Eur Respir J 2002; 20: 813–818
DOI: 10.1183/09031936.02.00289702
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0903-1936

Vaccination of COPD patients with a pneumococcus type 6B tetanus toxoid conjugate vaccine

S. Jonsson*, G. Vidarsson*,#, H. Valdimarsson*, G. Schiffman¶, R. Schneerson+, I. Jonsdottir*

Vaccination of COPD patients with a pneumococcus type 6B tetanus toxoid conjugate vaccine. S. Jonsson, G. Vidarsson, H. Valdimarsson, G. Schiffman, R. Schneerson, I. Jonsdottir. ©ERS Journals Ltd 2002.

ABSTRACT: This paper examines how pneumococcal type 6B polysaccharide conjugated to tetanus toxoid (Pn6B-TT) compares to a 23 valent pneumococcal vaccine (pneumococcal polysaccharide (PPS)-23) with respect to immunogenicity and serum opsonic activity in patients with chronic obstructive pulmonary disease (COPD).

Patients with COPD aged 55–75 yrs were vaccinated with Pn6B-TT (n=10) or with PPS-23 (n=9). Healthy young adults (HA) were vaccinated with Pn6B-TT as controls. Total antibodies to serotype 6B polysaccharide were measured by radioimmunoassay and immunoglobulin (Ig)G antibodies by enzyme-linked immunosorbent assay. Opsonic activity was measured by a phagocytosis assay using human neutrophils as effector cells.

The patient groups were comparable by age, smoking history, lung function and use of steroids. COPD patients vaccinated with Pn6B-TT or PPS-23 showed an increase in IgG antibodies and a nonsignificant increase in opsonic activity. This was similar to the increase in IgG and opsonic activity seen in HA. There was a significant correlation between antibody levels and opsonic activity in COPD patients vaccinated both with Pn6B-TT and PPS-23.

Pneumococcal antibodies have been shown to confer protection from infection. The results of the present study indicate that protective immunity can be expected in elderly chronic obstructive pulmonary disease patients vaccinated with conjugate vaccines.

Eur Respir J 2002; 20: 813–818.

*Depts of Immunology and Medicine, Landspitali - University Hospital, Reykjavik, Iceland, #Dept of Immunology, University Medical Centre Utrecht (UMCU), Utrecht, The Netherlands, ¶Dept of Microbiology and Immunology, State University of New York Science Center at Brooklyn, Brooklyn, NY, USA, +Laboratory of Developmental and Molecular Immunology, National Institute of Child Health and Human Development, Bethesda, MD, USA.

Correspondence: I. Jonsdottir, Dept of Immunology, Landspitali - University Hospital, Hringbraut, 101 Reykjavik, Iceland.
Fax: 354 5601943
E-mail: ingileif@landspitali.is

Keywords: Antibodies, chronic obstructive pulmonary disease, opsonophagocytosis, pneumococcus, vaccination

Received: October 25 2001
Accepted after revision: April 12 2002

The study was funded by the Science Fund of the Icelandic Research Council and The Icelandic Ministry of Health and Social Security.

Infections due to *Streptococcus pneumoniae* continue to be an important cause of morbidity and mortality among elderly individuals with a variety of chronic diseases [1–3]. Pneumococci are the most frequent causes of community-acquired pneumonia, which has a mortality rate of 5–10%, despite modern antimicrobial therapy and intensive care [4, 5]. Although the risk to patients with chronic obstructive pulmonary disease (COPD) has been disputed [6, 7], a prospective study showed that patients with chronic pulmonary, renal and cardiac disease comprised the most significant risk groups for pneumococcal pneumonia and its complications [2].

Vaccination with pneumococcal polysaccharide (PPS) stimulates antibody production [8] and is protective in healthy adults [9, 10], but immunogenicity is low in certain risk groups [11]. Several prospective randomised studies have failed to show protection against the development of pneumonia [2, 12, 13], but their validity has been in doubt because of the lack of statistical power [14]. Case-control studies seem to indicate efficacy in preventing bacteraemia, which complicates 15–25% of hospitalised pneumonia cases [10], but have the disadvantage of possible selection bias. The increasing spread of penicillin-resistant pneumococci worldwide has added to the need for a vaccine of undisputed efficacy [15, 16]. To increase immunogenicity, protein-conjugated PPS vaccines have been developed [17]. Pneumococcal conjugate vaccines have been shown to be immunogenic [17–20] and highly effective against invasive disease in children unresponsive to PPS [20].

In the present study, antibody responses and opsonic activities were compared in the sera of elderly patients with COPD, vaccinated either with pneumococcal type 6B polysaccharide conjugated to tetanus toxoid (Pn6B-TT) or with PPS-23. An increased antibody response of healthy adults to this experimental vaccine compared to PPS was previously reported [17], but the vaccine had not been tested in at-risk adults. The antibody responses and opsonic activity of sera from healthy adults immunised with

Pn6B-TT were also studied as a control group. The safety and immunogenicity of Pn6B-TT after repeated vaccinations has been reported [18, 19].

Materials and methods

Informed consent was obtained from the patients and healthy adult volunteers and the protocol was reviewed and approved by the Ethics Committees of the National University Hospital and Reykjavik City Hospital in Reykjavik, Iceland (Assurance no. S-8172-01), the Medical Board of the National Institutes of Health, Bethesda, MD, USA (protocols OH93-CH-NO19 and OH93-CH-NO24), and the United States Food and Drug Administration (FDA) (BB-IND 1977) according to European and USA regulations.

Vaccines and vaccinees

Pn6B-TT was prepared at the Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, Bethesda, MD, USA (Lot 55683). Twenty-three valent PPS vaccine (Imovax) was obtained from Pasteur Mérieux, Lyon, France.

Vaccinees were injected with 0.5 mL of Pn6B-TT containing 12 μg of type 6B polysaccharide and 37.5 μg tetanus toxoid or with 0.5 mL of Imovax (Pasteur Mérieux) containing 25 μg of each of the 23 serotypes in the vaccine. Healthy adult volunteers aged 23–50 yrs (median 28.9 yrs) received one injection of Pn6B-TT (healthy adults (HA), n=15) or one dose of Imovax (controls, n=15). Ten elderly individuals with stable COPD received a single injection of 0.5 mL of Pn6B-TT (group A), and nine received a single injection of the PPS-23 Imovax (group B). Each subject had to meet all of the following entry criteria: 1) clinical diagnosis of COPD of any type; 2) aged 55–75 yrs; 3) forced expiratory volume in one second (FEV1) <1.5 $L \cdot s^{-1}$, FEV1/forced vital capacity <65%, and <10% response to bronchodilator; 4) no immunocompromising disease or a history of pneumococcal vaccination or infection with pneumococcus type 6B; 5) received no steroids or equivalent to <15 mg prednisolone per day. Blood samples were collected before and 1 month after the vaccination. Sera were kept in aliquots at -20°C for antibody measurements and at -70°C for analysis of opsonic activity. Antibody responses and opsonic activities were compared between the COPD patients and the healthy adults.

Antibodies

Immunoglobulin (Ig)G anti-Pn6B was measured by enzyme-linked immunosorbent assay (ELISA), according to the protocol recommended by the pneumococcal workshop at the Centers for Disease Control and Prevention, Atlanta, GA, USA, October 1994, with minor modifications [18]. In brief, ELISA plates (Costar, Cambridge, MA, USA) were coated with 10 $\mu g \cdot mL^{-1}$ Pn6B polysaccharide (American Type Culture Collection, Rockville, MD, USA) for 5 h at 37°C. Standard and test sera were diluted 1:25 and adsorbed with 50 $\mu g \cdot mL^{-1}$ of cell wall polysaccharide (CWPS) (Statens Seruminstitute, Copenhagen, Denmark) for 30 min at room temperature, prior to incubation in four two-fold dilutions for 2 h in the Pn6B-coated plates. Pn6B-IgG was detected by incubation with biotin-labelled monoclonal antibody HP-6043 (Hybridoma Reagent Laboratory, Baltimore, MD, USA) at 1:500 dilution, followed by incubation with alkaline phosphatase-labelled avidin (DAKO, Glostrup, Denmark) at 1:2,000 dilution for 1 h. The reaction was developed by p-nitrophenyl phosphate (Sigma, St Louis, MO, USA) and optical density read at 405 nm in a Titertek Multiscan Spectrophotometer (Flow Laboratories, Irvine, UK). IgG anti-Pn6B levels are expressed in $\mu g \cdot mL^{-1}$, calculated from a curve generated by serial dilutions of an in-house standard prepared from an adult post 23-valent PPS vaccination pool, calibrated against reference serum 89SF, provided by C.E. Frasch, US FDA, Rockville, MD, USA.

Total anti-Pn6B antibodies (Ab) were measured by radio-immunoassay (RIA) [21] and the results expressed in ng Ab $N \cdot mL^{-1}$ (conversion factor for ng Ab $N \cdot mL^{-1}$ to Ab concentration is 6.25).

Bacteria

Freeze-dried *S. pneumoniae* serogroup 6 was reconstituted in Todd Hewith broth and subcultured on sheep blood agar (37°C, 5% CO_2). By subtyping with specific monoclonal antibodies (Statens Seruminstitute), this strain was found to be of serotype 6A (after the study had been completed). Colonies were harvested and suspended in Tryptoset broth (Difco Laboratories, Detroit, MI, USA) for storage in -70°C. For radiolabelling, a culture with an initial density of 1×10^4 colony forming units $\cdot mL^{-1}$ was started in 5 mL of Rosswell Park Memorial Institute (RPMI) 1640 (GIBCO, Life Technologies GIBCO BRL, Paisley, UK), supplemented with 10% foetal calf serum (FCS; GIBCO) and 500 μCi of ^{3}H-labelled-lysine (Amersham, Amersham, UK), collected in mid-log phase by centrifugation at 2,200×*g* for 20 min, and washed in Hank's balanced salt solution (HBSS, GIBCO) containing 5% FCS. The labelled pneumococci were adjusted to 1.5×10^7 bacteria $\cdot mL^{-1}$ in HBSS with 5% FCS and used immediately. The viability and density was confirmed by plate colony counts for each experiment.

Phagocytes

Fresh polymorphonuclear leukocytes (PMNLs) were isolated from the peripheral blood of a healthy adult volunteer by dextran sedimentation followed by ficoll (Histopaque, Sigma) gradient centrifugation to remove mononuclear cells. The final concentration was adjusted to 1.5×10^6 PMNL $\cdot mL^{-1}$ HBSS. The blood donor was FcγRIIa-H131 homozygote (kindly genotyped by C.L. Anderson and J.M. Osborne, Ohio

State University College of Medicine, Columbus, OH, USA), and FcγRIII-NA1/NA2 heterozygote (typed using fluorescence staining with monoclonal antibodies (MAb) CLBgran11 and GRM1 and analysis by flow cytometry (FACS); the MAbs were a kind gift from M. de Haas and A.E.G. Kr. von dem Borne, CLB, Amsterdam, The Netherlands).

Opsonophagocytosis

Sera were assayed as described [22] with minor modifications, using fresh polymorphonuclear cells (PMN) and ^{3}H-labelled Pn6B without added complement [19]. Bacterial and PMN suspensions (150 μL of each, ratio of ~10:1) were mixed with test sera at a concentration predetermined to be in the sensitivity range of the assay [8, 22]. The total volume of 0.5 mL was incubated with rotation (250 rev·min^{-1}) for 30 min at 37°C. Controls for nonspecific binding (NSC; with all reactants except heat-inactivated FCS instead of human serum) and total bacteria input (TB; with all reactants) were included in each assay. The reaction was stopped by adding 2 mL of phosphate buffered saline + 0.02% NaN_3. The PMNs and the cell-associated bacteria (CAB) were pelleted by centrifugation at 160×*g*, except that TB was centrifuged at 2,200×*g*. After washing, cell pellets were resuspended in 0.5 mL of 1.25% deoxycholate and transferred to 4.5 mL of scintillation liquid (Hionic-fluor, Packard Bioscience, Meriden, CT, USA). The radioactivity (range 500–10,000 counts per min (cpm)) was measured in a liquid scintillation counter (Packard Bioscience) and per cent uptake of ^{3}H-labelled bacteria was calculated as:

$$\frac{(\text{cpm CAB} - \text{cpm NSC})}{(\text{cpm TB} - \text{cpm NSC})} \times 100\% \qquad (1)$$

Statistical analysis

A paired t-test was used on log-transformed values for comparison within groups and nonparametric signed-rank test when normal distribution was not obtained. For comparison between groups, a t-test was used except when normality failed or variance was unequal, then the Mann-Whitney rank-sum test was used. Pearson correlation was used to evaluate the relationship between opsonic activity and antibody concentration. A p-value of <0.05 was considered significant.

Results

Subjects

The two groups of patients with COPD were comparable with respect to age, smoking history and degree of functional impairment (table 1). Of the patients who received Pn6B-TT or PPS-23, the mean ages were 69 and 68 yrs, the mean % predicted values for FEV1 were 29% and 37% (p=0.09), and the duration of smoking was 46 yrs and 35 yrs, respectively (p=0.80). Five of 10 patients who received Pn6B-TT were being treated with steroids compared to three of 10 patients who received PPS-23.

Table 1. – Characteristics of vaccinated patients with chronic obstructive pulmonary disease

Vaccine group	Age mean	% pred FEV1	Smoking yrs	Steroids n
A: Pn6B-TT[#]	69	29	46	5/10
B: PPS-23[¶]	68	37	35	3/9
p-value	0.67	0.09	0.80	0.46

FEV1: forced expiratory volume in one second; Pn6B-TT: pneumococcal type 6B polysaccharide conjugated to tetanus toxoid; PPS-23: pneumococcal polysaccharide 23. [#]: n=10; [¶]: n=9. A p-value of <0.05 was considered significant.

Antibodies

Before vaccination, the geometric mean (GM) total Pn6B antibody level in the sera of the two patient groups were 466 and 398 ng Ab N·mL^{-1}, respectively, and above the level considered to be protective [21]. Table 2 shows the GM antibody levels of each group before and after vaccination by the two antibody measuring methods. As a whole, the groups responded with a highly significant rise in antibody levels to both vaccines. There was no difference in the postvaccination levels of IgG or total antibody in the COPD patients receiving Pn6B-TT or the PPS-23. Two patients in each group had very low prevaccination levels of antibody and no rise after vaccination, whereas one patient in group A and two in group B had very high prevaccination levels and no further increase after vaccination. This was found both for total and IgG antibodies and demonstrates the individual variability in natural immunity and the response to vaccination. This lack of response did

Table 2. – Antibodies to serotype 6B pneumococcal polysaccharide (anti-Pn6B) before and after vaccination of chronic obstructive pulmonary disease (COPD) and healthy adults (HA)

	Group		
	A	B	C
Vaccine	Pn6B-TT	PPS-23	Pn6B-TT
IgG μg·mL^{-1}			
Pre	2.39	2.99	2.45
Post	4.69	5.73	7.32
p-value	0.0138	0.0231	0.0017
Total ng Ab N·ml^{-1}			
Pre	466	398	340
Post	1323	1159	1373
p-value	0.0127	0.0143	0.0001

Pn6B-TT: pneumococcal type 6B polysaccharide conjugated to tetanus toxoid; PPS-23: pneumococcal polysaccharide 23; IgG: immunoglobulin G; Ab: antibody. A: COPD, n=10; B: COPD, n=9; C: healthy adults, n=15. A p-value of <0.05 was considered significant.

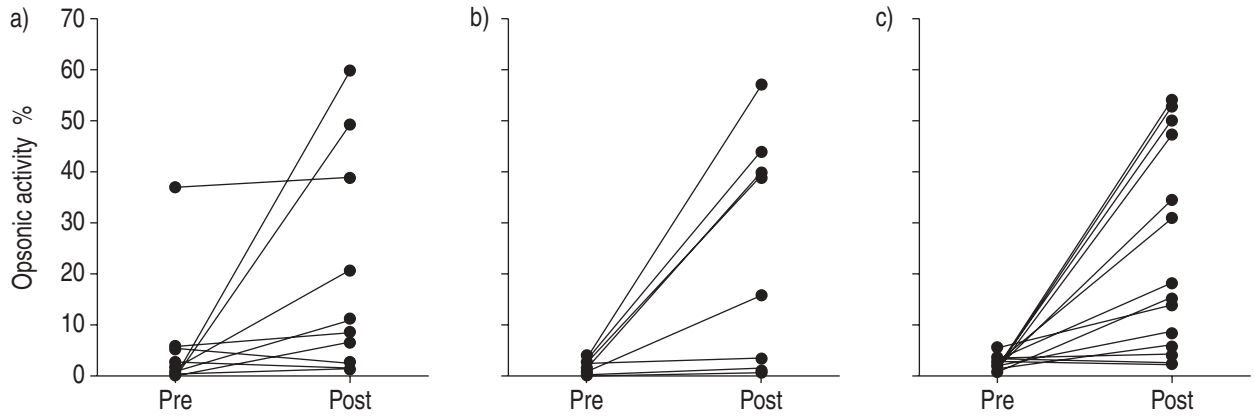

Fig. 1. – Opsonic activity measured as % uptake of radiolabelled pneumococci by polymorphonuclear cells, mediated by pre- and postvaccination sera from a) chronic obstructive pulmonary disease (COPD) patients vaccinated with pneumococcal type 6B polysaccharide conjugated to tetanus toxoid (Pn6B-TT) (p=0.066), b) COPD patients vaccinated with pneumococcal polysaccharide-23 (p=0.094), or c) healthy adults vaccinated with Pn6B-TT (p=0.001). A p-value of <0.05 was considered significant.

not relate to steroid intake. All other individuals had a substantial increase in antibody levels after vaccination. Healthy adult volunteers injected with Pn6B-TT responded with a significant rise in total and IgG antibodies (table 2). The HA group had a similar GM prevaccination level as both COPD groups, but slightly higher postvaccination levels; 7.32 *versus* 4.69 and 5.73 μg IgG·mL^{-1} and 1,373 *versus* 1,323 and 1,159 ng Ab N·mL^{-1}, respectively. The differences were not statistically significant.

Unlike the authors' previous experience with a Pn6A [17], the response to the polysaccharide and the Pn6B-TT conjugate was comparable to that seen with PPS-23 in the present study. This prompted a reanalysis of this vaccine lot. No disintegration of the conjugate was detected, but the concentration of the conjugate was found to be only half of the original concentration (the rest was found attached to the vials). This could be related to the lesser antibody response.

Opsonisation

The sensitivity range of the opsonisation assay is narrow and opsonisation reaches a plateau of ~60% uptake at high serum antibody concentration. The 5% serum concentration was in the sensitivity range of the assay and was chosen for measurements of opsonic activity of adult sera.

In agreement with the rise in anti-6B antibodies, many of the COPD patients vaccinated with PPS-23 or with Pn6B-TT showed a vaccine-induced increase in opsonic activity (fig. 1), although not significant for either group. In the healthy adult volunteers vaccinated with Pn6B-TT, a highly significant increase in opsonic activity was observed. The GM postvaccination opsonisation activities were similar in both COPD groups (p=0.811), but there was a marked individual variation in all groups.

When the postvaccination antibody levels were compared to the opsonising activity of sera as measured in the phagocytosis assay, an excellent correlation was found. Figure 2 shows the relationship between

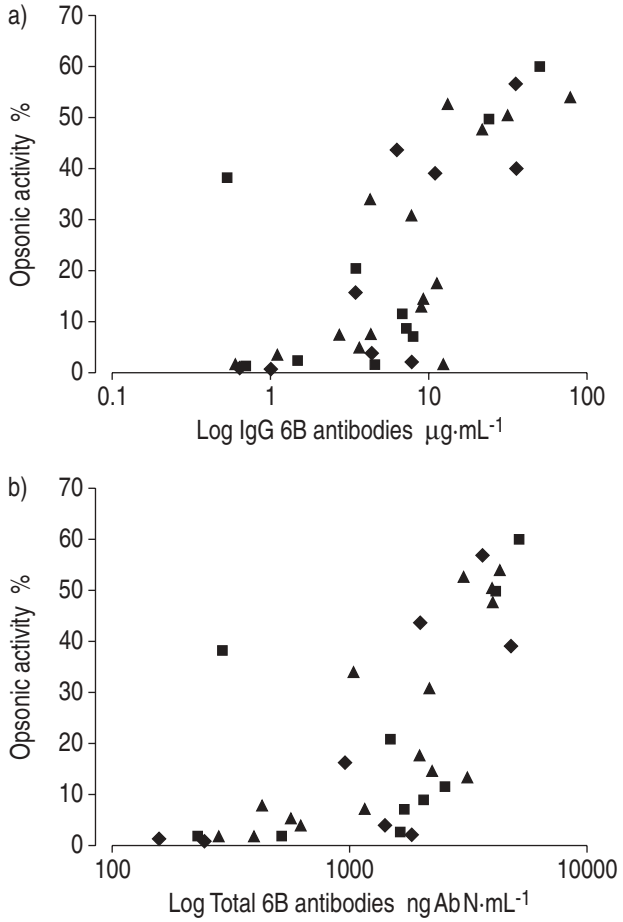

Fig. 2. – Relationship between opsonic activity (% uptake of pneumococci) and a) 6B immunoglobulin G (IgG) antibody levels (r=0.702, p<0.001) or b) 6B total antibody levels in postvaccination sera (r=0.779, p<0.001) from chronic obstructive pulmonary disease (COPD) patients vaccinated with pneumococcal type 6B polysaccharide conjugated to tetanus toxoid (Pn6B-TT) (■), COPD patients vaccinated with pneumococcal polysaccharide-23 (◆) or healthy adults vaccinated with Pn6B-TT (▲). Ab: antibody. A p-value of <0.05 was considered significant.

opsonic activity and the postvaccination antibody levels for anti-Pn6B in ng Ab $N \cdot mL^{-1}$ as measured by RIA and in μg $IgG \cdot mL^{-1}$ measured by the ELISA for all COPD and HA subjects. The correlation between opsonic activity and antibody levels was high for total antibodies using RIA values ($r=0.779$, $p<0.001$) and for IgG antibodies ($r=0.702$, $p<0.001$) measured by ELISA. The relationship between postvaccination IgG and opsonisation was analysed among COPD patients according to the vaccine given. There was a highly significant correlation in the Pn6B-TT group ($r=0.783$, $p=0.007$), and a slightly lesser one in the PPS-23 group ($r=0.749$, $p=0.020$). Similar results were obtained by RIA, which correlated significantly with opsonisation both in the Pn6B-TT group ($r=0.713$, $p=0.021$) and in the PPS-23 group ($r=0.768$, $p=0.026$). The antibody measurements, therefore, reflect functional activity.

Discussion

In the present study, the authors compared the antibody response and opsonic activity of sera from two groups of patients with COPD, one vaccinated with Pn6B-TT and the other with PPS-23. Both groups were found to respond with significant rises in antibody levels comparable to that seen in a group of HA vaccinated with Pn6B-TT. The majority of individuals responded to the vaccinations with a marked increase in antibody levels; however, in each group of patients there were individuals who did not respond, demonstrating the individual variability in the response to vaccination with these antigens.

The rise in antibody levels measured as IgG or total antibody was accompanied by a corresponding increase in opsonic activity of sera indicating the formation of a functionally active antibody. There was a similar correlation between postvaccination IgG or total antibody and opsonic activity for the group vaccinated with Pn6B-TT or PPS-23. In previous studies the authors demonstrated that the antibodies produced in response to vaccination with Pn6B-TT are primarily IgG1 [19], which is known to activate complement readily.

In an earlier study of healthy individuals, greater antibody production had been shown following vaccination with Pn6B-TT compared with PPS [17]. A booster effect could not be elicited following repeated injections with the Pn6B-TT conjugate vaccine [17]. Similar results were obtained by others in a study of older adults using CRM197 conjugate where the magnitude of antibody responses to the conjugate vaccine and PPS-23 were similar, and no booster effect could be found after re-injection with PPS-23 following CRM197-conjugate vaccine [23]. However, in a study of patients with Hodgkin′s disease primed with heptavalent outer membrane protein conjugate, a strong booster effect following subsequent vaccination with PPS-23 was seen for five of the six serotypes tested [24]. These findings suggest that immune responses to conjugate vaccines in adults may be dependent on the carrier protein to which the PPS is conjugated, the vaccination schedule, vaccine constituents and/or related to host factors.

Pneumococcal serotype-specific opsonic activity of sera may be a more direct indicator of the protective potential of an experimental vaccine than serum antibodies alone. Protection from pneumococcal infection depends primarily on opsonisation of the bacteria by type-specific serum antibodies and complement. The present authors have shown for several pneumococcal serotypes that in adults vaccinated with polysaccharide vaccine, opsonic activity of sera correlated best with IgG anti-PPS [8], while antibodies to the pneumococcal CWPS had little opsonic activity [22]. Human antibody responses to PPS in adults have been reported to be primarily of the IgG2 subclass [25], which does not readily activate complement unless at high concentrations or high epitope density [21, 26, 27]. Furthermore, PPS are T-cell independent antigens of type 2 that do not generate immunological memory. Antibodies produced following vaccination of children and healthy adults using experimental PPS conjugated to proteins are primarily of the IgG1 subclass, which readily activates compliment. Such a T-cell dependent characteristic and a booster response have been demonstrated in children and patients with Hodgkin′s disease using protein-conjugated pneumococcal polysaccharide vaccines [20, 24].

In a recently reported efficacy study in children, a heptavalent pneumococcal conjugate vaccine was highly efficacious against invasive disease [20]. These findings offer hope that similar advances can be achieved for the large population of adults at risk for serious pneumococcal infections.

The controversy regarding the efficacy of PPS-23 in adults at risk has probably reduced the usage of this vaccine in many parts of the world [3]. Further studies are needed to test the immunogenicity and efficacy of pneumococcal conjugate vaccines in adults at risk. The ability to elicit booster responses after repeated injections also requires further study. Such studies could provide valuable information on how to improve immunity against this important pathogen and to resolve the controversy regarding the utility of pneumococcal vaccination in the elderly and chronically ill.

In previous studies of pneumococcal immunity among patients with COPD, these individuals have been shown to have higher levels of antibodies than healthy adults presumably as a result of frequent antigen exposure [6, 21]. The antibody response to vaccination with PPS has usually been comparable to that of healthy controls, but levels have shown a more rapid decline with time [28, 29]. According to the present study and previous reports [30], the antibody response to vaccination does not appear to be influenced by the prevaccination antibody level or the age of the recipient.

In the present study, it has been demonstrated that pneumococcal type 6B polysaccharide conjugated to tetanus toxoid induced production of functional antibodies in elderly patients with chronic obstructive pulmonary disease. The results indicate that protective immunity can be expected in elderly chronic obstructive pulmonary disease patients vaccinated with

protein-conjugated polysaccharide vaccines. Repeated injections might be beneficial in this age group, although this remains to be shown. Considering that serotype 6B is one of the least immunogenic pneumococcal polysaccharides, it is anticipated that the response to the other serotypes will be better and conjugate vaccines will prove to be more effective against pneumococcal disease in adults at risk.

Acknowledgements. The authors wish to thank G. Ingolfsdottir for excellent technical assistance and J.B. Robbins for expert advice.

References

1. Austrian R. Some observations on the pneumococcus and on the current status of pneumococcal disease and its prevention. *Rev Infect Dis* 1981; 3: S1–S17.
2. Simberkoff MS, Cross AP, Al-Ibrahim M, *et al.* Efficacy of pneumococcal vaccine in high-risk patients. Results of a Veterans Administration Cooperative Study. *N Engl J Med* 1986; 315: 1318–1327.
3. Fedson DS. Pneumococcal vaccination in the United States and 20 other developed countries, 1981–1996. *Clin Infect Dis* 1998; 26: 1117–1122.
4. Austrian R, Gold J. Pneumococcal bacteremia with specific reference to bacteremic pneumococcal pneumonia. *Ann Intern Med* 1964; 60: 759–776.
5. Hook EW III, Horton CA, Schaberg DR. Failure of intensive care unit support to influence mortality from pneumococcal bacteremia. *JAMA* 1983; 249: 1055–1057.
6. Davis AL, Aranda CP, Shiffman G, Christianson LC. Pneumococcal infection and immunologic response to pneumococcal vaccine in chronic obstructive pulmonary disease. *Chest* 1987; 92: 204–212.
7. Willliams JH, Moser KM. Pneumococcal vaccine and patients with chronic lung disease. *Ann Intern Med* 1986; 104: 106–109.
8. Bardardottir E, Jonsson S, Jonsdottir I, Sigfusson A, Valdimarsson H. IgG subclass response and opsonization of *Streptococcus pneumoniae* after vaccination of healthy adults. *J Infect Dis* 1990; 162: 482–488.
9. Austrian R, Douglas RM, Schiffman G, *et al.* Prevention of pneumococcal pneumonia by vaccination. *Trans Assoc Am Physicians* 1976; 89: 184–194.
10. Landesman SH, Schiffman G. Assessment of the antibody response to pneumococcal vaccine in high-risk populations. *Rev Infect Dis* 1981; 3: S184–S197.
11. Örtqvist Å, Hedlund J, Burman LÅ, *et al.* Randomised trial of 23-valent pneumococcal capsular polysaccharide vaccine in prevention of pneumonia in middle-aged and elderly people. *Lancet* 1998; 351: 399–403.
12. Forrester HL, Jahnigen DW, LaForce FM. Inefficacy of pneumococcal vaccine in a high-risk population. *Am J Med* 1987; 83: 425–430.
13. Shapiro ED, Berg AT, Austrian R, *et al.* The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. *N Engl J Med* 1991; 325: 1453–1460.
14. Fedson DS. The clinical effectiveness of pneumococcal vaccination: a brief review. *Vaccine* 1999; 17: 85–90.
15. Kristinsson KG. The epidemiology of penicillin-resistant pneumococci (in Swedish). *Nordisk Medicin* 1996; 111: 103–108.
16. Nuorti JP, Butler JC, Crutcher JM, *et al.* An outbreak of multidrug-resistant pneumococcal pneumonia and bacteremia among unvaccinated nursing home residents. *N Engl J Med* 1998; 338: 1861–1868.
17. Schneerson R, Robbins JB, Parke JC Jr, *et al.* Quantitative and qualitative analyses of serum antibodies elicited in adults by *Haemophilus influenzae* type b and pneumococcus type 6A capsular polysaccharide-tetanus toxoid conjugates. *Infect Immun* 1986; 52: 519–528.
18. Sigurdardottir ST, Vidarsson G, Gudnason T, *et al.* Immune responses of infants vaccinated with serotype 6B pneumococcal polysaccharide conjugated with tetanus toxoid. *Pediatr Infect Dis J* 1997; 16: 667–674.
19. Vidarsson G, Sigurdardottir ST, Gudnason Th, *et al.* Isotypes and opsonophagocytosis of pneumococcus type 6B antibodies elicited in infants and adults by an experimental pneumococcus type 6B-tetanus toxoid vaccine. *Infect Immun* 1998; 66: 1–6.
20. Shinefield HR, Black S. Efficacy of pneumococcal conjugate vaccines in large scale field trials. *Pediatr Infect Dis J* 2000; 19: 394–397.
21. Landesman SH, Smith PM, Schiffman G. Pneumococcal vaccine in elderly patients with COPD. *Chest* 1983; 84: 433–435.
22. Vidarsson G, Jonsdottir I, Jonsson S, Valdimarsson H. Opsonization and antibodies to capsular and cell wall polysaccharides of *Streptococcus pneumoniae*. *J Infect Dis* 1994; 170: 592–599.
23. Douglas CP, Anderson EL, Lottenbach K, Mink CM. Reactogenicity and immunogenicity of a protein-conjugated pneumococcal oligosaccharide vaccine in older adults. *J Infect Dis* 1996; 173: 1014–1018.
24. Chan CY, Molrine DC, George S, *et al.* Pneumococcal conjugate vaccine primes for antibody responses to polysaccharide pneumococcal vaccine after treatment of Hodgkin′s disease. *J Infect Dis* 1996; 173: 256–258.
25. Barrett DJ, Ayoub EM. IgG2 subclass restriction of antibody to pneumococcal polysaccharides. *Clin Exp Immunol* 1986; 63: 127–134.
26. Bredius RG, de Vries CE, Troelstra A, *et al.* Phagocytosis of *Staphylococcus aureus* and *Haemophilus influenzae* type B opsonized with polyclonal human IgG1 and IgG2 antibodies. Functional hFc gamma RIIa polymorphism to IgG2. *J Immunol* 1993; 151: 1463–1472.
27. Burton DR, Woof JM. Human antibody effector function. *Adv Immunol* 1992; 51: 1–84.
28. Kraus C, Fischer S, Ansorg R, Hüttemann U. Pneumococcal antibodies (IgG, IgM) in patients with chronic obstructive lung diseases 3 years after pneumococcal vaccination. *Med Microbiol Immunol* 1985; 174: 51–58.
29. Konradsen HB. Quantity and avidity of pneumococcal antibodies before and up to five years after pneumococcal vaccination of elderly persons. *Clin Infect Dis* 1995; 21: 616–620.
30. Musher DM, Groover JE, Graviss EA, Baughn RE. The lack of association between aging and postvaccination levels of IgG antibody to capsular polysaccharides of *Streptococcus pneumoniae*. *Clin Infect Dis* 1995; 22: 165–167.