absorbed serum pairs (Table 2).

Absorption with polysaccharide 22F also reduced antibody concentrations in sera from infants at 3, 6, and 12 months of age (Fig. 1). Although infants had much lower antibody concentrations in nonabsorbed sera, the absorp-

FIG. 1. Antibody concentrations (conc) in absorbed and nonabsorbed samples from mother-infant pairs. The values in parentheses are % reduction due to absorption.
tion ratios were similar to those observed in maternal and cord sera.

**DISCUSSION**

Our results show that absorption of sera from unimmunized pregnant Chilean women with polysaccharide 22F significantly decreased the mean concentrations of antibody to all 10 serotypes tested. Our results for unimmunized mothers are consistent with previous studies showing that the degree of reduction of antibody concentrations after absorption with 22F polysaccharide could be as high as half or even 90% of the initial antibody concentration for some serotypes (7, 16).

As expected, a similar effect of absorption with 22F polysaccharide was observed in cord blood and in samples obtained from infants at 3 months of age. These results for infants were expected since IgG antibodies found in cord blood and early infancy are maternal antibodies transmitted across the transplacental barrier (1, 8). Transmission rates for both absorbed and nonabsorbed antibodies ranged from 0.34 to 0.65. These results confirm prior observations of significantly lower antibody concentrations in the cord blood than in the mother despite significantly greater total IgG concentrations in cord blood than in maternal blood (1, 6, 8). The lower ratio may be explained by the fact that antipneumococcal antibodies are predominantly IgG2 antibodies, which are not transported across the placenta as well as IgG1 antibodies, as shown in a previous study on transplacental transmission of serotype-specific pneumococcal antibodies in a Brazilian population (5). The ratio of transplacental transmission of maternal antibodies was not affected by absorption with polysaccharide 22F, suggesting that serotype-specific antibodies and nonspecific antibodies are transported equally across the placenta.

A study performed in São Paulo, Brazil, using the same ELISA method and reference sera revealed that transplacental transmission of antibodies from unimmunized mothers against serotypes 3 and 14 was close to 100%. In contrast, the transmission of antibodies to serotypes 1, 6B, and 9V ranged from 77 to 83%, suggesting a predominant IgG2 response to these serotypes (8). Maternal antibody concentrations were measured at the time of delivery in the Brazilian study. Our study reported maternal immunity assessed at the beginning of the third trimester of pregnancy. The timing of the maternal sampling is likely to explain this difference. Total antibodies to several pneumococcal serotypes were found to decrease during pregnancy in a small series of patients from the first trimester to samples obtained predelivery (10). Therefore, lower maternal antibody concentrations at the time of delivery are likely to increase transmission ratios.

The decrease in antibody concentration effected by absorption with 22F polysaccharide is higher for antibodies developed after contact with heterologous pneumococcal serotypes or in the absence of detectable contact with pneumococci (17, 18, 9) than for antibodies developed after a specific infection or after immunization (19). This may explain the rather large decrease in antibody concentrations observed in sera from our unimmunized study population.

Our results are important to consider since sera from unimmunized individuals may have lower opsonophagocytosis than expected on the basis of the antibody concentration obtained by ELISA (2, 13). Absorption of sera with pneumococcal polysaccharide type 22F removes nonspecific cross-reactive antibodies and improves the correlation between the antibody concentration obtained by ELISA and opsonophagocytic assays, which in turn seem to better correlate with protection against pneumococcal infection (7, 17, 24). Our results suggest that future studies of maternal immunity and transplacental transmission of pneumococcal antibodies need to be done with absorption of both maternal and cord blood sera with 22F polysaccharide.

There is conflicting evidence about whether nonspecific antibodies are present in infants, with one study showing that these antibodies were not present (7) while another showed that they were present, although in a lower concentration than in adults (20). These differences could be due to the fact that nonspecific antibodies may appear in some populations but not others. Our study shows significantly decreased antibody concentrations in samples from unimmunized infants at 3, 6, and 16 months of age absorbed with polysaccharide 22F. These results suggest that most of these antibodies are of maternal origin initially. Antibodies that do develop at 6 and 12 months of age may be developed in response to subclinical pneumococcal infections with pneumococci with heterologous serotypes, even with no detectable contact with pneumococci (11, 23). Our results will provide an important backdrop for future studies of antibodies produced by pregnant women (16, 14, 12) and/or infants (17, 9).

In conclusion, our study shows that absorption with a heterologous polysaccharide, such as serotype 22F, removes a significant amount of serotype-specific antibodies, when measured by ELISA, from unimmunized, pregnant mothers and from antibodies in the cord blood of their offspring and during the first year of life. A third-generation antibody assay (22) that includes absorption with 22F polysaccharide should be used in all studies of maternal immunity and transplacental transmission of IgG antibodies since it changes the results of antibody concentrations measured in both mothers and infants.

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