Chapter 5a

Pneumococcal conjugate vaccine primes for polysaccharide inducible IgG2 antibody response in children with recurrent Otitis Media

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Abstract

Children with recurrent episodes of otitis media can have a deficient IgG2 antibody response to polysaccharide antigens. We have vaccinated 5 otitis-prone children with heptavalent pneumococcal conjugate vaccine. While the patients did show an IgG1 antibody response to all pneumococcal serotypes included in the conjugate vaccine, the IgG2 response, especially to serotypes 6B, 9V, 19F and 23F, was poor. However, vaccination with a 23-valent polysaccharide vaccine 6 months after conjugate vaccination did induce an 11.5-163 fold increase in IgG2 anti-polysaccharide antibody titers. It can be concluded that a specific IgG2 anti-polysaccharide antibody deficiency can be overcome by priming with a pneumococcal conjugate vaccine, followed by a booster with a polyvalent polysaccharide vaccine.
Introduction

Acute otitis media (AOM) is the most frequently occurring bacterial infection in infants and young children. By the age of three years, 71% of children have had one or more episodes of AOM [1]. *Streptococcus pneumoniae* is the main pathogen in bacterial AOM, involved in at least 50-60% of the cases. The defense against infections with these bacteria, characterized by their polysaccharide capsule, depends primarily on antibodies against the capsular polysaccharide.

The peak incidence of childhood AOM is between 6-18 months of age, which matches the period after maternally derived antibodies have disappeared and before onset of antibody formation to polysaccharide antigens. Up to 5% of all children are highly susceptible to infections with *Streptococcus pneumoniae*, manifested in frequently recurrent episodes of AOM. This condition, termed otitis prone, can persist beyond the age of 2 years. Otitis-prone children are suggested to be immunologically different from age matched healthy children in their inability to mount an adequate IgG2 antibody response to *S. pneumoniae* [2]. We previously described a group of children with frequent recurrent mucosal respiratory tract infections. These children showed low to absent IgG2 and/or IgA anti-pneumococcal polysaccharide antibody responses following vaccination with pneumococcal polysaccharide vaccine, despite normal serum IgG2 and IgA immunoglobulin levels [3]. Recently, D’Hooghe et al. observed in a group of patients with otitis media with effusion (OME), significantly lower IgG2 and/or IgA anti-pneumococcal polysaccharide antibody responses following vaccination with pneumococcal polysaccharide vaccine compared to age-matched, healthy controls (Dhooge et al, manuscript in preparation). In all these three studies, the IgG1 anti-polysaccharide antibody responses was normal. These observations suggest that IgG2 anti-polysaccharide antibodies are required for clinical protection against AOM.

Polysaccharide conjugate vaccines have been developed to change the nature of the anti-polysaccharide antibody response into a T cell dependent response and thus induce responsivity early in life. Pneumococcal conjugate vaccines (PCV) indeed have been shown to be immunogenic in infants [4-6] and to induce immunological memory [6]. PCV induce primarily an IgG1 type anti-polysaccharide antibody response [6]. It remains to be determined whether IgG1 antibodies only will be sufficient to prevent mucosal infections like AOM in an otitis prone childhood population. In view of above arguments, the induction of IgG2 or IgA anti-
pneumococcal polysaccharide antibodies may be necessary for optimal protection against mucosal respiratory tract infections.

Based on the results of a study of O'Brien [6] and our own -unpublished- observations, we hypothesized that priming with a conjugate vaccine, followed by vaccination with a pneumococcal polysaccharide vaccine might induce an increased IgG antibody response and favor a shift towards an IgG2 type antibody response. We here present the results of a preliminary study in five otitis-prone children vaccinated with a pneumococcal conjugate vaccine, followed by booster vaccination with a pneumococcal polysaccharide vaccine.

Methods

Patient population

The patients included in this study were referred to the Department of Immunology of our hospital by their pediatrician or otolaryngologist because of recurrent AOM with otorhea for at least 6-8 episodes per year. All five patients were Caucasian and older than two years of age (2.1 - 12.5 years at time of conjugate vaccination). The episodes of AOM were characterized by signs of pain, irritability, new otorhea and additional symptoms like fever ( > 38.5 °C) and concomitant signs of upper respiratory tract infection. The diagnosis AOM was confirmed when patients also had otoscopic signs typical for AOM (Casselbrant,1992). None of the patients had anatomic abnormalities, nor suffered from recognized disease entities like cystic fibrosis or granulocytopenia. Total serum immunoglobulin levels (IgG and IgG subclasses, IgA and IgM) were normal for age. In two patients total IgE levels were increased (300 and 53 U/l, respectively). Complement-activity was normal as were antibody responses to tetanus and diphtheria toxoid. Two of the patients had previously (2 and 7.5 years respectively before conjugate) received a 23-valent pneumococcal polysaccharide (PnPS) vaccine (Pneumovax, Merck, Sharp and Dohme, Haarlem, The Netherlands), containing 25 µg of purified type-specific capsular polysaccharides of 23 pneumococcal serotypes (1, 2, 3, 4, 5, 6B, 8, 9N, 9V, 10A, 12F, 14, 15B, 17F, 18C, 19F, 20, 22F, 23F, 33F) to assess their capacity to respond to polysaccharide antigens. Both patients mounted an IgG1 and IgM anti-pneumococcal polysaccharide response; no IgG2 (post vaccination titers <20 U/ml for 6/8 and 8/8 tested serotypes respectively) and hardly any IgA anti-polysaccharide antibodies were induced.
Written parental consent was obtained before immunization intramuscularly with a heptavalent pneumococcal conjugate vaccine (PCV, containing pneumococcal polysaccharides serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, conjugated either to CRM\_197 (Wyeth Lederle Vaccines and Pediatrics, Rochester, NY) or conjugated to meningococcal outer membrane protein (OMP; Merck Research Laboratories, Blue Bell, PA). Latter PCV was given in the context of a compassionate use protocol. A booster vaccination with 23-valent PnPS vaccine was given 6 months after the vaccination with PCV. Serum was drawn before and 2-4 weeks after each vaccination and stored at -80 °C until use.

**Determination of type specific anti-polysaccharide antibodies.**

IgG1-, IgG2-, IgA- and IgM-type serum antibody levels to the seven pneumococcal polysaccharides included in the conjugate vaccine and to two pneumococcal polysaccharides not included in the PCV (serotypes 1 and 12), were measured by ELISA as described previously [3]. Pre- and postvaccination serum samples were analyzed simultaneously. All serum samples were preincubated overnight with excess free common cell wall polysaccharide (CPS) to remove anti-CPS antibodies. A standard serum from a normal non-vaccinated adult was included in every ELISA-run as a control.

Antibody concentrations of patient samples were expressed relative to a reference adult hyperimmune plasma pool [7]. The latter was assigned 100 U/ml (100%) for each serotype.

**Results**

**Anti-pneumococcal antibody responses following PCV**

Vaccination with PCV induced a significant (2.7 - 43 fold) increase in IgG1 antibody levels for all pneumococcal serotypes included in the vaccine (4, 6B, 9V, 14, 18C, 19F and 23F; see Table 1). Mean IgG2-anti-pneumococcal polysaccharide antibody levels after vaccination with PCV remained <20 U/ml for all serotypes, except for serotype 14. The reason is that one patient mounted an extremely high IgG2 anti-PS14 antibody response (1968 U/ml). The IgA antibody response was >20 U/ml for 4 of the serotypes included in the PCV. As expected, antibody levels to pneumococcal serotypes not included in the PCV (serotypes 1 and 12) did not change (Table 1).
Table 1. Serotype specific antibody response to pneumococcal conjugate vaccine in children with recurrent otitis media:

Mean antibody levels to pneumococcal serotypes included in the PCV (4, 6B, 9V, 14, 18, 19F and 23F) and 2 non-PCV serotypes (1 and 12) before and after vaccination with PCV. The range in antibody titers and fold increase in titer is indicated between brackets.

**Antibody responses following pneumococcal polysaccharide vaccination**

Six months after vaccination with the PCV, patients were booster vaccinated with a 23-valent PnPS vaccine. This booster vaccination with non-conjugated polysaccharides now induces a clear-cut IgG2 antibody response, illustrated for serotypes 9V and 23F in Figure 1. Among the 7 PCV serotypes, post PnPS IgG2 titers to 6B and 23F are the lowest, although for both serotypes the fold increase in IgG2 antibodies is >10 (Table 2).
Table 2. Antibody response to polyvalent pneumococcal polysaccharide vaccine in PCV primed patients.

Mean antibody levels to pneumococcal serotypes included in the PCV (4, 6B, 9V, 14, 18, 19F and 23F) and 2 non-PCV serotypes (1 and 12) before and after vaccination with PCV. The range in antibody titers and fold increase in titer is indicated between brackets.

Booster vaccination with the PnPS vaccine also induced a substantial IgA antibody response and a further increase in IgG1-anti-pneumococcal polysaccharide antibodies (Figure 1 and Table 2). The antibody titers reached after vaccination with PnPS against serotypes not included in the PCV (1 and 12) were considerably lower.
Discussion

Polyvalent pneumococcal polysaccharide vaccines normally induce IgM, IgG1, IgG2 and IgA antibodies of which especially in adults and older children IgG2 is the predominant isotype. Although it has been demonstrated that anti-polysaccharide antibodies confer protection against infections with encapsulated bacteria, the actual contribution of the various isotypes to host defense is unknown. Patients with an IgA and/or IgG2 deficiency have an increased susceptibility for mucosal infections with encapsulated bacteria. We previously described a childhood population with recurrent mucosal upper respiratory tract infections, where encapsulated bacteria like *S. pneumoniae* are a major pathogen. Striking was the low to absent IgG2 and IgA antibody response: 43% of all children had defective IgG2 antibody responses and 22% also low IgA antibody responses following vaccination with the 23-valent PnPS vaccine [3]. Therefore, the inability to make IgG2- and IgA-type anti-pneumococcal polysaccharide
antibodies appears to define a clinical entity of high susceptibility for mucosal infections with bacteria like *S. pneumoniae*.

The frequent occurrence of infections with encapsulated bacteria in infants and young children reflects the ontogeny of the components of host defense [8;9]. The existing polysaccharide vaccines are of limited use in infants and young children because for many polysaccharide antigens, adequate antibody titers are not attained until the age of 18-24 months or later [10]. However, covalent conjugation of a protein carrier to bacterial capsular polysaccharides induces upon immunization a T cell dependent antibody response and priming for an anamnestic or secondary response [10-12]. Pneumococcal conjugate vaccines (PCV) have recently been shown to be safe and immunogenic in healthy infants and young children [13-15]. They are known to prime the vaccinees to respond to a nonconjugated polysaccharide vaccine [14;15]. Furthermore, secondary immunization with pneumococcal polysaccharide vaccine after priming with a conjugate vaccine, elicited in those healthy infants and young children an increase in both IgG1 and IgG2 subclasses [6].

In children with recurrent mucosal respiratory tract infections, an aberrant response to pneumococcal polysaccharide vaccines is frequently observed [3;16]. The data in this study indicate that the specific IgG2 anti-polysaccharide antibody deficiency in patients with recurrent respiratory tract infections, can be overcome by vaccination with PCV followed by booster vaccination with a polyvalent pneumococcal polysaccharide vaccine. Otitis prone children as well as other patient groups at risk for invasive infections with encapsulated bacteria therefore might benefit from such a vaccination schedule.

The mechanism of how a PCV can prime for a PnPS inducible IgG2 response is unknown. It would be desirable if priming would also occur for serotypes not included in the PCV. Our data on the PnPS booster vaccination induced IgG2 response to serotypes 1 and 12 are not conclusive in that respect. A larger group of patients, as well as a control group not primed with PCV, are required to address this issue.
References


