PCV13 induced IgG responses in serum associate with serotype-specific IgG in the lung.

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Summary: This study found that immunisation with PCV13 elicits serotype-specific IgG responses, with serum IgG levels to correlate with those measured in bronchoalveolar lavage sample, but shows poor immunogenicity against serotype 3 in both sites.

Abstract

Pneumococcal conjugate vaccine efficacy is lower for non-invasive pneumonia than invasive disease. In this study, participants were vaccinated with PCV13 or HepA (control). Bronchoalveolar lavage samples were taken between 2-6 months and serum at 4- and 7-weeks post vaccination. In the lung, anti-capsular IgG levels were higher in the PCV13 group compared to control for all serotypes, except 3 and 6B. Systemically, IgG levels were elevated in the PCV group at 4-weeks for all serotypes, except 3. IgG in BAL and serum positively correlated for nearly all serotypes. PCV13 shows poor immunogenicity to serotype 3, implying lack of protective efficacy.

Clinical trial registration with ISRCTN: 45340436

Introduction

Colonisation of the human nasopharynx with *Streptococcus pneumoniae* (the pneumococcus, Spn) is a frequent and immunising event, but local/distal tissue invasion leads to a spectrum of diseases, including pneumonia. Community acquired pneumonia (CAP) is a leading cause of death across all economic settings (1).

The licenced pneumococcal conjugate vaccines, PCV7, PCV10 and PCV13 are designed to elicit anti-capsular immune responses to some the most prevalent serotypes causing disease. Currently either PCV10 or PCV13 are included in childhood immunisation programmes of many countries, whereas a pneumococcal polysaccharide vaccine (PPV23) is more commonly recommended for older adults and high-risk groups Despite vaccinations, two PCV13-included serotypes, 3 and 19A, have continued to be detected in colonisation studies in fully PCV13 vaccinated children (2). Serotype 3 is especially important in adults, where it makes up a significant amount of remaining pneumococcal disease, the majority of which is community acquired pneumonia (CAP) (3). PCV13 efficacy against CAP has been reported to be lower compared to invasive pneumococcal disease (IPD), with a study finding 45% vaccine efficacy for a first episode of vaccine type non-bacteraemic, non-invasive CAP, compared to 75% for IPD (4). Assessment of the efficacy of PCVs against CAP is more complicated than IPD, due to diagnostic challenges, lower specificity of the endpoint measuring efficacy to CAP and presence of other respiratory pathogens causing CAP. Estimated lower vaccine efficacy could be the result of reduced humoral responses to PCV13 in the lung mucosa, as failure of local immunoglobulin production in the lung has been associated with increased nosocomial pneumonia in ventilated patients (5). Herein, we described the anti-capsular polysaccharide (CPS) IgG responses in the lung lining fluid of healthy adults in response to PCV13 vaccination and compared the levels of anti-CPS IgG to 6B induced by experimental nasal pneumococcal colonisation or immunisation.

Methods

Healthy, non-smoking participants aged 18-50 years old were enrolled into a randomised controlled trial previously described (6). Ethical approval was given by the Northwest-Liverpool East Research Ethics Committee (REC) reference number 12/NW/0873. Participants were randomised to receive the PCV13 (Prevenar-13, Pfizer n=49) or hepatitis A vaccine (Avaxim, Sanofi Pasteur MSD n=50). Serum samples were collected at baseline, 4- and 7-weeks post-vaccination (PV). 5 weeks following vaccination they were inoculated intranasally with live pneumococcus, serotype 6B, as part of an

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Experimental Human Pneumococcal Challenge study to test efficacy of the vaccine against nasal colonisation (7).

A subset (n=19, HepA= 10 and PCV13= 9) consented to research bronchoscopy and this subset is the focus of this paper (Table S1). Bronchoalveolar lavage (BAL) samples were collected at a singular time point between 2-6 months post vaccination (Figure S1A), using a procedure previously described (8). In addition, a subset of non-vaccinated (PCV13 or PPV23) participants (n=63), enrolled in different experimental human pneumococcal challenge studies (15/NW/0146, 14/NW/1460) were stratified based on carriage status to assess the effect of nasopharyngeal pneumococcal colonisation on lung mucosa.

IgG levels to capsular polysaccharides of all vaccine serotypes were measured in both serum and BAL samples using the WHO standardised ELISA method, as previously described (9). IgG to 6A was not measured due to cross reactivity with 6B (10). Briefly, ELISA plates were coated with 5 μg/ml of each purified CPS (Statens Serum Institut). BAL supernatant was used undiluted, whereas serum samples were used in three 1:3 serial dilutions, starting from 1:50. Antigen-specific antibodies were detected by goat anti-human IgG (1/4,000; Fc-specific)-alkaline phosphatase (Sigma). Optical density was measured at 405 nm using FLUOstar Omega plate reader (BMG Labtech, UK).

Multiplex opsonophagocytosis assays (MOPA) were performed in serum samples, using the WHO standard method, as previously described (11). Three cassettes of pneumococcal bacteria, containing four pneumococcal serotypes each resistant to a different antibiotic (Table S2) (covering all PCV13 serotypes except serotype 3), were incubated with serial dilutions of human serum then further incubated with baby rabbit complement and HL-60 cells. Each well was spotted onto 4 blood agar plates and each plate was overlaid with agar containing an antibiotic before overnight incubation, allowing only the correspondingly resistant pneumococcal strain to grow. Opsonophagocytic activity was calculated by determining the concentration of serum at which 50% of bacteria were killed.

Statistical analysis

Antibody titres from control and PCV13 vaccinated subjects were compared using the Mann–Whitney U-test when two groups were compared. Friedman test with Dunn's multiple comparisons test was performed when three groups were compared. The serum and BAL IgG titres were converted to a log10 base and analysed for correlation with linear regression by the Pearson's correlation coefficient using the statistical software R and prism8. Significance was set at $P < 0.05$.

Results

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PCV13 elicits antibody responses in the lung lining fluid against all serotypes, except serotype 3

To investigate whether PCV13 elicits antibody responses in the lung of vaccinated subjects, we used research bronchoscopy to sample the human lung within 6 months post vaccine administration. Levels of anti-CPS IgG in BAL were statistically significantly higher in the PCV13 vaccinated group (n= 9) compared to control group (n= 10) for all vaccine-antigens, except serotype 3 and 6B (Figure 1A). The highest fold difference in BAL IgG levels between the two groups was measured for serotype 23F, whereas the lowest significant difference was measured for serotype 19F (Table S2). Anti-CPS IgG to 23F and 19F were respectively 8.4x and 1.8x higher in the PCV13 group compared to control (median:8.4x, IQR:2.8x-12.6x and median:1.8x, IQR: 1.4x -5.1x). Both HepA and PCV13 subjects were inoculated with Spn6B at 5 weeks post vaccination, which resulted in 8/10 (80%) and 1/9 (11%) carriage in HepA and PCV group, respectively (Table S1). Titres of BAL IgG to CPS-6B between experimentally induced pneumococcal carriers (Spn+, n=8) in the control group and non-carriers (Spn-, n=8) in the PCV13 vaccinated group did not differ significantly (p=0.32) (Figure 1B). This boosting effect of nasopharyngeal pneumococcal colonisation in the lung was also observed in a separate non-vaccinated cohort, where Spn carriers (n=31) had 4.3x higher levels of IgG to CPS-6B in the BAL compared to non-carriers (n=32) up to 6-months post colonisation (median= 3.51, IQR: 1.19-11.74 in Spn+ vs median= 0.81, IQR: 0.62-1.11 in Spn-) (Figure 1B).

In the analysis of the IgG titres with time, levels of anti-capsular IgG in BAL were sustained at relatively higher levels in PCV vaccinated subjects compared to the controls for up to 4 months post-vaccination for most serotypes, although they decreased over time (Figure S1B). A steeper drop in antibody levels was observed in serotypes that had some of the lowest fold rises compared to the control vaccinated subjects (9V, 19A, 19F) (Figure S1B).

In blood, baseline levels of anti-CPS IgG increased for all measured vaccine-antigens at 4-weeks post-vaccination in PCV13 subjects, except serotype 3 (1.25x fold-increase, IQR: 1.06x – 1.51x) (p= 0.129) (Table 1) (Figure 1C). Serum anti-CPS IgG to nearly all serotypes, but serotype 3, also increased from baseline to 7 weeks post-vaccination. Raised antibody titres decreased from 4 weeks to 7 weeks post-vaccination for all the vaccine antigens. This drop was most profound for anti-CPS IgG to 19A, which decreased by 32% over this time-period (4-wk, median:9.06 µg/ml [IQR:3.40 -20.02] vs 7-wk, median: 6.17 µg/ml [IQR: 3.15- 14.10], p= 0.019). Likewise, in PCV13 subjects, opsonophagocytic killing increased at 4-weeks post vaccination compared to baseline for most vaccine-antigens, with killing capacity declining over time (7-weeks post vaccination) (Figure S1C).

Correlation of anti-CPS IgG titres in BAL and paired serum samples at 7-weeks post vaccination in the PCV vaccinated group demonstrated a positive association for all serotypes, except 9V, with a moderate strength of association for most serotypes (Figure 1D).

Discussion

This study demonstrates that vaccination with PCV13 elicits IgG responses to vaccine-antigens not only systemically but also in the lung mucosa and that pneumococcal colonisation with Spn6B may have a boosting effect in the lung, similar to that induced by intramuscular immunisation. Post vaccination, lung IgG levels against the vaccine-antigens, although waning over time, were still higher in the PCV13 group for some serotypes compared to control counterparts for up to 4 months. In serum, PCV13 elicited robust serotype-dependent IgG responses. Heightened serum IgG titres began to decline after 4 weeks post-vaccination, although they did not differ statistically between 4- and 7-weeks post vaccination. This phenomenon was observed for most measured vaccine serotypes, except serotype 3, in which PCV13 did not induce antibody responses either systemically or in the lung mucosa, implying diminished immunogenicity.

Many studies have reported reduced PCV13 ability to elicit antibody responses against serotype 3 in serum (3) and have suggested that higher IgG concentrations may be required to protect against this serotype (12). As a result, there is some debate regarding the effectiveness of PCV13 against serotype 3 disease. PCV13's reduced immunogenicity to serotype 3 may reflect the high circulation of serotype 3 throughout the PCV13 implementation. Clearly, serotype 3 differs from other serotypes, and its ability to evade current vaccine strategies is of concern. On the other hand, PCV13 induced robust IgG responses in blood and lung for serotype 19A. Some possible factors contributing to serotype 19A emergence are high rates of 19A carriage, penicillin non-susceptibility and capsular switching (13).

Overall, our blood and BAL findings are in agreement with data from Malawi, whereby vaccination with a 7-valent PCV (PCV7), which induces antibody responses to serotypes 4, 6B, 9V, 14, 18C, 19F and 23F,significantly increased capsular specific IgG to four serotypes (6B, 14, 19F and 23F) in serum and BAL of both HIV positive and negative Malawian adults (5). In this study, no significant difference was observed between antibody responses to Spn6B in BAL between PCV13 and control vaccinated subjects, as both groups were challenged with 6B at 5-weeks after vaccination. The pneumococcal challenge resulted in disproportionally higher pneumococcal colonisation rates in the control group (80% in control vs 11% in PCV group) and elicited similar levels of lung antibodies to CPS of6B in the colonised, control vaccinees compared to non-colonised, PCV vaccinees. As opposed to a study of

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inhaled delivery of PPV23 (14), nasopharyngeal pneumococcal colonisation induced mucosal immune responses in the lung of colonised subjects. This is in consistence with previous work from our group which has shown that human pneumococcal colonisation has an immunogenic effect in the lung by seeding the lung mucosa with pneumococcal specific CD4$^+$ T cells (15). In this study, cellular responses, such as tissue resident polysaccharide specific B cells, following PCV13 vaccination were not measured. However, as memory B cells are an important aspect of lung immune defences, future studies are needed to focus on the role of resident B cells in the lung in response to vaccination and the local IgG production- knowledge that will contribute to better understand the relationship of PCVs, and the lower vaccine efficacy witnessed against CAP compared to IPD

Some of the limitations of this study are the small sample size and the difference in timing of sample collection between serum and BAL samples, which may have affected the strength of correlation between these two sites. Despite this, the study shows an appropriate response to pneumococcal conjugate vaccine in lung fluid up to 4 months post-vaccination. Also, the positive association between systemic and lung IgG suggests antibody diffusion from blood to mucosa, providing assistance to the site of infection. These finding work against the notion that poor humoral responses at the lung mucosa play a role in reduced vaccine efficacy for most serotypes, except serotype 3. Our findings imply that discrepancies in vaccine efficacy against pneumonia could be due to several other factors, such as viral coinfection causing CAP, diminished lung resident cell function during such coinfections or challenges to ascertain pneumonia aetiology. On the other hand, lack of immunogenicity against serotype 3 supports numerous data on reduced vaccine-efficacy for this serotype. Additionally, the study encourages the idea of developing a live attenuated pneumococcal vaccine for inhaled delivery for the prevention of pneumonia.

Authors contribution

E.M and D.M.F conceived and designed the study.

E.M, D.McL, A-S.W, S.J analysed and interpreted the data.

A.H.W and A.C assisted in clinical procedures and recruitment.

D.McL wrote the first draft of the paper

E.M, A-S.W, D.G, R.S.H, S.B.G., A.C and D.M.F commented on and approved the paper.

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Declaration of Interests

The authors declare no competing interests.

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Table 1: Systemic IgG to CPS for the vaccine serotypes. Median fold-increase at 4- and 7-weeks post vaccination from baseline (BL) and interquartile range (IQR) per serotype per vaccinated group (PCV, n=9 and HepA, n=10). For comparison of IgG levels between baseline and 4- or 7- weeks p adjusted is shown as calculated after correction for multiple comparisons using Dunn's test.

Serotype	PCV13 Fold-change 4wks/BL (IQR)	P adjusted	PCV13 Fold-change 7wks/BL (IQR)	P adjusted	HepA Fold-change 4wks/BL (IQR)	P adjusted	HepA Fold-change 7wks/BL (IQR)	P adjusted
1	3.28 (1.76 – 5.91)	0.001	1.63 (1.24 – 4.16)	0.330	0.99 (0.83-1.07)	>0.99	0.93 (0.89-0.98)	0.38
3	1.25 (1.06 – 1.51)	0.129	1.25 (0.85 – 1.54)	>0.99	0.93 (0.84-1.07)	>0.99	0.90 (0.83-1.09)	>0.99
4	4.18 (3.05 – 5.64)	<0.0001	2.57 (2.41 – 3.84)	0.023	0.87 (0.80-0.95)	0.58	0.82 (0.75-1.0)	0.08
5	4.74 (3.46 – 13.83)	<0.0001	4.71 (2.90 – 16.08)	0.012	1.13 (0.93-1.33)	>0.99	1.16 (1.01-1.35)	0.14
6B	4.94 (3.11 – 17.51)	0.009	6.45 (3.30 – 12.91)	0.017	0.81 (0.76-0.97)	0.72	1.62 (1.0-4.18)	0.72
7F	2.97 (2.27 – 14.29)	<0.0001	2.92 (1.88 – 10.70)	0.012	0.91 (0.87-1.03)	>0.99	1.07 (0.80-1.20)	>0.99
9V	7.60 (5.32 – 8.53)	<0.0001	5.55 (3.91 – 7.35)	0.023	0.86 (0.65-1.08)	0.87	0.97 (0.61-1.0)	0.23
14	9.96 (8.58 – 13.85)	<0.0001	7.53 (6.16 – 11.59)	0.023	0.92 (0.77-0.95)	0.38	0.86 (0.71-0.93)	0.020
18C	11.46 (3.73 – 16.17)	0.001	8.15 (3.55 – 11.21)	0.076	0.84 (0.81-0.93)	0.38	0.86 (0.81-0.98)	0.14
19A	4.44 (2.70 – 7.80)	0.003	3.10 (1.76 – 4.92)	0.21	0.81 (0.68-0.89)	0.13	0.75 (0.64-0.89)	0.075
19F	2.85 (2.35 – 3.60)	0.001	2.67 (2.16 – 4.18)	0.076	0.75 (0.71-0.81	0.004	0.81 (0.79-0.90)	0.231
23F	3.53 (2.17 – 5.45)	<0.0001	2.81 (2.14 – 4.36)	0.012	1.15 (1.03-1.21	0.23	1.17 (0.96-1.43)	0.23

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Figure 1: A) Levels of IgG to capsular polysaccharide (anti-CPS IgG) for 12 pneumococcal serotypes in the bronchoalveolar lavage (BAL) fluid collected between 2 to 6 months post vaccination with either PCV13 (n=9) or HepA (n=10). Each dot represents an individual. Error bars depict medians with IQR. Mann Whitney test was used. *p<0.05, **p<0.01. **B)** From left to right: Levels of IgG to capsular polysaccharide of Spn6B measured in the BAL fluid of HepA/Spn+ (n=8), PCV13/Spn- (n=8), Spn+ (n= 31) and Spn- (n= 32) up to 6 months post pneumococcal challenge. Each dot represents an individual. Error bars depict medians with IQR. P value is shown on the graph. Mann Whitney test was used **C)** Fold change of serum anti-CPS IgG from baseline (pre-vaccination) at 4- and 7-weeks post vaccination measured against 12 vaccine-serotypes. Boxplots depict median and IQR. Dashed line represents fold-change equal to 1. Friedman test, following Dunn's multiple comparison test was used. **D)** Correlation of anti-CPS IgG levels in serum at 7-weeks post vaccination with the paired anti-CPS IgG levels detected in BAL sample the day of bronchoscopy. Spearman correlation was used and linear regression line with 95% confidence interval (grey shedding) are shown.

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Figure 1