Pneumococcal vaccination in older adults induces antibodies with low opsonic capacity and reduced antibody potency

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Abstract

The primary mode of prevention of adult disease from *Streptococcus pneumoniae* is vaccination with anti-capsular polysaccharide vaccine; however, its effects are less in the targeted older population than in young persons. Few studies have examined the mechanism behind this limited effectiveness. We have measured antibody concentrations and opsonization titers for multiple serotypes amongst both old adults and young, healthy controls. To avoid specificity problems associated with pneumococcal antibody ELISA, we absorbed the serum samples with c-polysaccharide and capsular polysaccharide of 22F type. Antibody concentrations were found to be similar for six out of the seven tested serotypes, while opsonization titers were significantly higher in six out of seven serotypes in the younger population. Antibody potency, as measured by the ratio of opsonization titer to antibody concentration, was found to be significantly higher for the younger subjects for all serotypes. We conclude that, while all ages of adults make similar concentrations of antibodies in response to pneumococcal vaccine, the effectiveness of those antibodies is significantly reduced in the older adult population.

Keywords

Streptococcus pneumoniae; vaccine; efficacy

1. Introduction

Streptococcus pneumoniae infection remains a major cause of morbidity and mortality amongst older adults [1], and it is associated with a substantial loss of independence [2]. Prevention through vaccination has been stressed as a mechanism to reduce disease burden [1,3]. Unfortunately, the effectiveness of the currently approved 23-valent pneumococcal polysaccharide vaccine (PPV23) becomes reduced as a person's age increases above 75 years of age [4,5]. The original studies [6,7] that led to vaccine approval were performed on younger, healthier adults compared to the population currently recommended to receive pneumococcal vaccine.

It has been hypothesized that this decrement in vaccine protective efficacy might be due to an impaired immune response to vaccination in older adults. It has been previously reported that older adults have a less effective antibody response to PPV23 than do their younger counterparts [8–10]. Because anti-pneumococcal antibodies provide protection by opsonizing the bacteria, these studies investigated not only antibody production but also the opsonic

potential of the immune sera from older adults compared to younger adults. However, due to difficulties with the assays, opsonic capacities were measured for a limited number of serotypes and led to variable results [8,10]. Also, none of these studies used the currently accepted, third generation enzyme linked immunosorbent assay (ELISA) [11,12] for quantitation of antibody response. Older-generation ELISAs were limited because they measured some antibodies that are not capsule-specific [13], limiting the reliability of reported antibody titers. It is therefore difficult to definitively determine with the available data if older adults' poor vaccine immune response is due to less production of antibody or if the poor response is due to poor effectiveness of the produced antibodies.

To our knowledge, this is the first study to compare PPV23 antibody response between younger and older adults using the third-generation ELISA[11]. We will also examine the functionality of those serotype-specific antibodies produced using a multiplexed opsonization assay. The multiplexed assay allows us to measure opsonization of multiple serotypes across a large number of samples in an efficient manner. This type of large-scale testing of opsonization has not been feasible until recent advances in the opsonization assay were developed [14], thus the existing literature for studies on this scale is limited [8,15]. Our goal is to determine whether older adults actually produce fewer antibodies and to examine if those antibodies produced have reduced functionality.

2. Materials and Methods

Study preparations

Licensed pneumococcal polysaccharide vaccine (PPV23) was 23-valent vaccine from either Wyeth (PNU-IMMUNE® 23, Wyeth-Lederle Lab., Pearl River NY) or Merck (Pneumovax®, Merck, Whitehouse Station NJ) and contained 25 mcg of each serotype polysaccharide per 0.5 mL dose.

Clinical trial design

Older adult subjects were taken from the control-group subset of a previously reported randomized, double-blind, placebo-controlled multicenter trial of protein conjugated pneumococcal vaccine in community-dwelling elderly adults [16]. This group included subjects 65 years of age or older (median age 72 years). Subjects were excluded if they were high-risk (i.e. immunocompromised, splenectomized, or with advanced renal, hematologic or hepatic disease) or if they had received PPV23 within the last 5 years. This control-group subset received the usual dose of 0.5 mL of PPV23. Sera used in this analysis were drawn 28 days post vaccination.

Younger adult subjects were healthy volunteers, who were 45 years of age or younger. These subjects were recruited from laboratory research personnel from large university medical centers. Most subjects performed clinical duties and were not exposed to pneumococci in their work. All subjects had not previously received PPV23 and received 0.5 mL of PPV23 and serum samples were drawn approximately 28 days post vaccination.

Laboratory analysis

Enzyme Linked Immunoassay for IgG anti-PS antibody—The amount of IgG anti-capsular PS antibody was determined by a "sandwich" type 3rd generation pneumococcal antibody ELISA [11] using fresh solutions prepared with water from a Milli-Q UF water purification system (Millipore, Bedford MA) to minimize background signals. Briefly, the wells of medium binding microtiter plates were coated at 37°C with pre-determined concentration (usually 1–10 µg/ml) of capsular PS (ATCC, Rockville, MD) for 5 hr in PBS with 0.02% NaN_3. Next, the plates were washed with TBS containing 0.1% Brij-35 (Sigma,

St. Louis, MO). All serum samples (20 µl) were pre-absorbed with 5 µg C-PS (Statens Serum Institut, Copenhagen, Denmark) and 10 µg 22F-PS in a total volume of 1 ml of PBS containing 0.05% Tween-20 for 30 min at room temperature. Serum pool 89-SF was absorbed only with C-PS and used as the standard (Food and Drug Administration, Bethesda, MD). The pre-absorbed serum samples and 89-SF were serially diluted and aliquots were added to the PS coated microtiter wells. The plates were incubated for 2 hrs at room temperature and washed as described above. Next, alkaline phosphatase-conjugated goat antibody specific for human IgG (Biosource, Camarillo, CA) in PBS with 0.02% NaN_3 was added for 2 hr at room temperature. The amount of enzyme conjugate immobilized to each well was determined with the addition of the *p*-nitrophenyl phosphate substrate (Sigma) in diethanolamine buffer and incubation at room temperature for 2 hr. The reactions were stopped with NaOH, and the optical density at 405 nm and 690 nm was measured using an ELISA microplate reader. The amount of antibody was determined by comparing the optical densities for each of the diluted serum samples to the optical density curve constructed with the standard sample (89-SF) at multiple dilutions.

Multiplexed quadruple-serotype opsonophagocytic killing assay (MOPA4)—
MOPA4 was performed as described previously [14]. Briefly, opsonization assay target bacteria were prepared for 7 serotypes (serotypes 4, 6B, 7F, 9V, 14, 18C, 19F, and 23F). Each target bacteria has been made to be resistant to one of 4 antibiotics (optochin, streptomycin, spectinomycin, and trimethoprim) but susceptible to the other three. Frozen aliquots of target pneumococci were thawed, washed twice with opsonization buffer B {Hanks' balanced salt solution (HBSS) with Mg/Ca, 0.1% gelatin, and 10% fetal bovine serum} by centrifugation ($12,000 \times g$, 2 minutes), and diluted to the proper bacterial density (~2×10^5 cfu/ml of each serotype). Equal volumes of 4 bacterial suspensions that were chosen to be analyzed together, were pooled. All serum samples were incubated at 56°C for 30 minutes before serial dilutions in Opsonization Buffer B. Serially diluted serum (20 µl/well) was mixed with 10 µl of bacterial suspension in each well of round bottom 96-well plates (Corning Inc., Corning, NY). After 30 minute incubation at RT with shaking (mini orbital shaker, Bellco Biotechnolgy, Vineland, NJ) at 700 rpm, 10 µl of 3–4 week-old-rabbit complement (PelFreeze Biologicals, Rogers, Arkansas) and 40 µl of HL60 cells (4×10^5 cells) were added to each well. HL60 cells were differentiated to granulocytes by culturing in RPMI-1640 with 10% fetal bovine serum and 1 % L-glutamine and 0.8% dimethylformamide at a starting density of 4×10^5 cells/ml for 5–6 days. Plates were incubated in a tissue culture incubator (37°C, 5% CO_2) with shaking at 700 rpm. After a 45 minute incubation, plates were placed on ice for 10–15 minutes and an aliquot of the final reaction mixture (10 µl) was spotted onto 4 different THY agar plates (Todd-Hewitt broth with 0.5 % yeast extract and 1.5% agar). When the fluid was absorbed into the agar, an equal volume of an overlay agar (THY with 0.75% agar and 25 mg/L of 2,3,5-tripheynyltetrazolium chloride) containing one of the four antibiotics was applied to each THY agar plate. After an overnight incubation at 37°C, the number of bacterial colonies in the agar plates was enumerated. Opsonization titers were defined as the exact serum dilution that kills 50% of bacteria, which was estimated by interpolating results. A detailed protocol is posted on a website (www.vaccine.uab.edu). To reduce analytical variations, all the samples were analyzed using identical reagent lots and within a short period of time.

Statistical Analysis—Antibody concentrations, opsonization titers, and the ratio of opsonization titers to antibody concentrations (as a measure of antibody potency) were graphically represented using reverse cumulative distribution curves (RCDC). Antibody potency was defined as the opsonization titer divided by the antibody concentration for each serotype. Geometric mean concentrations (GMC) for ELISA, geometric mean titers (GMT) for opsonization, and geometric mean potency (GMP) for antibody potency were calculated for each serotype for both age groups. A two-tailed Mann-Whitney U test was used to analyze

differences between groups because data were non-normally distributed. Statistical analysis was conducted using NCSS and PASS (Number Cruncher Statistical Systems, Kaysville, Utah).

3. Results

A total of 102 subjects were analyzed, 44 in the younger adults group and 58 in the older adults group. The RCDCs for serotype specific antibody concentrations are shown in Figure 1, and a summary of the mean concentrations, 95% confidence intervals (CI), and calculation of statistical differences are shown in Table 1. Antibody concentrations were similar for six out of the seven serotypes, with serotype 19F revealing higher antibody concentrations in the younger adult group as compared to the older adult group (GMC 9.14 µg/mL [CI 6.79-12.31] vs. 5.24 µg/mL [CI 3.99-6.88], $p = 0.01$).

Opsonization titers were determined for each subject for each serotype. The RCDCs for each serotype specific opsonization titer are demonstrated in Figure 2. Numerical results are summarized in Table 1. Opsonization titers were significantly higher in the younger adult group in six of the seven serotypes, with serotype 18C opsonization titers being similar between the two groups (1343 [CI 721-2505] vs. 933 [CI 500-1740], $p = 0.66$).

Measurements of antibody potency were made by dividing the opsonization titer by the antibody concentration for each serotype. Antibody potency RCDCs are shown in Figure 3 and the data is numerically shown in Table 1. Antibody potency was significantly higher in younger subjects for all seven serotypes ($p<0.05$ for all serotypes).

4. Discussion

To examine the functionality of the antibodies present after a PPV23 vaccination, we have correlated antibody concentration with opsonization indices as a measure of antibody potency for a large number of samples for many different serotypes. To our knowledge, this is the first report comparing the functionality of antibody response to PPV23 between younger and older adults using the third-generation ELISA assay method. Our data demonstrate that older adults (65 years and older) make similar quantities of anti-capsular pneumococcal antibodies as do younger adults (45 years old and younger), but that those antibodies are less functional and do not opsonize bacteria as efficiently. These findings are consistent with clinical data that have demonstrated limited vaccine efficacy amongst the older adult population [4,17–20]. It suggests that the antibodies produced in old adults may not have the same potency as those elicited in younger adults.

Our study has some limitations. First, subjects were administered vaccine produced by two different manufacturers. It is possible that the vaccines from the different manufacturers could stimulate different amounts of antibody or more functional antibody, and potentially alter our results. However, comparison of antibody responses between the two different vaccines revealed no differences in antibody titers (data not shown). Additionally, subjects were recruited for the younger group at a separate time from the older adults as they were not part of the same original study. Though the dates of collection were different, all samples were analyzed together to minimize technical differences in data generation. However, these differences are likely of limited importance because our conclusion is based on the differences between the two independent measures (ELISA vs. opsonization assay) of antibody response.

Our findings significantly extend the findings of previous studies. For instance, prior studies by Rubins [9,10] also showed inconsistent production of antibodies after vaccination. However, Rubins et al. only examined opsonization titers for one serotype, limiting the ability to make generalized conclusions based on that result. Romero-Steiner et al. have also reported

that old adults produce less functional antibodies than do young adults [8]. However, the most significant limitation to all of these studies is that they employed the second generation ELISA, which could have over estimated antibody concentrations for old adults and may have reduced apparent functionality of antibodies. In addition, the Romero-Steiner study had a limited control group (n=12), potentially reducing the ability to make generalizations based on its findings. Our data, based on a large number of samples and serotypes, clearly demonstrate that even with similar antibody concentrations, there is significant functional limitation of those antibodies as evidenced by the universally reduced antibody potency.

We believe this definitive demonstration of low opsonic capacity of post-vaccination sera from old adults is critically important. It demonstrates that opsonization assay results are a more reliable predictor of the immune status than ELISA results. This conclusion would be expected since anti-capsule antibodies provide protection by opsonizing pneumococci for phagocytes. This conclusion is also supported by clinical experience as well. For instance, a conjugate vaccine induces young children to produce antibodies that are cross-reactive to serotype 19A by ELISA, but no cross-reactivity is seen when measured by an opsonization assay [21]. Dramatic increases in 19A prevalence since the introduction of the conjugate vaccine [22,23] clearly demonstrate that the opsonization assay is a more reliable indicator of immunity than is ELISA. In the studies of HIV infected adults and children [24,25], the two assays produced different results and opsonization assay results are now widely accepted as the true measure of immune status. Since opsonization assay is now quite easy to perform and has become largely standardized [26], we believe that the opsonization assay is an important analytical tool for studying pneumococcal vaccines among old adults.

There are several possible explanations for the poor antibody potency observed in the older adult subjects. It has been shown that older adults express a different antibody V region repertoire of both the light chains [27] and heavy chains [28] in response to pneumococcal vaccination. Whether these changes are functionally significant has not been well established, though this possibility certainly warrants further investigation. It has also been shown that there are significant changes in antibody glycosylation with aging [29–31] with a greater proportion of agalactosyl glycoforms of IgG produced in older subjects. Deglycosylation has further been shown to lead to a decrease in antibody binding to antigen [32], though this effect has not been specifically demonstrated for antibodies to pneumococcal capsule. Further, it has been shown that there are distinct populations of antibody-producing B cells, B-1 cells, that change with aging [33]. These B-1 cells, which may also exist in humans [34], produce antibodies that may enhance the specific B-cell immune response to an antigen challenge. Because the number of B-1 cells decrease with age, it is plausible that the reduction in antibodies produced by these cells could result in production of less effective anti-capsular antibodies.

We have demonstrated that while older adults tend to produce similar quantities of antibody in response to PPV23, the quality of those antibodies is different than those of younger subjects. We have extended the findings of prior studies which were limited by the use of older, less accurate procedural methods. Future work should be aimed at identifying the cause of this reduction in antibody function so that future vaccines can be designed to produce more effective antibodies in this at-risk population.

Acknowledgements

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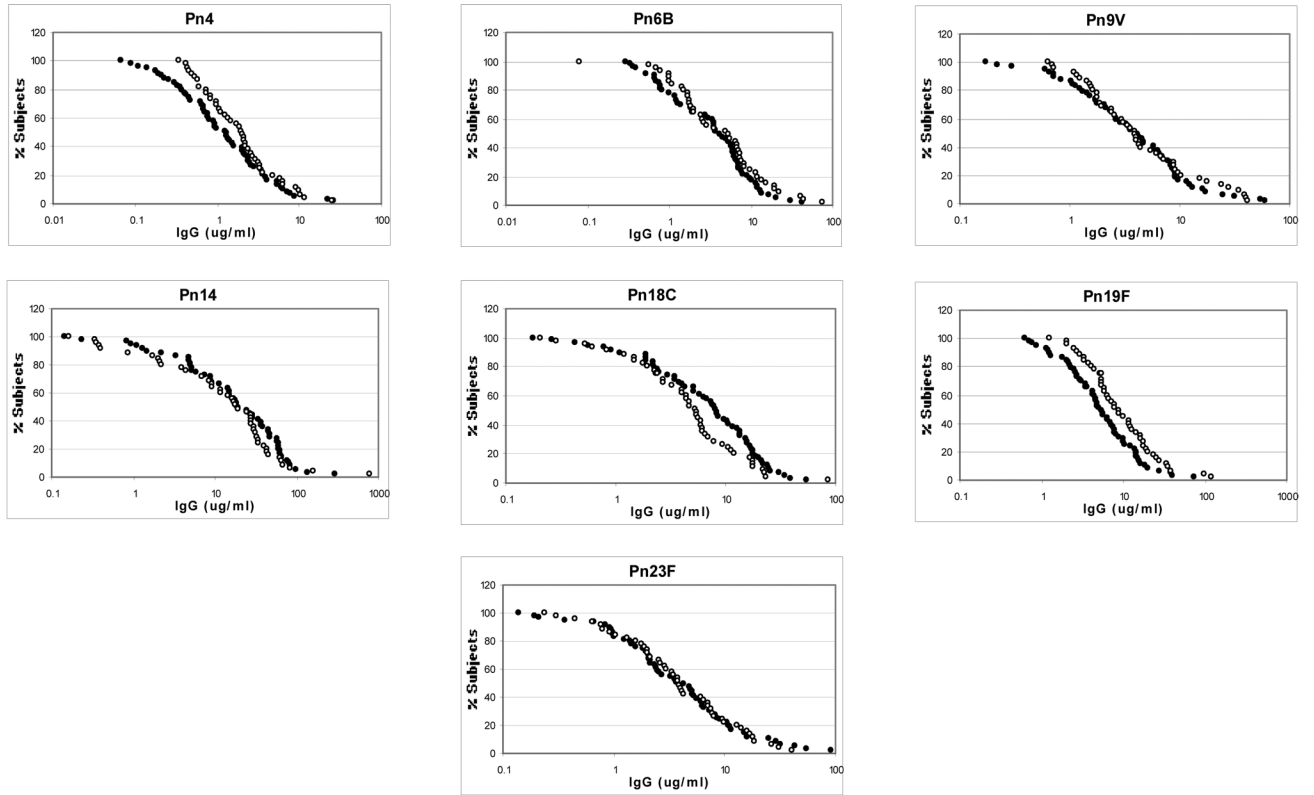
Figure 1.
Reverse cumulative distribution curves for antibody concentration
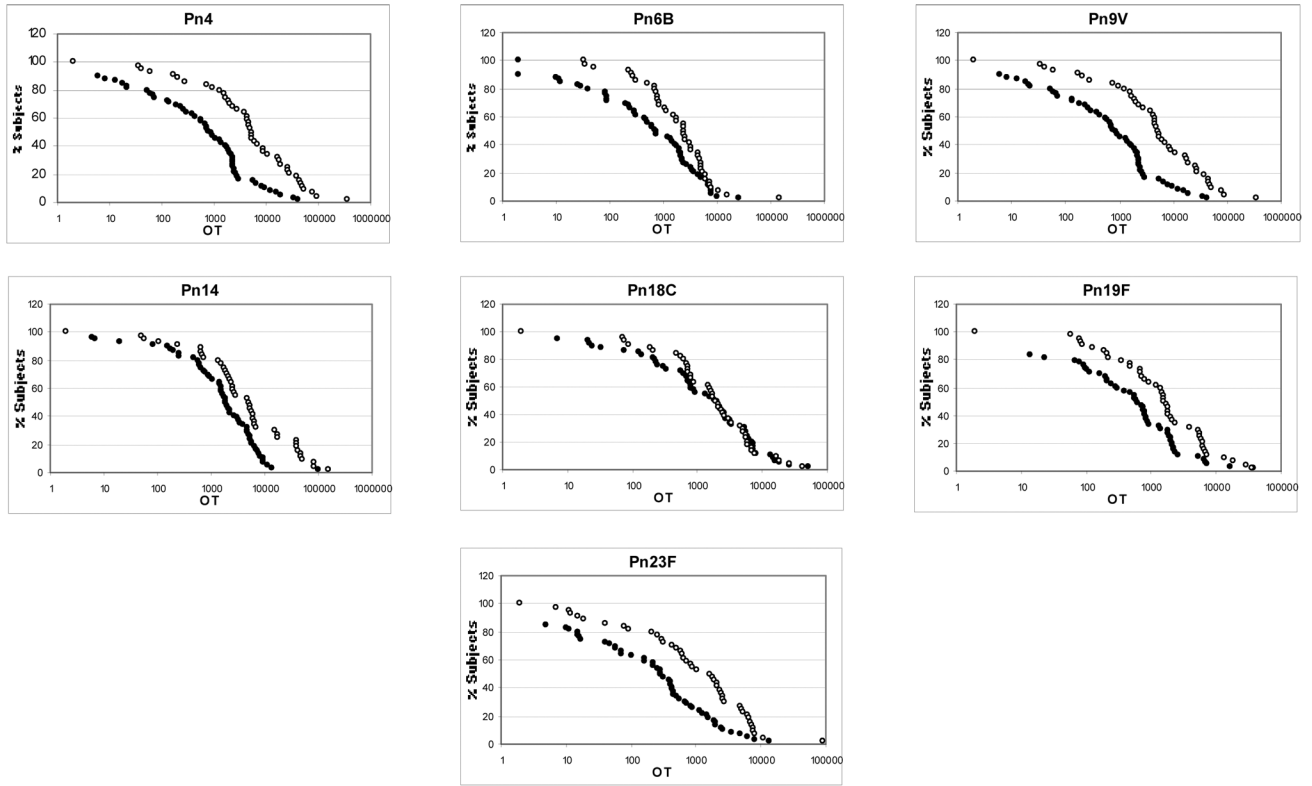Open circle = young adults
Closed circle = older adults

Figure 2.
Reverse cumulative distribution curves for opsonization titers
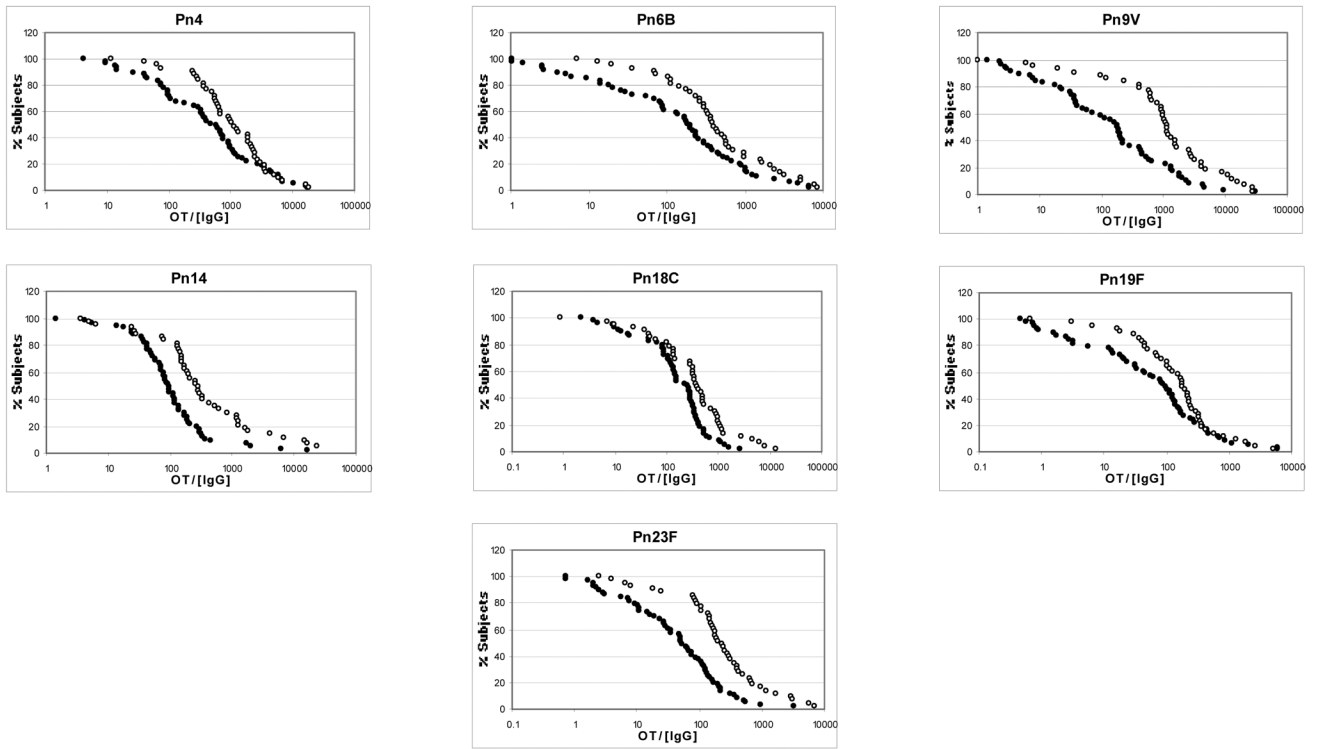Open circle = young adults
Closed circle = older adults

Figure 3.
Reverse cumulative distribution curves for antibody potency
Open circle = young adults
Closed circle = older adult

Table 1

Geometric Means for Antibody Concentration, Opsonization Titers, and Antibody Potency

Serotype	Age	IgG conc (ug/ml)			Opsonic titer (OT)			Antibody potency (OT/[IgG])			Antibody needed for 1:8 Ops. Index (ng/ml)[f]
		GMC[a]	95% CI[d]	Sig[e]	GMT[b]	95% CI[d]	Sig[e]	GMP[c]	95% CI[d]	Sig[e]	
4	Young	1.84	1.35–2.51	NS[g]	1735	1074–2804	p<0.01	969	612–1536	p=0.02	7.7
	Old	1.15	0.82–1.63		453	250–821		393	233–665		16.6
6B	Young	4.04	2.71–6.01	NS	1707	1046–2787	p<0.01	405	247–665	p<0.01	21.0
	Old	3.16	2.29–4.35		377	188–756		119	67–214		43.6
9V	Young	4.25	3.01–6.01	NS	3957	1921–8151	p<0.001	917	464–1810	p<0.0001	7.2
	Old	3.56	2.58–4.94		411	203–832		115	61–217		45.3
14	Young	11.45	6.65–19.70	NS	3715	1888–7309	p<0.01	343	177–665	p<0.0001	30.6
	Old	15.53	10.29–23.43		1250	717–2179		80	49–131		92.9
18C	Young	4.46	3.09–6.42	NS	1344	721–2505	p=0.09	310	174–550	p=0.02	21.5
	Old	6.33	4.57–8.77		933	500–1740		149	100–221		36.6
19F	Young	9.14	6.79–12.31	p=0.01	1254	707–2223	p<0.01	135	81–227	p=0.03	45.0
	Old	5.24	3.99–6.88		266	133–532		51	28–93		89.9
23F	Young	3.79	2.63–5.47	NS	766	377–1554	p<0.001	204	119–349	p<0.0001	41.3
	Old	3.69	2.59–5.26		147	76–285		40	25–64		160.0

[a]GMC = Geometric mean concentration

[b]GMT = Geometric mean titer

[c]GMP = Geometric mean potency

[d]CI = Confidence interval

[e]Sig = Statistical significance

[f]Amount of antibody needed for 1:8 opsonization titer was obtained by dividing the antibody concentration (ng/ml) by the opsonization index and multiplying by 8. The median value was shown for each group.

[g]NS = Not significant