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Rapid kinetics of serum IgA after vaccination with Prevnar®13 followed by Pneumovax®23

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Abstract

Streptococcus pneumoniae is a causative agent of community-acquired pneumonias. The recommendations of the 2012 Advisory Committee on Immunization Practices include vaccination with Prevnar®13 (protein-polysaccharide conjugate vaccine; PCV), followed by Pneumovax®23 (polysaccharide-based vaccine; PSV) in adults 65+ or the immunocompromised. In this experiment, a group of 4 healthy volunteers were vaccinated with PCV followed by PSV 60 days later. ELISAs were optimized to study kinetics of IgA, IgM, total IgG and its four subclasses against 14 polysaccharides of the pneumococcal capsule. Although this is a small sample, results from volunteers consistently showed that rapid induction of monomeric IgA followed by rapid decline is typical for both vaccines. IgA was not detected after PSV vaccination in those serotypes present in PCV, suggesting the population of B cells secreting IgA is not renewed within 60 days of activation by PCV. In contrast to mice, human neutrophils expressed a functional receptor for the constant region of monomeric IgA. Thus, the role of IgA early in the human immune response should be further investigated.

1. Introduction

Streptococcus pneumoniae is a gram-positive diplococcus and a causative agent of community-acquired pneumonias. Infection with pneumococcus can be prevented upon vaccination with the polysaccharide-based Pneumovax®23 (PSV) or the protein-polysaccharide-conjugate Prevnar®13 (PCV). The targets for these vaccines are polysaccharides that form the pneumococcal capsule. PSV contains 23 serotypes that represent 85–90% of serotypes involved in adult invasive diseases [1]. Since this vaccine is composed solely of polysaccharides, it is believed that PSV induces a T cell independent response [2]; thus, it should not induce B cell memory. PCV contains 12 serotypes present in PSV in addition to serotype 6A. These serotypes are conjugated to protein, thus PCV induces a T cell dependent response and B cell memory [3].

The Advisory Committee on Immunization Practices recommends the use of PCV followed by PSV in adults 65+ or the immunocompromised [4]. Protection by these vaccines correlates with the level of opsonizing anti-capsule antibodies in serum [5, 6]. Various schedules of pneumococcal immunization have been empirically tested with differing results [7, 8]. A systematic review of PSV found that, due to limitations in design and execution and the possibility of detrimental effects, there is only moderate evidence that PSV should be used regularly in HIV-infected adults [7]. Another trial involving HIV-infected patients found that PCV was initially more effective than PSV, but that this difference declined over time. This study also found that antibody response to PSV and PCV depended on levels of CD4^{+} T cells [8]. Nevertheless, the population of B cells involved in the induction of the antibody response to these vaccines has not been thoroughly determined [9, 10]. The population of B cells mainly involved in an antibody response is correlated with the subclass of antibodies induced, and thus the type of antibodies detected could assist in identifying the target B cell population [11]. Several researchers have looked at a few isotypes in the antibody response against pneumococcal vaccines, such as IgM or IgG2 [12]. Herein, the kinetics of different antibody isotypes (4 subclasses of IgG, IgA, and IgM) are shown against 14 polysaccharide serotypes present in the pneumococcal capsule after vaccination with PCV followed by PSV.

2. Materials and methods

2.1. Vaccination schedule

Four healthy human volunteers, one female and three males aged 50–60 years, were vaccinated with PCV (Lot # H41948, Pfizer). Sixty days later the volunteers were vaccinated with PSV (Lot #7002253600, Merck). Both vaccines were

administered by intramuscular injection in the deltoid muscle. Blood samples were taken at days 0, 14, 28, 59, 74, 88, 119, and 177 post-vaccination. Serums were kept at −80 °C until use. This protocol was approved by Wright State University IRB. Informed consent was obtained for each volunteer.

2.2. Optimization of ELISAs

This technique is a modification of the WHO protocol for the detection of anti-pneumococci IgG antibodies in serum [13]. The reaction volume was 50 uL. Briefly, concentrations of polysaccharides ranging from 10 ug to 10 ng per well were tested. The signal was consistently detected at concentrations as low as 0.1 ug per well. Therefore, a concentration of 1 ug per well was used. All serotypes were obtained through American Tissue Cell Culture (ATCC). Polysaccharides were incubated at 37 °C for 5 h in Immunolon® ELISA Plates and stored at 4 °C for no longer than 1 week before use.

The following positive control serums were used to optimize the ELISAs: reference serum 007sp (For review, see ref. [14]), serum from a 45-year-old male at 41 days post vaccination with PSV (serum C1), and serum from a 59-year-old woman vaccinated with PCV followed by PSV at 29 days post-vaccination with PCV (serum C2).

Serums were absorbed in a solution containing PBS pH 7.2, 0.02% N3Na, 5 ug/mL cell wall (Statens Serum Institut, Copenhagen, Denmark), and 5 ug/mL of capsular polysaccharide 22F (ATCC) for 45 min to decrease background, as described [13, 14]. A 1:25 dilution of the absorbed serums was tested with polyclonal anti-Ig-total. The washing solution for all steps in the ELISA was 10 mM Tris Buffered Saline (TBS)/0.1% Brij 3 times followed by 10 mM TBS 3 times and monoclonal mouse anti-human antibodies against total IgG (Clone G18-145), IgG1 (Clone HP6070), IgG2 (Clone G18-21), IgG3 (Clone HP6047), IgG4 (Clone HP6025), IgM (Clone G20-127), and IgA1/IgA2 (Clone G20-359). The optimal dilution for these antibodies was 1:100 with a 60 min incubation at room temperature. The biotinylated antibodies were detected using streptavidin conjugated to alkaline phosphatase (Thermo Scientific, Waltham, MA). The best signal was found at 2 ug/mL after room temperature incubation for 60 min. The presence of alkaline phosphatase was detected with incubation for 30 min at 37 °C with a *p*-nitrophenylphosphate solution (Thermo Scientific). The enzymatic reaction was stopped with 3 M NaOH. Optical densities were measured using a Tecan NanoQuant Infinite M200 Pro plate reader at 405 nm.

2.3. Kinetics of antibody response

ELISAs for serotypes 2, 8, 9N, 10A, and 12F (present only in PSV); 6A (present only in PCV); 1, 3, 6B, 9 V, 14, 19A, 19F, and 23F (present in both vaccines) were

tested for all donors following the optimized protocol described above. Optical densities were expressed as geometric means and standard error was calculated using Excel (Microsoft).

3. Results

There was no significant level of response detected for IgG1, IgG3 and IgG4, therefore the kinetics for these subclasses are not reported (data not shown). Fig. 1 shows the kinetics of IgA and IgG2 antibodies against serotypes present solely in PCV or PSV. All serotypes show similar peak times for IgA and IgG2 at 14 days post vaccination. After this peak IgA levels rapidly decline, whereas IgG2 levels continue to rise for most serotypes throughout the remainder of the experimental period. Although serotype 9 N is not present in the PCV vaccine, there was a small increase in the titer of antibodies detected against this serotype.

Fig. 2 shows the kinetics of IgA and IgG2 antibodies against serotypes present in both PCV and PSV. As seen in serotypes present solely in PSV or PCV, IgA levels peak at 14 days post vaccination and rapidly decline. In contrast to serotypes present only in PSV, IgG2 levels peak as early as 14 days post vaccination and as late as 28 days post vaccination, but remain steady throughout the experiment. In this system, no booster effect was detected for either isotype with the exception of IgG2 for serotype 14. In addition, no significant levels of IgA were detected in serotypes 3 or 14. As shown in Fig. 3, IgM and IgA antibodies display similar kinetics in serotypes present in only PSV and in serotypes present in both vaccines. Both isotypes peak at 14 days post vaccination and rapidly decline. No significant
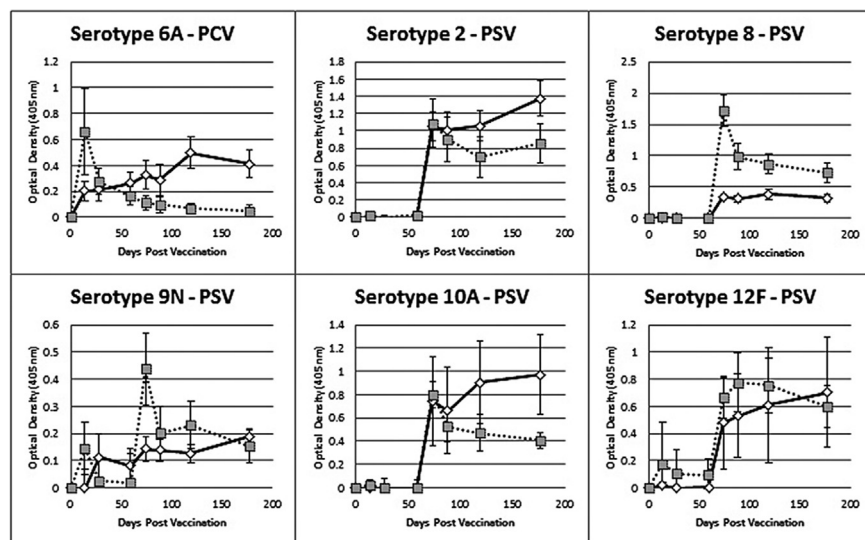

Fig. 1. Comparison of kinetics of IgA and IgG2 in serotypes present in either Prevnar®13 (PCV) or Pneumovax®23 (PSV). Volunteers were vaccinated with PCV at time 0 followed by PSV 60 days later. Geometric means of IgA (gray squares) and IgG2 (open circles) are shown.
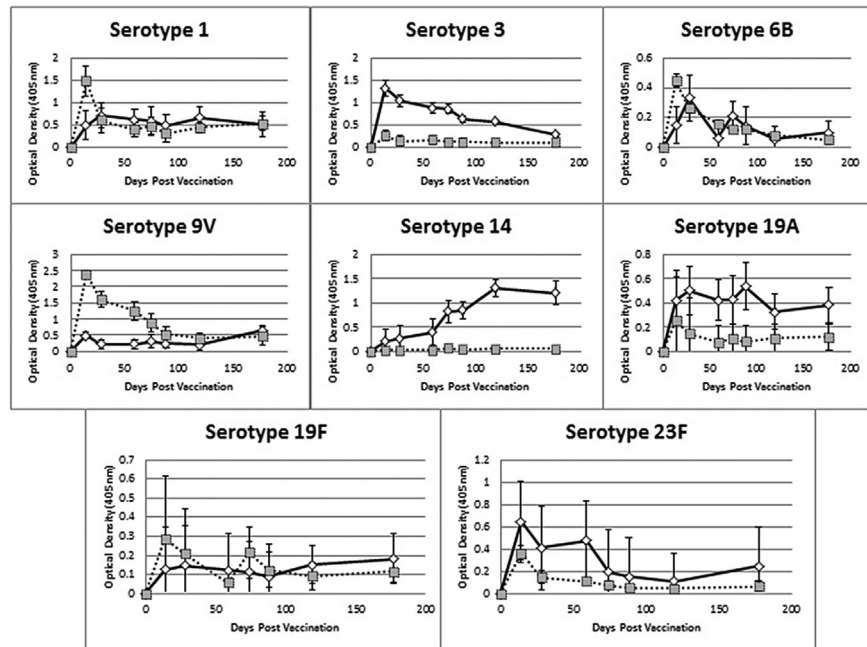
Fig. 2. Comparison of kinetics of IgA and IgG2 in serotypes present in both Prevnar[®]13 (PCV) and Pneumovax[®]23 (PSV). Volunteers were vaccinated with PCV at time 0 followed by PSV 60 days later. Geometric means of IgA (gray squares) and IgG2 (open circles) are shown.

increase in IgA levels was observed after introduction of PSV for those serotypes present in both vaccines.

4. Discussion

The antibody response was evaluated after vaccination with PCV followed by PSV, focusing on IgG2, IgA, and IgM as a possible indication of the B cell population targeted by the vaccines. IgG2 did not show a second peak in serotypes present in both vaccines, and instead remained steady or declined. With the exception of serotype 14, no apparent booster effect was observed. Reports of a booster of the antibody response after one dose of PCV followed by PSV are conflicting [15, 16]. One possibility that may explain these differing results is the interval between doses. In the report in which they found a booster effect, the interval between doses was 1 year [15] whereas the interval between doses in the study that did not find a booster effect was 6 months [16]. The interval in this study was 2 months. The difference in the interval between the two doses is an area that should be further explored.

There was a small increase detected in the titer of IgA and IgG2 antibodies against serotype 9 N after administration of the PCV vaccine despite this serotype not being present in this vaccine. This increase in titer of antibody could be due to cross-reaction with serotype 9 V which is present in the PCV vaccine. Other
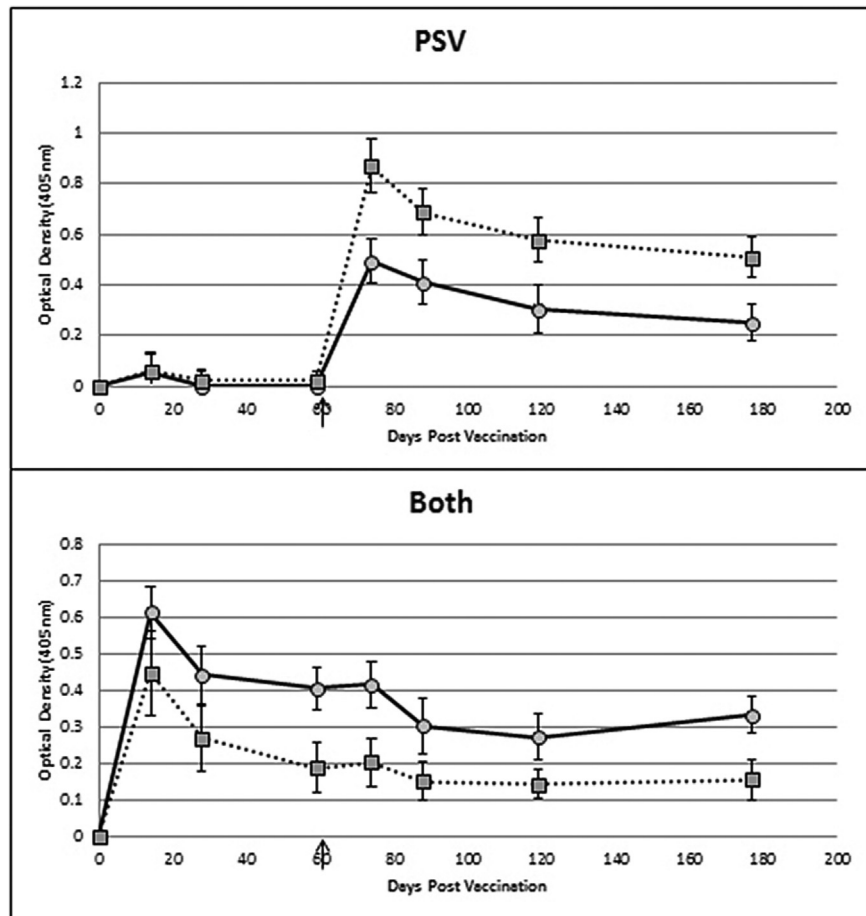
Fig. 3. Comparison of kinetics of IgM and IgA in serotype groups in PSV and in both PSV and PCV. Volunteers were vaccinated with PCV at time 0 followed by PSV 60 days later. The figure shows the kinetics of the average of serotypes present solely in the polysaccharide vaccine (PSV) or in both, the polysaccharide and conjugate vaccines (both). Average of geometric means of IgA (gray squares) and IgM (gray circles) are shown.

researchers have recently demonstrated a booster effect to serotype 9 N in children vaccinated with the 7-valent PCV followed by the PPV vaccine [17].

A significant finding of this study was that IgA initially showed similar kinetics to both IgG2 and IgM, but rapidly declined following its peak. These kinetics were very similar to IgM, but there was no detection of IgA after vaccination with PSV in those serotypes present in both vaccines. This suggests that the population of activated B cells secreting these IgA antibodies is not renewed within 60 days of activation by PCV, which is the period between vaccinations. In humans, monomeric serum IgA plays an important role in opsonophagocytosis in pneumococcal infection [18], although it is unclear which populations of B cells secrete these antibodies [2, 9, 10, 19, 20]. The mouse model has previously been used to study the immune response against pneumococcal infection and

vaccination. However, mouse myeloid cells do not express a functional CD89 molecule, the Fc receptor for IgA [21]. The difference between the mouse and human immune systems stresses the importance of dissecting the human antibody and B cell responses to pneumococcal vaccines to determine which populations of B cells would induce a high titer and long-lasting antibody response.

A limitation of this study is the small number of volunteers however, the results from all volunteers consistently showed that rapid induction of monomeric IgA followed by rapid decline is typical for both vaccines. Fast response to capsular serotypes suggests that this isotype is produced by an innate-like B cell population in humans. The role of IgA against encapsulated bacteria should be further investigated in order to better understand the early human immune response.

Declarations

Author contribution statement

Rebecca R. Crowther; Christine Collins; Cheryl Conley; Osvaldo J. Lopez: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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