The Journal of Microbiology, December 1998, p.303-307
Vol. 36, No. 4

Human Antibody Responses to Capsular Polysaccharides of *Streptococcus pneumoniae* 6B, 14, and 19F

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(Received June 5, 1998 / Accepted December 2, 1998)

Human antibody responses to *Streptococcus pneumoniae* 6B, 14, and 19F capsular polysaccharide were analyzed. Thirty-one healthy young adults were immunized with the pneumococcal 23-valent PS vaccine. Serum samples were obtained from them before and 1 month after vaccination. The amounts of total antibody, heavy chain and light chain isotypes were determined by enzyme-linked immunosorbent assay (ELISA). Vaccination increased the total levels of anti-6B, anti-14, and anti-19F PS antibodies by 3.4-fold, 3.8-fold and 4.1-fold, respectively. Some individuals showed great variations in antibody responses to different serotypes of PS. The IgG antibody was predominant in the responses to the three PSs, and most of the IgG anti-PS antibodies were IgG2 isotype. There was no significant difference in the κ and λ responses.

Key words: Antibody, isotype, *Streptococcus pneumoniae*

Streptococcus pneumoniae is an encapsulated Gram-positive bacterium. It is a significant pathogen causing pneumonia, meningitis, bacteremia and otitis media especially in young children, old adults and immunocompromised patients (1). Although approximately 90 serotypes have been identified by their distinct polysaccharide (PS) capsules, it has been estimated that less than 20 serotypes account for 90% of about 40,000 deaths caused per year in the United States by the pneumococcal infection in adults (3). Due to the fact that the invasive infections are associated with high rates of mortality even with the appropriate antibiotic treatment and the prevalence of antibiotic-resistant *S. pneumoniae* is on the increase (4, 8), there is a need for effective vaccines against *S. pneumoniae*. Most of the vaccines are designed to induce antibodies to the capsular PSs, which are known to be protective against invasive pneumococcal diseases by inducing complement-dependent opsonophagocytosis (18). The current pneumococcal vaccine consists of 23 purified capsular PSs (13). This vaccine appears to be 60 to 80% effective in preventing the invasive disease in the adults (6), but children under 2 years of age respond poorly to most of the pneumococcal PS antigens of the vaccine (1). To overcome this drawback of the PS vaccine, protein-conjugated pneumococcal PS vaccines are being developed and evaluated for immunogenecity and safety in various populations including young children (2, 15).

Studies of the human antibody repertoire to *Haemophilus influenzae* type (Hib) PS have shown that the antibody fine specificity, avidity, and protective capacity can correlate with the expression of particular clones and to the antibody structures (7, 11). Moreover, different Hib PS vaccines elicit antibody populations with distinct patterns of antibody structure and disparate functional capacities (14). Antibody responses to the pneumococcal PSs have not yet been analyzed completely. Defining the human antibody responses to various pneumococcal PSs would provide the basic data needed for evaluating the vaccination efficacy of the pneumococcal PS antigens. It will also contribute to the understanding of differences between the individuals and/or populations in their abilities to respond to the various the pneumococcal PS vaccines. In addition, pneumococcal antibody repertoire could also be used for the study of genetic determinants and The somatic forces dictating the expression of the human antibody repertoire to PS antigens. In this study, the human antibody responses to 3 major serotypes (6B, 14, and 19F) of the pneumococcal capsular PSs were analyzed following immunization with 23-

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Table 1. Antibody values of pneumococcal reference serum 89-SF[a]

PS serotype	Total antibody (μg/ml)	Isotype (μg/ml)						
		IgG	IgM	IgA	IgG1	IgG2	κ	λ
6B	24.3	16.9	3.0	1.5	1.69*	15.2*	17.6*	6.7*
14	37.0	27.8	1.2	1.9	(2.78)	(25)	(26.8)	(10.2)
19F	18.8	13.0	3.2	2.0	(1.30)	(11.7)	(13.6)	(5.17)

[a] Antibody values without any marking were initially assigned by Lederle-Praxis Biologicals. The IgG values have been confirmed at FDA. Values marked with * are from Park *et al.* (12). Values in parentheses are arbitrarily assigned on the assumption that anti-14 and anti-19F antibodies of 89-SF have the same isotype composition as anti-6B antibodies and thus must be considered highly provisional.

valent pneumococcal PS vaccine.

Materials and Methods

Reagents

Purified cell wall polysaccharide (C-PS) was purchased from Statens Serumin stitut (Copenhagen, Denmark). The serotype 6B, 14, and 19F pneumococcal capsular PSs were purchased from American Type Culture Collection (Rockville, MD, USA). Pneumococcal reference serum, 89-SF was obtained from Dr. Frasch (Center for Biologics Evaluation and Research, Rockville, MD) Anti-human immunoglobulins (Igs)-alkaline phosphatase conjugate (A3313) and anti-mouse IgG-alkaline phosphatase conjugate (A2429) were purchased from Sigma (St. Louis, MO, USA). Anti-human isotype specific monoclonal antibodies were from Dr. Nahm (Rochester University, NY, USA).

Vaccination

Thirty-one healthy young adults were received. a single intramuscular injection of polyvalent pneumococcal vaccine (PNEUMO 23, Pasteur-Merieux Serums & Vaccines, France). Serum samples were collected at the time of immunization and 1 month later.

Antibody ELISA

ELISA was used to determine the total antibody and the isotype levels of the anti-pneumococcal PS sera (12). Microplates were coated with 5 μg/ml pneumococcal PS (6B, 14, or 19F) in phosphate-buffered saline (PBS) and left overnight. The plates were washed with PBS containing 0.1% Tween 20 and incubated with blocking solution (PBS containing 1% bovine serum albumin and 0.05% Tween 20) for 30 minutes. Serum samples, diluted 50 fold using blocking solution containing 10 μg/ml of C-PS, were added to the wells, serially diluted and then incubated for 2 hours. Wells were washed four times, followed by the addition of alkaline phoaphatase-conjugated anti-human Igs. After incubation at room temperature for 2 hours, the wells were washed and p-nitrophenyl phosphate in diethanolamine buffer (pH 9.8) was added. Absorbance at 405 nm was read with a microplate reader. All the incubations in ELISA were carried out at room temperature. Antibody levels were calculated from a standard curve generated with reference serum 89-SF. Assigned antibody values of 89-SF are shown in Table 1.

For the determination of isotype levels of the anti-pneumococcal PS antibodies, each of the anti-human isotype-specific monoclonal antibodies (Table 2) was added to the microplate wells (1 μg/ml) following incubation with the serum samples. Afer incubation for 2 hours, the wells were washed and anti-mouse IgG alkaline phosphatase conjugate was added.

Table 2. Anti-human isotype-specific monoclonal antibodies

Ab	HG7	HG11	HP	δADA	HA1	HK2	HL1
Specificity	IgG	IgG1	IgG2	IgM	IgA	Kappa	Lambda

Results and Discussions

Total serotype-specific antibody in anti-pneumococcal PS sera

Serum samples from 31 young adults were analyzed for their serotype-specific antibody levels using 89-SF as the standard. Total antibody levels of pre- and postimmune sera are summarized in Table 3. Although the mean antibody levels of preimmune sera to the 3 serotypes were substantially higher than 1.9

Table 3. Total serotype-specific antibody levels of sera before and after vaccination

	Geometric mean of Ab concentration (μg/ml)		
	6B	14	19F
Preimmune	5.59	5.65	6.25
Postimmune	19.15	21.59	25.36
Fold increase	3.4	3.8	4.1

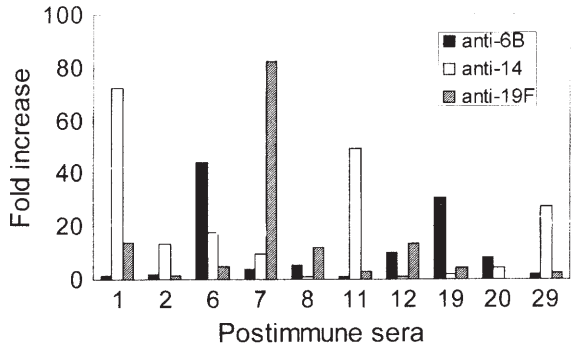

Fig. 1. Variations of fold increase among postimmune sera. Postimmune sera which have more than 10-fold differences in fold increases to the three different PS serotypes are shown here.

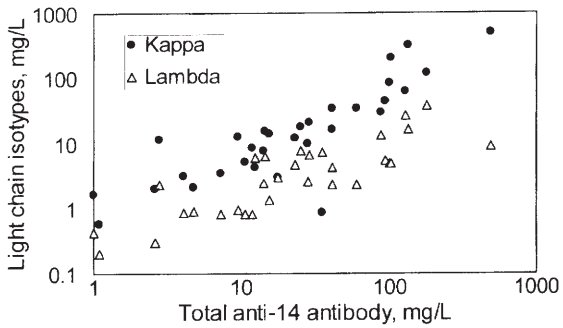

Fig. 2. Relationship between total anti-14 antibody and light chain isotypes. Correlation coefficients for κ, λ are 0.851, and 0.835, respectively.

μg/ml, the estimated 'protective level in humans' (16), some preimmune sera showed antibody levels less than 1.9 μg/ml (10 to 26% of tested sera depending on the PS serotype). The protective level of an antibody may depend on many factors including antibody affinity, antibody isotype and PS serotype, and the level of an antibody required to protect against a mild disease, such as otitis media, is likely to be different from that required to prevent more severe diseases, such as pneumonia or meningitis. Anyway the existence of young adults whose antibody levels are extremely low in preimmune sera raises a necessity of a comprehensive study which could clearly define the range of people to whom pneumococcal vaccination should be recommended.

Vaccination increased the total levels of anti-6B, anti-14, and anti-19F PS antibodies by 3.4-fold, 3.8-fold and 4.1-fold, respectively. The increase was statistically significant for all the three PS serotypes ($P<0.025$, two-tailed t test). However, several individuals showed great variations in antibody responses to different PS serotypes. In 10 out of the 31 postimmune sera, there were more than 10-fold differences in fold increases of antibody levels to the different PS serotypes and the PS showing the highest response differed between individuals (Fig. 1). These results extend previous reports (9, 10) in which although mean titers of antibody response against each capsular PS increased significantly, some individuals failed to show any response to certain serotypes. And the data strongly indicate that each PS antigen of the polyvalent anti-pneumopcoccal PS vaccine may have different immunogenecities in different individuals. As different antibody response could result in different vaccine efficacy, multivalent PS vaccines should be carefully tested for the immunogenecity of each vaccine component.

Distribution of light chain isotypes

In the analysis of light chain isotypes, κ and λ constituted 55% and 45% of anti-14 antibodies both in the preimmune and the postimmune sera. No significant difference was observed in κ and λ expression ($P=0.378$ in preimmune sera; 0.297 in postimmune sera, two-tailed t test). Similar results were observed also in the anti-6B and anti-19F PS antibody responses. And the levels of κ and λ anti-pneumococcal PS antibody responses were highly correlated with that of total antibody response (Fig.

Table 4. Isotype levels of sera before and after vaccination

PS	Serum	Geometric mean of Ab concentration (μg/ml)						
		IgG	IgG1	IgG2	IgM	IgA	κ	λ
6B	Preimmune	2.98	0.27	3.32	2.85	0.21	2.22	1.62
	Postimmune	10.6	0.77	10.0	4.41	0.99	6.29	5.73
	Fold increase	3.6	2.9	3.0	1.6	4.6	2.8	3.5
14	Preimmune	3.53	0.24	3.69	1.07	0.23	2.96	2.43
	Postimmune	16.2	0.44	13.9	2.17	0.92	9.84	8.08
	Fold increase	4.6	1.8	3.8	2.0	4.0	3.3	3.3
19F	Preimmune	4.53	0.46	3.97	2.54	0.39	3.83	3.14
	Postimmune	16.0	0.96	13.5	4.02	1.88	15.5	11.7
	Fold increase	3.5	2.1	3.4	1.6	4.8	4.0	3.7

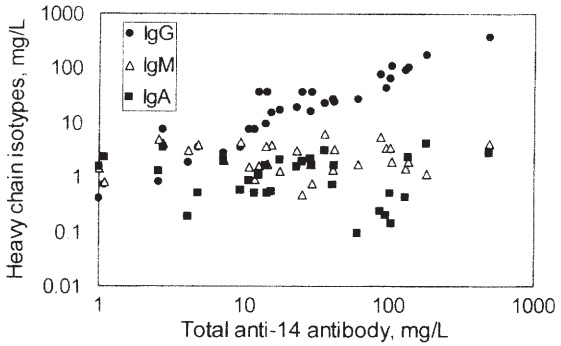

Fig. 3. Relationship between total anti-14 antibody and heavy chain isotypes. Correlation coefficients for IgG, IgM, and IgA are 0.942, 0.069, and -0.145, respectively.

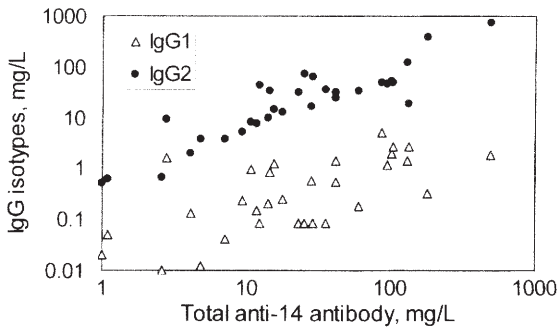

Fig. 4. Relationship between total anti-14 antibody and IgG subclasses. Correlation coefficients for IgG1 and IgG2 are 0.661 and 0.899, respectively.

2 and Table 5) regadrless of the serotypes. These results suggest that there are no particular dominance of κ or λ isotype in the light chain expression of human anti-pneumococcal antibody responses. Tarrand *et al.* (17) showed that antibodies of κ isotype are preferentially expressed in the human antibody responses to Hib PS, which has similar structure with pneumococcal 6B PS. The mechanism which make different immune responses to these structurally similar PSs is not clear now and needs further research.

Distribution of heavy chain isotypes

Isotype levels of pre- and postimmune sera are summarized in Table 4. In the anti-6B antibodies of preimmune sera, the majority of antibodies were IgG (49%) and IgM (48%). In the cases of the anti-14 and anti-19F antibodies, IgG was dominant (≥ 61%) with susbtantial amounts of IgM (≥22%). Fold increase by vaccination was higher for IgG (≥ 3.5) and IgA (≥4.0) than IgM (≤2.0) isotypes. IgG antibodies (≥66%) accounted for majority of the total anti-PS antibodies in the postimmune sera. In addition, the level of the IgG anti-pneumococcal PS antibody response was highly correlated with that of total antibody response (Fig. 3 and Table 5) regadrless of the serotypes.

IgG2 isotype constituted most (≥84% of IgG) of the IgG antibody responses in the postimmune sera (Table 4). and IgG2 levels were highly correlated with the total antibody levels in anti-pneumococcal PS antibody responses (Fig. 4 and Table 5). These results extend the previous data (12) in which human anti-6B antibody responses were mediated mainly by IgG2 isotype and suggest that restriction to IgG2 isotype in human antibody response is a common property of the various pneumococcal PSs.

To be protective against the bacterial infections, the antibodies should be able to elicit effector functions such as complement activation and/or opsonization. IgG2 has been claimed to be inefficient in activating complement and binding to Fc receptors on phagocyte surfaces (18). But some studies (5) have shown that IgG2 can activate complement at high epitope densities. IgG2 has also been reported to bind to LR allotype of FcγRIIa (19). Thus IgG2 anti-pneumococcal PS antibodies could induce the production of C3b and might be able to do their protective function by opsonizing the bacteria together with C3b.

Acknowledgments

This work was supported by a research grant (961-0506-052-2) from the Korea Science and Engineering Foundation. We especially thank Dr. Nahm of Rochester University for providing anti-hu-

Table 5. Correlation coefficients between antibody isotype and total antibody.

PS	Serum	Correlation coefficient						
		IgG	IgG1	IgG2	IgM	IgA	Kappa	Lamda
6B	Postimmune	0.913	0.684	0.919	0.033	0.569	0.915	0.789
	Preimmune	0.898	0.620	0.920	-0.012	0.321	0.669	0.757
14	Postimmune	0.942	0.661	0.899	0.069	-0.145	0.851	0.835
	Preimmune	0.933	0.799	0.978	0.535	-0.140	0.941	0.848
19F	Postimmune	0.847	0.533	0.828	-0.085	-0.033	0.756	0.649
	Preimmune	0.904	0.764	0.971	-0.050	-0.241	0.940	0.797

man isotype-specific monoclonal antibodies.

References

1. **Alonsode Velasco, E., A.F.M. Verheul, J. Verhoef, and H. Snippe.** 1995. *Streptococcus pneumoniae*: virulence factors, pathogenesis, and vaccines. *Microbiol. Rev.* **59**, 591-603.
2. **Anderson, E.L., D.J. Kennedy, K.M. Geldmacher, J. Donnelly, and P.M. Mendelman.** 1996. Immunogenecity of heptavalent pneumococcal vaccine in infants. *J. Pediatr.* **128**, 649-653.
3. **Austrian, R.** 1984. Pneumococcal infections, p. 257-288. *In* R. Germanier (ed.), Bacterial vaccines. Academic Press Inc., Orlando, Florida.
4. **Breiman, R.F., J.C. Butler, F.C. Tenover, J.A. Elliott, and R.R. Facklam.** 1994. Emergence of drug-resistant pneumococcal infections in the United States. *J. Amer. Med. Assoc.* **271**, 1831-1835.
5. **Burton, D.R. and J.M. Woof.** 1992. Human antibody effector function. *Adv. Immunol.* **51**, 1-54.
6. **Butler, J.C., R.F. Breiman, J.F. Campbell, H.B. Lipman, C.V. Broome, and R.R. Fcklam.** 1993. Pneumococcal polysacchride vaccine efficacy: an evaluation of current recommendations. *JAMA* **270**, 1826-1831.
7. **Granoff, D.M., P.G. Shackelford, S.J. Holmes, and A. H. Lucas.** 1993. Variable region expression in the antibody responses of infants vaccinated with *Haemophilus influenzae* type b polysaccharide-protein conjugates: description of a new λ light chain-associated idiotype and the relation between idiotypic expression, avidity, and vaccine formulation. *J. Clin. Invest.* **91**, 788-796.
8. **Lee, H.J., J.Y. Park, S.H. Jang, J.H. Kim, E.C. Kim, and K.W. Choi.** 1995. High incidence of resistance to multiple antimicrobials in clinical isolates of *Streptococcus pneumoniae* from a university hospital in Korea. *Clin. Infect. Dis.* **20**, 826-835.
9. **Mufson, M.A., H.E. Krause, G. Schiffman, and D.F. Hughey.** 1987. Pneumococcal antibody levels one decade after immunization of healthy adults. *Am. J. Med. Sci.* **293**, 279-284.
10. **Musher, D.M., M.J. Luchi, D.A. Watson, R. Hamilton, and R.E. Baughn.** 1990. Pneumococcal polysaccharide vaccine in young adults and old bronchitics: determination of IgG responses by ELISA and the effect of adsorption of serum with non-type-specific cell wall polysaccharide. *J. Infect. Dis.* **161**, 728-735.
11. **Nahm, M.H., K.H. Kim, P. Anderson, S.V. Hetherington, and M.K. Park.** 1995. Functional capacities of clonal antibodies to *Haemophilus influenzae* type b polysaccharide. *Infect. Immun.* **6**, 2989-2994.
12. **Park, M.K., Y. Sun, J.V. Olander, J.W. Hoffmann, and M.H. Nahm.** 1996. The repertoire of human antibodies to the carbohydrate capsule of *Streptococcus pneumoniae* 6B. *J. Infect. Dis.* **174**, 75-82.
13. **Robbins, J.B., R. Austrian, C.J. Lee, S.C. Rastogi, G. Schiffman, J. Henrichsen, P.H. Makela, C.V. Broome, R.R. Facklam, R.H. Tiesjema, and J.C. Parke, Jr.** 1983. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. *J. Infect. Dis.* **148**, 1136-1159.
14. **Schlesinger, Y. and D.M. Granoff.** 1992. Avidity and bactericidal activity of antibody elicited by different *Haemophilus influenzae* type b conjugate vaccines. *JAMA* **267**, 1489-1494.
15. **Shelly, M.A., H. Jacoby, G.J. Riley, B.T. Graves, M. Pichichero, and J.J. Treanor.** 1997. Comparison of pneumococcal polysaccharide and CRM197-conjugated pneumococcal oligosaccharide vaccines in young and elderly adults. *Infect. Immun.* **65**, 242-247.
16. **Schiffman G.** 1983. Pneumococcal vaccine: a tool for the evaluation of the B-cell function of the immune system. *Proc. Soc. Exp. Biol. Med.* **174**, 309-315.
17. **Tarrand, J.J., M.G. Scott, P.A. Takes, and M.H. Nahm.** 1989. Clonal characterization of the human IgG antibody repertoire to *Haemophilus influenzae* type b polysaccharide. Demonstration of three of V regions and their association with H and L chain isotype. *J. Immunol.* **142**, 2519-2526
18. **Van de Winkel, J.H.J. and P.J.A. Capel.** 1993. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol. Today* **14**, 215-221.
19. **Vioarsson, G., I. Jonsdottir, S. Jonsson, and H. Valdimarsson.** 1994. Opsonization and antibodies to capsular and cell wall polysaccharides of *Streptococcus pneumoniae. J. Infect. Dis.* **170**, 592-599.