INTRODUCTION

Pneumonia remains the leading global cause of death among children under age five, killing more than 900,000 children in 2013 and accounting for 15% of all child deaths [1]. *Streptococcus pneumoniae* (pneumococcus) is the most common cause of severe pneumonia. Pneumococcus also causes sepsis and meningitis and is one of the leading causes of bacterial otitis media (OM). In addition, pneumococci cause significant morbidity and mortality in elderly adults.

There are two main limitations of the currently available pneumococcal vaccines that are based on polysaccharide antigens. Firstly, despite a clear overall benefit of polysaccharide conjugate vaccines (PCVs), increasing pneumococcal disease caused by non-vaccine serotypes, through serotype emergence or replacement, has been well documented in many countries where PCVs have been used for several years [2]. The introduction of PCVs covering additional serotypes (PCV-10 and PCV-13) has helped to reduce serotype replacement in the short term [3]. The issue of serotype replacement is potentially of particular relevance for low-income countries where there is a broader spectrum of serotypes that cause disease [4, 5]. Secondly, PCVs are difficult to manufacture and therefore relatively expensive, which, without considerable financial assistance, limits their affordability and accessibility for low-income countries [6].

Pneumococcal vaccines that are more affordable and provide either protection against serotypes prevalent in the developing world or, ideally, broad protection across all pneumococcal serotypes would be highly desirable. A relatively new approach is to move away from using polysaccharide antigens to target conserved surface proteins common to most or all pneumococcal strains. One conserved protein, the cholesterol-binding haemolytic cytotoxin pneumolysin (Ply), is closely associated with the development of invasive disease and inflammation [7]. Non-toxic Ply mutants have been shown to give protection in animal models [8]. The most advanced protein vaccine candidates, including combinations of recombinant antigens and killed whole cell preparations, have been tested clinically, including Phase 2 trials in infants and elderly adults [9, 10]. By their nature

these vaccines have multiple mechanisms of action and the effect on nasopharyngeal colonisation is under investigation in regions of high carriage rates in Africa [11] and Asia.

The objective of this Phase 1 trial was to investigate in adults the safety and immunogenicity of a new, novel pneumococcal vaccine at 3 dose levels. PnuBioVax®, described in this manuscript, is a vaccine produced from genetically-modified *S. pneumoniae* TIGR4 in whom the Ply gene has been mutated to remove toxicity whilst retaining immunogenicity [12]. The bacteria are subjected to stress during fermentation, induced by a temperature shift from 30°C to 37°. This temperature shift is designed to mimic the translocation of *S. pneumoniae* from the nasopharynx to the circulatory system and from a commensal to an invasive phenotype thereby upregulating proteins that may be relevant for provoking protective immune responses as a consequence of infection. Following detergent extraction and ion exchange chromatography, the final vaccine includes multiple protein antigens, including PspA and non-toxic Ply and is formulated without additional adjuvant. PnuBioVax is under development as a vaccine against *S. pneumoniae* infection and offers the potential for broad-based protection against a wide range of pneumococcal strains and lower production costs compared to PCVs.

2. METHODOLOGY

2.1 Study vaccine

PnuBioVax is a sterile liquid formulated in 40 mM Tris, 150 mM NaCl, pH 8.0 at a protein concentration of 1 mg/mL. For 50 and 200 µg dose levels, the vaccine (PnuBioVax: Batch 15100) was diluted with 40 mM Tris, 150 mM NaCl, pH 8.0 prior to administration. This diluent also served as the placebo. PnuBioVax is non-adjuvanted. Vaccine and placebo were administered at a 0.5 mL volume by intramuscular injection. The trial was performed at a single site, Simbec Research Limited.

2.2 Study participants & study design

The trial (SPV-001) was a first-in-human randomised, placebo-controlled, parallel group, double-blind, dose escalation trial to evaluate the safety and immunogenicity of PnuBioVax administered on three occasions 28 days apart, at 50, 200 and 500 µg in adult subjects. Thirty six healthy males and females, aged 18 to 40 years, were recruited and divided into three cohorts of 12 subjects (9 receiving PnuBioVax and 3 placebo). Subjects were allocated to treatment groups according to a randomisation code using the PROC PLAN procedure of SAS® version 9.1.3.

After the first dose and at pre-determined times on each dosing day and follow-up visit, a safety review was conducted for dose escalation purposes. Dose escalation only proceeded following satisfactory review of the blinded day 8 safety data that included adverse events (AEs), routine laboratory assessments, vital signs, lymph node assessment, injection site reactions and concomitant medication from at least 9 evaluable subjects in the preceding cohort.

All subjects received at least one PnuBioVax or placebo dose and these constitute the safety population. Prior to unblinding, protocol deviations were identified in 7 subjects that were considered likely to affect the scientific interpretation of immunogenicity data. Therefore the immunogenicity analysis is based on the per protocol population (29 subjects).

The trial protocol and all relevant amendments, together with subject information and consent documents were reviewed and approved by Wales Research Ethics Committee 2. Clinical Trials Authorisation was obtained from the Medicines and Healthcare Regulatory Agency (MHRA). The clinical trial was performed in accordance with the Declaration of Helsinki (Brazil, 2013) and the principles of Good Clinical Practice.

2.3 Safety and reactogenicity assessment

The primary assessment was based on the incidence and severity of all treatment emergent adverse events (TEAEs). Secondary assessments were (i) The incidence and severity of common systemic vaccine related AEs (anorexia, nausea/vomiting, diarrhoea, headache, fatigue, myalgia, fever); (ii) The incidence and severity of injection site reactions (pain, tenderness, erythema, induration,

pruritus); and (iii) Changes in laboratory parameters (biochemistry, haematology and urinalysis) or physical examination from day 1 (baseline) to day 64 that were considered to be clinically significant.

2.4 Immunogenicity assessment

2.4.1 Anti-PnuBioVax IgG

Sera were prepared from blood samples taken on days 1 (pre-dosing), 29 (post dose 1), 57 (post dose 2) and 85 (post dose 3) to assess IgG antibody responses by ELISA. The assay was qualified and conducted by Simbec Research. Briefly, 96-well plates were coated with PnuBioVax, washed, and incubated with serially diluted duplicate samples of test serum. Bound antibody was detected with peroxidase-conjugated donkey anti-human IgG and developed with TMB. Absorbance values were plotted against the reciprocal value of the serum dilution, using a 4 parametric logistic regression with a semi-logarithmic scale to obtain titration curves for each sample. The end-point titre was determined as the titre where the absorbance was equal to a pre-determined cut-point of 0.5.

The proportion of subjects who developed an immune response to PnuBioVax were determined. An immune response was defined in two ways. Firstly, as at least a 2 fold increase in antibody titre from the baseline. Secondly, to compare the change in immune responses in PnuBioVax immunised subjects with any change observed in placebo subjects at the same time point. A response was defined as a change that was ≥2 x standard deviation (SD) of the placebo change at the same time point.

2.4.2 Multiplexed electrochemiluminescence (ECL) assay using MSD technology

This exploratory investigation measured antibody production against specific pneumococcal proteins at days 1 and 85. This procedure was conducted at UCL (University College London) GOS Institute of Child Health, London and utilises MesoScale Discovery (MSD, Rockville, MD, USA). The serum IgG antibodies to pneumococcal protein antigens were measured using a direct binding electrochemiluminescence- based multiplex assay [13] on customised 8 antigen plates. Antibody

interaction with immobilised recombinant antigen was converted into ECL signal and read using an ECL detector. ECL signal is relative to antibody concentration.

2.4.3 Ply neutralisation assay

The ability of anti-Ply antibodies, raised against the non-toxic Ply, to inhibit haemolysis was also investigated. Samples containing antibody were serially diluted and added to a lysate prepared from whole *S. pneumoniae* (WT) TIGR4 which has been subjected to multiple rounds of freeze/thawing. Lysates were also prepared from *S. pneumoniae* 6B and 11A. Lysates, which contains native Ply, was first titrated to obtain a dilution at which maximum lysis of a fixed number of red blood cells (RBC) was achieved. IgG prepared from human sera samples was incubated in the presence of the lysate (at the selected concentration) and then added to RBC and incubated. Plates were centrifuged to pellet the remaining RBC and supernatant transferred to a 96 well plate and absorbance (OD_{450}) measured. Sera concentration was plotted against absorbance and an end-point titre, defined as 50% inhibition of the maximum lysis, obtained.

2.5 Statistical methods

AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA) version 18.1. The incidence of TEAE and vaccine-related TEAEs was summarised by organ system, preferred term, severity and relationship to study drug. Abnormal laboratory safety, vital signs and 12-lead ECG data were tabulated. Absolute and change from baseline haematology, biochemistry and vital signs parameters were summarised descriptively. Lymph node and injection site reaction assessments were also summarised by frequency for each category.

Anti-PnuBioVax IgG titre responses were summarised descriptively and a comparison between treatments carried out using an analysis of covariance (ANCOVA) model with fixed effect for treatment and the log transformed baseline as a covariate. Fold increase in antibody titres were summarised and a ratio of the geometric means between the baseline and post-baseline visit

calculated. A comparison of the mean fold increase was carried out between treatments using an analysis of variance (ANOVA) model with fixed effect for treatment. Adjusted least squares means (LS Means) from the ANOVA model were also calculated, along with the 95% confidence intervals (CI).

From the fold increase in antibody titres, the proportion of subjects who developed an immune response to PnuBioVax, were summarised by frequency for each response category.

Ply neutralisation titres were compared between treatments using the fold increase in titre between day 85 and baseline samples for each subject and summarised by frequency.

3. RESULTS

3.1 Study participants

Subject demographics are summarised in Table 1. Of note is the higher percentage of male compared to female volunteers enrolled in the trial.

Table 1 Subject demographics

3.2 Safety and reactogenicity

In 36 subjects, there were a total of 87 TEAEs in the PnuBioVax and placebo groups (Table 2), including 31 that were classified as common vaccine-related AEs. There were no serious AEs. The common vaccine-related AEs occurred in 13 PnuBioVax (48%) and 2 placebo (22%) subjects.

Table 2 Treatment-emergent adverse events (TEAEs)

The most commonly occurring TEAE was headache. This was the only TEAE classified as a common systemic vaccine related TEAE that was reported (Table 3). When the number of subjects reporting any TEAE was compared, a higher % of subjects reported TEAEs following PnuBioVax when compared to placebo (21 (77.8%) vs 6 (66.7%), with a tendency towards an increase in the incidence of moderate headache with increasing dose of PnuBioVax (0 (0.0%), 2 (22.0%), 3 (33.3%) and 5 (55.6%) for placebo, 50, 200 and 500 µg PnuBioVax, respectively), although the majority were considered unlikely to be or unrelated to the vaccine. When the number of subjects reporting any TEAE was compared, a higher % of subjects reported TEAEs following PnuBioVax when compared to placebo (21 (77.8%) vs 6 (66.7%) with a tendency towards an increase in the incidence of moderate headache with increasing dose of PnuBioVax (0 (0.0%), 2 (22.0%), 3 (33.3%) and 5 (55.6%) for placebo, 50, 200 and 500 µg PnuBioVax, respectively), although the majority were considered unlikely to be or unrelated to the vaccine.

Table 3 Common vaccine related AEs

Injection site reactions (mostly pain and tenderness graded mild or moderate) were commonly reported, in particular in the 200 µg and 500 µg PnuBioVax groups. 2 subjects withdrew on the basis of moderate or severe reactions.

Across the entire assessment period (days 1 - 64), a higher number of subjects who received PnuBioVax reported pain (25 (92.6%) vs 4 (44.4%)) and tenderness (22 (81.5%) vs 1 (11.1%)) when compared to placebo. The pain and tenderness was mostly mild in intensity (grade 1) in that it did not interfere with activity and caused mild discomfort to touch.

Table 4 Injection site reactions

On each dosing day, there appeared to a delayed onset in pain and tenderness, with higher % subjects reporting pain and tenderness at 4, 8 and 20 hours post-dose when compared to 1 and 2 hours post-dose. Moderate (grade 2) pain and tenderness was reported by 4 (14.8%) subjects who received PnuBioVax, 2 (22.2%) following 200 µg and 2 (22.2%) following 500 µg. By day 7 post each dose, all reactions reported as moderate had subsided with the exception of one subject who recorded moderate pain and tenderness on day 29, which progressed to severe on day 31 resulting in them being withdrawn from the trial.

No subject reported any severe (grade 3) or potentially life threatening (grade 4) injection site reactions at their scheduled visits.

Across the entire assessment period (days 1 - 64), the incidence of erythema, induration and swelling was low, with 2 (7.4%) subjects who received PnuBioVax reporting mild transient erythema, both after 500 µg and 4 (11.1%) subjects reporting mild induration and swelling, of which 1 (11.1%) received placebo and 3 (11.1%) received 500 µg PnuBioVax. No subject reported any moderate (grade 2), severe (grade 3) or potentially life threatening (grade 4) erythema or induration and swelling following PnuBioVax or placebo. No subject reported pruritus at the injection site following PnuBioVax or placebo.

There were no vaccine related clinically significant changes in biochemistry, physical examination, lymph node assessment, vital signs or 12 lead ECG parameters during the trial.

3.3 Immunogenicity

3.3.1 Anti-PnuBioVax IgG titres

32 subjects received 3 immunisations of which 29 were considered 'per-protocol' prior to unblinding. Total IgG response to PnuBioVax was measured by ELISA. For the per protocol population, IgG endpoint titres are presented as geometric mean concentrations (with 95%

confidence intervals) for groups at days 1 (baseline), 29, 57 and 85 (Table 5). As expected, significant baseline titres were found in the adult subjects and therefore data were analysed by comparing titres post- and pre-immunisation as a ratio (fold increases). In addition, the number of subjects in each group achieving an immune response, as defined in Methodology, is reported (Table 5).

The fold-increase was statistically significantly higher for 200 and 500 μg PnuBioVax vs 50 μg PnuBioVax and placebo at each timepoint post-immunisation. Comparing with placebo at day 85, p values for the 200 and 500 μg groups were 0.0004 and <0.0001, respectively. There was no evidence of a difference in fold-increase between 200 and 500 μg PnuBioVax or 50 μg PnuBioVax and placebo.

Combining the 200 and 500 μg groups, 12 (100%) and 11 (91.7%) subjects who received PnuBioVax exhibited a fold increase in IgG titre of ≥2 SD of placebo on days 57 (after 2 doses) and 85 (after 3 doses). Similarly in these groups, 3 (25%) and 7 (58.3%) subjects exhibited a ≥2 fold increase in IgG titre on days 57 and 85.

Table 5 IgG response to PnuBioVax in per-protocol population

3.3.2 Antibody responses against pneumococcal proteins

29 subjects were evaluated in the per protocol population. Antibody responses against pneumococcal antigens PspA, Ply, PsaA, PiaA, the pilus proteins RrgB and RrgA were measured by MSD assays (Table 6).

All subjects in PnuBioVax 50, 200 and 500 μg dose groups had increases of ≥2 fold against the vaccine type pilus proteins RrgBT4 and RrgAT4 (TIGR4 protein sequences). Strong responses were also seen against the heterologous non-vaccine type pilus protein RrgB6B (serotype 6B protein sequence). Greater than 50% of subjects in the 200 and 500 μg dose groups (n=12) achieved a ≥2

fold increase in ECL signal against the antigens PspA2 (family 2 protein sequence), Ply and PiaA, and 5 out of 12 had responses against the PsaA protein.

Table 6 Antibody responses against pneumococcal proteins.

The number of subjects achieving a ≥2 fold increase from day 1 (baseline) to day 85 in antibody titres against individual pneumococcal proteins by ECL assay.

3.3.3 Ply neutralisation

Anti-Ply antibodies were purified and analysed to confirm functional activity in the haemolysis inhibition assay. Results using the TIGR4 lysate are shown in Table 7. In the 200 and 500 μg groups (n=12), 50% of subjects achieved a ≥2 fold increase in endpoint titre (defined as 50% inhibition of haemolysis); in comparison, in the placebo group (n=9), no subjects recorded an increase of ≥2 fold from baseline levels. Using antibodies from subjects in the 200 μg group, the same levels of Inhibition of haemolysis by lysates prepared from *S.pneumoniae* 6B and 11A were obtained.

Table 7 Number of subjects achieving a ≥2 fold increase from day 1 (baseline) to day 85 in Ply neutralisation titres

4. DISCUSSION

In this first investigation PnuBioVax in humans, the vaccine was considered safe and well tolerated following intramuscular injection of 50, 200 or 500 μg on 3 occasions to healthy adult subjects. There was a tendency towards more frequent and more severe injection site reactions with higher doses and an increased incidence of headache as the dose increased. Of particular note was the

absence of other typical vaccine-related adverse events such as anorexia, nausea/vomiting, diarrhoea, fatigue, myalgia (non-injection site) or fever. It can be concluded that vaccine safety would not preclude clinical investigation of PnuBioVax in target populations.

The antibody titre data demonstrated measurable IgG (specific for PnuBioVax) pre-dose at baseline. In adults this was predicted and is the result of previous exposure to *S. pneumoniae* both as infants during the acquisition of natural immunity and continued exposure into adulthood. Titres at 28 days following the final PnuBioVax immunisation were used to calculate the fold increase to account for the varying baseline titres. In the small subject numbers in this trial, results indicated that significant increases in IgG titres were achieved at the 200 and 500 μg dose levels. In contrast to polysaccharide-based pneumococcal vaccines, there is no definition of a protective immune response for novel protein-based vaccines, such as PnuBioVax.

Post-immunisation results demonstrate increases in antibody levels against several pneumococcal antigens. Importantly, antibodies against the non-toxic Ply were capable of neutralising the haemolytic activity of native Ply derived from several pneumococcal strains. There is evidence that neutralization of circulating Ply in mice can significantly attenuate cardiac injury during invasive pneumococcal disease (IPD) [14].

A multi-antigen vaccine such as PnuBioVax may be effective in several modes of action. Antibodies directed against ABC transporter pneumococcal proteins, PiaA and PsaA, have the potential to interfere with nutrient uptake [15, 16], whereas antibodies against the pilus proteins, RrgB and RrgA, may block adhesion [17, 18]. The role of PspA is to inhibit complement deposition on the pneumococcal surface and therefore antibodies to PspA may promote the opsonisation and killing of pneumococci [19].

Healthy adults are not generally susceptible to IPD and there are several lines of evidence that this protection is provided by naturally acquired IgG against protein antigens and not, as previously thought, largely dependent on antibodies against capsular polysaccharide antigens [20]. The young

healthy population in this trial had significant baseline IgG against protein antigens present in PnuBioVax and this response was boosted by PnuBioVax immunisation. As the main target group for a broadly protection pneumococcal vaccine is the paediatric population with low natural immunity, the immunogenicity provided by PnuBioVax in this group requires investigation in Phase 2 studies.

Conflict of interest

CE, SH, YP, MJ, AM, CC, CB are employees of ImmunoBiology Ltd. DG has received consultancy fees from ImmunoBiology Ltd and is an NIHR Senior Investigator. DG and PB's laboratory have been in receipt of research grants from ImmunoBiology Ltd.

Author's contributions

CE, SH, MJ and CB participated in study design, execution of the study, review of data and editing of the manuscript.

Acknowledgements

The authors thank Sarah Shelley and April Rowland for clinical trial management, together with investigators, staff and volunteers at Simbec Research Ltd. We are grateful to Professor Jeremy Brown for supporting much of the early pre-clinical development of PnuBioVax and for advice and encouragement.

5. REFERENCES

[1] O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. Lancet. 2009;374:893-902.

[2] Moore MR, Link-Gelles R, Schaffner W, Lynfield R, Lexau C, Bennett NM, et al. Effect of use of 13-valent pneumococcal conjugate vaccine in children on invasive pneumococcal disease in children

and adults in the USA: analysis of multisite, population-based surveillance. The Lancet Infectious Diseases. 2015;15:301-9.

[3] Feikin DR, Kagucia EW, Loo JD, Link-Gelles R, Puhan MA, Cherian T, et al. Serotype-specific changes in invasive pneumococcal disease after pneumococcal conjugate vaccine introduction: a pooled analysis of multiple surveillance sites. PLoS Med. 2013;10:e1001517.

[4] Manoharan A, Manchanda V, Balasubramanian S, Lalwani S, Modak M, Bai S, et al. Invasive pneumococcal disease in children aged younger than 5 years in India: a surveillance study. The Lancet Infectious Diseases. 2017;17:305-12.

[5] Jaiswal N, Singh M, Das RR, Jindal I, Agarwal A, Thumburu KK, et al. Distribution of serotypes, vaccine coverage, and antimicrobial susceptibility pattern of Streptococcus pneumoniae in children living in SAARC countries: a systematic review. PLoS One. 2014;9:e108617.

[6] Hargreaves JR, Greenwood B, Clift C, Goel A, Roemer-Mahler A, Smith R, et al. Making new vaccines affordable: a comparison of financing processes used to develop and deploy new meningococcal and pneumococcal conjugate vaccines. The Lancet. 2011;378:1885-93.

[7] Marriott HM, Mitchell TJ, Dockrell DH. Pneumolysin: a double-edged sword during the host-pathogen interaction. Curr Mol Med. 2008;8:497-509.

[8] Alexander JE, Lock RA, Peeters CC, Poolman JT, Andrew PW, Mitchell TJ, et al. Immunization of mice with pneumolysin toxoid confers a significant degree of protection against at least nine serotypes of Streptococcus pneumoniae. Infect Immun. 1994;62:5683-8.

[9] Prymula R, Pazdiora P, Traskine M, Ruggeberg JU, Borys D. Safety and immunogenicity of an investigational vaccine containing two common pneumococcal proteins in toddlers: a phase II randomized clinical trial. Vaccine. 2014;32:3025-34.

[10] Leroux-Roels I, Devaster JM, Leroux-Roels G, Verlant V, Henckaerts I, Moris P, et al. Adjuvant system AS02V enhances humoral and cellular immune responses to pneumococcal protein PhtD vaccine in healthy young and older adults: randomised, controlled trials. Vaccine. 2015;33:577-84.

[11] Odutola A, Ota MO, Antonio M, Ogundare EO, Saidu Y, Foster-Nyarko E, et al. Efficacy of a novel, protein-based pneumococcal vaccine against nasopharyngeal carriage of Streptococcus pneumoniae in infants: A phase 2, randomized, controlled, observer-blind study. Vaccine. 2017;35:2531-42.

[12] Kirkham LA, Kerr AR, Douce GR, Paterson GK, Dilts DA, Liu DF, et al. Construction and immunological characterization of a novel nontoxic protective pneumolysin mutant for use in future pneumococcal vaccines. Infect Immun. 2006;74:586-93.

[13] Turner P, Turner C, Green N, Ashton L, Lwe E, Jankhot A, et al. Serum antibody responses to pneumococcal colonization in the first 2 years of life: results from an SE Asian longitudinal cohort study. Clin Microbiol Infect. 2013;19:E551-8.

[14] Alhamdi Y, Neill DR, Abrams ST, Malak HA, Yahya R, Barrett-Jolley R, et al. Circulating Pneumolysin Is a Potent Inducer of Cardiac Injury during Pneumococcal Infection. PLoS Pathog. 2015;11:e1004836.

[15] Brown JS, Ogunniyi AD, Woodrow MC, Holden DW, Paton JC. Immunization with components of two iron uptake ABC transporters protects mice against systemic Streptococcus pneumoniae infection. Infect Immun. 2001;69:6702-6.

[16] Gor DO, Ding X, Briles DE, Jacobs MR, Greenspan NS. Relationship between surface accessibility for PpmA, PsaA, and PspA and antibody-mediated immunity to systemic infection by Streptococcus pneumoniae. Infect Immun. 2005;73:1304-12.

[17] Harfouche C, Filippini S, Gianfaldoni C, Ruggiero P, Moschioni M, Maccari S, et al. RrgB321, a fusion protein of the three variants of the pneumococcal pilus backbone RrgB, is protective in vivo and elicits opsonic antibodies. Infect Immun. 2012;80:451-60.

[18] Hilleringmann M, Giusti F, Baudner BC, Masignani V, Covacci A, Rappuoli R, et al. Pneumococcal pili are composed of protofilaments exposing adhesive clusters of Rrg A. PLoS Pathog. 2008;4:e1000026.

[19] Ren B, Li J, Genschmer K, Hollingshead SK, Briles DE. The absence of PspA or presence of antibody to PspA facilitates the complement-dependent phagocytosis of pneumococci in vitro. Clin Vaccine Immunol. 2012;19:1574-82.

[20] Wilson R, Cohen JM, Reglinski M, Jose RJ, Chan WY, Marshall H, et al. Naturally Acquired Human Immunity to Pneumococcus Is Dependent on Antibody to Protein Antigens. PLoS Pathog. 2017;13:e1006137.