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Antibody Response to a Seven-Valent Pneumococcal Conjugated Vaccine in Patients with Ataxia-Telangiectasia

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Immunodeficiency is a characteristic feature of ataxia-telangiectasia (A-T). Humoral immunodeficiency generally consists of hypogammaglobulinemia and impaired antibody response to bacterial and viral antigens. We previously observed defective antibody response to 23-valent pneumococcal polysaccharide vaccine (PPV) in 96% of 29 patients with A-T. In this study, we investigated the antibody response to a seven-valent pneumococcal conjugate vaccine, PCV7, in 14 patients with A-T. IgG antibody levels to four pneumococcal serotypes, 6B, 14, 19F, 23F, which were included in PCV7, were measured by ELISA in pre- and postimmunization serum samples. Antibody titers against each individual *Streptococcus pneumoniae* serotype was considered to be positive when serotype specific pneumococcal antibody titer was higher than 10% (>10 U/mL) of the reference plasma pool level. However, when the fold increase (FI) in postimmunization antibody titer was less than two, the subject was determined to be unresponsive to the given serotype. The values were compared with the results obtained in age- and ethnic-matched children after one dose of PPV. Only two patients produced antibodies to one serotype each; one to serotype 19 with a fold increase of <2, and the other to serotype 23F with a fold increase of 5.7 based on the above criteria, although the differences between pre- and postvaccine antibody titers for serotypes 14, 19, and 23 appeared to be statistically significant. In conclusion, A-T patients failed to respond to one dose of PCV7 vaccine. Two or more doses of conjugated vaccine may be required to recruit the help of T lymphocytes in A-T patients.

KEY WORDS: Ataxia-telangiectasia; conjugated pneumococcal vaccine; antibody response.

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INTRODUCTION

Ataxia-telangiectasia (A-T) is an autosomal recessive multisystem disease characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, increased radiosensitivity, predisposition to lymphoid malignancies, and variable T and B cell immunodeficiency (1–4). The leading cause of death in A-T is chronic sinopulmonary infections most probably due to immunodeficiency. *Streptococcus pneumoniae* is one of common causes of respiratory infections. It is the most important cause of childhood mortality particularly in developing countries according to the WHO estimates. Rapidly increasing antimicrobial resistance further increases the significance of pneumococcal diseases. Therefore, preventive approaches toward pneumococcal infections have been implemented. Immunization with pneumococcal polysaccharide vaccine (PPV) is one of these measures. However, because of immaturity of the immune system, it is not effective in children younger than 2 years of age in whom 80% of systemic pneumococcal disease develops. To circumvent the poor immunogenicity of PPV in children, several conjugated vaccines have been developed through coupling the pneumococcal polysaccharide (PP) antigen with a protein carrier that converts the T cell-independent antigen to a T cell-dependent one. These T cell-dependent pneumococcal antigens can induce antibody production at a very early age (5–7). Immunization with conjugate vaccine may be an option for patients who fail to mount an adequate response to polysaccharide vaccine (8). The seven-valent vaccine (PCV7) with diphtheria CRM197 carrier protein has recently been licensed and included in the routine immunization schedule of childhood in the United States (**7**). PCV7 is, now, recommended for universal use in children under 2 years of age, at the ages of 2, 4, and 6 months with a booster dose at 12–15 months of age (7). In children over 5 years of age, there are limited safety and immunogenicity

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data for PCV. Studies of small numbers of children with sickle cell disease and HIV infection suggest that PCV7 is safe and immunogenic in children up to 13 years old (7). Therefore, administration of a single dose of 23-valent PPV or PCV7 is acceptable in susceptible children older than 5 years of age. In A-T, antibody production in response to bacterial and viral antigens is generally reduced, although there is considerable variation (2, 4, 9–11). Previously, we evaluated the B-cell function in 29 patients with A-T, as assessed by IgG antibody production to six pneumococcal serotypes after immunization with a 23-valent PPV. The majority of those patients showed defective antibody production (12). Therefore, we conducted a study to investigate the antibody response to a PCV7 in A-T patients. This is the first report on the safety and immunogenicity of a conjugated pneumococcal vaccine in A-T patients.

SUBJECTS AND METHODS

Patients

Antibody production against PCV7 was determined in 14 patients with A-T (age range 7–20 years, median 13 years). Twelve of these patients were studied previously with respect to antibody production in response to unconjugated pneumococcal vaccine (Pneumo 23, Pasteur-Merieux). Serum IgG levels were within normal limits in all patients. IgG2 concentration was measured in 11 patients and low levels were found in six of them (54%). Health, age, and gender matched 40 subjects from the same ethnic population were included in the control group. The study was approved by the Institutional Review Board at the Hacettepe University Faculty of Medicine. Patients consented prior to the study. Blood was drawn by venipuncture before and after 4 weeks of immunization with PCV7. Serum samples were stored at -80°C until the analysis, and pre- and postimmunization samples were assayed simultaneously.

The Study Vaccine

The study vaccine, PCV7 (Prevnar; Wyeth Lederle Vaccines, Pearl River, NY), contained polysaccharide antigens for the seven most common serotypes of *Streptococcus pneumoniae* (4, 6B, 9V, 14, 18C, 19F, 23F). The vaccine was administered intramusculary according to manufacturer's instructions.

ELISA for Measurement of Antipneumococcal Antibodies

IgG antibody levels to four pneumococcal serotypes, 6B, 14, 19F, 23F, which were included in both PCV7 and PPV, were measured by ELISA in pre- and postimmunization serum samples. Ninety-six well microtiter plates were coated with capsular polysaccharide antigens provided from American Type Culture Collection, ATCC, Rockville, MD. All serum samples were preincubated overnight with CWPS (species-specific pneumococcal common cell wall polysaccharide) (C-polysaccharide purified by Statens Serum Inst, Denmark) to eliminate the antibodies against cell wall polysaccharides. Antibody concentration to each serotype was expressed as the percentage of concentration in the reference serum, the hyperimmune plasma pool (US Pneumococcal Reference serum FDA7 CBER, Bethesda, MD) in units per milliliter where the reference plasma pool concentration represents 100 U/mL for each serotype. Antibody titer against each individual *Streptococcus pneumoniae* serotype was considered to be positive when serotype specific pneumococcal ab titer was higher than 10% ($>$10 U/mL) of the reference plasma pool level (12). However, when the fold increase (FI) in postimmunization antibody titer was less than two, the subject was determined to be unresponsive to the given serotype. The values were compared with the results obtained in age- and ethnic-matched children after one dose of PPV. The serotype-specific antibody concentrations were converted to gravimetric units (μg/mL) according to IgG assignments of reference serum pool (13). In order to confirm the consistency of the results, pre- and post-immunization pneumococcal antibody titers were also kindly studied in the laboratory of Dr G Rijkers (Department of Immunology, University Hospital for Children, Utrecht, the Netherlands).

Statistical Analysis

Geometric mean concentrations (GMC) of antibody titers against each serotype were calculated. Pre- and postimmunization antibody titers were compared by Wilcoxon signed ranks test.

RESULTS

The vaccine was safe and well tolerated in all patients. Pre- and postimmunization antibody titers with one dose of PCV7 and GMCs against individual pneumococcal serotypes (6B, 14, 19F, 23) are shown in Figs. 1 and 2, and in Table I. The difference between pre- and postantibody titers in response to PCV7 in patients with A-T, and between pre- and postantibody titers in response to PPV in patients with A-T and in the control group (reported in ref. (12)) were compared by Wilcoxon signed ranks test. With PCV7, the differences were significant

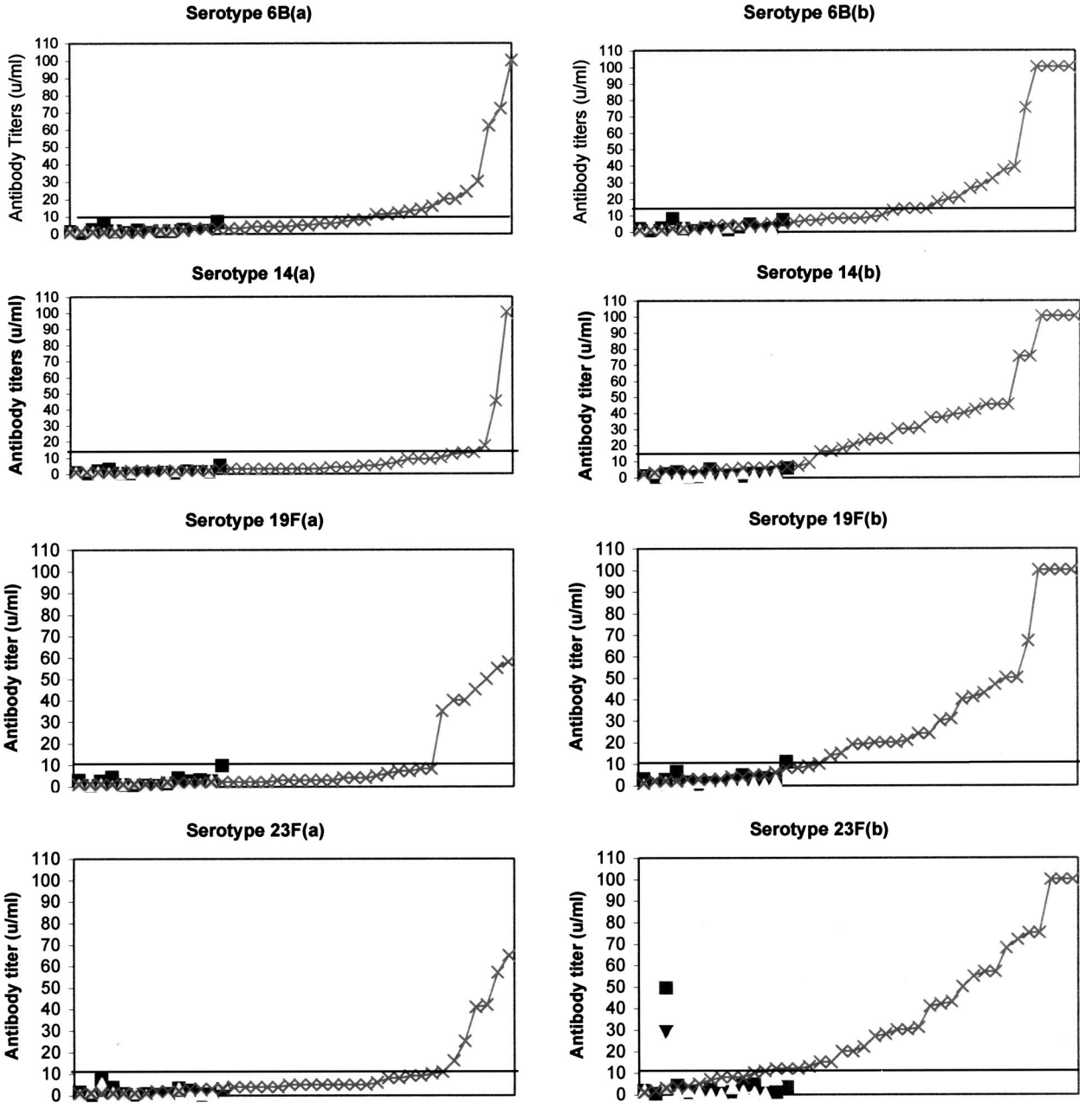
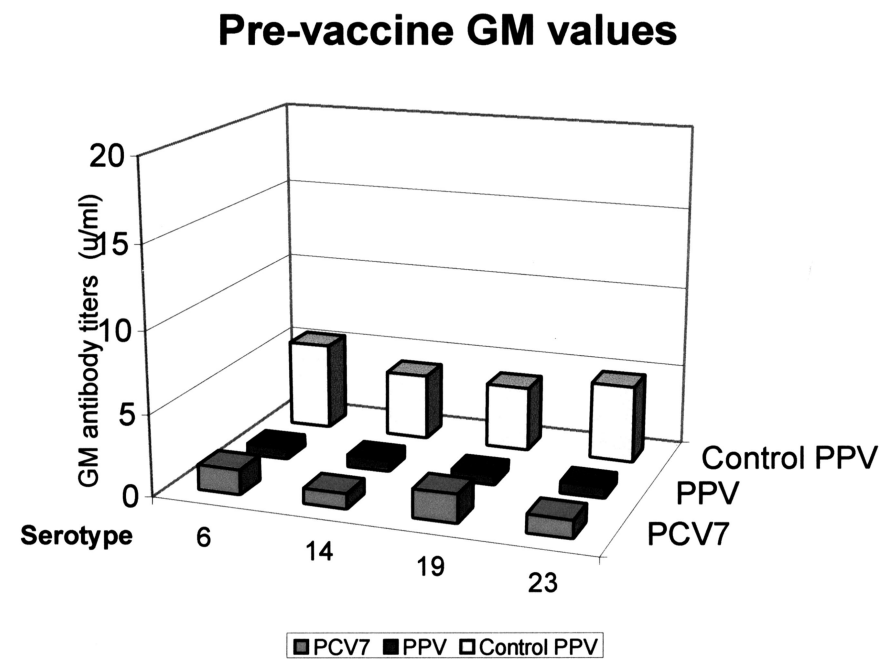


Fig. 1. a) Pre- and b) postimmunization antibody titers (u/mL) to individual serotypes in response to one dose of PCV7 and PPV. ■, PCV7 Values obtained with seven-valent pneumococcal conjugate vaccine in patients; ▼, PPV Values obtained with 23 valent pneumococcal polysaccharide vaccine in patients; ×, Control PPV Values obtained with 23 valent pneumococcal polysaccharide vaccine in controls.

between pre- and postvaccine antibody titers for serotypes 14, 19, and 23, whereas the difference was not significant for serotype 6 ($p = 0.53$). However, only two out of 14 patients with A-T produced an antibody titer of >10 U/mL to one serotype; one patient to serotype 19 with a FI < 2, and the other patient to serotype 23F with a FI of 5.7. Thus, on the basis of the criteria for antibody response, the latter patient was considered to have positive antibody response to one serotype present in PCV7. This patient had responded to the same serotype after PPV but the titer was somewhat lower. The former patient had not produced any antibody to any serotype after PPV. After PCV7, one patient had no detectable polysaccharide antibodies to any serotype. Overall, one of the two A-T patients who did not have any detectable antibody postimmunization with unconjugated PPV was observed to mount an antibody



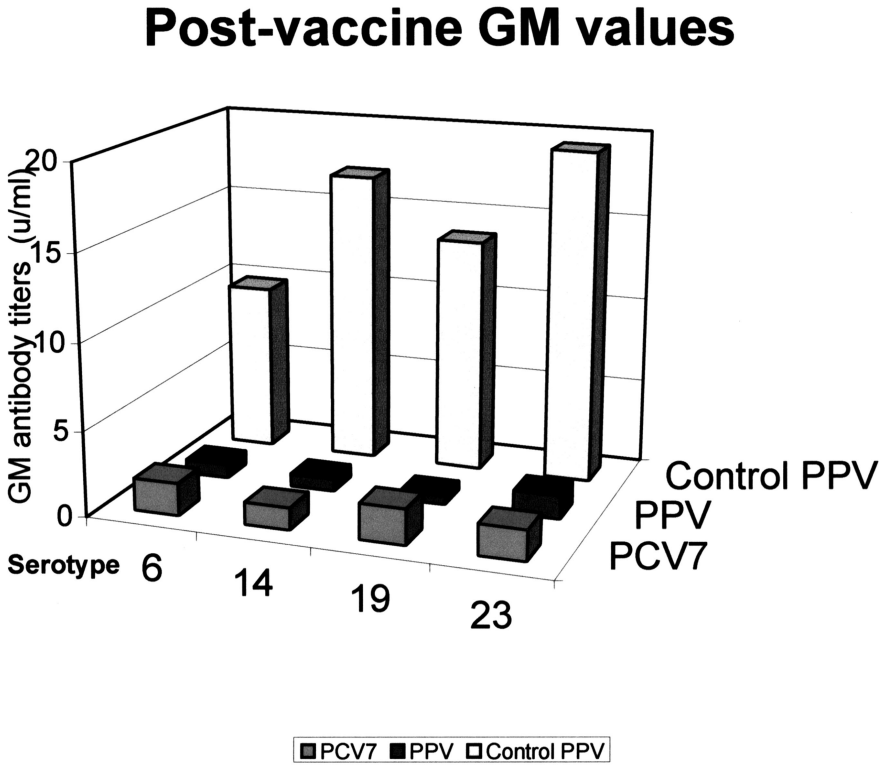


Fig. 2. Geometric means of pre- and postimmunization antibody titers (u/mL) to individual serotypes in response to one dose of PCV7 and PPV. PCV7, PPV, and Control PPV values as in Fig. 1 on page 413.

Table I. Antibody Titers to Four Pneumococcal Serotypes in Response to PCV7 and PPV in Patients with A-T and in the Control Group

	Patient group				Control group	
	PCV7 ($N = 14$)		PPV ($N = 12$)[a]		PPV ($N = 40$)[a]	
Serotype 6B						
GM (u/mL)	1.57	1.89	0.71	0.81	5.41	9.8*
Range	0.4−7.0	0.4−7.3	0.1−2.8	0.1−3.1	1–100	1–100
GM (μg/mL)	0.26	0.32	0.12	0.14	0.91	1.66
Range	0.07−1.2	0.07−1.35	0.02−0.47	0.02−0.53	0.17–16.9	0.17–16.9
Serotype 14						
GM (u/mL)	0.93	1.32*	0.76	0.75	4.1	17.1*
Range	0.2−5.3	0.2−5.8	0.1−2.8	0.1−3.2	1–100	2–100
GM (μg/mL)	0.26	0.37	0.21	0.21	1.11	4.7
Range	0.07−1.35	0.06−1.61	0.02−0.53	0.03−0.97	0.27–27.8	0.56–27.8
Serotype 19F						
GM (u/mL)	1.76	2.1*	0.64	0.53	4.04	14.7*
Range	0.4−9.8	0.4−11	0.4−11	0.6−11	1–58	1–100
GM (μg/mL)	0.23	0.27	0.08	0.07	0.52	1.79
Range	0.07−1.27	0.08−4.01	0.02−0.33	0.03−2.43	0.13–7.54	0.13–13.0
Serotype 23 F						
GM (u/mL)	1.1	1.78*	0.7	1.36	4.83	19.4*
Range	0.4−7.3	0.4−49.5	0.1−5.8	0.1−30	1–65	1–100
GM (μg/mL)	0.09	0.14	0.06	0.11	0.39	1.57
Range	0.02−0.59	0.08−4.01	0.01−0.47	0.01−0.59	0.08-5.26	0.08–8.1

Note. PCV, values obtained with seven-valent pneumococcal conjugate vaccine; PPV, Values obtained with 23-valent pneumococcal polysaccharide vaccine; GM, geometric mean; Pre-vac: prevaccine; Post-vac: postvaccine. Values >100 u/mL were accepted as 100. Serotype specific IgG antibody concentrations of reference serum for serotypes 6B, 14, 19F, and 23F are 16.9, 27.8, 13.0, and 8.1 μg/mL, respectively.
[a] Reported in ref. (12).
* $p < 0.005$.

response to some serotypes postimmunization with PCV7 although the antibody titers were very low.

The results from Dr Rijkers' laboratory were comparable to ours (data not shown).

Serum IgG2 concentration was measured in 11 patients and low or undetectable levels were found in six of them (54%). However, there was no correlation between serum IgG2 levels and antibody production.

DISCUSSION

Immunodeficiency of both cellular and humoral immune systems is one of the landmarks of A-T. In A-T, the humoral immune system shows variable impairment with complete or partial deficiencies of IgA, IgE, IgG, and IgG subclasses, whereas the proportion of total B cells in the peripheral blood usually remains normal or elevated (2, 4, 9, 10). However, the immune defects developing in patients with A-T are still poorly characterized. Illegitimate joining during V(D)J recombination, intrinsic B-lymphocyte switch defects, or the lack of T-cell-derived switch factors, defect in intracellular signal transduction in T- and B-lymphocytes, inability to carry out correct processing, and trafficking of antigens through Golgi apparatus have been proposed to explain the immunodeficiency (2, 14–17). Khanna *et al.* (18) demonstrated that signaling through the B-cell antigen receptor induced by anti-immunoglobulin cross-linking is impaired in EBV transformed A-T B-lymphocytes. This study suggested that ATM (ataxia-telangiectasia mutated), the defective gene in A-T, might also function in this signaling pathway. However, Speck *et al.* (19) found no evidence of any defect in B-cell receptor signal transduction in A-T B cells.

The antibody responses to bacterial and viral antigens are generally reduced in A-T patients (2, 4, 9, 10). In a study by Weemaes *et al.*, the mean increases in antibody titers to blood group substance, *E. coli*, Vi antigen and tularemi antigen were significantly less than in the control group (9). The primary IgG, IgM, and IgA antibody responses to HPH (helix pommatia haemocyanin), a T-cell dependent antigen, were found to be defective in patients with A-T, whereas secondary responses to diphteria, tetanus, and polio vaccine were normal (9). In another study, weak antibody response to tetanus and polio antigen was reported (10). In five AT patients, absent or low antibody titers to various viruses were found (4). *In vitro*, lymphocytes from AT patients produced less anti-influenza antibody than lymphocytes from normal controls did (11). We observed defective antibody response to 23-valent PPV in 96% of the 29 patients with A-T in our previous study (12). Antibody response to PCV7

immunization in the present study was also poor. However, few patients who did not respond to PPV produced detectable antibodies post PCV7 immunization. In addition, with PCV7, differences between pre- and postvaccination antibody titers for serotypes 14, 19, and 23 were statistically significant although most of the postvaccination antibody titers did not reach the criteria for positive antibody responsiveness. Conjugation of polysaccharides to protein carriers has been a significant improvement in vaccine strategy since this approach facilitates the generation of antibody response by converting a T-independent antigen to a T-dependent antigen. In older patients, although conjugated vaccine has been shown to be efficient (20), the response may not be as good as expected because of the probable low immunity to the carrier protein in this age group (21). Studies in animals and children have shown that previous exposure to the carrier protein may improve the immunogenicity of glycoconjugate vaccines (22, 23). Therefore, prior immunization with the carrier protein antigen is considered as a legitimate approach to enhance the immunogenicity.

In summary, A-T patients, older than 7 years, failed to respond one dose of PCV7 vaccine. However, two or more doses of conjugated vaccine may be required to recruit the help of T-lymphocytes, or a recent boost with the carrier protein may be helpful as suggested for older adults.

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