Anticapsular Polysaccharide Antibodies and Nasopharyngeal Colonization with Streptococcus pneumoniae in Infant Rats

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To evaluate the effect of passive immunization with anticapsular antibodies on nasopharyngeal carriage, two models of Streptococcus pneumoniae colonization were developed in infant rats. In a direct inoculation model, 3- to 4-day-old infant rats were intranasally inoculated with $2 \times 10^5$ cfu of S. pneumoniae type 3 or $6 \times 10^5$ cfu of S. pneumoniae type 23F. In an intralitter transmission model, 2 infant rats were intranasally inoculated with $10^6$ cfu of pneumococcus type 3 or type 19F and placed in a cage containing 10 infant rats. Pretreatment with bacterial polysaccharide immune globulin led to a significant reduction in colonization of contact animals with S. pneumoniae type 3 or 19F in the intralitter transmission model ($P < .05$). No effect of immune globulin could be demonstrated in the direct inoculation model. These results indicate that systemic anticapsular antibodies conferred significant protection against nasopharyngeal acquisition by intralitter spread of S. pneumoniae type 3 and 19F.

The nasopharynx is the main reservoir for polysaccharide encapsulated bacteria, such as Streptococcus pneumoniae, Haemophilus influenzae type b (Hib), and Neisseria meningitidis. Exposure to these organisms, as in day care centers or after close contact with infected persons, has been shown to be a risk factor for the development of invasive disease. Invasive S. pneumoniae disease usually occurs within 1 month of acquisition of a new serotype [1]. Immune-mediated prevention of nasopharyngeal colonization may therefore lead to an important reduction in the incidence of invasive pneumococcal disease.

A dramatic reduction in the incidence of invasive Hib disease was noted after the introduction of conjugate Hib vaccines in 1988. This effect has been attributed to protection not only from bacteremia but also from nasopharyngeal carriage, thereby protecting unimmunized children from exposure (“herd immunity”). Two possible mechanisms have been postulated for the reduction of colonization. First, IgA-mediated mucosal immunity may directly prevent colonization. Children immunized with conjugate Hib vaccine express secretory IgA Hib polysaccharide antibodies in saliva [2]. In infant rats, intra-
nasal administration of secretory IgA prevented nasopharyngeal colonization [3]. Second, IgG antibodies, derived from serum, may also prevent colonization. Kauppi et al. [3] showed that high doses of intraperitoneally administered IgG anti–Hib polysaccharide antibodies reduced nasopharyngeal colonization in infant rats. We hypothesized that passive immunization with polysaccharide globulin would prevent or reduce nasopharyngeal colonization with S. pneumoniae. We developed two infant rat models of S. pneumoniae nasopharyngeal colonization to examine the effect of systemic human antibody directed against pneumococcal polysaccharide.

Materials and Methods

Animals. Outbred, virus-free pregnant female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were allowed to deliver in our animal housing facilities. Infant pups from all litters were randomly distributed among cages, so that each breeder had a litter of 10–12 pups.

Bacteriologic methods. Strains of S. pneumoniae types 3, 19F, and 23F were stored in Todd-Hewitt broth supplemented with 0.5% yeast extract (Difco, Detroit) and 20% glycerol at −70°C. Inocula for intranasal challenge were prepared by growing S. pneumoniae to mid–log phase in 10 mL of Todd-Hewitt broth supplemented with 0.5% yeast extract. Bacteria were diluted in 0.5% low-gelling-point agarose to the desired inoculum concentration and briefly stored on ice until intranasal inoculation.

Nasopharyngeal cultures were performed on 5% sheep blood agar supplemented with 2.5 mg/L gentamicin (Becton Dickinson Microbiology Systems, Cockeysville, MD). Plates were incubated overnight at 37°C, and colonies were counted 24 h later. S. pneumoniae was identified by colonial morphology and, when necessary, optochin disk susceptibility.

Animal models. For the intranasal inoculation model, infant rats were randomized to be pretreated with saline or with 10 or 50 μg of bacterial polysaccharide immune globulin (BPIG, lot 8; Massachusetts Biologics Laboratory, Jamaica Plain, MA) administered subcutaneously on study day 0. BPIG is a hyperimmune globulin obtained from adults immunized with 23-valent pneumococcal vaccine, 4-valent meningococcal vaccine, and Hib vaccine, as described previously [4]. BPIG contains >95% IgG, trace amounts of IgM and IgA, and 150 mg/mL nonspecific antibody. At 24 h later, each infant rat was challenged with 10 mL of S. pneumoniae type 3 (≈2 × 10⁶ cfu) or type 23F (6 × 10⁵ cfu) suspension in the right nostril atraumatically with a micropipette.

Pneumococcal nasal colonization of the animals was assessed 24 h later. Sterile, nonbacteriostatic saline was atraumatically placed in the right nostril of each infant rat. Approximately 10 mL of expelled fluid was then collected using a micropipette and plated on gentamicin-supplemented sheep blood agar plates. Colonization was defined as at least 1 cfu of S. pneumoniae per 10 μL of nasal fluid. Recovery of ≤50 and >50 cfu/10 μL was considered light and heavy colonization, respectively.

For the intralitter transmission model, each cage of infant rats was randomized to be pretreated with normal saline or BPIG. Two infant rats in each cage were randomly selected as the “index cases” of pneumococcal colonization and were not pretreated. The remaining 8–10 infant rats per cage were designated as “contacts.”

One day before intranasal inoculation (day 0), contact animals from each cage received 0.5 mL of normal saline or BPIG (corresponding to 50 μg) subcutaneously, according to the treatment assignment of the cage. The index cases received no treatment. At 24 h after treatment of contact animals (day 1), the index cases were challenged with 10 mL of S. pneumoniae type 3 suspension (~10⁵ cfu) or type 19F (~2 × 10⁵ cfu) in the right nostril atraumatically. Nasal inoculation of index cases was performed only once. Contacts were not challenged with S. pneumoniae.

On days 1 and 7, blood samples were obtained from all contact animals for measurement of anticapsular antibody concentration. The distal tail vein was cleansed with isopropyl alcohol and punctured with a sterile lancet. Blood (10 mL) was obtained from each contact and samples from 5 pups/cage were pooled. Pneumococcal nasal colonization of the index cases and contact animals was assessed daily for 10 days as described above.

Anticapsular antibody ELISA. Serum type 19F antipneumococcal IgG was measured by ELISA using the method of Quataert et al. [5], which conforms to the World Health Organization/Centers for Disease Control and Prevention consensus assay. Samples were preabsorbed with C polysaccharide absorbent (gift of Porter Anderson) and calculated versus the US standard reference serum 89SF (Center for Biological Evaluation and Review, US Food and Drug Administration) assigned 13.0 μg/mL. Type 3 assays were slightly modified, using a coating of methylated human serum albumin and type 3 pneumococcal polysaccharide at 4°C [6]. The standard 89SF was assigned 4.0 μg/mL. IgG anti–type 3 polysaccharide using this method. Using these methods, the concentration of anti–type 19 polysaccharide in BPIG was 231 μg/mL and the concentration of anti–type 3 polysaccharide was 72 μg/mL. The lower limit of detection in sera was 0.1 μg/mL for both serotypes.

Statistical methods. The differences in colonization frequency between saline- and BPIG-treated infant rats and the proportion of animals with light and heavy colonization were compared using Fisher’s exact test. P < .05 was considered significant.

Results

Intranasal inoculation model. A total of 36 infant rats were intranasally inoculated with 2 × 10⁶ cfu of S. pneumoniae type 3 (12 animals/treatment group: saline or 10 or 50 μg of BPIG). All animals were heavily colonized (>50 cfu/10 μL) with S. pneumoniae 24 h later. In another experiment, 24 infant rats were intranasally inoculated with a lower inoculum (6 × 10⁵ cfu) of S. pneumoniae type 23F (12 animals received saline, and 12 received 10 μg of BPIG). All animals in both groups were heavily colonized with S. pneumoniae 24 h later. Thus pretreatment with BPIG had no effect on the frequency or density of colonization with S. pneumoniae type 3 or 23F in this model.

Intralitter colonization model with type 3 S. pneumoniae. Eight cages were studied. A total of 38 infant rats were treated with 50 μg of BPIG, and 37 were treated with saline 1 day before inoculation of the index animals. All 16 index animals had growth of S. pneumoniae from nasal aspirate cultures on day 1 and remained colonized.

The number of contacts that became colonized after exposure to index animals was significantly higher in the saline-
treated animals than the BPIG-treated animals on days 6–10 ($P < .05$ at all time points; figure 1). On day 10, 35 (92%) of 38 animals were colonized in the saline-treated group versus 18 (49%) of 37 in the BPIG-treated group ($P < .001$, relative risk = 0.53, 95% confidence interval = 0.37–0.74). When only colonized animals were analyzed, the percentage of heavily colonized (>50 cfu/10 µL) animals on days 6–10 was significantly higher in saline-treated than in BPIG-treated animals ($P < .05$; figure 2). On day 6, for example, 16 (64%) of 25 colonized saline-treated animals were heavily colonized with $S. pneumoniae$, and 9 (36%) had light colonization; conversely, 3 (25%) of 12 colonized BPIG-treated animals were heavily colonized, whereas the remaining 9 had light colonization ($P = .04$). Similar results were found on days 8 and 10 ($P < .05$; figure 2).

In BPIG-treated animals, the geometric mean type 3 capsular antibody concentration from pooled blood on day 1 was 8.22 mg/mL and on day 7 was 1.44 mg/mL. No type 3 capsular antibody was detected in saline-treated contact animals on days 1 or 7 (<0.1 µg/mL).

**Figure 1.** Frequency of nasopharyngeal colonization with $S. pneumoniae$ type 3 in infant rats treated with saline or bacterial polysaccharide immune globulin (BPIG). Differences in colonization frequency at days 6–10 are significant ($P < .001$, Fisher’s exact test).

**Figure 2.** Comparison of density of colonization with $S. pneumoniae$ type 3 in colonized infant rats treated either with saline or bacterial polysaccharide immune globulin (BPIG) on days 6, 8, and 10 following exposure. Differences in proportion of saline- and BPIG-treated animals with light (<50 cfu/10 µL) and heavy (>50 cfu/10 µL) colonization density are significant each day ($P < .05$, Fisher’s exact test).
**Intralitter colonization model with *S. pneumoniae* type 19F:**
Four cages were studied: 20 animals received 50 μg of BPIG, and 18 received saline. On day 10, 6 of 20 of the BPIG-treated animals versus 14 of 18 saline-treated animals were colonized with *S. pneumoniae* type 19F (*P* = .05). No difference in the density of colonization was noted between BPIG- and saline-treated animals. In BPIG-treated animals, the geometric mean type 19F antcapsular antibody concentration from pooled blood on day 1 was 18.0 mg/mL. No type 19F antcapsular antibody was detected in saline-treated contact animals on day 1 (<0.1 μg/mL).

**Discussion**

In this study, we evaluated systemic pneumococcal antcapsular antibody for prevention of nasal colonization with *S. pneumoniae* in the infant rat. In our direct intranasal inoculation model, no reduction in the frequency or density of nasal colonization with *S. pneumoniae* type 3 or 23F occurred after treatment with antcapsular antibody. In contrast, in the intralitter transmission model, systemic pneumococcal antcapsular antibody provided significant protection from acquisition of *S. pneumoniae* type 3 and 19F. At the serum concentrations achieved, antcapsular antibody reduced the frequency of colonization almost 2-fold. Furthermore, in colonized animals, antcapsular antibody also effectively reduced the concentrations of *S. pneumoniae* type 3 in nasal washes in BPIG-treated animals compared with that in saline-treated animals. These results suggest that systemic antcapsular antibody protects against intralitter spread of mucosal colonization with *S. pneumoniae*.

Previous animal models that have been studied to evaluate *S. pneumoniae* colonization have relied primarily on direct inoculation of mucosal surfaces in CBA/N mice and in chimpanzees [7, 8]. These models require a relatively large inoculum (10³–10⁵ cfu) and thus may not adequately reflect the conditions of colonization in humans. In contrast, in our infant rat intralitter transmission model, acquisition of colonization resulted from contact with other colonized animals. This model may thus have greater relevance to the acquisition of *S. pneumoniae* colonization in humans.

In animal models of Hib infection, the capacity of systemic antibodies to prevent colonization has been investigated. In an intralitter transmission model, passively administered burro antibody to Hib did not protect against nasopharyngeal colonization, although antibody levels were not measured [9]. Conversely, serum concentrations of human anti–Hib antibody >7 mg/mL prevented nasal colonization in an infant rat model [3]. These conflicting results may be due to different systemic antibody concentrations achieved in each study. Human studies of nasopharyngeal colonization with Hib provide further evidence for the role of serum antibodies in preventing colonization. Polysaccharide vaccine against Hib produced serum anti–Hib antibody concentrations ≤1 mg/mL and did not protect against Hib colonization in children [10]. In contrast, the conjugate Hib vaccine elicited much higher serum antibody concentrations (11.8–66.7 mg/mL after booster doses) and reduced colonization [11, 12]. Prevention of nasopharyngeal colonization may therefore be dependent on higher serum concentrations.

Reduction of nasal carriage of *S. pneumoniae* may also be related to serum concentrations of antipneumococcal antibody. Unlike polysaccharide pneumococcal vaccines, a heptavalent conjugate pneumococcal vaccine significantly reduced nasopharyngeal carriage of vaccine-type pneumococci after 2 doses of vaccine in children [13]. Although *S. pneumoniae* type 3 is not included in current conjugate vaccines, it is notable that the serum concentrations of antibody that resulted in reduced colonization with serotype 3 in the intralitter model were similar to concentrations achieved for other serotypes following two or three doses of conjugate pneumococcal vaccines [13, 14].

The intralitter model of *S. pneumoniae* colonization has some limitations. First, serum concentrations of passively administered antibody diminish rapidly over time. In infant rats, accelerated clearance of human antibody occurs after 7 days, either as a result of stimulation of anti–human IgG antibody or because of antibody neutralization by capsular antigen at mucosal sites [15]. Using our model, it may be difficult to determine a minimum protective serum antibody concentration from passive immunization experiments, as this model may underestimate the protective effect of antcapsular antibodies in humans. Second, conjugate vaccines may reduce colonization by both mucosal and systemic immunologic responses. Both secretory IgA– and serum-derived IgG, for example, are detectable in the saliva of children after administration of conjugate Hib vaccine [3]. Our model does not address the contribution of secretory IgA in the prevention of nasal colonization with *S. pneumoniae*. Additionally, we used passive immunization with immune globulin derived from donors immunized with pure polysaccharide pneumococcal vaccine. Therefore, issues such as boosting and antibody subclass production following immunization with conjugate pneumococcal vaccines could not be addressed in this passive immunization model. Finally, it is uncertain whether our findings with *S. pneumoniae* types 3 and 19F would apply to other pneumococcal serotypes.

Our study demonstrates that systemic IgG antibody to pneumococcal polysaccharide reduces the intralitter transmission of *S. pneumoniae* types 3 and 19F. Therefore, IgG antibodies may be sufficient to reduce pneumococcal nasopharyngeal carriage in humans. This suggests that the phenomenon of herd immunity may occur after mass immunization with a conjugate pneumococcal vaccine, and has important ramifications for children in day care or those exposed to infected persons. This model could serve to study potential vaccine candidates, such as pneumococcal proteins, and establish whether these vaccines would protect against nasopharyngeal carriage.

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References


