Randomized Controlled Study of the Safety and Immunogenicity of Pneumococcal Vaccine Formulations Containing PhtD and Detoxified Pneumolysin with Alum or Adjuvant System AS02\textsubscript{V} in Elderly Adults

Karlis Pauksens,\textsuperscript{a} Anna C.Nilsson,\textsuperscript{b} Magalie Caubet,\textsuperscript{c} Thierry G. Pascal,\textsuperscript{c} Pascale Van Belle,\textsuperscript{c} Jan T. Poolman,\textsuperscript{c}\textsuperscript{*} Pierre G. Vandepapelière,\textsuperscript{c}\textsuperscript{*} Vincent Verlant,\textsuperscript{c} Peter E. Vink\textsuperscript{d}

Department of Infectious Diseases, Uppsala University Hospital, Akademiska Sjukhuset, Uppsala, Sweden\textsuperscript{a}; Department of Clinical Sciences, Malmö Infectious Disease Research Unit, Lund University, Lund, Sweden\textsuperscript{b}; GlaxoSmithKline Vaccines, Rixensart, Belgium\textsuperscript{c}; GlaxoSmithKline Vaccines, King of Prussia, Pennsylvania, USA\textsuperscript{d}

Six vaccine formulations containing AS02\textsubscript{V}, or alum (aluminum phosphate [AlPO\textsubscript{4}]) adjuvant with pneumococcal proteins, pneumococcal histidine triad D (PhtD), and/or detoxified pneumolysin (dPly), either as a polysaccharide carrier in an 8-valent pneumococcal conjugate vaccine (8PCV) or as free (unconjugated) proteins, were evaluated in adults 65 to 85 years of age. In this phase I observer-blind study, 167 healthy subjects were randomized to receive two doses (days 0 and 60) of 10 or 30 \( \mu \)g PhtD-dPly plus AS02\textsubscript{V}, or alum, 8PCV plus AS02\textsubscript{V}, or alum, or one dose (day 0) of 23-valent polysaccharide pneumococcal vaccine (23PPV) as a control (placebo on day 60). The safety, reactogenicity, and antibody-specific responses to these vaccines were evaluated. No vaccine-related serious adverse events were reported. The incidences of solicited local and specific general (fatigue and myalgia) symptoms tended to be higher in the AS02\textsubscript{V} groups than in other groups. Anti-PhtD and anti-Ply antibody responses were observed in all groups except the control group. One month post-dose 2, the anti-PhtD and anti-Ply antibody geometric mean concentrations tended to be higher with AS02\textsubscript{V} than with alum, higher with a dose of 30 \( \mu \)g than with 10 \( \mu \)g for PhtD-dPly and higher with 30-\( \mu \)g PhtD-dPly formulations than with conjugated PhtD and dPly (8PCV) formulations. Functional antibody responses, measured by an opsonophagocytic activity assay, tended to be higher with 8PCV than with 23PPV. In conclusion, vaccine formulations containing free or conjugated PhtD and dPly had acceptable reactogenicity and safety profiles in elderly adults. Immune responses were enhanced with an AS02\textsubscript{V}-adjuvanted formulation containing free 30-\( \mu \)g PhtD-dPly compared to those with alum adjuvant and conjugated proteins. (This study has been registered at ClinicalTrials.gov under registration no. NCT00756067.)

The incidence of invasive pneumococcal disease among elderly adults (\( \geq \)65 years of age) is higher than in younger adult age groups (1), and due to a deterioration in immune function with advancing age, the responses to pneumococcal vaccination in this age group can be inadequate (2). This is of concern because the incidence of community-acquired pneumonia is also increased in elderly adults (3), presenting a significant burden on health care systems that is anticipated to increase in the future. For example, in the United States, pneumococcal pneumonia-related hospitalizations are projected to almost double by 2040 (4). The 23-valent polysaccharide pneumococcal vaccine (23PPV) is recommended for use in elderly adults, but its efficacy against nonbacteremic pneumonia is debatable (5–9). In addition, as pneumococcal polysaccharides are T-cell-independent antigens, 23PPV does not induce any immunological memory response (10).

Pneumococcal conjugate vaccines (PCVs), in which pneumococcal polysaccharides are conjugated to a protein carrier, elicit a better priming response in the adult population than 23PPV (11). The 13-valent PCV is available for use in adults, and its efficacy against pneumococcal pneumonia in individuals \( \geq 65 \) years of age is being evaluated in the Community-Acquired Pneumonia Immunization in Adults (CAPiTA) trial (12). However, its benefit might be restricted by the reduced prevalence of vaccine serotypes in elderly adults as a result of 13-valent PCV use in pediatric vaccination programs (9, 13, 14). Also, pneumococcal serotypes vary widely with regard to geographic patterns and age (15), limiting the coverage of conjugate vaccines.

Immune responses against conserved \textit{Streptococcus pneumoniae} proteins have the potential to offer serotype-independent coverage against pneumococcal diseases (16–19). Pneumococcal histidine triad protein D (PhtD) and pneumolysin (Ply) are under investigation as potential vaccine candidates. PhtD is a pneumococcal cell surface protein that appears to have multiple functions, including involvement in metal ion homeostasis, evasion of complement deposition, and adherence of bacteria to host cells (20, 21). Ply is a cholesterol-dependent cytolysin produced by \textit{S. pneumoniae} and a key virulence factor exerting cytolytic effects that contribute to lung injury, permeability edema, and neuronal damage (22, 23). Due to the cytolytic effects of native Ply, detoxified Ply (dPly) has been developed as a vaccine candidate (P. Hermand et al., unpublished data).
The inclusion of adjuvants has been shown to enhance the magnitude and affect the type of antigen-specific immune responses to vaccines in various populations (24). AS02v is an adjuvant system that consists of an oil-in-water emulsion combined with two potent immunostimulants, 3-O-desacyl-4′-monophosphoryl lipid A (MPL) and the saponin QS-21 (Quillaja saponaria Molina, fraction 21; Antigenics, Inc., Lexington, MA, USA) (25). An AS02v-adjuvanted PhtD-dPly vaccine was shown to protect against pneumococcal pneumonia in animal models (21, 26). In studies of elderly adults and adults 18 to 45 years of age, improved immune responses were seen with a PhtD vaccine containing the AS02v adjuvant system compared to those with an alum adjuvant, and the reduced immune response to vaccines among elderly adults was partially restored to the level of vaccine-induced response observed in younger adults (27). The inclusion of the adjuvant system AS03 also enhanced immune responses to an investigational PhtD-dPly vaccine containing nontypeable Haemophilus influenzae protein D in adults 18 to 40 years of age (28).

This study was conducted in healthy elderly adults to examine the safety, reactogenicity, and immunogenicity of six different investigational pneumococcal vaccine formulations containing AS02v or alum adjuvant with PhtD and dPly proteins either conjugated to pneumococcal polysaccharide in an 8-valent PCV (8PCV) or as free proteins.

MATERIALS AND METHODS

Study design and participants. This was a phase 1 observer-blind randomized controlled study of 167 healthy adults 65 to 85 years of age conducted at three study sites in Sweden (University Hospital, Uppsala; University Hospital, Orebro; and Malmö University Hospital, Malmö) between 19 September 2008 and 28 May 2009 (registered at ClinicalTrials.gov under registration no. NCT00756067). All participants gave written informed consent to the study, which was approved by the Medical Product Agency in Sweden and the regional ethics committee in Uppsala and was conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki. Exclusion criteria included previous vaccination against S. pneumoniae or with a MPL/QS-21-containing vaccine, vaccination with a diphtheria or tetanus toxoid-containing vaccine within the last month, use of any investigational product or other vaccine (except the influenza vaccine) within the last 30 days, administration of immunomodifying drugs within the last 6 months, and administration of blood products within the last 3 months. Subjects were also not eligible to take part in the study if they had bacterial pneumonia or invasive pneumococcal disease <3 years before the first dose or if they had a history of a bleeding disorder or immunodeficient condition, malignancy in the last 5 years, or a history of alcohol or drug abuse. Serious neurologic or mental disorders, active infection, clinically significant anemia, and pulmonary, cardiovascular, hepatic, or renal abnormalities were also exclusion criteria.

The subjects were randomized to receive (at days 0 and 60) vaccinations with 10 μg PhtD-dPly plus AS02v (PhtD-dPly10/AS02v, group), 10 μg PhtD-dPly plus alum (aluminum phosphate [AlPO4]) (PhtD-dPly10/alum group), 30 μg PhtD-dPly plus AS02v (PhtD-dPly30/AS02v group), 30 μg PhtD-dPly plus alum (PhtD-dPly30/alum group), 8PCV plus AS02v (8PCV/AS02v group), and 8PCV plus alum (8PCV/alum group). A control group received 23PPV (Pneumovax; Sanofi Pasteur MSD, Lyon, France) at day 0 and a placebo (saline solution) at day 60. The treatment allocation at each site was performed using a central randomization system on the Internet, which used a minimization algorithm accounting for subject age (65 to 70 or >70 years) and study center.

As this was the first study for each of the above candidate vaccine formulations in humans, study enrollment was conducted in two steps (Fig. 1), with first enrollments in the PhtD-dPly10/alum, PhtD-dPly10/AS02v, and 8PCV/alum groups. Enrollment in the control group was split between the two steps. The observer was blinded to the study groups during the two steps, meaning that the participants and those responsible for study endpoint evaluation were unaware of subjects’ individual vaccination assignments but were open with respect to which vaccine formulations were administered for each of the two steps. For the first doses at each step, over the first 5-day period, a maximum of three subjects per step were vaccinated to allow for close safety observations; after this 5-day period, there was no daily limitation of the number of subjects vaccinated.

Before proceeding to the second doses at step 1 and the first doses for the step 2 groups (PhtD-dPly30/alum, PhtD-dPly30/AS02v, and 8PCV/AS02v), a safety evaluation of the step 1 groups was conducted. Similarly, before proceeding to the second doses at step 2, safety evaluations of both doses for the step 1 groups and first doses for the step 2 groups were completed. These evaluations were conducted by a safety review committee composed of a biostatistician, a clinical physician, and a safety physician at GlaxoSmithKline (GSK) Vaccines who were otherwise not involved in the trial. The committee membership selection ensured that study-related personnel remained blinded to vaccination assignment until the study was completed. The committee reviewed unblinded safety data collected within 7 days of each dose.

The primary objective of the study was to evaluate the safety and reactogenicity profiles of the candidate pneumococcal vaccines when administered to elderly adults. Secondary objectives included the evaluation

FIG 1 Study design. Enrollment was conducted in two steps. Before administration of the second dose at step 1 and first-dose administration at step 2, a safety evaluation was completed for the groups administered the first dose at step 1. The safety of both doses at step 1 and first doses at step 2 were also evaluated before second-dose administration at step 2. The control group received a 23-valent polysaccharide pneumococcal vaccine (23PPV) at day 0 with placebo (saline solution) at day 60. All other groups were administered two doses of PhtD-dPly (10 or 30 μg) or 8-valent pneumococcal conjugate vaccine (8PCV), each with AS02v or alum adjuvant. The numbers in parentheses indicate the number of subjects in a particular group.
Pneumococcal Vaccines with PhtD and dPly Proteins

May 2014 Volume 21 Number 5 cvi.asm.org

of antibody responses induced, per dose, against PhtD and dPly proteins (as free proteins and as carrier proteins for pneumococcal polysaccharides) by candidate formulations adjuvanted with alum or AS02v. The opsonophagocytic activities (OPA) of antibodies against the pneumococcal serotypes included in the 8PCV formulations were also evaluated, as well as the antibody response against protein D (data not shown).

**Vaccines.** All study vaccine formulations except 23PPV were developed and manufactured by GSK Vaccines (Rixensart, Belgium). The AS02v-adjuvanted vaccines (8PCV and the 10-µg and 30-µg PhtD-dPly vaccines) were supplied as freeze-dried pellets in monodose vials to be reconstituted with a liquid formulation of AS02v adjuvant, which was supplied in prefilled syringes. The alum-adjuvanted vaccines (8PCV and the 10-µg and 30-µg PhtD-dPly vaccines) were supplied as a liquid adsorbed on alum in monodose vials.

The 8PCV contained pneumococcal serotypes 19A and 22F (each 5 µg polysaccharide) conjugated to the carrier proteins dPly (15 µg) and PhtD (22 µg), respectively; serotypes 4, 5, 7F, and 14 were conjugated to nontypeable *H. influenzae* protein D (each 3 µg polysaccharide), serotype 18C (3 µg) to tetanus toxoid, and serotype 19F (3 µg) to diphtheria toxoid.

The 23PPV contained serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 35F (each 25 µg polysaccharide) and was supplied in prefilled syringes. The saline placebo was supplied in monodose vials.

All study vaccines were to be stored at 2 to -8°C. The vaccines were administered by intramuscular injection (0.5 ml) into the deltoid region of the nondominant arm. The participants were observed closely for >30 min after vaccination.

**Reactogenicity and safety assessment.** Solicited local (injection site pain, redness, and swelling) and general (fatigue, fever, gastrointestinal symptoms, headache, malaise, and myalgia) adverse events (AEs) were recorded by the participants on diary cards during the 7-day follow-up (days 0 to -6) after each vaccination. Unsolicited AEs were recorded for 31 days (days 0 to -30) after each vaccination. Serious AEs (SAEs) were reported throughout the study, which were defined as any medical event resulting in death, a life-threatening event, or an event causing disability or requiring hospitalization or prolongation of hospitalization. The durations, causality, and outcomes of the AEs were recorded. All solicited local reactions were considered to be causally related to vaccination. For other AEs, an assessment of causal relationship to vaccination was based on the clinical judgment of an investigator and was classified as either possible or not causally related. AE and SAE intensity were scored on a scale from 1 to 3 (mild to severe). Grade 3 solicited fever was defined as an oral temperature of >39.5°C. Grade 3 solicited swelling or redness was defined as a symptom diameter of >50 mm. All other grade-3 AEs/SAEs were defined as symptoms preventing normal daily activity.

Blood samples for hematologic and biochemical analysis and urine samples for urinalysis were taken before the first vaccination and after the first (days 1, 7, 30, and 60) and second vaccine doses (days 61, 67, and 90).

**Immunogenicity assays.** Blood samples were taken before the first vaccination and at postvaccination intervals up to 30 days after the second vaccine dose (day 90). All blood samples were processed and stored appropriately until analyzed at the laboratories of GSK Vaccines (Rixensart, Belgium).

The anti-PhtD and anti-Ply antibody concentrations were measured at days 0 (before the first vaccine dose), 30, and 90 using a multiplex assay based on Luminex technology, which allows the concentrations of several specific antibodies to be determined in any blood serum sample. As antigen is coated on the surface of color-coded beads, multiple antigens were able to be tested simultaneously in the same wells. The cutoff for the multiplex assay was 391 Luminex units (LU)/ml for PhtD and 599 LU/ml for Ply. The OPA was measured for each pneumococcal serotype by an opsonophagocytic killing assay using an HL60 cell line (29). The results were presented as the dilution of blood serum (opsonic titer) able to sustain 50% killing of live pneumococci under the assay conditions.

**Statistical analysis.** The primary objective of the study was to evaluate the safety of the candidate pneumococcal vaccines. As this was the first study of each vaccine formulation in humans, the planned number of participants in each group was restricted to 24. The study was descriptive, with limited statistical power.

The safety analysis was conducted on the total vaccinated cohort (TVC). If the difference in the number of subjects in the TVC and according-to-protocol (ATP) cohort was >10% of the subjects in more than one group, the safety analysis was also conducted on the ATP cohort for safety. The ATP cohort was defined as the subjects who met all eligibility criteria, complied with the procedures defined in the protocol, met no elimination criteria during the study, and received at least one vaccine dose. No comparative analysis was conducted for safety; any described trends were based on observed values and should be interpreted with caution.

All evaluable participants for whom immunogenicity data were available were included in the ATP cohort for an analysis of immunogenicity. Anti-PhtD and anti-Ply geometric mean antibody concentrations (GMCS) and OPA geometric mean titers (GMTs) for each pneumococcal serotype were calculated with 95% confidence intervals (CIs). The anti-PhtD and anti-Ply antibody responses 30 days after each vaccine administration were compared between the groups in exploratory analyses using a two-way analysis of covariance (ANCOVA) model on log-transformed data that included the vaccine formulation (PhtD-dPly10, PhtD-dPly30, or 8PCV) and type of adjuvant (alum or AS02v) as a fixed effect and the prevaccination log-transformed concentration as a regressor. The Tukey-Kramer adjustment method for multiplicity was applied. The absence of adjuvant by formulation interaction was tested at 10% and added in the model if significant (*P* < 0.10). The group GMC ratio and its 95% CI were computed from group contrast in the model if the interaction was statistically significant (*P* < 0.10) or if at least one of the main factors (formulation or adjuvant) was statistically significant (*P* < 0.05). Depending on the statistical significance, the GMC ratios compared either treatment groups or pooled treatment groups (by dose or adjuvant type).

The immune responses against each pneumococcal serotype were compared between groups (8PCV/alum, 8PCV/AS02v, and PPV23) using a one-way ANCOVA model that included the groups as a fixed effect and the prevaccination log-transformed titer as a regressor. The group GMT ratio and its 95% CI versus 23PPV were calculated from group contrast in the model if the overall group effect was statistically significant (*P* < 0.05).

In the analyses of antibody GMC or GMT ratios of one group over another, 95% CIs not including 1 indicated that a significant difference might exist. The results of the exploratory group comparisons should be interpreted with caution, as they were not adjusted for the number of endpoints, and statistically significant findings might have occurred by chance. Analyses were performed using Statistical Analysis System (SAS) versions 9.1 and 9.2 and StatXact 7 PROCs.

**RESULTS**

**Study population.** A total of 167 subjects were enrolled and vaccinated in the study; 126 were included in the ATP cohort for safety and 125 in the ATP cohort for immunogenicity. The reasons for exclusion from the ATP cohorts are provided in Fig. 2. Among the reasons, 32 subjects were excluded from a single study site because the vaccine formulations were exposed to a temperature deviation (−0.5°C for 38 h) and, contrary to study protocol, were administered to participants. Second doses were not permitted for these study participants. Separate analyses were conducted for this excluded population.

Four participants withdrew from the study because of an SAE not considered to be related to vaccination (one in the PhtD-dPly30/AS02v group and one in the PhtD-dPly30/alum group because of pulmonary thrombosis and prostate cancer, respectively) or a protocol violation (one in the PhtD-dPly30/AS02v group and one in the 8PCV/alum group because of high fever at vaccination and high haptoglobin level, respectively). The demographic char-
acteristics of all groups were comparable with respect to mean age, and all participants were white, while the female-to-male ratio varied per treatment group (Table 1).

Reactogenicity and safety. For the solicited local and general AEs, there was no consistent protein-dose effect (Fig. 3 and 4). During the 7-day postvaccination period, the most frequently reported solicited local AE in each group was pain (Fig. 3). Overall, the incidence of each solicited local AE (any and grade 3) tended to be higher in the AS02V groups than in the other groups, with the highest incidences of redness and swelling in the 8PCV/AS02V group. An exception was the incidence of pain in the 8PCV/alum group, which was similar to the incidence in the 8PCV/AS02V group and within the same range as in the PhtD-dPly/AS02V groups.

The most frequently reported grade-3 solicited local AE in each group was redness or swelling. Grade-3 redness and swelling were reported more frequently with AS02V than with the alum adjuvant, with the exception of grade-3 swelling in the PhtD-dPly10 groups (Fig. 3). There were few reports of grade-3 pain (one report after the second dose of PhtD-dPly30/AS02V, and one report after each dose of 8PCV/AS02V).

Fatigue, headache, and myalgia were the most frequently reported solicited general AEs (Fig. 4). Incidences of fatigue and myalgia tended to be higher in the AS02V groups. Overall, there were few reports of grade-3 solicited general AEs (each reported by a maximum of one subject per group). No cases of fever of >39.5°C were reported.

The percentages of subjects reporting at least one unsolicited AE in the 31-day postvaccination period were in the same range (37.5 to 52.2%) in all groups (Table 2). Nasopharyngitis (25 subjects) and headache (8 subjects) were the most frequently reported unsolicited AEs. At least one grade-3 unsolicited AE was reported by 4 to 17% of the subjects in each group (Table 2). None of the grade-3 unsolicited events was reported in more than one subject in each group. At least one unsolicited AE that was assessed by the investigator to be causally related to vaccination was reported by 4.0 to 29.2% of the subjects (Table 2). Each type of related unsolicited event was reported by only one subject in each group, apart from injection site pruritus (3 subjects in the PhtD-dPly30/AS02V group) and warmth (4 subjects in the 8PCV/AS02V group).

SAEs were reported in one subject in each of five groups (no reports in the PhtD-dPly10/AS02V and 8PCV/alum groups). No SAEs were considered by the investigator to be causally related to vaccination. No fatal SAEs were reported. All SAEs resolved, except one (prostate cancer), which was ongoing at the end of the study.

The results of the ATP cohort for safety were consistent with those of the TVC. For the cohort excluded due to vaccine temperature deviation, the safety data did not differ substantially from the results for the ATP cohort (data not shown).

Immunogenicity. The results for the ATP cohort for immunogenicity were consistent with those of the TVC and the cohort excluded due to vaccine temperature deviation (data not shown).

---

**TABLE 1 Demographics of participants (total vaccinated cohort)**

| Participant characteristic | PhLD-dPly10/AS02V (23) | PhLD-dPly10/alum (24) | PhLD-dPly30/AS02V (24) | PhLD-dPly30/alum (24) | 8PCV/AS02V (24) | 8PCV/alum (23) | 23PPV (25) |
|---------------------------|------------------------|-----------------------|------------------------|------------------------|----------------|----------------|-------------|
| Age (mean ± SD) (yr)      | 71.1 ± 5.75            | 70.0 ± 5.03           | 70.2 ± 4.67            | 69.9 ± 4.45            | 70.0 ± 4.06    | 70.4 ± 5.39    | 70.2 ± 4.71  |
| Age range (yr)            | 65–82                  | 65–82                 | 65–82                  | 65–82                  | 65–80          | 65–84          | 65–84       |
| Female/male (%)           | 52.2/47.8              | 50.0/50.0             | 62.5/37.5              | 75.0/25.0              | 62.5/37.5      | 56.5/43.5      | 64.0/36.0   |
(i) Immunogenicity of pneumococcal proteins dPly and PhtD. Before vaccination, all subjects in all groups were seropositive for antibodies against Ply (antibody concentration, ≥599 LU/ml) and PhtD (≥391 LU/ml). Apart from the control 23PPV group, anti-Ply and anti-PhtD antibody responses were observed after the first dose in all groups (Fig. 5). One month after the second dose, anti-Ply antibody GMCs tended to be higher with the 30-µg PhtD-dPly than with the 10-µg formulations (Fig. 5A); anti-Ply antibody GMCs also tended to be higher with AS02v than with alum adjuvant. Anti-Ply antibody GMCs with the 8PCV formulations (containing conjugated 22 µg PhtD and 15 µg dPly) were generally lower than those observed with PhtD-dPly/AS02v formulations containing free protein (Fig. 5A). The exploratory analysis showed formulation (PhtD-dPly30 versus PhtD-dPly10 versus 8PCV) and adjuvant effects that were statistically significant after each dose (see Table S1 in the supplemental material). Significantly higher anti-Ply antibody GMCs were observed for PhtD-dPly30 than for PhtD-dPly10, both PhtD-dPly formulations than for 8PCV, and with the AS02v, than the alum adjuvant (see Table S2 in the supplemental material).

Similar trends as those for anti-Ply antibodies were observed for anti-PhtD antibody responses (Fig. 5B; see also Tables S1 and S3 in the supplemental material).

(ii) Immunogenicity of pneumococcal serotypes. Eight pneumococcal serotypes (4, 5, 7F, 14, 18C, 19A, 19F, and 22F) were common to 8PCV and the 23PPV control vaccine. For serotype 19A conjugated to dPly and serotype 22F conjugated to PhtD, pneumococcal serotype OPA GMTs tended to be higher for both 8PCV groups than for the control 23PPV group 1 month after the first vaccine dose (Fig. 6). Similar trends were observed for the other serotypes conjugated to protein D, tetanus toxoid, or diphtheria toxoid (data not shown). OPA GMTs post-dose 2 were generally lower than those post-dose 1 but were consistently higher than those prevaccination.

Exploratory analysis showed significant formulation effects for serotypes 19A and 22F (see Table S4 in the supplemental material). For serotype 19A, the OPA GMTs were significantly higher with 8PCV/alum versus 23PPV after each vaccine dose, while for serotype 22F, the OPA GMTs were significantly higher for the 8PCV/AS02v group than for the 23PPV group (see Table S5 in the supplemental material). Also, for serotype 19A, the OPA GMT tended to be higher for 8PCV/alum than for 8PCV/AS02v, while for serotype 22F, the reverse trend was observed (Fig. 6B). For serotype 22F, no increase in the OPA GMT was observed in the 8PCV/AS02v group from post-dose 1 to post-dose 2, while slight decreases were observed in the 8PCV/alum and 23PPV groups (Fig. 6B).

For the other serotypes, 1 month after the first vaccine dose, an exploratory analysis showed OPA GMTs were significantly higher in the 8PCV/alum group versus the 23PPV group for serotypes 4,
FIG 4 Incidences of solicited general adverse events, with 95% confidence intervals, within the 7-day follow-up after each vaccine dose and overall per subject (total vaccinated cohort).
7F, and 18C and in the 8PCV/AS02V group for serotype 18C (see Table S5 in the supplemental material). After the second vaccine dose, the OPA GMTs were significantly higher for serotype 4 in the 8PCV/AS02V group and serotype 18C in both 8PCV groups. For vaccine formulations not containing pneumococcal polysaccharides, serotype-specific OPA responses in the PhtD-dPly groups did not increase from the prevaccination levels (Fig. 6). However, for the PhtD-dPly30/AS02V group, the OPA GMT for serotype 14 was in the same range as GMTs in the 8PCV and 23PPV groups after each dose (data not shown).

**DISCUSSION**

Highly conserved *S. pneumoniae* proteins are being investigated as part of a development plan for new vaccines that offer increased pneumococcal coverage (30). The conserved pneumococcal proteins PhtD and dPly have both been shown in earlier studies to elicit functional antibodies (21,31–35) and provide protection against pneumococcal infection in animal models (21, 26, 35–38). This phase I study evaluated six different experimental formulations of pneumococcal vaccines containing PhtD and dPly as free or conjugated proteins, as well as with AS02V or alum adjuvant. The study was conducted with elderly adults, a population for whom the efficacy of available pneumococcal vaccines is unclear (5–9, 13, 14).

No vaccine-related SAEs were reported during the study, and all vaccine formulations were well tolerated. Pain was the most common solicited local AE in all groups, and fatigue and myalgia were the most common general AEs. There were few reports of grade-3 general AEs, with no reports of fever of >39.5°C. In the groups that received vaccines containing the AS02V adjuvant system, there was a trend toward a higher incidence of solicited local (any and grade 3) AEs and of specific solicited general AEs (fatigue and myalgia) compared to with the groups that received vaccines containing alum or the control 23PPV vaccine. Previous studies of adults ≥65 years of age and younger adults administered PhtD vaccines reported a tendency toward higher reactogenicity with AS02V- than with alum-containing formulations (27). An increase in reactogenicity was also observed in a study of a vaccine containing PhtD, dPly, and protein D administered with the AS03 adjuvant system compared to with antigen alone (28).

Humoral immune responses were demonstrated to the pneumococcal proteins in the groups administered vaccines containing free nonconjugated PhtD and dPly proteins, with the greatest increases in antibody GMCs with higher protein doses and the AS02V adjuvant system. The observation of enhanced IgG responses with AS02V compared to with the alum adjuvant was consistent with studies of other vaccines using MPL- and QS-21-containing adjuvant systems (39), including the previous study on

---

**TABLE 2** Percentage of subjects reporting unsolicited adverse events during the 31-day postvaccination period (total vaccinated cohort)

| AE group                                      | % (95% confidence interval) of subjects reporting unsolicited AEs for the indicated vaccination group (n) |
|-----------------------------------------------|---------------------------------------------------------------------------------------------------|
| Any unsolicited AE                            | 43.5 (23.2–65.5) 50.0 (29.1–70.9) 37.5 (18.8–59.4) 41.7 (22.1–63.4) 45.8 (25.6–67.2) 52.2 (30.6–73.2) 52.0 (31.3–72.2) |
| Grade 3 unsolicited AE                        | 13.0 (2.8–33.6) 8.3 (1.0–27.0) 8.3 (1.0–27.0) 8.3 (1.0–27.0) 16.7 (4.7–37.4) 17.4 (5.0–38.6) 4.0 (0.1–20.4) |
| Unsolicited AE related to vaccination         | 4.3 (0.1–21.9) 12.5 (2.7–32.4) 20.4 (7.1–42.2) 4.2 (0.1–21.1) 29.2 (12.6–51.1) 13.0 (2.8–33.6) 4.0 (0.1–20.4) |

---

**FIG 5** Anti-Ply (A) and anti-PhtD (B) antibody geometric mean concentrations (GMCs) before and 30 days after the first (day 30) and second (day 90) vaccine doses (ATP cohort for immunogenicity).
PhtD vaccination in elderly adults (27). Anti-PhtD and anti-Ply antibody responses were also observed in the groups administered 8PCV, which contained 22 μg PhtD and 15 μg dPly as protein conjugates for two serotypes. Depending on the adjuvant used, the effects of the adjuvant on dPly and PhtD seemed to vary depending on whether these proteins were conjugated to polysaccharide. The immune responses to dPly and PhtD in the 8PCV/alum group were comparable to those following 10 μg dPly-PhtD or in between 10 μg and 30 μg dPly-PhtD vaccination. Considering that the doses of Ply and PhtD in 8PCV (15 μg and 22 μg) were between the doses in the dPly-PhtD free-protein formulations (10 μg and 30 μg), this suggests that in the context of an alum-adjuvanted formulation, dPly and PhtD have similar immunogenicity potentials when conjugated to polysaccharide. However, in context of an AS02V formulation, the effect of adjuvantation seemed to differ depending on whether dPly and PhtD were conjugated to polysaccharide: the free-protein formulations (10 μg and 30 μg dPly-PhtD combined with AS02V) induced higher antibody concentrations than immunization with dPly and PhtD conjugated to polysaccharide (8PCV combined with AS02V). Similarly, the increase in GMC post-dose 2 compared to post-dose 1 seemed to be less pronounced when dPly and PhtD were conjugated to polysaccharide in the presence of AS02V compared to those with free proteins adjuvanted with AS02V.

The functional antibody responses induced by 8PCV in terms of the OPA titers against each of the pneumococcal conjugated polysaccharides were comparable to or higher than the responses elicited with 23PPV containing high-dose plain polysaccharides. This was consistent with the OPA responses reported 1 month after a single dose of the 13-valent PCV or PPV23 administered to adults ≥70 years of age (14). For most groups and serotypes, OPA responses were not detected with the free-protein formulations; however, for serotype 14, some OPA response above baseline seemed to occur with the high-dose free-protein AS02V formulation. The tendency toward higher OPA responses against pneumococcal serotypes with 8PCV/AS02V versus 23PPV might therefore have been due to not only an anti-polysaccharide response but also to some synergy between anti-protein and anti-polysaccharide responses.

In the 8PCV formulations, new conjugates (serotypes 19A and 22F) were evaluated that used pneumococcal proteins as carriers. Both conjugates were shown to be immunogenic, as demonstrated by increased OPA GMTs after immunization. However, the OPA titers obtained for each serotype showed no consistent adjuvant effect. The reasons why AS02V adjuvant did not improve the antibody responses of the 8PCV formulation to polysaccharides or to the carrier proteins remain unclear, although the sample size (~25 subjects per group) may be too small to observe a clear difference. This descriptive study was not designed or powered to detect this kind of effect. The measured OPA response seemed to be driven primarily by the polysaccharide component of the conjugate, as low or no OPA responses were detected with the formulations containing dPly and PhtD only (the free-protein formulations). While the study design does not allow definitive conclusions to be drawn on whether the conjugation of 19A and 22F to dPly and PhtD, respectively, increased the immunogenicity of the polysaccharide, the results of this study suggest such an effect. Indeed, the 8PCV formulations containing 5 μg of 19A and 22F polysaccharide per dose tended to induce higher OPA GMTs after immunization than those with 23PPV, which contains 25 μg of polysaccharide per dose. For serotype 19A, the OPA GMT was significantly higher with 8PCV/alum than with 23PPV, while for serotype 22F, the OPA GMT was significantly higher with 8PCV/AS02V. There was no decrease in the OPA GMT for serotype 22F in the 8PCV/AS02V group from post-dose 1 to post-
dose 2, while slight decreases were observed in the 8PCV/alum and 23PPV groups. For all other serotypes in each group, OPA GMTs post-dose 2 tended to be lower than those post-dose 1, apart from the OPA GMT for serotype 18C (conjugated to tetanus toxoid) in the 8PCV/AS02a group, which showed a slight increase after the second vaccine dose. This indicates that a single dose of 8PCV is sufficient for an OPA GMT response since, with the immunization schedule tested, a second dose did not generally increase the response.

The limitations of this study include its small sample size, and the elimination of subjects who were administered a vaccine exposed to a temperature deviation reduced the ATP immunogenicity cohort size further. However, as the primary objective of the study was to assess the safety of the vaccine formulations, immunogenicity analyses were descriptive, with limited statistical significance. The ability to make firm conclusions on immune responses induced by PhdT and dPly proteins or 8PCV serotypes is therefore restricted. Another limitation is the multiplicity of antigens in the formulations, which does not allow definitive conclusions to be drawn on the roles of individual components. Also, the study only examined the short-term safety and reactogenicity profiles of the vaccine formulations.

In this study, vaccine formulations containing free or conjugated PhdT and dPly proteins had acceptable reactogenicity profiles, and no safety concerns were raised when they were administered to elderly adults. Two vaccine doses induced immune responses against both proteins, with the highest responses with the free-protein formulation containing 30 μg PhdT-dPly and the AS02a adjuvant system. Functional antibody OPA responses against pneumococcal serotypes 19A and 22F were observed with 8PCV and were similar to those of or higher than those of the control 23PPV. These results suggest that PhdT and dPly proteins might be used in future vaccine formulations as carrier proteins for polysaccharides. The study indicates that the inclusion of the AS02a adjuvant system enhanced immune responses against free PhdT and dPly proteins but did not consistently enhance responses to conjugated proteins or polysaccharide compared to with the alum adjuvant. These results will help guide the development of the next generation of pneumococcal vaccines.

ACKNOWLEDGMENTS

GlaxoSmithKline Biologicals S.A. was the funding source and was involved in all stages of study conduct and analysis. GlaxoSmithKline Biologicals S.A. also funded all costs associated with the development and the publishing of this article. All authors had full access to the data, and the corresponding author was responsible for submitting the publication.

We thank all trial participants, as well as the clinicians, nurses, and laboratory technicians at the study centers, for their contributions. We thank principal investigator Hans Holmberg for his contribution to this study. We also thank Anne Dhoest (independent medical writer) for writing the clinical report, Julie Salize for study coordination, Nathalie De Schrevel for Luminex analyses, and Dominique Wauters for OPA analyses (all employed by GSK Vaccines), as well as Lisa Allamassey (Keyrus on behalf of GSK Vaccines) for performing the statistical analysis, Wouter Houthoofd and Shirin Khalili (XPE Pharma & Science on behalf of GSK) for publication management, and Joanne Knowles (independent medical writer) for initial drafting of the manuscript and the incorporation of comments received from the authors on behalf of the GSK group of companies. M.C., T.G.P., P.V.B., V.V., and P.E.V. are employees of the GSK group of companies; V.V. and P.E.V. own GSK stock and stock options. J.T.P. and P.G.V. were employees of the GSK group of companies during the time of the study. K.P. and A.C.N. received funding from GlaxoSmithKline Biologicals S.A. to perform the clinical trial reported here, and K.P. also received funding from Pfizer to perform clinical trials outside this work.

REFERENCES

1. Muhammad RD, Oza-Frank R, Zell E, Link-Gelles R, Narayan KM, Schaffner W, Thomas A, Lexau C, Bennett NM, Farley MM, Harrison LH, Reingold A, Hadler J, Beall B, Klugman KP, Moore MR. 2013. Epidemiology of invasive pneumococcal disease among high-risk adults since the introduction of pneumococcal conjugate vaccine for children. Clin. Infect. Dis. 56:e59–e67. http://dx.doi.org/10.1093/cid/cis971.

2. Westerink MA, Schroeder HW, Jr, Nahm MH. 2012. Immune responses to pneumococcal vaccines in children and adults: rationale for age-specific vaccination. Aging Dis. 3:51–67.

3. Welte T, Torres A, Nathwani D. 2012. Clinical and economic burden of community-acquired pneumonia among adults in Europe. Thorax 67:71–79. http://dx.doi.org/10.1136/thx.2009.129502.

4. Wroe PC, Finkelstein JA, Ray GT, Linder JA, Johnson KM, Rifas-Shiman S, Moore MR, Huang SS. 2012. Aging population and future burden of pneumococcal pneumonia in the United States. J. Infect. Dis. 206:1179–1182. https://doi.org/10.1093/infdis/jis212.

5. Jackson LA, Neuzil KM, Yu O, Benson P, Barlow WE, Adams AL, Hanson CA, Mahoney LD, Shay DK, Thompson WW, Vaccine Safety Datalink. 2003. Effectiveness of pneumococcal polysaccharide vaccine in older adults. N. Engl. J. Med. 348:1747–1755. http://dx.doi.org/10.1056/NEJMoa022678.

6. Mobberley SA, Holden J, Tatham DP, Andrews RM. 2008. Vaccines for preventing pneumococcal infection in adults. Cochrane Database Syst. Rev. (1): CD000422. http://dx.doi.org/10.1002/14651858.CD000422.pub2.

7. Huss A, Scott P, Stuck AE, Trotter C, Egger M. 2009. Efficacy of pneumococcal vaccination in adults: a meta-analysis. CMAJ 180:48–58. http://dx.doi.org/10.1503/cmaj.080734.

8. Mushker DM. 2012. Editorial commentary: should 13-valent protein-conjugate pneumococcal vaccine be used routinely in adults? Clin. Infect. Dis. 55:265–267. http://dx.doi.org/10.1093/cid/cis644.

9. Ridda I, Mushker DM. 2012. Is there a potential role for protein-conjugate pneumococcal vaccine in older adults? Australs. Med. J. 5:231–235. http://dx.doi.org/10.4066/AMJ.2012.1160.

10. World Health Organization. 2008. 23-valent pneumococcal polysaccharide vaccine. WHO position paper. Wkly. Epidemiol. Rec. 83:373–384.

11. Clutterbuck EA, Lazarus R, Yu LM, Bowman J, Bateman EA, Diggle L, Angus B, Peto TE, Beverley PC, Mant D, Pollard AJ. 2012. Pneumococcal conjugate and plain polysaccharide vaccines have divergent effects on antigen-specific B cells. J. Infect. Dis. 205:1408–1416. http://dx.doi.org/10.1093/infdis/ijs212.

12. Hak E, Grobbee DE, Sanders EA, Verheij TJ, Bolkenbaas M, Huijts SM, Gruber WC, Tansey S, McDonough A, Thoma B, Patterson S, van Alphen AJ, Bonten MJ. 2008. Rationale and design of CAPITA: a RCT of 13-valent conjugated pneumococcal vaccine efficacy among older adults. Neth. J. Med. 66:378–383.

13. Vila-Corcoles A, Ochoa-Gondar O. 2013. Preventing pneumococcal disease in the elderly: recent advances in vaccines and implications for clinical practice. Drugs Aging. http://dx.doi.org/10.1007/s40266-013-0060-5.

14. Centers for Disease Control and Prevention. 2012. Licensure of 13-valent pneumococcal conjugate vaccine for adults aged 50 years and older. MMWR Morb. Mortal. Wkly. Rep. 61:394–395.

15. Scott JA, Hall AJ, Dagan R, Dixon JM, Eyken SJ, Fenoll A, Hortal M, Jette LP, Jorgensen JH, Lamonte F, Latorre C, Macfarlane JT, Shlaes DM, Smart LE, Taunay A. 1996. Serogroup-specific epidemiology of Streptococcus pneumoniae: associations with age, sex, and geography. Clin. Infect. Dis. 22:973–981. http://dx.doi.org/10.1093/clinids/22.6.973.

16. Lipsitch M, Whitney CG, Zell E, Kajjalainen T, Dagan R, Malley R. 2005. Are anticapsular antibodies the primary mechanism of protection against invasive pneumococcal disease? PLoS Med. 2:e15. http://dx.doi.org/10.1371/journal.pmed.0020015.

17. Malley R. 2010. Antibody and cell-mediated immunity to Streptococcus pneumoniae: implications for vaccine development. J. Mol. Med. (Berl.) 88:135–142. http://dx.doi.org/10.1007/s00109-009-0579-4.

18. Moffitt KL, Malley R. 2011. Next generation pneumococcal vaccines.

Pneumococcal Vaccines with PhdT and dPly Proteins

May 2014 Volume 21 Number 5 cvlasm.org 659

Downloaded from http://cvl.asm.org on July 19, 2018 by guest
26. Denoël P, Anderson PW. 2012. Serotype-independent pneumococcal experimental vaccines that induce cellular as well as humoral immunity. Proc. Natl. Acad. Sci. U. S. A. 109:3623–3627. http://dx.doi.org/10.1073/pnas.1121383109.

27. Plump tér CD, Ogunti y AD, Paton JC. 2012. Polyhistidine triad proteins of pathogenic streptococci. Trends Microbiol. 20:485–493. http://dx.doi.org/10.1016/j.tim.2012.06.004.

28. Godfroid F, Hermand P, Verlant V, Denoël P, Poolman JT. 2011. Preclinical evaluation of the Ph protein as potential cross-protective pneumococcal vaccine antigens. Infect. Immun. 79:238–245. http://dx.doi.org/10.1128/IAI.00378-10.

29. Marriott HM, Mitchell TJ, Dockrell DH. 2008. Pneumolysin: a double-edged sword during the host-pathogen interaction. Curr. Mol. Med. 8:497–509. http://dx.doi.org/10.2174/156652408785747924.

30. Lucas R, Czikora I, Sridhar S, Zemskov E, Gorshkov B, Siddaramappa U, Osęgale A, Lawson J, Verin A, Rick FG, Block NL, Pillich H, Romero M, Leustik M, Chakraborty T. 2013. Mini-review: novel therapeutic strategies to blunt actions of pneumolysin in the lungs. Toxins. (Basel) 5:1244–1260. http://dx.doi.org/10.3390/toxins5071244.

31. Garçon N, Leroux-Roels G, Cheng W. 2011. Vaccine adjuvants, p 89–113. In Garçon N, Cunningham AL, Stanbery LR (ed), Understanding modern vaccines: perspectives in vaccinology. Elsevier BV, Amsterdam, The Netherlands.

32. Vandenbroucke P, Horsmans Y, Morris P, Van Mechelen M, Janssens M, Koutousoúkou M, Van Belle P, Clement F, Hanon E, Wettendorff M, Garçon N, Leroux-Roels G. 2008. Vaccine adjuvant systems containing monophosphoryl lipid A and QS21 induce strong and persistent humoral and T cell responses against hepatitis B surface antigen in healthy adult volunteers. Vaccine 26:1375–1386. http://dx.doi.org/10.1016/j.vaccine.2007.12.038.

33. Denoël P, Philipp MT, Doyle L, Martin D, Carletti G, Poolman JT. 2011. A protein-based pneumococcal vaccine protects rhesus macaques from pneumonia after experimental infection with Streptococcus pneumoniae. Vaccine 29:5495–5501. http://dx.doi.org/10.1016/j.vaccine.2011.05.051.

34. Leroux-Roels I, Devaster JM, Leroux-Roels G, Verlant V, Henckaerts I, Moris P, Hermand P, Van Belle P, Poolman JT, Vandepapelière P, Horsmans Y. 2013. Adjuvant system AS02, enhances humoral and cellular immune responses to pneumococcal protein PhdT vaccine in healthy young and older adults: randomised, controlled trials. Vaccine, in press. http://dx.doi.org/10.1016/j.vaccine.2013.10.052.

35. Berglund J, Vink P, Tavares Da Silva F, Lestrade P, Bouteiraux D. 2014. Safety, immunogenicity and antibody persistence following an investigational Streptococcus pneumoniae and Haemophilus influenzae tri-protein vaccine in a phase 1 randomized controlled study in healthy adults. Clin. Vaccine Immunol. 21:56–65. http://dx.doi.org/10.1128/CVI.00430-13.

36. Romero-Steiner S, Libotti D, Pais LB, Dykes J, Anderson P, Whitin JC, Keyserling HL, Carlone GM. 1997. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against Streptococcus pneumoniae using differentiated HL-60 cells. Clin. Diagn. Lab. Immunol. 4:415–422.

37. Bors D, Hausdorff W, Fierens F, Godfroid F, Verlant V. 2012. Progress towards development of a protein-based pneumococcal vaccine. p.27. 8th International Symposium on Pneumococci and Pneumococcal Diseases, Iguacu Falls, Brazil, 11 to 15 March 2012.

38. Adamou JE, Heinrichs JH, Erwin AL, Walsh W, Gayle T, Dormitzer M, Dagan R, Brehaw YA, Barren P, Lathigra R, Langermann S, Koenig S, Johnson S. 2001. Identification and characterization of a novel family of pneumococcal proteins that are protective against sepsis. Infect. Immun. 69:949–958. http://dx.doi.org/10.1128/IAI.69.2.949-958.2001.

39. Khan MN, Pichichero ME. 2012. Vaccine candidates PhdT and PhE of Streptococcus pneumoniae are adhesins that elicit functional antibodies in humans. Vaccine 30:2900–2907. http://dx.doi.org/10.1016/j.vaccine.2012.02.023.

40. Kirkham LA, Kerr AR, Douce GR, Paterson GK, Dilts DA, Liu DF, Mitchell TJ. 2006. Construction and immunological characterization of a novel nontoxic protective pneumolysin mutant for use in future pneumococcal vaccines. Infect. Immun. 74:586–593. http://dx.doi.org/10.1128/IAI.74.2.586-593.2006.

41. Kametchou T, Bologa M, Hopfer R, Neveu D, Hu B, Sheng X, Corde N, Pouzet C, Zimmermann G, Gurunathan S. 2013. Safety and immunogenicity of the pneumococcal pneumolysin derivative PlyD1 in a single-antigen protein vaccine candidate in adults. Vaccine 31:327–333. http://dx.doi.org/10.1016/j.vaccine.2012.11.005.

42. Salha D, Szeto J, Myers L, Claus C, Sheung A, Tang M, Lijutic B, Hanwell D, Ogilvie K, Ming M, Messham B, van den Dobbelsteen G, Hopfer R, Ochs MM, Gallichan S. 2012. Neutralizing antibodies elicited by a novel detoxified pneumolysin derivative, PlyD1, provide protection against both pneumococcal infection and lung injury. Infect. Immun. 80:2212–2220. http://dx.doi.org/10.1128/IAI.06348-11.

43. Alexander JE, Lock RA, Peeters CG, Poolman JT, Andrew PW, Mitchell TJ, Hansman D, Paton JC. 1994. Immunization of mice with pneumolysin toxoid confers a significant degree of protection against at least nine serotypes of Streptococcus pneumoniae. Infect. Immun. 62:5683–5688.

44. Paton JC, Lock RA, Lee CJ, Li JP, Berry AM, Mitchell TJ, Andrew PW, Hansman D, Boulnois GJ. 1991. Purification and immunogenicity of genetically obtained pneumolysin toxoids and their conjugation to Streptococcus pneumoniae type 19F polysaccharide. Infect. Immun. 59:2297–2304.

45. Ogunti y AD, Woodrow MC, Poolman JT, Paton JC. 2001. Protection against Streptococcus pneumoniae elicited by immunization with pneumolysin and CbpA. Infect. Immun. 69:5997–6003. http://dx.doi.org/10.1128/IAI.69.9.5997-6003.2001.

46. Garçon N, Van Mechelen M. 2011. Recent clinical experience with vaccines using MPL- and QS-21-containing adjuvant systems. Expert Rev. Vaccines 10:471–486. http://dx.doi.org/10.1586/erv.11.29.