MAJOR ARTICLE

Revaccination with a 23-Valent Pneumococcal Polysaccharide Vaccine Induces Elevated and Persistent Functional Antibody Responses in Adults Aged ⩾65 Years

Susan B. Manoff,[1] Charles Liss,[1] Michael J. Caulfield,[1] Rocio D. Marchese,[1] Jeffrey Silber,[1] John Boslego,[1,a] Sandra Romero-Steiner,[2] Gowrisankar Rajam,[2] Nina E. Glass,[2,a] Cynthia G. Whitney,[2] and George M. Carlone[2]

[1]Merck Research Laboratories, North Wales, Pennsylvania; and [2]Centers for Disease Control and Prevention, Atlanta, Georgia

Background. Older adults are at high risk of developing invasive pneumococcal disease, but the optimal timing and number of vaccine doses needed to prevent disease among this group are unknown. We compared revaccination with 23-valent pneumococcal polysaccharide vaccine (PN23) with primary vaccination for eliciting initial and persistent functional antibody responses.

Methods. Subjects aged ⩾65 years were enrolled. Functional (opsonic) and total immunoglobulin (Ig) G antibody levels were measured following either PN23 primary vaccination ($n = 60$) or revaccination 3–5 years after receiving a first PN23 vaccination ($n = 60$). Antibody against vaccine serotypes 4, 14, and 23F was measured at prevaccination (day 0), 30 days after vaccination, and 5 years after vaccination.

Results. By day 30, both primary vaccination and revaccination induced significant increases in opsonic and IgG antibody levels. Day 30 levels following revaccination were slightly lower but not significantly different than those after primary vaccination. Year 5 levels were similar in both groups and remained significantly higher than prevaccination levels for primary vaccination subjects. There was good agreement between postvaccination opsonic and IgG antibody levels.

Conclusions. Revaccination of older adults with PN23 was comparable to primary vaccination for inducing elevated and persistent functional and IgG antibody responses.

Streptococcus pneumoniae remains a leading cause of adult disease. In 2007, morbidity and mortality rates of invasive pneumococcal disease (IPD) in US adults aged ⩾65 years were 39.3 cases and 6.4 deaths per 100,000, respectively [1].

Pneumovax 23 (PN23; Merck) is comprised of the capsular polysaccharides of 23 *S. pneumoniae* serotypes. PN23 has been shown to be effective against IPD in several epidemiological and clinical studies [2–7] and to significantly reduce the mortality rate, length of stay, and rate of intensive care admissions among adults hospitalized with community-acquired pneumonia [8, 9]. PN23 serotypes account for a large proportion of penicillin-resistant IPD and represented 88% of IPD identified by a United States–based surveillance program in 1998 [10].

In the United States, the Advisory Committee on Immunization Practices (ACIP) recommends PN23 vacci-

Received 19 May 2009; accepted 25 September 2009; electronically published 20 January 2010.

[a] Present affiliations: PATH, Washington, DC (J.B.); Vanderbilt School of Medicine, Nashville, Tennessee (N.E.G.).

Reprints or correspondence: Dr Susan Manoff, Merck Research Laboratories, Merck, UG3CD-28, PO Box 1000, North Wales, PA 19454-1099 (susan_manoff@merck.com).

The Journal of Infectious Diseases 2010; 201:525–33

0022-1899/2010/20104-0009$15.00
DOI: 10.1086/651131

Potential conflicts of interest: Authors affiliated with Merck may potentially own stock and/or hold stock options in the company. Although this research was funded in part by a Collaborative Research Agreement (CID-04-M184-00) between Merck and the Centers for Disease Control and Prevention for the evaluation of opsonophagocytic activity, the government researchers are reporting the findings of the research without any conflict of interest or endorsement of any vaccine product.

Presented in part: the 5th International Symposium on Pneumococci and Pneumococcal Diseases, 2–6 April 2006, Alice Springs, Australia (abstract P010.10); the 12th International Congress on Infectious Diseases, Lisbon, Portugal, 15–18 June 2006 (abstract 41.032 and 53.002); the Infections Control World Organization: Vaccines, Immunization and Immunotherapy 5th World Congress (ICWO), Montreal, Canada, 6–9 November 2006; and the 6th International Symposium on Pneumococci and Pneumococcal Diseases, Reykjavik, Iceland, 6–8 June 2008 (abstract P3-145).

Financial support: Studies were supported by Merck.

nation for all adults aged ⩾65 years and for individuals aged ⩾2 years with risk factors for pneumococcal disease [11]. The ACIP recommends a single revaccination for persons with asplenia or immunocompromising conditions and for those aged ⩾65 years who have not received the vaccine within 5 years and were first vaccinated before the age of 65 years [11]. The ACIP does not currently recommend routine revaccination for the general population, due to a lack of published information regarding duration of protection and studies suggesting that persons may not respond as well to revaccination [12, 13].

Studies of the immune response following revaccination have been limited by small sample size or by the use of older, imprecise assays; none have evaluated functional (opsonic) antibody. This study compared the opsonic and total immunoglobulin (Ig) G antibody levels after PN23 primary vaccination to levels following revaccination, and assessed the correlation of opsonic and total IgG antibody at the postvaccination time points. Immune responses to three serotypes with a high disease prevalence were evaluated in adults aged ⩾65 years. This age group provided a conservative assessment of immunogenicity and represents the population most likely to be revaccinated.

METHODS

Study design. This report is an analysis of the antibody responses of a subset of subjects who enrolled into a study evaluating the safety and immunogenicity of primary vaccination and revaccination with PN23, as described elsewhere [14]. A total of 1008 subjects in 2 age groups (50–64 years and ⩾65 years) were enrolled in 1997–1998 and either (1) received their first vaccination with PN23 (primary vaccination group) or (2) had received documented PN23 vaccination 3–5 years previously and were revaccinated with PN23 (revaccination group). Eligible subjects were ambulatory, and any underlying chronic illnesses were in stable condition. Exclusion criteria for enrollment included immunosuppression, and a history of IPD. At baseline, data were collected to ascertain whether subjects had preexisting chronic cardiovascular disease, chronic pulmonary disease, or diabetes mellitus. All subjects received PN23.

This substudy was conducted to evaluate opsonic antibody responses to serotypes 4, 14, and 23F. These serotypes were selected as they are among the most common serotypes causing IPD in adults [15] and had a qualified opsonophagocytic killing (OPK) assay for evaluating opsonic antibody. Moreover, serotypes 14 and 23F are among those most commonly associated with penicillin resistance [10]. Serum samples were analyzed at 3 time points: day 0 (baseline), day 30 after vaccination, and year 5 after vaccination (to assess the initial and long-term antibody responses, respectively).

A subset of 120 participants (60 each in the primary vaccination and revaccination groups) was randomly selected from among the overall study subjects aged ⩾65 years who received PN23 on day 0 and who had serum from all substudy time points. Antibody responses to the 3 serotypes were measured in 2 assay formats: (1) an OPK assay to measure opsonic antibody, and (2) an enzyme immunoassay (EIA) to detect IgG antibody. The substudy utilized the same EIA used for the overall protocol [14]. Serum samples for the three time points were tested concurrently in both assay formats.

Laboratory methods. All serum samples were blinded to the laboratory staff. OPK assays were performed at the Centers for Disease Control and Prevention (CDC; Atlanta, GA) using a standardized assay [16] with serum that was not heat inactivated prior to assay. Titers are defined as the reciprocal of the highest serum dilution that results in ⩾50% killing of *S. pneumoniae* serotypes 4, 14, and 23F. The lower limit of detection for a positive OPK titer was 8. For the purpose of calculating GMTs, titers <8 were assigned a value of 4.

EIAs used to quantify the immune response to pneumococcal vaccines have undergone many refinements over the past 20 years. The current consensus "guidance protocol" for the EIA, which was accepted by the World Health Organization (WHO) in 2000, includes the use of 2 preabsorbents: pneumococcal cell wall polysaccharide (CPS) and pneumococcal polysaccharide (PS) from serotype 22F. These 2 preabsorbents are used to improve specificity by minimizing nonspecific signals that arise from antibodies directed against bacterial common antigens [17, 18]. Pneumococcal PS 22F was chosen for use in the "guidance protocol" since it is available through American Type Cell Culture and not likely to be included in future vaccines. Although this assay format was adopted by experts at a meeting held in 2000 at the WHO (Geneva, Switzerland), the group acknowledged that different assay protocols may be acceptable but it would be useful to select one well-characterized assay to provide guidance to new laboratories [17]. The EIA validated in 1999 by Merck [19] was developed prior to the "guidance protocol" and is consistent with the "Pneumococcal PS 22F" assay format in that C-polysaccharide and heterologous capsular polysaccharides 25 and 72 are used as absorbents. Merck chose different preabsorbents because PS 22F is a component of PN23, whereas PS 25 and 72 are not. Both EIA assays measure IgG antibody concentrations in μg/mL. A study comparing the Merck and "Pneumococcal PS 22F" assays showed moderate agreement between the formats [19].

Statistical analysis. Summary statistics (*n,* mean, 95% confidence intervals, and probability values) were employed to characterize the OPK and EIA responses by study group, serotype, and time period. Opsonic titers and IgG concentrations were transformed using natural logarithms prior to analysis. Confidence intervals for the OPK geometric mean titers (GMTs) and EIA geometric mean concentrations (GMCs) were

Table 1. Day 0, Day 30, and Year 5 Serotype-Specific Functional Antibody Geometric Mean Titers (GMTs; as Measured by Opsonophagocytic Killing [OPK]) and Total Immunoglobulin (Ig) G Geometric Mean Concentrations (GMCs; as Measured by Enzyme Immunoassay), for Subjects Aged ⩾65 Years after Primary Vaccination or Revaccination with 23-Valent Pneumococcal Polysaccharide Vaccine on Day 0

	Day 0			Day 30			Year 5		
Measure, serotype	Revaccination (n = 60)	Primary vaccination (n = 60)	P[a]	Revaccination (n = 58)	Primary vaccination (n = 60)	P[a]	Revaccination (n = 57)	Primary vaccination (n = 60)	P[a]
OPK GMT (1/dilution)									
Serotype 4	10.2	5.9	.051	34.0[b]	47.4[b]	.468	40.3[b]	37.2[b]	.781
Serotype 14	300.9	56.4	<.001[c]	570.1[b]	1072.4[b]	.040[c]	299.8	265.0[b]	.723
Serotype 23F	39.4	13.6	.006[c]	135.9[b]	217.7[b]	.322	161.3[b]	161.3[b]	>.99
EIA GMC, μg/mL									
Serotype 4	1.4	0.4	<.001[c]	2.6[b]	3.9[b]	.107	1.6	1.6[b]	.935
Serotype 14	7.5	2.8	<.001[c]	10.2[b]	14.7[b]	.060	6.6[b]	6.6[b]	.998
Serotype 23F	2.7	0.9	<.001[c]	4.4[b]	4.6[b]	.859	2.6	2.1[b]	.402

NOTE. n Values denote the no. of subjects with evaluable serologic test results at given time point (per protocol).

[a] P values are for the comparisons of the primary and revaccination groups.

[b] Statistically significantly different from day 0 within treatment group ($P < .05$).

[c] Statistically significant difference between primary and revaccination groups ($P < .05$).

calculated assuming a normal distribution of the natural logarithm transformed data. To compare subjects receiving revaccination versus subjects receiving primary vaccination with respect to OPK titers and IgG concentrations at each time period, a geometric mean (GM) ratio and probability value employing the t distribution were calculated [20]. OPK and EIA antibody distributions were described at each time point using reverse cumulative distribution (RCD) plots. A linear regression model was fit to quantify the agreement between the OPK and IgG levels.

Ethical conduct. The study protocol, amendments, and informed consents were reviewed and approved by an institutional review board at each investigative site and written informed consent was obtained from each participant before enrollment. Substudy activities were approved by an institutional review board at the CDC.

RESULTS

Characteristics of the substudy subjects. The substudy included 120 subjects, 60 each in the revaccination and primary vaccination groups. The groups were similar by gender (overall, 48% male and 52% female), race/ethnicity (the majority of subjects were white), and age (mean age, approximately 71 years for both groups; range, 65–84 years for the revaccination group and 65–88 years for the primary vaccination group). The groups were also comparable by the baseline prevalence of chronic diseases. Among revaccination and primary vaccination subjects, chronic cardiovascular disease was reported by 47% and 53%, chronic pulmonary disease by 17% and 12%, and diabetes mellitus by 3% and 7%, respectively; at least 1 of these conditions was reported by 60% of both groups. The groups differed by the percentage of subjects who had ever smoked (58% in the revaccination group, compared with 45% in the primary vaccination group).

The substudy groups were similar to the overall study groups by demographic characteristics. Slight differences were observed between the substudy and the overall study groups in baseline health conditions; at least 1 such condition was reported in the overall revaccination and primary vaccination groups by 71% and 65%, respectively. Slight differences were also observed between the substudy and overall study groups in ever smoking at baseline, which was reported in the overall revaccination and primary vaccination study groups by 53% and 54%, respectively [14].

Response patterns of serotype-specific functional (OPK) and IgG (EIA) antibody. Table 1 contains the substudy GMs of OPK and EIA levels at day 0, day 30, and year 5 for serotypes 4, 14, and 23F. Data (with 95% confidence intervals) are presented graphically in Figure 1. The findings were consistent for all 3 serotypes. The baseline OPK and EIA values were higher for the revaccination group than for the primary vaccination group; in the majority of cases, the 95% confidence intervals around the baseline values did not overlap. Following both revaccination and primary vaccination, the OPK and EIA levels significantly increased from day 0 to day 30. The day 30 OPK and EIA GM levels and mean-fold rises from day 0 (data not shown) were slightly lower for revaccination subjects than for primary vaccination subjects. There was a statistically significant reciprocal relationship between prevaccination OPK and

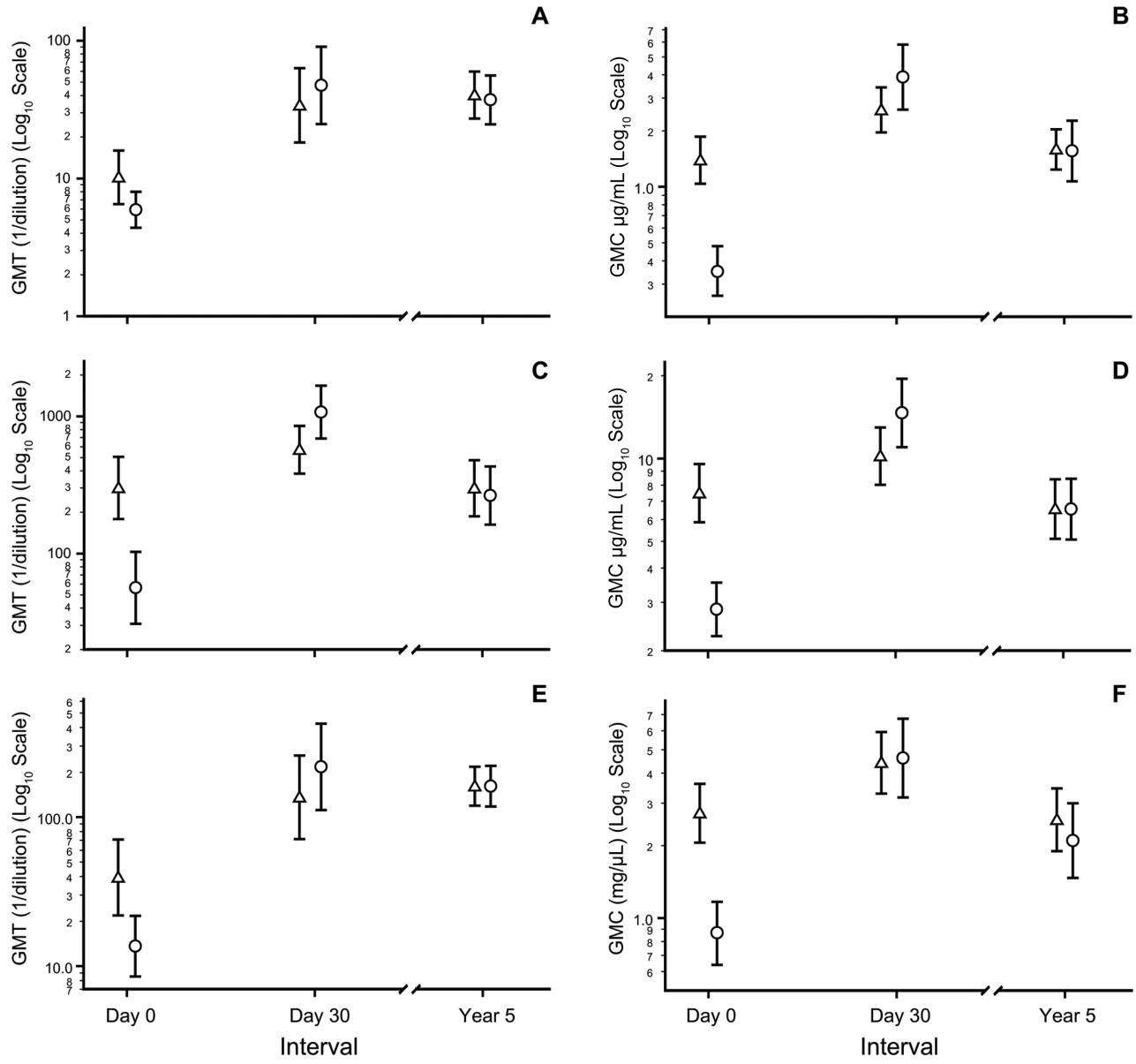

Figure 1. Geometric mean concentrations (GMCs) and titers and 95% confidence intervals as measured by opsonophagocytic killing *(A, C,* and *E)* and enzyme immunoassay *(B, D,* and *F)* for subjects aged ⩾65 years receiving primary vaccination *(circles)* and revaccination *(triangles)* for serotypes 4 *(A* and *B),* 14 *(C* and *D),* and 23F *(E* and *F).*

EIA levels and postvaccination responses in both substudy groups; higher day 30 levels correlated with lower prevaccination concentrations (data not shown). The 95% confidence intervals around the day 30 mean values for OPK and EIA antibody overlapped for the revaccination and primary vaccination groups. Importantly, at year 5, there were no differences between the revaccination and primary vaccination groups in either OPK or EIA levels. Moreover, year 5 responses for the revaccination and primary vaccination groups were significantly higher (confidence intervals did not overlap) than responses for the primary vaccination subjects at baseline (day 0) when they were vaccine naïve. In the revaccination group, OPK GMTs for serotypes 4 and 23F were significantly higher at year 5 than at baseline. The patterns in EIA levels for the substudy groups were consistent with those observed for the overall study groups (data not shown) [14].

Figure 2 contains the RCD plots of OPK titers for serotypes 4, 14, and 23F. Similar patterns were observed for all 3 serotypes. At day 0, the antibody curves for the revaccination group were shifted to the right of the curves for the primary vaccination group, reflecting the higher baseline antibody levels for the revaccination group. Day 30 curves for the revaccination and

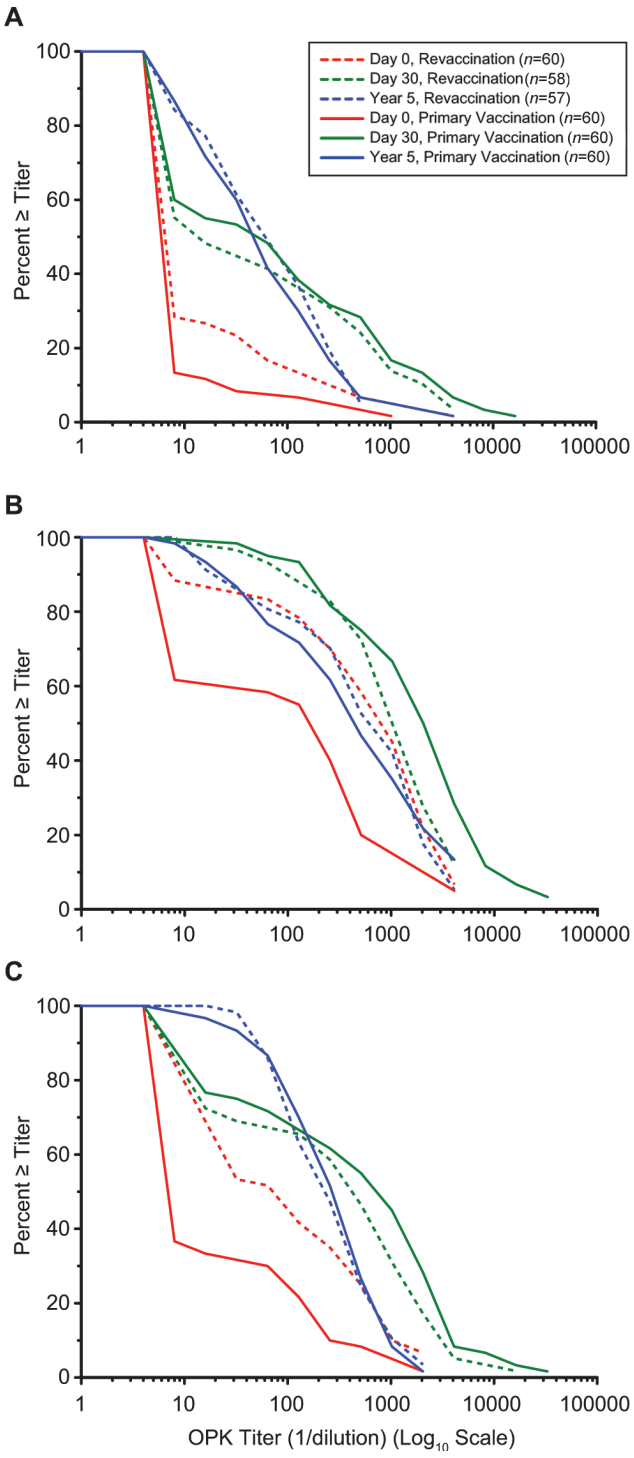

Figure 2. Reverse cumulative distribution plot of opsonophagocytic killing titers at day 0, day 30, and year 5 for primary vaccination and revaccination subjects aged ⩾65 years for serotypes 4 *(A)*, 14 *(B)*, and 23F *(C)*.

primary vaccination groups were shifted to the right of the day 0 values, indicating the increase in opsonic antibody titers from day 0. Day 30 distributions for the revaccination group were shifted to the left of those in the primary vaccination group, although the curves were generally close together. At year 5, OPK antibody distributions nearly overlapped for the revaccination and primary vaccination groups. Moreover, the year 5 levels for both treatment groups remained higher than the levels for unvaccinated subjects (day 0 levels for primary vaccination subjects).

Table 2 shows the percentage of subjects in the two substudy groups with detectable OPK (titer, ⩾8). This level was chosen as a point of reference and does not correspond to a seroprotective level, since such a correlate has not been established. Similar patterns were observed for all 3 serotypes. At baseline, the percentage of subjects with detectable titers was higher for revaccination subjects than for primary vaccination subjects. From day 0 to day 30, this percentage increased in both groups, and was similar for the 2 groups at day 30. From day 30 to year 5, the percentage either remained the same or increased in both groups; at year 5, the percentage was similar in both groups.

Figure 3 contains an RCD plot of IgG antibody concentrations at day 0 and day 30 for serotype 4. At day 0, the antibody curve for the revaccination group was shifted to the right of the curve for the primary vaccination group, reflecting the higher baseline antibody levels for the revaccination group. Both day 30 curves were shifted to the right of the day 0 curves, indicating the increase in antibody levels from day 0. Day 30 curves for the substudy groups overlapped at lower antibody concentrations, but began to diverge at higher antibody concentrations. The primary vaccination group achieved the highest antibody concentrations. Similar IgG antibody distribution patterns were seen with the other 2 serotypes (not shown). For the 3 serotypes assessed, the fold rise in antibody levels from day 0 to day 30 was lower for the revaccination group than for the primary vaccination group (data not shown); this was consistent with the statistically significant reciprocal relationship between prevaccination levels and postvaccination responses described earlier.

Table 3 shows the percentage of subjects at the day 30 time point with serotype-specific antibody (EIA) concentrations of ⩾0.5, ⩾1.0, and ⩾5.0 μg/mL. These levels were chosen as points of reference and do not correspond to a seroprotective level since such a correlate has not been established. The results indicate that at day 30, the percentage of subjects with GMCs ⩾0.5 and ⩾1.0 μg/mL were similar in the revaccination and primary vaccination groups. An analysis of the distribution of antibody responses provides a partial explanation for the moderately lower day 30 GMC values observed for serotypes 4 and 14 in the revaccination groups relative to the primary vaccination subjects. Only at higher levels such as 5.0 μg/mL did the primary vaccination group percentages exceed those of the revaccination group.

When considering all substudy subjects together, there is

Table 2. Percentage of Subjects with Opsonophagocytic Killing (OPK) Titers ⩾8 for Serotypes 4, 14, and 23F at Day 0, Day 30, and Year 5 for Subjects Aged ⩾65 Years after Primary Vaccination or Revaccination with 23-Valent Pneumococcal Polysaccharide Vaccine on Day 0

	Percentage of subjects with OPK titer ⩾8					
	Revaccination			Primary vaccination		
Serotype	Day 0 (n = 60)	Day 30 (n = 58)	Year 5 (n = 57)	Day 0 (n = 60)	Day 30 (n = 60)	Year 5 (n = 60)
4	28	55	84	13	60	87
14	88	97	100	62	98	98
23F	53	72	100	37	77	98

NOTE. Data are percentage of subjects who achieved the stated antibody concentration level. A titer of ⩾8 was chosen as a point of reference and does not necessarily correspond to a seroprotective level, because such a correlate has not been established in adults.

considerable agreement between the initial and long-term responses to vaccination measured by the OPK and IgG assays. For all 3 serotypes, the correlation coefficients for the day 30 levels ranged between 0.54 and 0.70. For all 3 serotypes, the correlation coefficients for the year 5 levels ranged between 0.52 and 0.56. All correlations were statistically significant ($P < .05$).

DISCUSSION

Prior to the widespread use of antibiotics, pneumococcal pneumonia was sometimes treated with passively transferred serotype-specific antisera. This therapy reduced the death rate of pneumococcal pneumonia by ~50% [21, 22]. Early administration was particularly effective. When given within 24 h after the onset of serotype 1 pneumonia, treatment with antisera cleared bacteremia, shortened illness duration, and reduced mortality to 5%, one-sixth of the standard death rate of 33% [22]. These results indicate that functional antibodies provide protection against IPD in humans. *S. pneumoniae* is not susceptible to antibody and complement-mediated lysis due to the thicker peptidoglycan layer present in gram-positive bacteria. Antibody plus complement opsonize the organisms rendering them susceptible to uptake and killing by phagocytic cells. The importance of opsonic antibodies is illustrated in a study by Musher et al [23], who showed that serum obtained from hospitalized patients with bacteremic or nonbacteremic pneumococcal pneumonia contained serotype-specific IgG that was poorly opsonic compared with antibodies from healthy adults.

The present study was conducted to measure, in a subset of subjects enrolled in a larger clinical trial, serotype-specific opsonic and total IgG antibody responses after revaccination or primary vaccination with PN23. The revaccination group, who had been vaccinated 3 to 5 years previously, had significantly higher day 0 OPK and EIA antibody levels than the primary vaccination group, consistent with the persistence of anticapsular antibody following PN23. Thirty days after vaccination, both revaccination and primary vaccination induced statistically significant increases in opsonic and IgG antibody to the serotypes tested. Elevated antibody levels were long-lasting. Five years following both revaccination and primary vaccination, mean OPK and EIA antibody levels had declined from the initial response, but remained significantly higher than levels for the primary-vaccination subjects when they were vaccine naïve.

The magnitude of the day 30 results reported here agree with the postvaccination IgG concentrations and are generally higher than the OPK titers previously reported by Romero-Steiner et al [24] for adults between 63 and 89 years old. The OPK assays for both studies were performed in the same laboratory. The difference in OPK titers may be explained as Romero-Steiner and colleagues studied nursing home residents, who may have been more frail than the ambulatory population enrolled here.

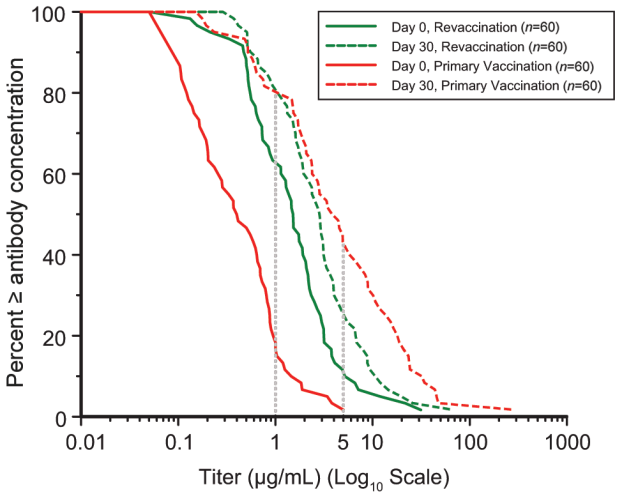

Figure 3. Reverse cumulative distribution plot of immunoglobulin G antibody (enzyme immunoassay) concentrations at day 0 and day 30 for primary and revaccination subjects aged ⩾65 years (serotype 4).

Table 3. Percentage of Subjects (Age, ⩾65 Years) at Day 30 Achieving Serotype-Specific Immunoglobulin G Antibody Concentrations ⩾0.50, ⩾1.00, and ⩾5.00 μg/mL

	Revaccination subjects (n = 60)			Primary vaccination subjects (n = 60)		
Serotype	⩾0.50 μg/mL	⩾1.00 μg/mL	⩾5.00 μg/mL	⩾0.50 μg/mL	⩾1.00 μg/mL	⩾5.00 μg/mL
4	95	80	25	92	80	42
14	100	100	72	100	100	80
23F	98	87	50	93	78	52

NOTE. Data are percentage of subjects who achieved the stated antibody concentration level. These concentration levels were chosen as a point of reference and do not necessarily correspond to a seroprotective level, because such a correlate has not been established in adults.

In the present study, the primary and revaccination groups reached comparable OPK and IgG levels at day 30, even though some of the observed GM values were slightly lower for the revaccination group than for the primary vaccination group (the point estimates for the groups were within 2-fold for the OPK and ~30% for the EIA GMs). The lower levels following revaccination were explained, at least in part, by the higher baseline antibody levels observed in the revaccination group, as higher day 30 levels were correlated with lower prevaccination concentrations. As a result, the fold-rises from baseline to day 30 were lower in the revaccination group than in the primary vaccination group. These results suggest that fold rise is not a good indicator of immune response after revaccination.

The observation of slightly lower day 30 GM antibody levels following revaccination is consistent, in direction and magnitude, with published EIA findings of studies that evaluated the immunogenicity of PN23 and earlier (lower-valent) formulations in adults [12, 13, 25–28]. Unlike these earlier studies, our study compared antibody functionality, distributions, and levels at the initial postvaccination immune response and beyond.

The distributions of OPK and IgG responses reported here reveal that differences in day 30 antibody levels are primarily driven by individuals with responses that exceed higher antibody levels. At day 30, similar proportions of the revaccination and primary vaccination groups had OPK titers ⩾8. Although the OPK RCD curves for the revaccination group were shifted to the left of the primary vaccination group, both curves were generally close together. Similar proportions of both groups had day 30 IgG antibody concentrations >0.5 and >1.0 μg per mL; only at higher concentrations, such as 5.0 μg per mL, did the primary vaccination group percentages exceed those of the revaccination group. Consistent with this, the day 30 IgG RCD curves overlapped at lower distributions and diverged at higher antibody concentrations.

At year 5, the OPK and EIA antibody mean levels and distributions were similar for the primary vaccination and revaccination groups, and the mean levels were significantly higher than for vaccine-naive subjects. These results agree with findings of the overall study, in which the differences in day 30 IgG levels resolved by years 1 to 2 for all 8 serotypes tested, and IgG levels through year 5 remained higher than in vaccine-naive persons for 7 of the 8 serotypes tested. Our results also agree with previous studies of earlier PPVs showing that some antibody persisted 4–6 years after primary vaccination for the serotypes studied [12, 27, 29–31] and as long as 10 years for serotypes 7F and 8 [31]. In the present study, the year 5 OPK RCD curves for serotypes 4 and 23F were shifted to the right of the day 30 curves at lower titer levels for both study groups. One possible explanation for this finding is exposure to other pneumococcal serotypes, resulting in the development of cross-reactive antibody with less functional activity.

Although a seroprotective threshold has not been established for adults, it is reasonable to project that persons with higher antibody levels would be at less risk for pneumococcal disease than persons with lower levels. This concept is supported by reports demonstrating an inverse correlation between age-specific seroprevalence rates of bactericidal antibody and age-specific disease rates for *S. pneumoniae* [32] and data showing that passive antibody therapy can prevent *S. pneumoniae* disease in persons with primary antibody deficiencies [33].

Consistent with previous reports in adults [18, 34–37], we observed generally good correlation between OPK and EIA antibody levels in postvaccination serum samples from older adults. It has been noted that this type of correlation can decrease as age increases, particularly in the very elderly [24]. However, employing an EIA with a second preabsorbant in addition to CPS increases assay specificity and the correlation with opsonic antibody results [18]. These findings suggest that the results of current pneumococcal binding assays do not provide falsely elevated estimates of the immune response in ambulatory adults aged ⩽88 years.

A limitation of the present analysis is that a subset of vaccine serotypes were evaluated; however, the serotypes assessed here are among the most common serotypes causing IPD in adults [15], and 2 of the serotypes evaluated are among the most common serotypes associated with penicillin resistance [10]. A second limitation is that a subset of study participants and time points were evaluated; however, the substudy and overall study groups were comparable by baseline characteristics and antibody response patterns. The overall study results are presented in a companion paper [14].

Overall, the results of this subset analysis demonstrate that first and second doses of PN23 induce capsule-specific opsonic and IgG antibodies in an older-adult population with a high prevalence of common chronic diseases, including risk factors for pneumococcal disease. At the year 5 time point, antibody

levels are comparable in the 2 groups, and remain higher than those in unvaccinated subjects.

Acknowledgments

We thank Patricia Holder (CDC; Atlanta, GA) for quality assurance and data management of OPK titers, Richard D. McFetridge and Jon Stek for editorial comments on the manuscript, and the members of the Pneumococcal Revaccination Study Group that participated in the conduct of this study. Members Pneumococcal Revaccination Group include: Renee White (VA Medical Center; Houston, TX); Mark Goldberg, Toni Goldberg, Peggy Porter-Helbig, and Christine Ore'Giron (Saguaro Research; Tucson, AZ); Richard Tucker, Bradley Cromar, and Anita Ashenbrenner (Wenatchee Valley Clinic; Wenatchee, WA); Gregory Poland, Robert M. Jacobson, Shelly Roberts, and Delana Weiss (Mayo Medical Center; Rochester, MN); Burton Lazar and Chris Bielke (Portland Clinic; Portland, OR); Delmar Greenleaf, John Allen, Cheryl Foltos, and Janet Wilkerson (Bend Memorial Clinic; Bend, OR); and Joseph Bertino, Anne Nafzeger, Anne Menhenick, and James B. Harbison (Bassett Healthcare; Cooperstown, NY).

References

1. Centers for Disease Control and Prevention. Active Bacterial Core Surveillance (ABCs) Report, Emerging Infections Program Network, *Streptococcus pneumoniae*, 2007. http://www.cdc.gov/ncidod/dbmd/abcs/survreports/spneu07.pdf.
2. Sims RV, Steinmann WC, McConville JH, King LR, Zwick WC, Schwartz JS. The clinical effectiveness of pneumococcal vaccine in the elderly. Ann Intern Med **1988**; 108:653–657.
3. Butler FC, Breiman RF, Campbell JF, Lipman HB, Broome CV, Facklam RR. Pneumococcal polysaccharide vaccine efficacy: an evaluation of current recommendations. JAMA **1993**; 270:1826–1831.
4. Shapiro ED, Berg AT, Austrian R, et al. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. N Engl J Med **1991**; 325:1453–1460.
5. Jackson LA, Neuzil KM, Yu O, et al. Effectiveness of pneumococcal polysaccharide vaccine in older adults. N Engl J Med **2003**; 348:1747–1755.
6. Spindler C, Hedlund J, Jasir A, Normark BH, Örtqvist Å. Effects of a large-scale introduction of the pneumococcal polysaccharide vaccine among elderly persons in Stockholm, Sweden. Vaccine **2008**; 26:5541–5546.
7. Moberley SA, Holden J, Tathan DP, Andrews RM. Vaccines for preventing pneumococcal infection in adults. Cochrane Database Syst Rev **2008**:CD000422.
8. Fisman DN, Abrutyn E, Spaude KA, Kim A, Kirchner C, Daley J. Prior pneumococcal vaccination is associated with reduced death, complications, and length of stay among hospitalized adults with community-acquired pneumonia. Clin Infect Dis **2006**; 42:1093–1101.
9. Johnstone J, Marrie TJ, Eurich DT, Majumdar SR. Effect of pneumococcal vaccination in hospitalized adults with community-acquired pneumonia. Arch Intern Med **2007**; 167:1938–**1943**.
10. Whitney CG, Farley MM, Hadler J, et al. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. N Engl J Med **2000**; 343:1917–**1924**.
11. Centers for Disease Control and Prevention. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep **1997**; 46(RR-8): 1–24.
12. Jackson LA, Benson P, Sneller VP, et al. Safety of revaccination with pneumococcal polysaccharide vaccine. JAMA **1999**; 281:243–248.
13. Torling J, Hedlund J, Konradsen HB, Ortqvist A. Revaccination with the 23-valent pneumococcal polysaccharide vaccine in middle-aged and elderly persons previously treated for pneumonia. Vaccine **2003**; 22: 96–103.
14. Musher DM, Manoff SB, Liss C, et al. Safety and antibody response, including antibody persistence for 5 years, after primary vaccination or revaccination with pneumococcal polysaccharide vaccine in middle-aged and older adults. J Infect Dis **2010**; 201:516–24 (in this issue).
15. Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin Infect Dis **2000**; 30:100–121.
16. Romero-Steiner S, Libutti D, Pais LB, et al. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. Clin Diagn Lab Immunol **1997**; 4:415–422.
17. Wernette CM, Frasch CE, Madore D, et al. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. Clin Diagn Lab Immunol **2003**; 10:514–519.
18. Concepcion NF, Frasch CE. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. Clin Diagn Lab Immunol **2001**;8: 266–272.
19. Marchese RD, Jain NT, Antonello J, et al. Enzyme-linked immunosorbent assay for measuring antibodies to pneumococcal polysaccharides for the PNEUMOVAX 23 vaccine: assay operating characteristics and correlation to the WHO international assay. Clin Vaccine Immunol **2006**; 13:905–912.
20. Fleiss J. Statistical methods for rates and proportions. 2nd ed. New York: John Wiley & Sons, **1981**.
21. Casadevall A, Scharff MD. Serum therapy revisited: animal models of infection and development of passive antibody therapy. Antimicrob Agents Chemother **1994**; 38:1695–1702.
22. Cecil RL,. Effects of very early serum treatment in pneumococcus type 1 pneumonia. JAMA **1937**; 108:689–692.
23. Musher DM, Phan HM, Watson DA, Baughn RE. Antibody to capsular polysaccharide of *Streptococcus pneumoniae* at the time of hospital admission for pneumococcal pneumonia. J Infect Dis **2000**; 182:158–167.
24. Romero-Steiner S, Musher DM, Cetron MS, et al. Reduction in functional antibody activity against *Streptococcus pneumoniae* in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity. Clin Infect Dis **1999**; 29:281–288.
25. Mufson MA, Hughey DF, Turner CE, Schiffman G. Revaccination with pneumococcal vaccine of elderly persons 6 years after primary vaccination. Vaccine **1991**; 9:403–407.
26. Davidson MD, Bulkow LR, Grabman J, et al. Immunogenicity of pneumococcal revaccination in patients with chronic disease. Arch Intern Med **1994**; 154:2209–2214.
27. Musher DM, Groover JE, Rowland JM, et al. Antibody to capsular polysaccharides of *Streptococcus pneumoniae:* prevalence, persistence, and response to revaccination. Clin Infect Dis **1993**; 17:66–73.
28. Artz AS, Ershler WB, Longo DL. Pneumococcal vaccination and revaccination of older adults. Clin Microbiol Rev **2003**; 16:308–318.
29. Hilleman MR, Carlson AJ Jr, McLean AA, Vella PP, Weibel RE, Woodhour AF. *Streptococcus pneumoniae* polysaccharide vaccine: age and dose responses, safety, persistence of antibody, revaccination, and simultaneous administration of pneumococcal and influenza vaccines. Rev Infect Dis **1981**; 3(Suppl):S31–S42.
30. Mufson MA, Krause HE, Schiffman G. Long-term persistence of antibody following immunization with pneumococcal polysaccharide vaccine. Proc Soc Exp Biol Med **1983**; 173:270–275.
31. Mufson MA, Krause HE, Schiffman G, Hughey DF. Pneumococcal antibody levels one decade after immunization of healthy adults. Am J Med Sci **1987**; 293:279–284.
32. Sutliff W, Finland M. Antipneumococcic immunity reactions in individuals of different ages. J Exp Med **1932**; 55:837–852.
33. Keller MA, Stiehm ER. Passive immunity in prevention and treatment of infectious diseases. Clin Microbiol Rev **2000**; 13:602–614.
34. Anttila M, Voutilainen M, Jäntti V, Eskola J, Käyhty H. Contributions

of serotype-specific IgG concentration, IgG subclasses and relative antibody avidity to opsonophagocytic activity against *Streptococcus pneumoniae*. Clin Exp Immunol **1999**; 118:402–407.
35. Kolibab K, Smithson SL, Shriner AK, et al. Immune response to pneumococcal polysaccharides 4 and 14 in elderly and young adults: antibody concentrations, avidity, and functional activity. Immun Aging **2005**; 2:10.
36. Tarragó D, Aguilar L, Jansen WTM, et al. Dependence of correlation between antibody titres and opsonphagocytosis on pneumococcal serotype and patient morbidity in pre- and post-pneumococcal vaccination states. Clin Microbiol Infect **2007**; 13:369–376.
37. Waites KB, Canupp KC, Chen Y, DeVivo MJ, Nahm MH. Revaccination of adults with spinal cord injury using the 23-valent pneumococcal polysaccharide vaccine. J Spinal Cord Med **2008**; 31:53–59.