Vaccine 28 (2010) 3071–3075

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Short communication

Concomitant administration of recombinant PsaA and PCV7 reduces *Streptococcus pneumoniae* serotype 19A colonization in a murine model

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ARTICLE INFO

Article history:
Received 15 October 2009
Received in revised form 12 February 2010
Accepted 17 February 2010
Available online 4 March 2010

Keywords:
Pneumococcal vaccines
Non-interference
Expanded vaccine coverage

ABSTRACT

A murine colonization model was used to determine the effect of co-administering 7-valent polysaccharide-protein conjugate vaccine and pneumococcal surface adhesin A. Mice were challenged intranasally with either PCV7 serotypes, 4 or 14, or a non-PCV7 serotype, 19A. Post-challenge samples were evaluated for IgG antibody levels, opsonophagocytic activity, and nasopharyngeal colonization. No interference was observed between immune responses from the concomitant and individual immunizations. Concomitant immunizations reduced carriage for tested serotypes; largest reduction was observed for 19A. From these mouse studies, co-administering pneumococcal antigens appear to expand coverage and reduce colonization against a non-PCV7 serotype without inhibiting immunogenicity to other serotypes.

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1. Introduction

The introduction of 7-valent polysaccharide-protein conjugate vaccine (PCV7) in 2000 has successfully reduced the incidence of invasive pneumococcal disease (IPD) due to vaccine serotypes (VT) among young children and adults in the United States [1]. The decline in carriage of VT may have allowed non-vaccine serotypes (NVT) to fill the niche and cause disease, the phenomena known as serotype replacement [2–4]. By 2004, 88% of IPD among children <5 years old was due to NVT [2]. Of the NVT, serotype 19A was predominant [2]. Serotype 19A isolates were identified in IPD cases in the United States [5–7] and Korea [8] with increased non-susceptibility to antimicrobials. Even though serotype 19A was known to cause IPD prior to the use of PCV7 [2,9], clonal expansion of serotype 19A was also reported [10,11].

As a method to protect against serotype replacement disease, pneumococcal conjugate vaccines (PCV) are increasing in their valences [3,12,13]. The distribution of pneumococcus constantly changes and varies geographically, complicating the construction and implementation of new PCV [11,14]. Although pneumococcal (Pnc) polysaccharides are considered the major virulence factor, Pnc proteins in a vaccine formula could provide serotype-independent protection [14]. The evaluation of these protein-based vaccines, for the most part, has been limited to the mouse model [15]. Briles et al. observed enhanced reduction of nasopharyngeal colonization in mice immunized with the Pnc surface protein A (PspA) and Pnc surface adhesin A (PsaA) in comparison to mice immunized with PspA or PsaA alone [16]. PsaA, a common Pnc protein, has been shown to be immunogenic and reduce nasopharyngeal carriage in a mouse model [16–18]. Previous studies also showed that PspA mixed with pneumolysin or the combination of Pnc histidine triad proteins, PhtB (BVH-11) and PhtE (BVH-3) enhances the protection against pneumonia in the mouse model [19–22]. More than one mechanism of defending against infection is targeted as a result of combining proteins; however, no other pneumococcal antigen as of yet can elicit comparable protection to that of Pnc polysaccharides in conjugate form [22].

In our study, we co-administered PCV7 and rPsaA to increase serotype coverage of PCV7. We evaluated the immune responses and reduction in carriage of PCV7 serotypes 4 and 14, and non-PCV7 serotype 19A in mice.

2. Material and methods

2.1. Bacterial isolates and growth conditions

Streptococcus pneumoniae serotype 4 (CSF isolate DS2341-94), 14 (blood isolate D2232-92) and 19A (blood isolate DS3842-03) were used. All strains were provided by the Streptococcus Reference Laboratory at the Centers for Disease Control and Prevention. Serotypes were confirmed through latex agglutination and capsular swelling (Quellung reaction) tests [18]. For PCV7 serotypes 4 and 14, stocks were prepared as before [18,23]. To optimize colonization, the transparent phenotype of the serotype 19A strain

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0264-410X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2010.02.086

was isolated and enriched as described previously [24]. A transparent strain was used in accordance with the recommendations from the Pneumococcal Vaccine Animal Model Consensus Group and with previous studies on the appropriateness and effectiveness of transparent strains in the animal colonization model [15,25].

2.2. Mice

A total of 80 commercially acquired Swiss-Webster adult females (ND4), 6–8 week old (20–25 g), were used in each experiment. They were housed under standard conditions (25 °C, relative humidity ~40%; pathogen-free) with food and water available, *ad libitum* in filter-top cages. Mice were allowed to acclimate for a week prior to immunization.

2.3. Immunizations

Mouse immunization and challenge protocol were approved by the Animal Care and Use Committee (CDC, Atlanta, GA), which holds an accreditation from the American Association for the Accreditation of Laboratory Animal Care. Prevnar™ (PCV7) was obtained from Wyeth-Lederle, Pearl River, NY. rPsaA was the kind gift of Sanofi Aventis (Swiftwater, PA).

In keeping with previously established regimens for rPsaA [18] and PCV7 [26], a schedule of 3-doses was used for rPsaA and PCV7 in combination and for individual immunizations. Inoculations were given at 2-week intervals. One microgram of PCV7 was administered subcutaneously at each interval. rPsaA suspended in PBS with 6.3 mg/ml aluminum phosphate adjuvant was subcutaneously administered at 100 μg per dose initially and followed with 50 μg boosters. For combination immunizations (PCV7 + rPsaA), PCV7 and rPsaA were given as two separate inoculations. Mice which were unimmunized, immunized with aluminum phosphate adjuvant in PBS, and immunized with either rPsaA (in PBS plus aluminum phosphate adjuvant) or PCV7 alone served as controls. Sera were collected prior to immunizations, a week after the last dose, and 3–5 days after intranasal challenge. These collections were evaluated for Immunoglobulin G (IgG) levels by using enzyme-linked immunosorbent assays (ELISA) and for functional antibody by using an opsonophagocytic assay.

2.4. Measuring sera IgG antibody levels

Antigen-specific IgG levels were measured with ELISA. For the measurement of PsaA antibodies, an anti-PsaA ELISA described for human sera was followed with minor modifications [27]. A highly specific mouse monoclonal antibody, 8G12G11B10 (8G12), produced against native PsaA, served as the reference serum with a stock concentration of 8 mg/ml [28]. Pooled sera from mice immunized with two doses of 100 μg PsaA was used as the quality control and a goat anti-mouse horse peroxidase conjugate (Biorad Laboratories, Richmond, CA) was used for the enzyme-conjugate.

IgG antibodies specific to Pnc capsular polysaccharide (Ps) for serotypes 4, 14, or 19A were measured in the ELISA platform as described previously [26]. Pnc Ps used to coat ImmulonII plates (Dynex, Chantilly, VA) were purchased from ATCC (Manassas, VA). A heterologous Ps, serotype 22F, was added for absorption of cross-reactive antibodies [29,30]. Reference serum was previously generated by pooling post-immunization sera (two doses, 2 μg/each of conjugate vaccine) and given the arbitrary units of 100 U/ml [26]. Pooled sera from mice immunized with two doses of 1 μg PCV7 served as the quality control. Goat anti-mouse HRP conjugate was purchased from Southern Technologies (Birmingham, AL).

To measure total functional antibodies, a standard opsonophagocytic assay (OPA) described by Romero-Steiner et al. [31,32] was utilized. Titers were calculated as the reciprocal dilution at which ≥50% bacterial killing occurred in comparison to complement control wells. To assess differences in functional activity due to species specific phagocytic cells, an alternative OPA protocol using Raw 264.7, mouse monocytes (ATCC) and guinea pig complement (MP Biomedicals, Solon, OH) was also evaluated [15,33,34].

2.5. Nasopharyngeal carriage

A week after administering the last dose, mice were intranasally challenged with approximately 1×10^6 CFU of log phase *S. pneumoniae* serotype 4, 14, or 19A suspended in 10 μl PBS. Challenge doses were later confirmed by counting the overnight growth of a 10-fold serial diluted challenge inoculum [18]. Three to five days post-challenge, each mouse was euthanized and its nasopharyngeal (NP) cavity washed as described by Moreno et al. [26] and Wu et al. [35]. As seen in the study by Moreno et al., control mice significantly cleared pneumococci six days post intranasal challenge [26]. In this study, we found three to five days post-challenge to be the optimal time point in detecting a difference between control and immunized mice. NP washes (100 μl) were collected, diluted with equal volume of saline, and further serially diluted, 3-fold, an additional five times in a 96-well plate. Fifty microliters of each dilution was cultured on blood agar plates supplemented with 2.5 mg/L gentamicin. In preliminary studies, mice cleared serotypes 4 and 19A within 4 days and serotype 14 within 5 days post-challenge. Because of these results, NP washes were conducted 3 days post-challenge of serotype 4 or 19A and 4 days post-challenge with serotype 14. As previously defined, carriage values are the average count of Pnc colony-forming units (cfu) collected in 50 μl of nasal wash [18]. Counts were adjusted for dilution factors prior to averaging.

2.6. Statistical analysis

Antibody concentrations were calculated with a 4-parameter logistic equation (ELISA for Windows, CDC). Mean or geometric mean of OPA titers (with log-transformation) and colony counts were calculated. Significant differences, $P \leq 0.05$, were determined between two groups using Mann–Whitney rank sum test or *t*-test, within an experiment using one way analysis of variance on ranks, and for multiple pairwise comparisons using the Student–Newman–Keuls method (SigmaStat software version 2.0; Jandel scientific, Point Richmond, CA).

3. Results

3.1. IgG antibody response pre and post intranasal challenge

To examine the effect of PCV7 + PsaA co-administration on IgG antibody levels, mouse immune sera were assayed before and after challenge. With the anti-PsaA ELISA, both PCV7 + rPsaA and rPsaA immunized mice demonstrated an IgG response (Fig. 1a). Before and after intranasal challenge with any of the serotypes tested (serotype 4, 14, or 19A), the mean anti-PsaA concentrations for PCV7 + rPsaA and rPsaA immunized mice were not significant from each other (*P*-values, 0.27 and 0.21, respectively). Sera from unimmunized mice and mice immunized with either PBS/adjuvant (not shown) or PCV7 had no measurable amounts of anti-PsaA IgG.

With the anti-Pnc PS ELISA, the average IgG antibody concentrations were not statistically different for PCV7 immunized mice and PCV7 + rPsaA immunized mice no matter the serotype prior to and after challenge (Fig. 1b). Unimmunized mice and mice immunized with PBS/adjuvant (not shown) or rPsaA induced low IgG levels. In mice immunized with rPsaA alone, a higher IgG response to Pnc Ps