Low Cord Blood Type 14 Pneumococcal IgG1 but Not IgG2 Antibody Predicts Early Infant Otitis Media

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Type-specific IgG1 and IgG2 antibodies to Streptococcus pneumoniae capsular polysaccharides 14 and 19F were measured in cord blood samples from 425 neonates, to determine which antibody subclass was most strongly associated with otitis media (OM) during the first 6 months of life (early OM). Early OM was significantly associated with type 14 IgG1 antibody in the lowest antibody quartile (P = .055) but not with type 19F IgG1 antibody or with either IgG2 antibody. IgG1 and IgG2 antibodies were significantly intercorrelated for type 14 (r = .52, P < .001) and type 19F (r = .38, P < .001). Multivariate analysis revealed that having type 14 IgG1 antibody in the lowest quartile, child care attendance, and sibling and maternal OM history were independent risk factors for early OM. Although type-specific pneumococcal IgG2 antibody concentrations were significantly higher than IgG1 concentrations, IgG2 antibodies apparently are not protective against OM during early infancy.

Materials and Methods

The study population and measured outcomes have been described elsewhere [6]. The primary outcome measure was age at first OM episode. IgG1 and IgG2 subclass antibodies to types 14 and 19F polysaccharide were measured by use of a modification of the World Health Organization consensus ELISA protocol, as described by Quataert et al. [7]. Mouse monoclonals were used for the detection of antibody conjugates HP6069 for IgG1 and equal parts of HP6002 and HP6014 for IgG2 (HybriDima Reagent Laboratory, Baltimore, MD). The lower limits of detection were 0.07 and 0.04 µg/mL for type 14 IgG1 and IgG2, respectively, and 0.06 and 0.03 µg/mL for type 19F IgG1 and IgG2, respectively (authors' unpublished data).

Antibody concentration distributions were skewed; hence, antibody values were natural log-transformed, and geometric means were calculated. Antibody concentration also was modeled as a categorical variable by dividing the subjects into quartiles on the basis of antibody concentration. Infants were divided into 4 groups on the basis of their type 14 IgG1 antibody concentration. Kaplan-Meier analysis for the age at first OM revealed that the lowest quartile had distinctly earlier onset of OM, whereas the upper 3 quartiles showed considerable overlap among the age-to-first-OM curves. Therefore, the IgG1 type 14 groups were redefined by dividing the subjects into 2 groups, infants with antibody concentrations in the lowest and those with concentrations in the highest 3 quartiles. Groups were compared by the univariate log-rank test. This method was used with the other 3 antibody types.

Multivariate analysis with Cox’s proportional hazard model was done for age at first OM episode as a function of antibody levels, family OM history, child care attendance, and respiratory tract infection (RTI, defined as cold, upper respiratory infection, coryza, rhinorrhea, cough, nasopharyngitis, pneumonia, or bronchitis). Recurrent OM in siblings was defined as ≥3 episodes or as the placement of tympanostomy tubes. Child care attendance in the first 6
months of life and RTI were used as time-dependent variables for the multivariate approach, because the status of these 2 risk factors changed over time. At each time point when OM occurred, the model was evaluated for all the subjects who were still at risk for the event, and a determination was made as to the current value of the respective risk factor. A binary (yes/no) risk factor was considered positive for that subject only for the time prior to the event. Interactions between antibody levels and the other risk factors were tested. All statistical comparisons were considered significant at the .05 level.

Results

The 611 pregnant women who enrolled in this study gave birth to 627 offspring, 605 of whom completed some follow-up and 425 of whom contributed cord blood samples [6]. Most (97%) of the 425 infants with cord blood samples were white, 49% were male, and average birth weight was 3.3 kg; maternal mean and median ages were 31 years. At 2 weeks and 6 months, 80% and 39% of the infants were fully or partially breast-fed, respectively. By age 6 months, 48% of the infants attended child care. At 2 weeks and at 6 months, 12% and 13%, respectively, had a parent who smoked tobacco. Household income was ≤$20,000 for 3% and was >$60,000 for 30%. Recurrent OM history for siblings was documented for 26% of the 425 infants. Enrolled subjects without cord blood samples did not have significantly different demographic characteristics.

Not all the cord blood samples tested were included in the final analysis for one of the following reasons: the sample did not have complete clinical or demographic information, the sample was missing, or the antibody data did not meet quality control standards. As a result, the number of samples used in the final analysis is different for each of the 6 antibodies measured (table 1).

Univariate log-rank analysis with the Kaplan-Meier method revealed that early OM was associated with having type 14 IgG1 antibody in the lowest quartile (table 1; figure 1). Age at first OM episode was not associated significantly with type 14 IgG2 or with type 19F IgG1 or IgG2. The lowest antibody quartiles for type 14 IgG1 and IgG2 antibody were ≤0.10 µg/mL and ≤0.20 µg/mL, respectively; for type 19F IgG1 and IgG2, the lowest quartiles were ≤0.06 µg/mL and ≤0.63 µg/mL, respectively.

Early OM also was significantly associated with sibling history of recurrent OM (relative risk [RR], 2.06; log-rank P < .001), child care attendance during the first 6 months of life (RR, 1.99; P = .029), maternal recurrent-OM history (RR, 1.82; P = .027), but not paternal OM history, and RTI (RR, 8.81; P < .001).

Multivariate analysis revealed that, when we controlled for the influence of the other variables, anti-polysaccharide type 14 IgG1 antibody became even more significant in the prediction of early OM (P < .001). Child care attendance, sibling recurrent OM history, and maternal recurrent OM history were also more significant in the multivariate model. There were no

Table 1. Distribution of antibody to type 14 polysaccharide of Streptococcus pneumoniae and univariate correlation between age at first episode of otitis media (OM) and concentrations of type 14 anti-polysaccharide total IgG, IgG1, and IgG2 subclass antibodies in cord blood.

<table>
<thead>
<tr>
<th>Subclass</th>
<th>n</th>
<th>Geometric mean concentration</th>
<th>25th percentile</th>
<th>Range</th>
<th>95% CI</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>424</td>
<td>1.82</td>
<td>0.55</td>
<td>0.04–135.62</td>
<td>1.54–2.15</td>
<td>.030</td>
</tr>
<tr>
<td>IgG1</td>
<td>403</td>
<td>0.31</td>
<td>0.10</td>
<td>0.04–47.00</td>
<td>0.28–0.36</td>
<td>.055</td>
</tr>
<tr>
<td>IgG2</td>
<td>372</td>
<td>0.61</td>
<td>0.20</td>
<td>0.02–103.88</td>
<td>0.51–0.72</td>
<td>.310</td>
</tr>
</tbody>
</table>

NOTE. Geometric mean concentration, 25th percentile, and range are given in µg/mL. Correlations were determined by use of log-rank test, Kaplan-Meier method. CI, confidence interval.

a These data were previously reported by use of in-house antibody concentration assignments to US Food and Drug Administration reference serum 89SF [6]. With universal acceptance of 89SF antibody assignments reported by Quataert et al. [7], type 14 total IgG concentrations reported here are the result of transforming previously reported values to new 89SF assigned values on the basis of conversion factors reported in table 1 of [6].

b Correlation with age at OM onset.
significant interactions among type 14 IgG, child care, or sibling and maternal OM history.

Spearman correlations showed a significant correlation between type 14 IgG1 and IgG2 \( (r = .52; \ P < .001) \) and between type 19F IgG1 and IgG2 \( (r = .38; \ P < .001) \). Total type 14 IgG antibody also correlated with IgG1 \( (r = .51; \ P < .001) \), IgG2 \( (r = .61; \ P < .001) \), and the sum of IgG1 and IgG2 \( (r = .67; \ P < .001) \). Total type 19F IgG correlated with IgG1 \( (r = .31; \ P < .001) \), IgG2 \( (r = .48; \ P < .001) \), and the sum of IgG1 and IgG2 \( (r = .49; \ P < .001) \).

**Discussion**

This large, prospective maternal-infant study revealed that risk of early OM is associated significantly with low cord blood concentrations of IgG1, but not IgG2, antibodies to type 14 polysaccharide of *S. pneumoniae*. Prellner et al. [8] reported that a low serum concentration of type-specific anti-polysaccharide antibody during the first 3 years of life was associated with an increased risk of developing recurrent AOM. We also have reported that low concentrations of types 14 \( (P = .030) \) and 19F \( (P = .020) \) total IgG antibodies were associated with early OM [6]. Interactions among early OM and breast-feeding history, parental smoking habits, and low cord blood concentrations of types 3, 4, 6B, 18C, and 23F anti-polysaccharide total IgG antibodies were not significant [6].

The association of OM risk with low type 14 pneumococcal IgG1 antibody likely is due to the high frequency of infections with this serotype that occur during childhood. Among 3884 children <6 years old, type 14 accounted for 15% of AOM middle ear fluid isolates and was the serotype isolated most frequently from blood (29%) [9].

The association of OM risk with type 14 IgG1 and not IgG2 antibody may be due to increased transplacental transport of the IgG1 subclass or to greater functional activity of IgG1 antibodies. A study of *Haemophilus influenzae* type b vaccines administered during pregnancy showed that IgG1 antibodies against capsular polyribosylribitol phosphate (PRP) were transported more efficiently across the placenta than were IgG2 anti-PRP antibodies [10]. Ratios of neonatal to maternal IgG subclass anti-polysaccharide type 14 antibody reported by Gasparoni et al. [11] were 1.66 for IgG1 and 0.78 for IgG2, which demonstrates the active transport of IgG1. However, we found significantly lower absolute concentrations of IgG1 antibody to types 14 and 19F than of respective IgG2 concentrations in cord blood. Thus, the active transport of IgG1 antibody is not sufficient alone to explain the apparent protective effect of type 14 IgG1 antibody against OM in infants. Selective transport of IgG1 antibody to nasopharyngeal mucosal surfaces or to the middle ear, or greater functional opsonophagocytic activity of IgG1 than of IgG2 antibody could explain the observation and warrant further study.

The presence of immunoreactive contaminants in polysaccharide preparations used as the ELISA solid-phase matrix has been demonstrated by Yu et al. [12], who found that IgG antibodies to a novel epitope (i.e., non-type-specific antibodies) were detected by ELISA to types 4, 6B, 9V, 18C, 19F, and 23F, but not to type 14. This may explain the poor correlation of anti-polysaccharide pneumococcal antibodies other than type 14 with early OM. These contaminants could have the effect of artificially increasing the amount of type-specific antibody detected by ELISA and may explain the poor correlation of cord blood type 19F subclass antibodies with early OM.

The correlation of early OM risk with maternal and sibling recurrent OM history could reflect the known Gm(n) and Km(l) allelic regulation of IgG response to pneumococcal polysaccharide vaccine [13]. In addition, allotypic correlations within the subset of persons who have low concentrations of type 14 IgG1 antibody and early OM could be explored. Studying allotypic differences between those with high and low antibody concentrations may reveal other factors that affect immune response. Identifying the allotypic differences that correlate with susceptibility to middle ear infection could lead to improved screening and treatment options for OM.

The results of 2 recently reported clinical trials suggest that substantial infant protection against OM will not be achieved by administering pneumococcal conjugate vaccine during early infancy. A US study of 37,830 infants who were immunized at ages 2, 4, 6, and 12–15 months with a 7-valent pneumococcal conjugate vaccine (PNCRM197; Wyeth-Lederle Vaccines and Pediatrics, West Henrietta, New York) revealed only an 8.9% (95% confidence interval [CI], 5.8%–11.8%) reduction in the number of visits for clinically detected OM in PNCRM197 recipients, compared with controls, between 6 and 24 months old [4]. However, there was a 22.8% (95% CI, 6.7%–36.2%) reduction in recurrent OM \( (>5 \) episodes within 6 months, \( >6 \) within a year) and a 20.1% reduction in tympanostomy tube placement, compared with controls, after age 7 months.

A Finnish study of 1662 infants who were immunized on the same schedule and with the same vaccine as that used in the US study revealed an overall 6% (95% CI, −4% to 16%) reduction in the number of visits for AOM, irrespective of etiology, between ages 6.5 and 24 months [5]. However, there was a 34% (95% CI, 21%–45%) reduction in the rate of culture-confirmed pneumococcal AOM, irrespective of serotype, and a 57% (95% CI, 44%–67%) reduction in the rate of culture-confirmed, vaccine-type–specific AOM. Thus, infant immunization with a 7-valent pneumococcal conjugate vaccine, which has demonstrated immunogenicity in infants [14], is not likely to reduce the overall burden of early OM.

Administering polyvalent pneumococcal vaccine to women during pregnancy is an alternative method of protecting infants from early OM, if vaccine-induced maternal antibody is transferred effectively to the infant, as is the case with *H. influenzae* type b PRP vaccines given during pregnancy [10]. Concern has been expressed by some that maternal immunization might in-
terfere with the infant immune response to pneumococcal vaccine that is caused by transfer of idio­typic antibodies (i.e., infant immune tolerance). However, high levels of maternally derived anti-PRP antibody did not suppress infant immune response to PRP-T (PRP conjugated to tetanus toxoid) vaccine given at ages 4 or 14 months [15], which suggests that maternal pneumococcal immunization should be explored further as prophylaxis for early OM.

Acknowledgments

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References

5. Eskola J, Kilpi T, Finnish Otitis Media Study Group. Efficacy of a heptavalent pneumococcal conjugate vaccine (PncCRM) against serotype-spe-