Colostrum Obtained from Women Vaccinated with Pneumococcal Vaccine during Pregnancy Inhibits Epithelial Adhesion of \textit{Streptococcus pneumoniae}

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Prevention of nasopharyngeal colonization may reduce the burden of pneumococcal infection during infancy. Colostrum obtained from Gambian mothers who had been vaccinated with either Pneumovax II or Mengivax A&C (n = 8 per group) during pregnancy was examined for inhibition of adherence of \textit{Streptococcus pneumoniae} serotypes 6B and 14 to pharyngeal epithelial cells in vitro. Pneumococcal adherence was significantly reduced in the presence of breast milk (P \leq .0001 for \textit{S. pneumoniae} serotype 14; P = .036 for serotype 6B), independent of the concentration of secretory IgA antibodies. Maternal vaccination with polyvalent pneumococcal polysaccharide vaccine boosts the capacity of colostrum to inhibit adherence of pneumococci to pharyngeal epithelial cells. In breast-feeding populations, maternal vaccination might prevent pneumococcal disease in young infants.

During the early weeks of life, the newborn infant is immature and responds poorly to conventional vaccines, making it particularly vulnerable to infection. During this period, the infant relies on maternally derived, passively acquired antibodies initially obtained in utero, via transplacental transfer of IgG-class antibodies to the fetus, and, after delivery, as secretory antibodies, via breast-feeding; the quantity of antibodies transferred to the infant could be increased by maternal vaccination during pregnancy [1].

Worldwide, \textit{Streptococcus pneumoniae} is a leading cause of acute lower-respiratory-tract infections (ALRI) in children. Deaths from ALRI are most frequent during the first year of life and account for approximately one-third of deaths in children 1–11 months old, particularly in developing countries [2, 3]. Previous studies have examined how serum antibody concentrations in mother-infant pairs are affected after maternal vaccination with polyvalent pneumococcal polysaccharide (PP) during pregnancy, but little attention has been paid to the concentration and function of secretory antibodies in breast milk [4, 5]. Secretory antibodies play an important defensive role at mucosal surfaces, preventing the adherence, colonization, and penetrative spread of bacteria. Because the nasopharynx is the ecological niche of the pneumococcus, enhancement of the levels of specific secretory antibodies in breast milk should prevent adherence of the bacteria to the epithelial cells, thereby reducing colonization and the potential for invasion.

After maternal vaccination with either PP or meningococcal polysaccharide (MP) during late pregnancy, the capacity of colostrum to inhibit adherence of pneumococci to the infants’ pharyngeal epithelial cells in vitro was investigated. Of the 23 capsular polysaccharides contained in the vaccine, pneumococcal serotypes 6B and 14 were chosen for investigation, because they are 2 of the most commonly identified serotypes obtained from the nasopharynxes of Gambian children.

\textbf{Subjects, materials, and methods.} The present investigation was a double-blind study in which rural Gambian women recruited during the late second to early third trimester of pregnancy were randomly assigned to be vaccinated with either polyvalent PP (Pneumovax II; Pasteur Mérieux) or MP serogroups A and C (Mengivax A&C; Pasteur Mérieux). A single 0.5-mL dose of vaccine was administered intramuscularly in the deltoid. This was a substudy of secretory antibodies in breast milk, in which 56 women and 57 women, respectively, were assigned to the MP-vaccinated group and the PP-vaccinated group. Colostrum was available from 8 woman in each group and was analyzed in the present study. The MP-vaccinated group
Samples, which were processed as reported elsewhere [6], were diluted 1:1 with PBS:1% human serum albumin (Sigma) and incubated at 4°C for 24 h, with a 1-μg/mL concentration of cell-wall polysaccharide (Staten Seruminstitut, Copenhagen, Denmark), to remove any contaminating anti-cell wall antibodies. This mixture was centrifuged at 400g for 15 min, and the supernatants were collected for testing; 8 samples per study group were tested.

Lyophilized *S. pneumoniae* serotype 6B and serotype 14 polysaccharides (National Collection of Type and Cultures of the Public Health Laboratory, Colindale, London) were reconstituted in 1 mL of tryptic soy broth (Sigma), and ~0.1 mL of the resulting solution was transferred, by use of a Pasteur pipette, onto blood agar (BioMérieux) and incubated at 37°C for 18 h in 5% CO₂. Characteristic circular colorless colonies surrounded by a zone of α-hemolysis were produced and were harvested from the plate into 10 mL of Todd Hewitt broth (Difco). After incubation at 37°C for 3 h in 5% CO₂, 3.7 MBq of [6-3H]thymidine (Amersham Pharmacia Biotech) were added, and incubation continued for 1 more hour. Bacteria were subsequently washed 3 times, by being centrifuged at 1200g for 30 min, to remove all unbound [6-3H]thymidine. Bacteria were microscopically counted and were diluted in Hanks’ balanced salt solution (HBSS) (Sigma), to 2.8–4.0 × 10⁸ bacteria/mL (1.4–2.0 × 10⁹ bacteria/well).

A human pharyngeal epithelial-cell line derived from pleural-wash fluid from an adult woman with primary carcinoma of the pharynx [7], Detroit 562 (European Collection of Cell Cultures), was cultured at 37°C in 5% CO₂ and nutrient mixture RPMI 1640 (Sigma) supplemented according to the method described by Peterson et al. [7]. At confluence, the cells were recovered by the addition of 0.25% trypsin-EDTA (Sigma), were washed, and were transferred to sterile 96-well microtiter plates (Greiner Labortechnik), for the adhesion assay. The plates were cultured for an additional 24–48 h, to obtain confluent monolayers at a density of 1.5–2.0 × 10⁶ cells/mL (1.5–2.0 × 10⁵ cells/well), according to the method described by Anderson et al. [8].

Adherence of *S. pneumoniae* to human pharyngeal epithelial cells was assessed by a protocol described elsewhere [8], with minor modifications. Confluent monolayers in the microtiter plates were washed 3 times with 200 μL of sterile PBS/well, by use of a multichannel pipette, and 50 μL of diluted and preabsorbed sample or control (i.e., HBSS) was pipetted into the wells, according to the experimental design, followed by 50 μL of bacterial suspension. Each sample test was performed in triplicate. The assay plate was placed on a minishaker (Vari-Shaker Microtiter; Dynatech) and incubated for 30 min at 37°C. Nonadherent bacteria were removed by 4 cycles of washing with HBSS at 37°C. The cell monolayers were detached by exposure to 0.25% trypsin-EDTA (Sigma) and were harvested onto glass-fiber filter mats by use of an LKB Cell Harvester (Wallac). The air-dried filter mat was placed in a sealed polyethylene bag containing scintillation fluid, and the microtiter well-associated radioactivity was read by a β-plate reader (Liquid Scintillation Counter 1205 β-plate; Wallac). The intra-plate coefficient of variation for the measurement of adhesion of *S. pneumoniae* to human pharyngeal epithelial cells was 9.4%; the interplate coefficient of variation was 11.4%.

Both the level and the avidity of IgA anti-pneumococcal antibody in colostrum were determined by established ELISA methods [4, 9]. Adherence was expressed as the number of pneumococci bound per cell and was based on the amount of radioactivity associated with a known concentration of pneumococci. Data were analyzed by the SPSS statistical program (version 10 for Windows 98; SPSS). Differences between groups were tested by the independent-samples t test, with P < .05 used as the cutoff point for significance. Results are presented as the arithmetic mean (95% confidence interval [CI]).

**Results.** The mean adherence capacity of *S. pneumoniae* serotype 14, which was 200 bacteria/1 epithelial cell (95% CI, 184.9–215.1), was approximately double that of *S. pneumoniae* serotype 6B, which was 110 bacteria/1 epithelial cell (95% CI, 84.3–135.7) (figure 1). Both the concentration of specific secretory IgA (s-IgA) antibodies and the avidity of the antibodies were significantly higher in colostrum from PP-vaccinated mothers than in that from MP-vaccinated mothers (table 1). The adhesion of both *S. pneumoniae* serotypes, 14 and 6B, to pharyngeal epithelial cells was inhibited more effectively by colostrum from PP-vaccinated mothers than by that from MP-vaccinated mothers (figure 1); this difference was statistically significant for serotype 14 (P < .05) but not for serotype 6B (P = .247).

**Discussion.** Maternal vaccination with polyvalent PP vaccine was shown to enhance the capacity of colostrum to inhibit adherence of pneumococci to pharyngeal epithelial cells. The in vitro function of colostrum after vaccination of rural Gambian mothers during pregnancy was investigated as a surrogate measure of their milk’s immune activity in protecting their infants from nasopharyngeal colonization by *S. pneumoniae*. Maternal vaccination during pregnancy potentially offers an opportunity to boost the immune system of infants, not only via transplacental antibody transfer but also via secretory antibodies in colostrum. This is an approach that could potentially reduce the incidence of nasopharyngeal carriage in infants—and, consequently, the incidence of invasive pneumococcal disease.

Reduction in nasopharyngeal carriage has been achieved in children by the use of protein-conjugated pneumococcal vac-
cines. However, vaccination of infants is usually initiated when they are ∼2 months old and therefore does not offer protection during the first 2–3 months of life, a period during which 20%–30% of the burden of invasive pneumococcal disease during the first year of life occurs [3].

Adherence of pneumococci to the respiratory tract is a complex process. The pneumococcus has an adherence preference for sialylated saccharides. On human pharyngeal epithelial cells [8] and human type II lung cells [10] in vitro, the disaccharide unit GlcNAc-(β1→3)Gal has been shown to be a primary binding lectin for pneumococci. Protein molecules that bind to either sialic components on epithelial cells or the pneumococcal surface could possibly block this specific adherence. Inhibition of adherence to human nasopharyngeal epithelial cells by anti-pneumococcal surface-protein adhesin A (PsaA) has been reported in rabbit models [11], and, in Gambian infants, the presence of anti-PsaA antibodies in serum has been shown to correlate with protection against nasopharyngeal carriage of pneumococci [12]. Currently available pneumococcal vaccines have been shown to contain protein contaminants of the pneumococcal cell wall, such as PsaA and pneumococcal surface protein A [13]. There is a possibility that antibodies to these proteins, particularly to PsaA, may mediate inhibition of adhesion of pneumococci to epithelial cells.

The immune repertoire of human breast milk is broad and consists of cells, enzymes, cytokines, and immunoglobulins. Although classified into discrete categories, these substances are multifunctional; but there is a paucity of information concerning their in vivo functions. IgA antibody is the predominant immunoglobulin class in secretions such as breast milk; as a polymeric molecule with multiple epitopes that are able to cross-link antigens, s-IgA antibody is thought to play an important role at mucosal sites, which are the first line of defence against pathogens such as pneumococci.

### Table 1. Level and avidity of secretory IgA (s-IgA) antibody in colostrum after maternal vaccination during the late second to early third trimester of pregnancy, with either bivalent meningococcal polysaccharide (MP) serogroups A and C vaccine or polyvalent pneumococcal-polysaccharide (PP) vaccine.

<table>
<thead>
<tr>
<th>Vaccine (no. of mothers)</th>
<th>PP serotype 6B s-IgA</th>
<th>PP serotype 14 s-IgA</th>
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<tbody>
<tr>
<td></td>
<td>Level, U/mL</td>
<td>Avidity index</td>
</tr>
<tr>
<td>MP (8)</td>
<td>235.5 (191.7–279.3)</td>
<td>0.91 (0.49–1.26)</td>
</tr>
<tr>
<td>PP (8)</td>
<td>722.3 (646.1–798.5)</td>
<td>1.29 (0.94–1.63)</td>
</tr>
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</table>

**NOTE.** Data are arithmetic mean (95% confidence interval), on the basis of ELISA.

*P < .05, compared with MP.
Andersson et al. [14] have explored the antiadherence property of human breast milk and have observed that its capacity to inhibit attachment of pneumococci to human pharyngeal or buccal epithelial cells is independent of specific antibody content: inhibition of bacterial adherence was present after immunoadsorption of the immunoglobulin fraction and also was detected when breast milk from IgA-deficient women was tested. In the present study, because of an insufficient volume of breast milk, we were unable to perform IgA-adsorption studies.

A more recent report in which lactating women were vaccinated with PP vaccine has suggested that s-IgA antibody may mediate serotype-specific ex vivo killing of pneumococci in the presence of complement [15]. The mechanisms of complement activation and of phagocytosis initiation are unknown. It also is not clear how s-IgA antibody supports this functional activity at the mucosal surface. Although polymeric IgA antibody can activate the alternative complement pathway, IgA antibody does not bind C1q to activate the classic complement pathway [15]. Another study has demonstrated the killing of *S. pneumoniae* by serum IgA antibody [16]. Therefore, it is possible that s-IgA antibody may initiate complement activation on the surface of the bacteria via the alternative pathway and that binding to and uptake by phagocytes is mediated by complement receptors, rather than by IgA. In the present study, we did not evaluate the viability of pneumococci after adhesion to epithelial cells; however, the killing of bacteria by s-IgA antibody is unlikely to have contributed significantly to the inhibition of adherence, because (1) the latter did not correlate with s-IgA antibody content and (2) we did not store the specimens in a manner that would preserve complement activity.

The present study has clearly shown that maternal vaccination with polyvalent PP vaccine boosts the capacity of colostrum to inhibit pneumococcal adherence to pharyngeal epithelial cells. In breast-feeding populations, maternal vaccination with PP vaccine to prevent pneumococcal disease during early infancy would be cost saving and life saving. This approach warrants further investigation.

**Acknowledgment**

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**References**


