Immunization of Female Mice with Glycoconjugates Protects Their Offspring against Encapsulated Bacteria

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The immune system of the newborn is immature, and therefore it is difficult to induce protective immunity by vaccination in the neonatal period. Immunization of mothers during pregnancy against infections caused by encapsulated bacteria could thus be particularly attractive, as infants do not respond to polysaccharide (PS) antigens. Transmission of maternal vaccine-specific antibodies and protection of offspring against pneumococcal bacteremia and/or lung infection were studied in a neonatal murine model of pneumococcal immunization and infections. Adult female mice were immunized with native pneumococcal PS (PPS) of serotypes 1, 6B, and 19F or PPS conjugated to tetanus protein (Pnc-TT), and PPS-specific antibodies were measured in sera of mothers and their offspring. Effective transmission of maternal antibodies was observed, as PPS-specific immunoglobulin G levels in 3-week-old offspring of immunized mothers were 37 to 322% of maternal titers, and a significant correlation between maternal and offspring antibody levels was observed. The PPS-specific antibodies persisted for several weeks but slowly decreased over time. Offspring of Pnc-TT-immunized mothers were protected against pneumococcal infections with homologous serotypes, whereas PPS immunization of mothers did not protect their offspring, in agreement with the low titer of maternal PPS specific antibodies. When adult female mice were immunized with a meningococcal serogroup C conjugate vaccine (MenC-CRM), antibody response and transmission were similar to those observed for pneumococcal antibodies. Importantly, bactericidal activity was demonstrated in offspring of MenC-CRM-immunized mothers. These results demonstrate that this murine model of pneumococcal immunization and infections is suitable to study maternal immunization strategies for protection of offspring against encapsulated bacteria.

Infections caused by polysaccharide (PS)-encapsulated bacteria, such as Streptococcus pneumoniae (pneumococcus) and Neisseria meningitidis (meningococcus), are major causes of disease in infants and young children. Pneumococcus causes a substantial proportion of respiratory diseases in young children, in addition to severe invasive infections such as meningitis, sepsis, and pneumonia (3, 31). The meningococcus causes epidemics of meningitis and sepsis. The main burden of disease is in infants and young children, with an increased risk of outbreaks in adolescents (41).

To protect against infections early in life, vaccination strategies that rapidly induce protective immunity are needed, but due to immaturity and inexperience of the immune system of the newborn, immune responses are frequently weak and delayed, in particular for PS antigens (60). Whereas pneumococcal PS (PPS) and meningococcal serotype C PS (MenC-PS) vaccines are immunogenic and protective in healthy adults (13, 52, 58), they are not immunogenic in subjects at an early age (18, 48). By conjugation of PS antigens to protein carriers they become immunogenic in infants and children (4, 19, 50), and PS-protein conjugate vaccines are efficacious after immunization in infancy (7, 8; M. E. Ramsay, N. Andrew, E. B. Kaczmarski, and E. Miller, Letter, Lancet 357:195-196, 2001). To protect the very young against pneumococcal and meningococcal diseases, two strategies may be developed: neonatal and/or maternal immunization. As infants do not readily respond to PS antigens, maternal immunization could be a particularly attractive approach to protect against infections caused by encapsulated bacteria. During pregnancy, women are capable of mounting an adequate humoral immune response. Maternal pathogen-specific immunoglobulin G (IgG) antibodies are actively transported to the fetus during the third trimester of pregnancy; with enlargement of the placenta during the last 4 to 6 weeks of gestation, this active transport increases. The selective transport of IgG from mother to fetus is mediated by a specific IgG transport protein expressed in the placenta, FcRn, which is closely related in structure to major histocompatibility complex class I molecules (12, 61). FcRn is expressed in the yolk sacs (2, 10, 51) and intestines (10, 62) of neonatal mice and rats. IgG is thus transported across the yolk sac, and after birth, pups take up IgG from mothers’ milk through the intestinal epithelium. Serum IgG, particularly IgG1, levels of a full-term human neonate equal or exceed maternal IgG levels, and the duration of protection provided by maternal antibodies is determined by the titer of pathogen-specific protective antibodies present early after birth. Infants born with high antibody levels due to active immunization of the mothers may thus be protected for the time required for their immune system to respond adequately to vaccines (reviewed in reference 43). Safety and efficacy of maternal immunization for prevention of infectious diseases in infants has been reported,

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and prevention of neonatal tetanus by maternal immunization has proven successful in developing countries (66). Thus, PPS and MenC-PS or conjugate vaccines might be given before or during pregnancy to women at high risk or during periods of epidemics and endemicity.

Using an intranasal (i.n.) murine model of pneumococcal infections (54), we have shown that passive immunization with sera from infants vaccinated with pneumococcal conjugate vaccines can protect mice from bacteremia and pneumonia and protection was related to infant serum antibody titer and opsonic activity (29, 53). This pneumococcal infection model has been adapted to early life, and pneumococcal conjugate vaccines were shown to induce protective immunity against lethal pneumococcal infections in neonatal and infant mice (26). This early-life murine model was used to study transfer of maternal vaccine-induced antibodies through the placenta and from mother’s milk and protection against pneumococcal disease. Adult female mice were immunized before pregnancy with either native PPS or pneumococcal tetanus protein (TT) conjugate vaccines (Pnc-TT) of serotypes 1, 6B, and 19F, and transfer and persistence of maternal antibodies in their offspring were studied. At the age of 6 weeks, the offspring were challenged with homologous virulent pneumococci, and protection against pneumococcal infections was evaluated. The same protocol was used to study the transport and kinetics of maternal antibodies in offspring of female mice immunized with either MenC-PS or meningococcal conjugate vaccine (MenC-CRM). Serum bactericidal activity (SBA) was measured in offspring of mothers immunized with MenC vaccines to evaluate the protective capacity.

MATERIALS AND METHODS

Mice. Adult NMRI mice were obtained from M&B AS (Ry, Denmark). The mice were kept in micro-isolator cages with free access to commercial food pellets and water and housed under standardized conditions at the Institute of Experimental Pathology at Keldur (Reykjavik, Iceland) with regulated daylight, humidity, and temperature. Breeding cages were checked daily for newborns, and the pups were kept with their mothers until weaning at the age of 4 weeks. The animal experiments were authorized by the Experimental Animal Committee of Iceland and complied with animal welfare act 15/94.

Vaccines and adjuvant. PPSs of serotypes 1, 6B, and 19F were purchased from the American Type Culture Collection (Manassas, Va.). PPSs of serotypes 1, 6B, and 19F conjugated to tetanus protein (Pnc-TT) were produced by the Centre d’Immunologie Pierre Fabre (St. Julien en Genevois, France). Pnc-TT conjugates were synthesized with adipic acid dihydrazide as the linker. Serotype 1 PPS was conjugated to activated TT (32), whereas PPSs of serotypes 6B and 19F were activated before being coupled to the protein (33, 37). Conjugates were purified by gel filtration and stored at 4°C after addition of thimerosal at a final concentration of 100 μg/ml. Conjugates were analyzed for carbohydrate and protein with the anthrone and bichinonic assays, respectively. Covalence and absence of uncoupled protein were assessed by gel filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analyses. PPS/TT (wt/wt) ratios were determined to be 3.84, 0.78, and 1.04 for Pnc1-TT, Pnc6B-TT, and Pnc19F-TT conjugates, respectively. The mutant of Escherichia coli heat-labile enterotoxin LT-R72 adjuvant (22), native MenC-PS and MenC oligosaccharides conjugated to a diptheria toxoid mutant (MenC-CRM), was produced by Chiron Srl (Siena, Italy) (16). The saccharide/protein ratio (wt/wt) of MenC-CRM was 0.7.

Immunization and blood sampling. Adult (6-week-old) female mice were immunized subcutaneously with 5.0 μg of PPS or 0.5 μg of Pnc-TT of serotypes 1, 6B, and 19F. Pnc-TT of serotypes 6B and 19F were mixed with 5.0 μg of LT-R72 prior to immunization; other antigens were administered without adjuvant. Adult female mice were immunized with 10 μg of MenC-PS or 2.5 μg of MenC-CRM. The mice received a second dose of the same vaccines 2 weeks later. Unimmunized mice were used as controls. The mice were bled from the tail vein 1 week after delivery, and the offspring were bled weekly at 3 to 6 weeks of age for measurement of antibodies to PPS, TT, or MenC-PS in sera.

ELISA. Specific antibodies (IgG, IgG1, IgG2a, IgG2b, IgG3, and IgM) to PPSs of serotypes 1, 6B, and 19F were measured by enzyme-linked immunosorbent assay (ELISA) as described previously (25). In brief, microplates (MaxiSorp; Nunc AS, Roskilde, Denmark) were coated with 5 μg of PPS-1 or 10 μg of PPS-6B and PPS-19F (American Type Culture Collection) per ml of phosphate-buffered saline (PBS) and incubated for 5 h at 37°C. For neutralization of antibodies to cell wall PS (Statens Serum Institute, Copenhagen, Denmark), serum samples and standard were diluted 1:50 in PBS with sodium dodecyl sulfate (SDS) and incubated for 2 h at room temperature. The neutralized sera were serially diluted and incubated in duplicate in PBS-coated microtiter plates at room temperature for 2 h. Horseradish peroxidase-conjugated goat anti-mouse IgG, IgG1, IgG2a, IgG3, or IgM antibodies (Southern Biotechnology Associates Inc., Birmingham, Ala.) were diluted 1:5,000 in PBS-Tween and incubated for 2 h at room temperature for detection of bound antibodies. For development of the enzyme reaction, 3,3′,5,5′-tetramethylbenzidine peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was incubated for 10 min according to the manufacturer’s instructions, and the reaction was stopped by adding 0.18 M H2SO4. The absorbance was measured at 450 nm in an ELISA spectrophotometer (Titertek Multispan Plus MK II; ICN Flow Laboratories, Irvine, United Kingdom).

For detection of TT-specific antibodies, microtiter plates (MaxiSorp) were coated with 5.0 μg of purified TT (Aventis Pasteur, Marcy l’Etoile, France) per ml of 0.10 M carbonic buffer (pH 9.6) and incubated overnight at 4°C. After blocking of coated plates with PBS containing 1% bovine serum albumin (BSA; Sigma), duplicates of samples and standard were serially diluted in PBS-Tween and added to TT-coated plates and incubated for 2 h at room temperature. The detection of TT-specific antibodies and the development of the enzyme reaction were performed as described above.

MenC-PS-specific IgG antibodies were measured essentially as described elsewhere (21). Microtiter plates (MaxiSorp) were coated with 5.0 μg of purified meningococcal type C capsular PS (Chiron SrI) in PBS with methylated human serum albumin (5 μg/ml), and plates were incubated overnight at 4°C. The plates were then blocked with 1% (wt/vol) gelatin (BDH Chemicals Ltd., Poole, United Kingdom) in PBS (pH 7.2) and incubated for 3 h at 37°C. Following fixation with a solution containing 10% (wt/vol) saccharose (Merck, Darmstadt, Germany) and 4% (wt/vol) polyvinylpyrrolidone (Sigma) for 2 h at room temperature, the plates were dried and stored at 4°C until use. Serum samples and standard were serially diluted in PBS-Tween containing 1% BSA (Sigma) and incubated in duplicate in MenC-PS-coated microtiter plates overnight at 4°C. The detection of MenC-PS-specific antibodies and the development of the enzyme reaction were performed as described above.

Reference sera, obtained by hyperimmunization of adult mice with the same conjugate vaccines, were included on each microtiter plate. The titer of the referred serum, in ELISA units (EU) per milliliter, corresponds to the inverse of the serum dilution giving an optical density (OD) of 1.0. The titers of the test serum samples were calculated from the reference sera based on a minimum of four data points and parallelism between the serum samples and the reference curve. The interassay coefficient of variation was less than 10%, and the detection limit was 1.0 EU/ml. Results are expressed as mean log10 EU/ml ± standard deviation (SD). PBS-Tween was used for dilutions and washing, and 100-μl volumes were used in all incubation steps with three washings between.

Pneumococci and challenge of mice. The bacteria were maintained in Tryp-tose broth (Difco Laboratories, Detroit, Mich.) plus 20% glycerol (BUFa B.V., Uitgeest, The Netherlands) at −70°C. The day before challenge, stocks were plated on blood agar (Difco) and incubated at 37°C in 5% CO2 overnight. Isolated colonies were transferred to a heart infusion broth (Difco) with 10% horse serum, cultured at 37°C to log phase for 3.5 h, and resuspended in 0.9% sterile saline. Serial 10-fold dilutions were plated on blood agar to determine the inoculum’s density. At the age of 6 weeks the offspring of immunized female mice were challenged i.n. with 10 μl of virulent pneumococci: 1.0 × 107 CFU of serotype 1 (ATCC 6301), 4.3 × 107 CFU of serotype 6B (DS 2215), or 4.4 × 107 CFU of serotype 19F (ATCC 6319) as previously described (27, 29, 53, 54). The mice were sacrificed 24 h later, and pneumococcal bacteremia was determined as CFU per milliliter of blood and/or pneumococcal lung infection determined as CFU per milliliter of lung homogenate. Depending on the first dilution used, the detection limit was 2.0 CFU/ml of lung homogenate and 1.3 CFU/ml of blood.

SBA. Bacterial antibodies were titrated as previously described (5, 39). Briefly, N. meningitidis strain C11 was grown overnight in tryptose broth (starting from a frozen stock) with 5% CO2. Colonies were collected and used to inoculate 7 ml of Mueller-Hinton broth containing 0.25% glucose to

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reach an OD at 600 nm of 0.05 to 0.06. The culture was incubated for approximately 1.5 h at 37°C with 5% CO₂ with shaking until the OD at 600 nm reached 0.23 to 0.24. Bacteria were diluted in Gey’s balanced salt solution (Sigma) and 1% (wt/vol) BSA (Sigma) at the working dilution of 10⁵ CFU/ml. The total volume of the final reaction mixture was 50 μl, with 25 μl of serial twofold dilution of test serum, 12.5 μl of bacteria at the working dilution, and 12.5 μl of baby rabbit complement (final concentration, 25%). Controls included bacteria incubated with complement and immune sera incubated with bacteria and complement inactivated by heating at 56°C for 30 min. Immediately after the addition of the baby rabbit complement, the controls were plated on Mueller-Hinton agar plates using the tilt method (time 0). The plates were incubated for 18 h at 37°C with 5% CO₂, and the colonies corresponding to time 0 and time 1 were counted. The data are used to calculate the reciprocal serum dilution at which 50% of the bacteria are killed (50% titer).

Statistical analysis. Student’s t test and the nonparametric Mann-Whitney test were used to compare log antibody titers and numbers of CFU (log₁₀) between groups and time points. The Pearson correlation test was used. A P of <0.05 was considered statistically significant.

RESULTS

Immune responses of dams and transfer of maternal vaccine-specific IgG. To study the transmission of maternal pathogen-specific antibodies, adult female mice were immunized twice before pregnancy with native PPS or Pnc-TT of serotype 1, 6B, or 19F. Serotype 1 is highly immunogenic in mice, whereas serotypes 6B and 19F are less immunogenic, and therefore the mutant of E. coli heat-labile enterotoxin, LT-R72, was used as adjuvant for immunization with the conjugates of serotypes 6B and 19F. Unimmunized mice were used as controls. One week after delivery, mothers were bled, whereas offspring were bled weekly from 3 to 6 weeks of age, for measurement of vaccine-specific serum antibodies. Meningococcal and pneumococcal conjugate vaccines elicited much higher antibody responses than native PS (Fig. 1), both when the conjugates (Pnc6B-TT and Pnc19F-TT) were coadministered with LT-R72 and when they were given without adjuvant (Pnc-1-TT and MenC-CRM) as all the PSs. PS-specific IgG antibody levels in 3-week-old offspring were in general similar to or higher than those in their mothers 1 week postdelivery. Transfer of maternal antibodies was determined by calculation of EU per milliliter for each offspring as a percentage of the EU per milliliter of the respective conjugate-immunized mother. Thus, depending on the conjugate used for immunization of dams, the transfer ranged from 116 to 322% for Pnc1-TT, 96 to 115% for Pnc6B-TT, 37 to 59% for Pnc19F-TT, and 131 to 164% for MenC-CRM. Furthermore, there was a highly significant correlation between PS-specific IgG antibody titers in adult female mice and their offspring after immunization with either meningococcal C or pneumococcal conjugates of serotypes 1, 6B, and 19F, and a low standard deviation among the offspring of each dam was observed.
There was also a significant correlation between maternal and offspring IgG antibodies to the TT carrier of the pneumococcal conjugate (data not shown). These results demonstrate an active transfer of vaccine-specific maternal antibodies, which persisted in the offspring for several weeks and decreased slowly over time, $-1 \log_{10} \text{IgG EU/ml}$, during the last 3 weeks (Fig. 1). A similar pattern was observed for IgG antibodies to the carrier, TT (data not shown).

Maternal PS-specific isotypes transmitted to offspring. Immunization with pneumococcal conjugate vaccines without adjuvant elicits primarily IgG1. By adding the adjuvant LT-R72, the Th1-associated subclasses IgG2a and IgG3 are enhanced (25). Thus, immunization of mothers with Pnc19F-TT coadministered with LT-R72 enabled us to study the transmission of PPS-specific antibodies of various isotypes from mothers to offspring by calculating, for each isotype, the EU per milliliter for each offspring as a percentage of the EU per milliliter of the respective Pnc19F-TT-immunized mother (1 week after delivery). The results are shown as means $\pm$ SDs (error bars) of percent transfer of each antibody isotype to offspring of each of two mothers. The mothers were immunized twice before pregnancy with Pnc19F-TT with LT-R72.

(Fig. 2). There was also a significant correlation between maternal and offspring IgG antibodies to the TT carrier of the pneumococcal conjugate (data not shown). These results demonstrate an active transfer of vaccine-specific maternal antibodies, which persisted in the offspring for several weeks and decreased slowly over time, $-1 \log_{10} \text{IgG EU/ml}$, during the last 3 weeks (Fig. 1). A similar pattern was observed for IgG antibodies to the carrier, TT (data not shown).

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**FIG. 2.** Relationship between IgG antibodies to PPS for serotypes 1, 6B, and 19F and PS-IgG antibodies to MenC in sera from immunized mothers and their offspring. The mothers were immunized twice before pregnancy with PPS or Pnc-TT of serotype 1, 6B, or 19F. LT-R72 was coadministered with the conjugates of serotypes 6B and 19F. For meningococcus group C, mothers were immunized with MenC-PS or MenC-CRM. Offspring of unimmunized adult mice were controls. Samples were taken from dams 1 week after delivery and from 4-week-old offspring. Dams immunized with conjugates and their offspring (•), dams immunized with PS and their offspring (△), and controls (□) ($n = 8$/group) were evaluated. Individual lines with the same symbol represent offspring of individual dams. Error bars, standard deviations (SDs).

**FIG. 3.** Transmission of maternal PPS-specific antibody isotypes in sera from immunized mothers to their offspring. The percent transmission was determined by calculating for each isotype the EU per milliliter for each offspring (4 weeks of age) as the percentage of the EU per milliliter of the respective Pnc19F-TT-immunized mother (1 week after delivery). The results are shown as means $\pm$ SDs (error bars) of percent transfer of each antibody isotype to offspring of each of two mothers. The mothers were immunized twice before pregnancy with Pnc19F-TT with LT-R72.
immunization with Pnc19F-TT and LT-R72 adjuvant, as the relative proportion of the less effectively transferred antibodies IgG2a and IgG3 was higher than that following immunization with serotype 1 conjugate administered without adjuvant. As Pnc6B-TT is poorly immunogenic, PS-specific IgG2a and IgG3 titers were low or undetectable. Thus, the maternal antibodies primarily consisted of IgG1, which may account for the relatively high percentage of total PS-specific IgG transmitted compared to that of Pnc19F-TT-induced antibodies.

Protection against pneumococcal bacteremia and pneumonia. When offspring of dams immunized with PPS or Pnc-TT were 6 weeks old, they were challenged with virulent pneumococci of the homologous serotype 1, 6B, or 19F, and pneumococcal infections were evaluated 24 h after challenge as numbers of CFU/ml of blood and/or lungs depending on the serotype.

**Immunization of mother**

![Graphs showing protection against pneumococcal bacteremia and pneumonia](image)

FIG. 4. Protection of offspring (6 weeks old) of dams immunized with native (PPS) or conjugated (Pnc-TT) PS against bacteremia and pneumonia following i.n. challenge of pups with the homologous serotype. Dams were immunized twice before pregnancy with PPS-1 or Pnc1-TT (A and B), PPS-6B or Pnc6B-TT (C and D), and PPS-19F or Pnc19F-TT (E) and offspring of unimmunized mice were used as controls. Each bar represents results for offspring of a given dam, as the mean ± SD of numbers of CFU/ml (log_{10}) in blood (A and C) and lungs (B, D, and E) of offspring (n = 8/group) of PPS-immunized dams (white bars), Pnc-TT-immunized dams (black bars), or unimmunized dams (gray bars), 24 h after challenge with virulent pneumococci of homologous serotype. Detection limits are 1.3 for bacteremia and 2.0 for lung infection. Symbols: *, P < 0.05; **, P < 0.01; ***, P < 0.001 (compared to results for offspring of unimmunized dams).
TABLE 1. Serum bactericidal activity in adult female mice and their offspringa

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<th>Immunization and mother</th>
<th>SBA titerb for:</th>
<th>Offspring at age:</th>
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<td>Mother</td>
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<td>MenC-PS</td>
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<tr>
<td>Dam 1</td>
<td>16</td>
<td>&lt;4</td>
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<td>MenC-CRM</td>
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<td>Dam 1</td>
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* Dams were immunized twice before pregnancy with MenC-PS or MenC-CRM. Serum samples from mothers were obtained 1 week postdelivery and from offspring (n = 8 per group) when they were 4 and 6 weeks old. The sera from offsprings of each dam were pooled for SBA measurement.

b SBA titers for each of three immunized dams and their offspring (pooled sera) in the same order.

offspring of mothers immunized with either native PPS-1 (P < 0.001) or unimmunized controls (P < 0.001).

Challenge with S. pneumoniae of serotype 6B caused lung infection in offspring of unimmunized control mice (Fig. 4D), associated with low density of CFU in the blood (Fig. 4C). In contrast, pneumococci were not detected in the blood of offspring of Pnc6B-TT-immunized mothers (Fig. 4C). In addition, offspring of Pnc6B-TT-immunized dams had significantly reduced CFU in the lungs (P < 0.01 and P < 0.05) (Fig. 4D) compared to controls, whereas reduction of CFU in lungs following PPS-6B immunization was not significant.

As pneumococci of serotype 19F do not invade the blood after i.n. challenge in this mouse model (29), only lung infection was evaluated 24 h after challenge of 6-week-old offspring with this serotype. A high density of 19F pneumococci was detected in lungs of offspring of unimmunized control mice (Fig. 4E). Offspring of PPS-19F- and Pnc19F-TT-immunized mothers had significantly reduced numbers of CFU in lungs compared to controls (P < 0.001 and P < 0.01) (Fig. 4E). Still, the density of 19F pneumococci was high in the lungs of the offspring of immunized dams, and despite higher titers of IgG anti-19F in offspring of mice immunized with Pnc19F-TT than in those of mice immunized with PPS-19F, the numbers of 19F CFU in lungs was not significantly different between these groups of offspring. This is in agreement with previous findings, demonstrating that high levels of IgG anti-19F are needed to protect against 19F pneumococcal pneumonia in this mouse model (29).

Protection against meningococcal infection. To study the protective potential of maternal immunization with MenC vaccines, SBA was determined. SBA is the titer of heat-inactivated sera that induces killing of bacteria in the presence of complement. Sera from two out of three dams immunized with the MenC-CRM and pooled serum samples of their offspring obtained at the age of 4 or 6 weeks had detectable SBA (Table 1). The SBA of dams immunized with native MenC-PS was invariably low and undetectable in their offspring (Table 1).

While the immunogenicity of MenC-CRM was variable in adult female mice, those that developed measurable SBA titers efficiently transferred measurable SBA to their offspring, as evident at 4 and 6 weeks of age (Table 1). The high SBA titers in the dams also coincided with high IgG antibody titers measured by ELISA, as did the SBA and IgG titers in sera from their offspring at the age of 4 and 6 weeks (Table 1; Fig. 1).

DISCUSSION

In the present study, we used a neonatal mouse model of pneumococcal immunization and infections, previously shown to reproduce the main features of infant responses to pneumococcal conjugate vaccines (26). As expected, adult female mice immunized with two doses of conjugate developed higher levels of antibodies to PS than mice immunized with native PS. Here we demonstrate that there was an active transport of PS-specific maternal antibodies through the placenta and/or the gut from mothers’ colostrum and milk to the offspring. The maternal PS antibodies persisted for weeks and declined slowly. Using i.n. challenge of pups with a lethal dose of virulent pneumococci of three important pediatric serotypes, we also demonstrated that immunization of adult female mice with pneumococcal conjugates was able to protect their offspring from bacteremia caused by the homologous serotypes and significantly reduce the numbers of CFU in their lungs. In the present study, PPS-specific IgG1 and IgG3 antibodies were better transmitted from mothers to offspring than IgG2a. Our results are in contrast to those of studies on antibodies to group B streptococcus (GBS) and respiratory syncytial virus, in which effective transfer of all IgG subclasses was demonstrated (11, 46). Due to limited availability of serum, the antibody isotypes could not be measured until the offspring were 4 weeks old so the difference in half-lives of IgG subclasses should be considered (64). Similarly, human IgG2 is transmitted less efficiently over the human placenta than the other IgG subclasses (6, 15, 24, 57), probably due to lower binding specificity and affinity of FcRn for IgG2 (40).

The protective efficacy of maternal immunization with pneumococcal conjugate vaccines was evaluated for three important pediatric serotypes, previously shown to be virulent in this i.n. pneumococcal infection model (26, 27, 29, 53, 54). Challenge with serotype 1 pneumococci caused bacteremia and lung infection 24 h postchallenge in 6-week-old offspring of PPS-1-immunized and unimmunized control mice. In contrast, the titer of maternal antibodies was sufficient to protect the offspring of Pnc1-TT-immunized mothers from developing bacteremia. Furthermore, these offspring had a significantly reduced number of serotype 1 pneumococci in the lungs compared to offspring of mothers immunized with native PPS-1 or unimmunized controls. Comparable results were obtained after challenge with serotype 6B, which is less virulent in mice. Despite low density of CFU in the blood of offspring of unimmunized control mothers, a significant protection against bacteremia and reduced lung infection was demonstrated in offspring of Pnc6B-TT-immunized mothers. We previously demonstrated that relatively high titers of 19F IgG antibodies are needed to clear the lungs of adult mice challenged i.n. with pneumococcal serotype 19F in this pneumococcal infection model (29). Therefore, adult female mice were immunized...
with PPS-19F or Pnc19F-TT along with the adjuvant LT-R72, previously shown to enhance antibody response to pneumococcal conjugates in mice (25, 28). Significant reduction of numbers of CFU in lungs of offspring from Pnc19F-TT-immunized mice was observed compared to those in controls. Despite the high titer of PPS-19F-specific maternal antibodies the offspring had relatively high numbers of 19F pneumococci in their lungs 24 h after challenge. This is in agreement with observations from clinical studies, which resulted in an incomplete efficacy of 19F conjugate vaccines both against invasive pneumococcal disease (7) and otitis media (20).

Maternal immunization with pneumococcal conjugate vaccines has been studied in the chinchilla otitis media model. Significant reduction in both incidence ($P = 0.05$) and severity ($P < 0.01$) of experimental pneumococcal otitis media was demonstrated in the chinchilla pups, and maternal immunization was 82% effective at preventing mortality from invasive pneumococcal disease (23). Clinical trials showed maternal immunization with PPS vaccines in the third trimester of pregnancy to be safe, and transfer of vaccine-specific antibodies was demonstrated, both in developing and industrialized countries (34, 35, 44, 45, 49, 57; S. K. Obaro, Letter, Lancet 347:192-193, 1996). A trial of maternal immunization with 9-valent pneumococcal vaccines is currently being conducted in Minneapolis, Minn., to study the effect on otitis media. The trial also examines the effect of maternal antibodies on response to active immunization of the infant with 7-valent pneumococcal conjugate vaccines (17).

To extend our studies on maternal immunization against encapsulated bacteria, adult female mice were immunized with MenC-CRM, which elicited significantly higher MenC-specific antibodies, in two of three immunized dams, than native MenC-PS. The maternal antibodies were effectively transferred to the offspring and persisted for at least 6 weeks, although the titers declined $\sim 1 \log_{10}$ EU/ml over this period. SBA has been shown to correlate well with protection against meningococcal C disease, and measurements of SBA titer can thus be used as a surrogate for measuring protective capacity of MenC vaccination against meningococcal infection (9). It has been shown using a rabbit complement that an SBA titer between 8 and 64 indicates a protective immune response in humans (9). SBA was detected in dams immunized with MenC-CRM and in pooled sera of their offspring. The SBA in dams immunized with native MenC-PS was low and was not detectable in their 4-week-old offspring (Table 1). Thus, these results from maternal immunization against meningococcus are in agreement with our results on maternal immunization against pneumococci. Recently, maternal immunization with meningococcal A PS vaccine was shown to provide infants with significantly increased levels of MenA-specific IgG and oral IgA (56).

Our results are in agreement with the observation from the murine model for maternal immunization against GBS infection, which has been used extensively to study both immunogenicity in dams and efficacy of maternally transmitted antibodies in their neonates. Administration of an immunogenic GBS conjugate vaccine to female mice resulted in protection of most, if not all, of their pups, whereas little or no protection was seen among litters born to dams receiving native capsular PS or saline (38, 47, 65). In addition, this neonatal model has been used to study the therapeutic potential of GBS conjugate vaccine-induced human antibodies (30).

Maternally inherited antibodies may interfere with responses to vaccines administered in early infancy, but this depends on the vaccine (55, 59). Immunization of mothers during pregnancy could be especially attractive to provide protection of the newborn against infections caused by encapsulated bacteria, because infants do not readily respond to PS vaccines and antibody responses to PS tend to be short-lived (42). Interference of maternal antibodies in Haemophilus influenzae type b (Hib) vaccination has been reported (14). However, persistence of protective Hib antibodies without interference with the active antibody response has been shown in infants following passive-active immunization with high titers of bacterial PS immunoglobulins and Hib conjugate vaccine, also resulting in a dramatic decline in Hib disease (36, 63). A recent study suggested that maternal antibodies may interfere with infant responses to the primary series of pneumococcal conjugate vaccines, but the booster response was not affected. The authors concluded that a high preimmunization antibody titer does not interfere with the development of immunological memory (1).

Our results demonstrate that this murine model of lethal pneumococcal infections is suitable to study maternal immunization for protection of offspring against infections caused by encapsulated bacteria. Furthermore, it will be useful for the development of novel immunization strategies for protection against infections in early life.

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