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Age-Associated Differences in Immunoglobulin G1 (IgG1) and IgG2 Subclass Antibodies to Pneumococcal Polysaccharides following Vaccination

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Immunoglobulin G (IgG) subclass antibody responses to pneumococcal vaccines were determined for human subjects in four age groups. The ratios of IgG1/IgG2 antibody concentrations declined with advancing age for all five of the serotypes tested. Protein-conjugate vaccines elicited enhanced IgG antibody responses over plain polysaccharide vaccines in infants but not in adult groups.

Immunoglobulin G1 (IgG1) and IgG2 comprise about 90% of the total human serum IgG (5). Limited information is available concerning the IgG subclass composition of pneumococcal (Pn) antibodies generated following immunization with capsular polysaccharide vaccines (2, 12, 18). Individual responses tend to be oligoclonal and are usually restricted to IgG2 in adults and IgG1 in infants (3, 4, 12, 17). To examine whether differences in subclass antibody response to immunization with Pn vaccines exist between or within different age groups; we determined relative concentrations of IgG1 and IgG2 antibodies to five Pn serotypes in healthy infants, toddlers, preschool children, adults, and older adults before and after vaccination with licensed Pn polysaccharide (PS) vaccine (PV) or one of two investigational protein-conjugated Pn vaccines (CV).

(This work was presented in part at the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, October 1997, in Toronto, Ontario, Canada [13a].)

Serum specimens were obtained from 74 subjects enrolled in Pn vaccine immunogenicity studies at the Saint Louis University Center for Vaccine Development. Ten toddlers aged 12 to 15 months and 10 young adults aged 18 to 39 years received a single dose of licensed 23-valent Pn PV (Pneumovax-23; Merck & Co.) (PV1). Ten infants aged 6 to 10 weeks, 10 children aged 2 to 5 years, and 10 young adults aged 18 to 39 years received an investigational seven-valent Pn PS conjugated to the outer membrane protein of *Neisseria meningitidis* (Pn-OMP; Merck & Co.) (CV1). Infants in the CV1 vaccine group received a total of three doses of vaccine at 2, 4, and 6 months of age, and children aged 2 to 5 years received two doses of vaccine given 2 months apart. All other subjects received a single dose of vaccine. Twelve older adults aged 50 to 85 years received a licensed 23-valent Pn PV (Pnu-Imune; Wyeth-Lederle and Pediatrics, Pearl River, N.J.) (PV2), and 12 older adults aged 50 to 85 years received an investigational five-valent Pn PS conjugated to the carrier protein CRM_{197}, a nontoxic variant of diphtheria toxin (5VPn-CRM; Wyeth-Lederle and Pediatrics) (CV2). The licensed Pn PVs were comprised of 25 μg of purified PS of each of the same 23 capsular PSs per dose. The 7VPn-OMP vaccine contained 3.5 μg of type 6B PS, 2 μg of type 19F PS, 1.5 μg of type 9V PS, and 1 μg each of type 4, 14, 18C, and 23F PSs per dose. The 5VPn-CRM vaccine contained 10 μg of PS each for Pn serotypes 6B, 14, 18C, 19F, and 23F per dose. Prevaccination specimens were obtained from all subjects. Postvaccination specimens for infants and children receiving CV1 were obtained 1 month following administration of the final dose. For all other subjects, the postvaccination specimens were obtained 1 month postadministration of a single dose of CV or PV.

Relative concentrations of IgG1 and IgG2 antibodies to Pn serotypes 6B, 14, 18C, 19F, and 23F were determined by enzyme-linked immunosorbent assay (ELISA) in a cross-calibration adaptation of previously described Pn ELISA consensus methods (6, 7, 13). The U.S. standard human anti-*Haemophilus influenzae* type b (Hib) serum pool, lot 1983 (provided by Carl Frasch; Center for Biologics Evaluation and Review, Food and Drug Administration, Rockville, Md.), having defined concentrations of IgG subclass antibodies to Hib antigen (30.9 μg of IgG1 per ml and 16.1 μg of IgG2 per ml), was used as a reference serum. The assay was adapted as follows. Ninety-six-well Maxisorp microtiter plates (Nunc-Bacti; Fisher Scientific, St. Louis, Mo.) were coated with Hib oligosaccharide antigen conjugated to human serum albumin (HbO-HA; provided by Porter Anderson, University of Rochester, Rochester, N.Y.) at 2 μg/ml on the calibration side of the plate. The test sides of the plates were coated with type-specific Pn PS (American Type Culture Collection Manassas, Va.) at a coating concentration of 20 μg/ml. Study sera were preabsorbed with 10 μg of C-PS (C polysaccharide, the common antigen of *Streptococcus pneumoniae*) (Statens Seruminstitut, Copenhagen, Denmark) per ml at a 1:50 dilution and then serially diluted 1:2 for eight or more test dilutions to generate broad-range dose-response curves and ensure epitope excess in the ELISA system. Serial dilutions of the standard anti-Hib reference serum and a negative control serum were added to the calibration side of the plate. Study and control sera were added to the test side of the plate. The U.S. standard human anti-Pn reference serum, lot 89SF (14) (provided by C. Frasch) was included on

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the test side of each IgG2 ELISA plate as a positive control. A positive IgG1 anti-Pn PS control was prepared by pooling post-CV serum from nine infants with measurable IgG1 antibodies to all five Pn serotypes and was included on every IgG1 plate. International Union of Immunological Societies-documented (7–9) murine monoclonal antibodies specific for human IgG1 Fc (HP6069) and IgG2 Fc (HP6002) (obtained as biotin conjugates from the Hybridoma Reagent Laboratory, Baltimore, Md.) were used to detect serotype-specific subclass antibodies bound to the solid phase. IgG1 and IgG2 anti-Pn PS concentrations in study and control sera were estimated by interpolation from the standard anti-Hib dose-response curve and assigned microgram-per-milliliter equivalency units by using the reference line unit calculation mode of the Unitcalc data reduction software (15). Interassay coefficients of variation of $\leq$22% and parallelism between test and reference curves were maintained for all 10 cross-calibration systems. Antibody concentrations were logarithmically transformed, and geometric mean concentrations were compared by analysis of variance. Antibody concentrations that were less than the minimum quantifiable in the ELISA ($<$0.5 μg/ml) were assigned values of 50% of the minimum for statistical analyses. Comparisons of IgG1/IgG2 ratios were made by using Tukey's method of post-hoc pairwise comparisons. Frequency comparisons were made by using chi-square and Fisher's exact tests.

Geometric mean concentrations of IgG1 and IgG2 in serum are summarized by vaccine and age group in Tables 1 and 2. Infants and children mounted a predominantly IgG1 response, whereas adults and elder adults mainly responded with IgG2 antibodies regardless of the vaccine construct. The mean IgG1/IgG2 ratios following vaccination decreased stepwise with advancing age. IgG1/IgG2 ratios in older adults were more than 40-fold lower than those of infants for all serotypes tested after receipt of either vaccine formulation ($P < 0.05$) (Fig. 1). Although the IgG concentrations were low, IgG subclass ratios of

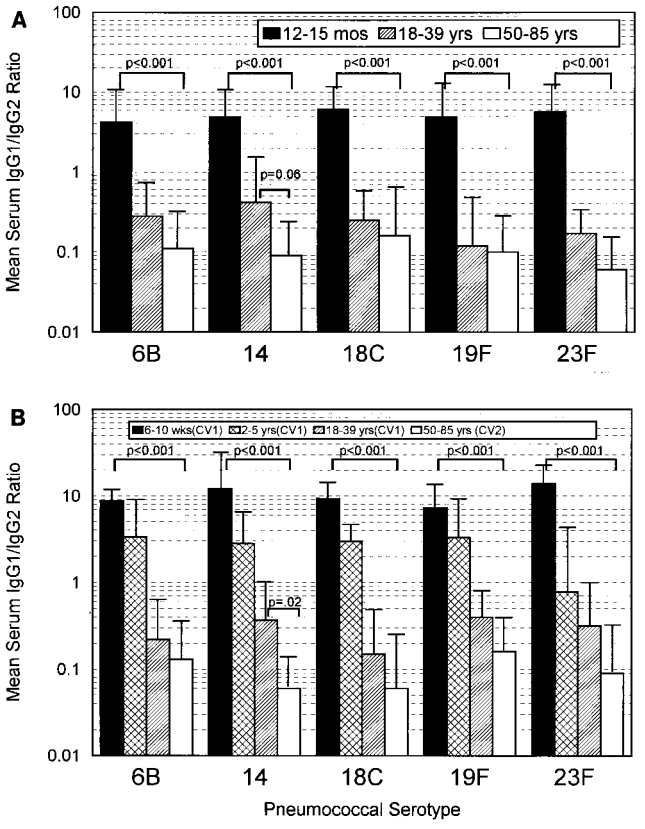

FIG. 1. Ratio of postvaccination geometric mean IgG1/IgG2 antibody responses in relation to age after immunization with licensed 23-valent Pn PV (A) or protein-conjugated Pn PV CV1 or CV2 (B).

TABLE 1. Serum IgG1 and IgG2 capsule-specific antibody responses to Pn PV by age[a]

Serotype, age (no. of persons)	IgG1 level		% ≥2-fold IgG1[b]	IgG2 level		% ≥ 2-fold IgG2
	Prevaccination	Postvaccination		Prevaccination	Postvaccination	
Pn6B						
12–15 mo (10)	0.85 (0.3–2.4)	1.18 (0.4–3.2)	10	0.25 (0.2–0.2)	0.28 (0.2–0.4)	0
18–39 yr (10)	0.31 (0.2–0.5)	0.47 (0.2–1.0)	20	0.80 (0.3–2.3)	1.70 (0.6–5.5)	40
50–85 yr (12)	0.27 (0.2–0.3)	0.36 (0.2–0.7)	8	1.17 (0.5–3.0)	3.26 (1.1–9.4)	42
Pn14						
12–15 mo (10)	1.08 (0.4–3.1)	1.51 (0.6–3.9)	20	0.25 (0.2–0.2)	0.31 (0.2–0.4)	0
18–39 yr (10)	0.32 (0.2–0.5)	1.93 (0.5–8.0)	40	0.44 (0.2–1.0)	4.65 (1.1–19.2)	80
50–85 yr (12)	0.37 (0.2–0.6)	0.42 (0.2–0.8)	8	1.16 (0.5–3.0)	4.92 (1.6–15.22)	50
Pn18C						
12–15 mo (10)	0.85 (0.3–2.5)	2.73 (1.2–6.5)	70	0.27 (0.2–0.3)	0.45 (0.3–0.7)	20
18–39 yr (10)	0.36 (0.2–0.5)	3.00 (1.0–8.7)	70	3.23 (1.3–7.8)	12.22 (4.7–31.8)	70
50–85 yr (12)	0.49 (0.2–1.0)	2.04 (0.6–7.2)	42	2.91 (1.0–8.8)	12.72 (5.5–29.5)	50
Pn19F						
12–15 mo (10)	0.93 (0.3–3.0)	1.23 (0.4–3.4)	20	0.25 (0.2–0.2)	0.25 (0.2–0.2)	0
18–39 yr (10)	0.29 (0.2–0.4)	0.28 (0.2–0.4)	0	0.74 (0.3–1.9)	2.29 (0.6–8.9)	40
50–85 yr (12)	0.26 (0.2–0.3)	0.38 (0.2–0.8)	8	1.01 (0.4–2.9)	3.75 (1.1–12.5)	58
Pn23F						
12–15 mo (10)	0.95 (0.3–2.6)	1.40 (0.6–3.3)	20	0.25 (0.2–0.2)	0.25 (0.2–0.2)	0
18–39 yr (10)	0.30 (0.2–0.4)	0.83 (0.4–1.9)	30	1.32 (0.5–3.6)	4.89 (1.9–12.4)	70
50–85 yr (12)	0.25 (0.2–0.02)	0.31 (0.2–0.5)	8	1.40 (0.5–3.8)	5.42 (2.0–14.7)	67

[a] Antibody levels are expressed as geometric mean concentrations in micrograms per milliliter (95% confidence intervals).
[b] Percentage of vaccine recipients whose antibody concentration increased by at least twofold.

TABLE 2. Serum IgG1 and IgG2 capsule-specific antibody responses to protein-conjugated Pn PV CV1 or CV2 by age[a]

Serotype, age (no. of persons)	IgG1 level		% >2-fold IgG1[b]	IgG2 level		% >2-fold IgG2
	Prevaccination	Postvaccination		Prevaccination	Postvaccination	
Pn6B						
6–10 wk (10)	0.35 (0.2–0.4)	3.82 (1.8–8.2)	90	0.25 (0.2–0.2)	0.44 (0.2–0.8)	30
2–5 yr (10)	0.35 (0.2–0.4)	2.77 (0.7–9.7)	70	0.27 (0.2–0.3)	0.83 (0.4–1.5)	50
18–39 yr (10)	0.29 (0.2–0.5)	0.41 (0.2–0.7)	10	0.47 (0.2–1.0)	1.83 (0.5–7.0)	30
50–85 yr (12)[c]	0.29 (0.2–0.7)	0.30 (0.2–0.4)	0	0.85 (0.3–2.2)	2.29 (0.8–6.6)	42
Pn14						
6–10 wk (10)	0.32 (0.2–0.6)	6.51 (2.2–19.5)	80	0.25 (0.2–0.2)	0.54 (0.3–1.1)	40
2–5 yr (10)	0.43 (0.2–1.0)	5.89 (3.2–10.8)	100	0.31 (0.2–0.5)	2.10 (0.7–6.3)	50
18–39 yr (10)	0.52 (0.2–1.0)	1.66 (0.5–5.0)	50	1.11 (0.4–3.3)	4.46 (1.9–10.3)	60
50–85 yr (12)	0.25 (0.2–0.2)	0.28 (0.2–0.4)	8	0.60 (0.2–1.6)	4.90 (1.6–14.8)	58
Pn18C						
6–10 wk (10)	0.34 (0.2–0.7)	4.29 (1.8–10.0)	90	0.25 (0.2–0.2)	0.46 (0.2–0.9)	30
2–5 yr (10)	0.37 (0.2–0.7)	8.25 (5.6–12.1)	100	0.55 (0.3–1.0)	2.77 (1.8–4.3)	80
18–39 yr (10)	0.36 (0.2–0.6)	0.60 (0.2–1.7)	20	1.93 (0.7–5.0)	4.00 (1.2–13.0)	40
50–85 yr (12)	0.31 (0.2–0.5)	1.56 (0.4–6.6)	50	2.42 (1.0–6.2)	24.9 (14.0–45.2)	92
Pn19F						
6–10 wk (10)	0.34 (0.2–0.7)	5.21 (2.4–11.1)	100	0.30 (0.2–0.5)	0.72 (0.3–1.6)	30
2–5 yr (10)	0.37 (0.2–0.7)	2.86 (1.0–7.9)	80	0.35 (0.2–0.5)	0.87 (0.4–1.8)	40
18–39 yr (10)	0.31 (0.2–0.5)	0.44 (0.2–0.9)	20	0.64 (0.3–1.4)	1.09 (0.4–2.9)	30
50–85 yr (12)	0.25 (0.2–0.2)	0.25 (0.2–0.2)	0	0.70 (0.3–1.8)	1.56 (0.6–4.0)	42
Pn23F						
6–10 wk (10)	0.34 (0.2–0.7)	4.02 (1.9–8.4)	90	0.30 (0.2–0.5)	0.29 (0.2–0.4)	10
2–5 yr (10)	0.37 (0.2–0.7)	0.74 (0.5–4.9)	50	0.31 (0.2–0.5)	0.95 (0.5–1.9)	50
19–39 yr (10)	0.31 (0.2–0.5)	0.73 (0.2–2.4)	30	0.83 (0.4–1.8)	2.22 (0.7–6.8)	40
50–85 yr (12)	0.33 (0.2–0.6)	0.64 (0.2–1.9)	17	1.32 (0.5–3.8)	7.08 (1.9–26.4)	58

[a] Antibody levels are expressed as geometric mean concentrations in micrograms per milliliter (95% confidence intervals).
[b] Percentage of vaccine recipients whose antibody concentration increased by at least twofold.
[c] Elder subjects received CV2; all others received CV1.

naturally acquired antibodies detected in prevaccination specimens showed trends between age groups that paralleled those of postvaccination specimens. Infants who received 7VPn-OMP were more likely to demonstrate a twofold-or-greater increase in IgG1 subclass antibodies to Pn serotypes 6B, 14, 19F, and 23F than toddlers who received unconjugated Pn PV (80 to 100% versus 10 to 20%, $P \leq 0.01$). The effect of enhanced immunogenicity of a protein-conjugate vaccine in infants was expected given previous immunogenicity studies (16). Enhanced immunogenicity was not seen in the young-adult group given the 7VPn-OMP vaccine, nor was it seen in the older-adult group given the 5VPn-CRM vaccine. The percentage of older adult subjects mounting a twofold-or-greater increase in IgG2 antibody to either vaccine was equivalent to that of the young-adult group for all of the Pn serotypes tested. In contrast, the percentage of older subjects mounting a twofold-or-greater increase in IgG1 antibodies tended to be lower than the percentage in the young-adult groups for Pn serotypes 6B, 19F, and 23F and was significantly lower for Pn serotype 14 ($P < 0.01$). The majority of the older adults tested made almost no detectable IgG1 antibodies to Pn serotypes 6B, 14, 19F, and 23F. However, half of the older adult subjects in both the CV and PV groups mounted a significant IgG1 response to Pn serotype 18C, demonstrating that some older adults retain the ability to generate an IgG1 response to selected Pn PS antigens. Interestingly, this was the only antigen for which significant increases in specific antibodies were detected in the infant group following receipt of unconjugated PV.

Data from these studies show age to be a significant factor in determining IgG subclass antibody responses to Pn vaccines. How these differences relate to protective efficacy in different age groups is unknown. Significant differences in avidity between antibodies elicited by different protein-conjugated Pn vaccines have been reported (1). Because the ELISA technique identifies both high- and low-avidity antibodies, the quantitative differences observed in these studies may not necessarily correlate with differences in functional capacity (10, 11). Age-associated differences with respect to subclass composition, avidity, and functional activity should be examined in order to develop a better understanding of the responses of different populations to Pn vaccines and to develop vaccine strategies suited to overcoming the various obstacles which are likely to be found in different age groups.

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