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Impaired IgG Antibody Production to Pneumococcal Polysaccharides in Patients with Ataxia–Telangiectasia

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Various factors seem to be etiologic in the susceptibility to sinopulmonary infections in ataxia–telangiectasia (A-T) patients, i.e., low serum and salivary IgA, low serum IgG2, and even aspiration of saliva. *S. pneumoniae* is a common pathogen responsible from pulmonary infections and impaired antibody response to polysaccharide antigens is seen in patients with IgG2 and IgA deficiency as well as patients with CVID and WAS. We studied IgG-type antibody production to six pneumococcal serotypes in 29 A-T patients by ELISA before and 3–4 weeks after pneumococcal vaccine. The response was considered positive when the antibody titer was >10 U/ml but weak when the titer was 10–20 U/ml. Twenty-two of 29 (76%) patients did not respond to any of the serotypes, 5 (17%) showed a positive response to one serotype, 1 (3.4%) to two serotypes, and 1 (3.4%) to four serotypes. With conversion to gravimetric units (ng IgG/ml) and >1800 ng/ml (300 ng Ab N/ml) considered a positive response, 5 of 29 (17.2%) patients showed a positive response (300 ng ab N/ml) to two or fewer serotypes. All patients tested produced IgG antibody to tetanus toxoid. Sixteen of 27 (59.3%) patients had low IgG2 and four (14.8%) had low IgG3 levels, while 18 (62.1%) of 29 patients had low serum IgA. No correlation was found either between serum Ig isotype levels and antipolysaccharide antibody response or between susceptibility to infection and antibody production. The mechanism responsible for disturbed antipolysaccharide (TI-2 antigen) antibody production in patients with A-T needs to be investigated. It may provide additional information on the function of the ATM gene product and be helpful in clarifying the role of B cells and contribution of T cells in TI-2 responses.

KEY WORDS: Ataxia–telangiectasia; pneumococcal polysaccharides; antibody; IgG subclass; IgA.

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 a variable degree of abnormalities of both cellular and humoral immunity (1). The latter include hypogammaglobulinemia, selective IgA (2, 3) and IgE (4) deficiency, and IgG subclass deficiency (5, 6). Reduced antibody responses both to bacterial antigens and to protein antigens have been reported (7–10). The defects in the primary antibody response are more pronounced than in the secondary response (9). Defects in cellular immunity include abnormal development of the thymus (10), a reduced *in vitro* T lymphocyte proliferative response (11, 12), and impaired generation of virus-specific MHC-restricted cytotoxic T cells (13). Frequent pulmonary infection is a prominent feature of A-T and may progress to bronchiectasis and pulmonary fibrosis severe enough to cause respiratory insufficiency and death. Although the severity of sinopulmonary infection tends to be associated with low levels of serum and salivary IgA, often in association with low levels of IgG2, a patient lacking serum and salivary IgA may be free of recurrent infections or a patient who suffers from severe progressive infection may show a normal immunoglobulin pattern (14–16). Even aspirations of saliva may be considered as another contributing factor leading to susceptibility to pulmonary infections in these patients.

It is known that *Streptococcus pneumonia* is a common pathogen responsible for pulmonary infections, and

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Table I. Numbers of A-T Patients and Controls with Positive Antibody to Each Serotype

Reference serum		ELISA, pneumococcal serotype	>1800 ng/ml[a]		>10 U/ml		Immunogenicity
Pneumococcal serotype	ng IgG Ab/ml		Patients	Controls	Patients	Controls	
3	2400	3	0/29	6/40	2/29 (1/29)[b]	33/40 (25/40)[b]	Strong
7F	5200	7F	1/29	26/40	4/29 (3/29)	37/40 (35/40)	Strong
23F	8100	23F	1/29	20/40	2/29 (2/29)	30/40 (22/40)	Weak
19F	13000	19F	2/29	23/40	2/29 (2/29)	24/40 (21/40)	Intermediate
6B	16900	6B	0/29	18/40	0/29 (0/29)	18/40 (12/40)	Weak
14	27800	14	2/29	28/40	1/29 (0/29)	24/40 (21/40)	Intermediate

[a] >300 ng Ab N/ml.
[b] Numbers in parantheses show the number of patients and controls with >20 U/ml Ab.

pneumococcal capsular polysaccharide antibody production has been reported to correlate with the serum IgG2 level (17). However, there are patients described in the literature with a defective antibody response to polysaccharide antigens while having normal serum IgG2 or with normal antibody production in the presence of IgG2 deficiency (18, 19–25). An impaired response to polysaccharide antigens has also been reported in some patients with IgA deficiency (21).

The aim of this study was to determine the role of antibody production capacity to pneumococcal polysaccharides in the susceptibility to recurrent sinopulmonary infections and to analyze the relationship between serum IgA and IgG2 levels and antibody production in A-T patients.

METHODS

Twenty-nine A-T patients aged between 4 and 19 years (median, 10 years; mean, 10.2 years) with normal serum IgG levels were included in the study.

Recurrent infection was defined as more than eight upper respiratory tract infections or at least four episodes of sinusitis per year and/or recurrent lower respiratory tract infection with or without chronic lung disease, bronchiectasis. Twenty-two of these patients suffered from frequent sinopulmonary infection, while seven did not. The patients were immunized with a 23-valent pneumococcal vaccine (pneumo 23, Pasteur Merieux) containing 25 μg of each purified type-specific capsular polysaccharide of 23 pneumococcal serotypes. Blood was drawn before and 3–4 weeks after immunization. All serum samples were stored at −80°C until used.

IgG antibody levels to six common pneumococcal serotypes—3, 7F (strong immunogenic), 14, 19F (intermediate immunogenic), 6B, and 23F (weak immunogenic)—were measured by ELISA in pre- and postimmunization serum samples. All sera were analyzed in duplicate and sera from individual subjects were analyzed in the same ELISA run. Microtiter plates were coated with capsular polysaccharide antigens obtained from the American Type Culture Collection (ATCC), Rockville, MD. All serum samples were preincubated overnight with species-specific pneumococcal common cell wall polysaccharide (CWPS) (C-polysaccharide purified; Statens Serum Institute, Denmark) to eliminate the antibodies to cell wall polysaccharides. Antibody concentrations are expressed as the percentage of reference serum; the hyperimmune plasma pool (U.S. Pneumococcal Reference serum FDA7 CBER, Bethesda, MD), as units per milliliter, where the reference plasma pool represents 100 U/ml for each serotype. We chose a minimal concentration of 10 U/ml as a positive postvaccination antibody titer but considered it as a weak response when the level was between 10 and 20 U/ml. This criterion for a positive antibody response was selected taking into account the results obtained in 40 healthy age-matched (5–15 years; median, 10 years; mean, 9.7 years) Turkish children (26). Type-specific pneumococcal antibody levels were converted to gravimetric units (ng/ml) using IgG assignments of reference serum 89S, revised as of 1998. A concentration of 1800 ng/ml antibody, which is equivalent to 300 ng Ab N/ml based on a conversion factor of 160 ng of antibody nitrogen (Ab/N)/ml to 1 μg/ml, was defined as an adequate IgG antibody response to an individual

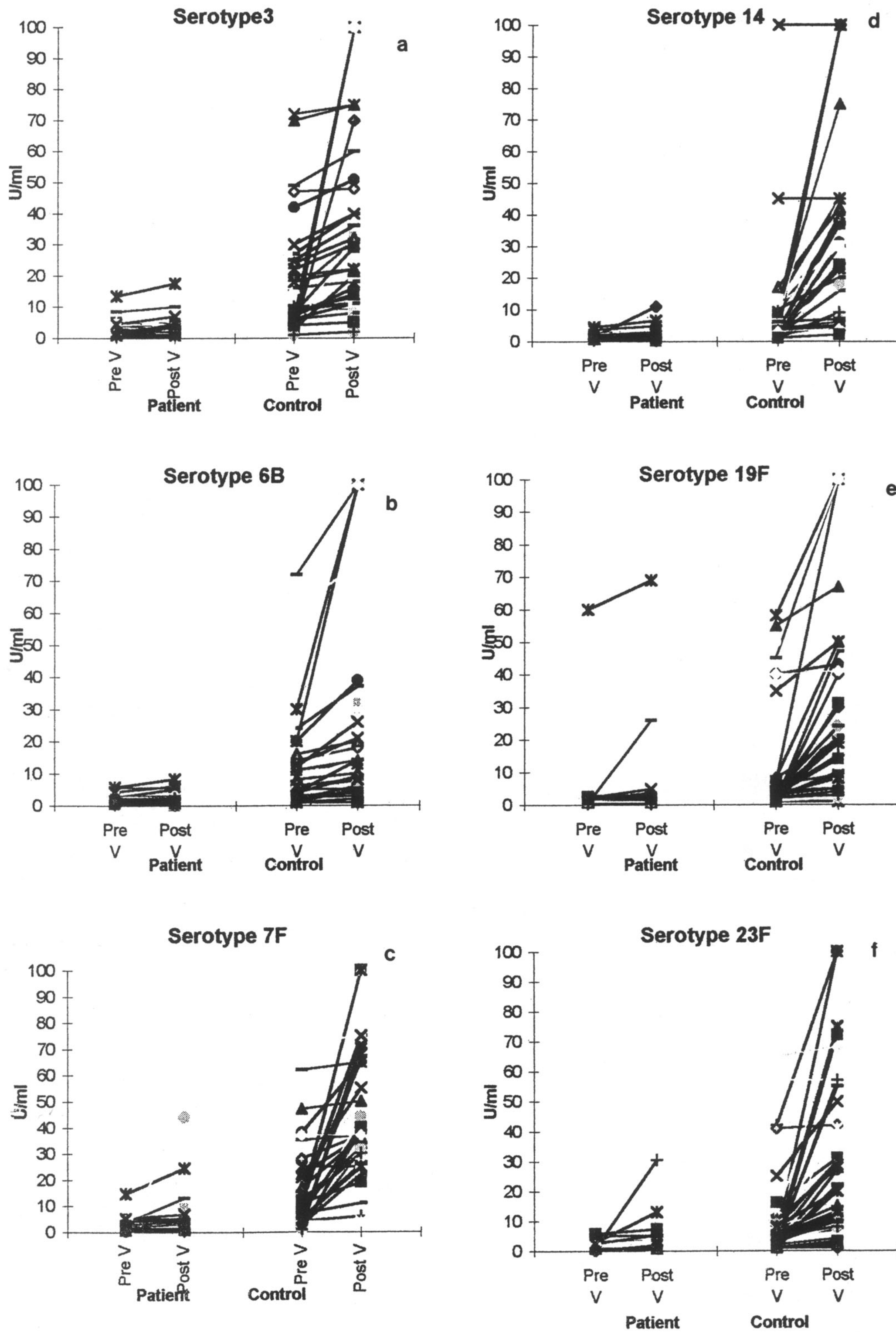

Fig. 1. Comparison of pre- and postimmunization antibody titers against six pneumococcal serotypes (U/ml; ELISA) in A-T versus healthy Turkish children (control group). Some lines represent more than one individual.

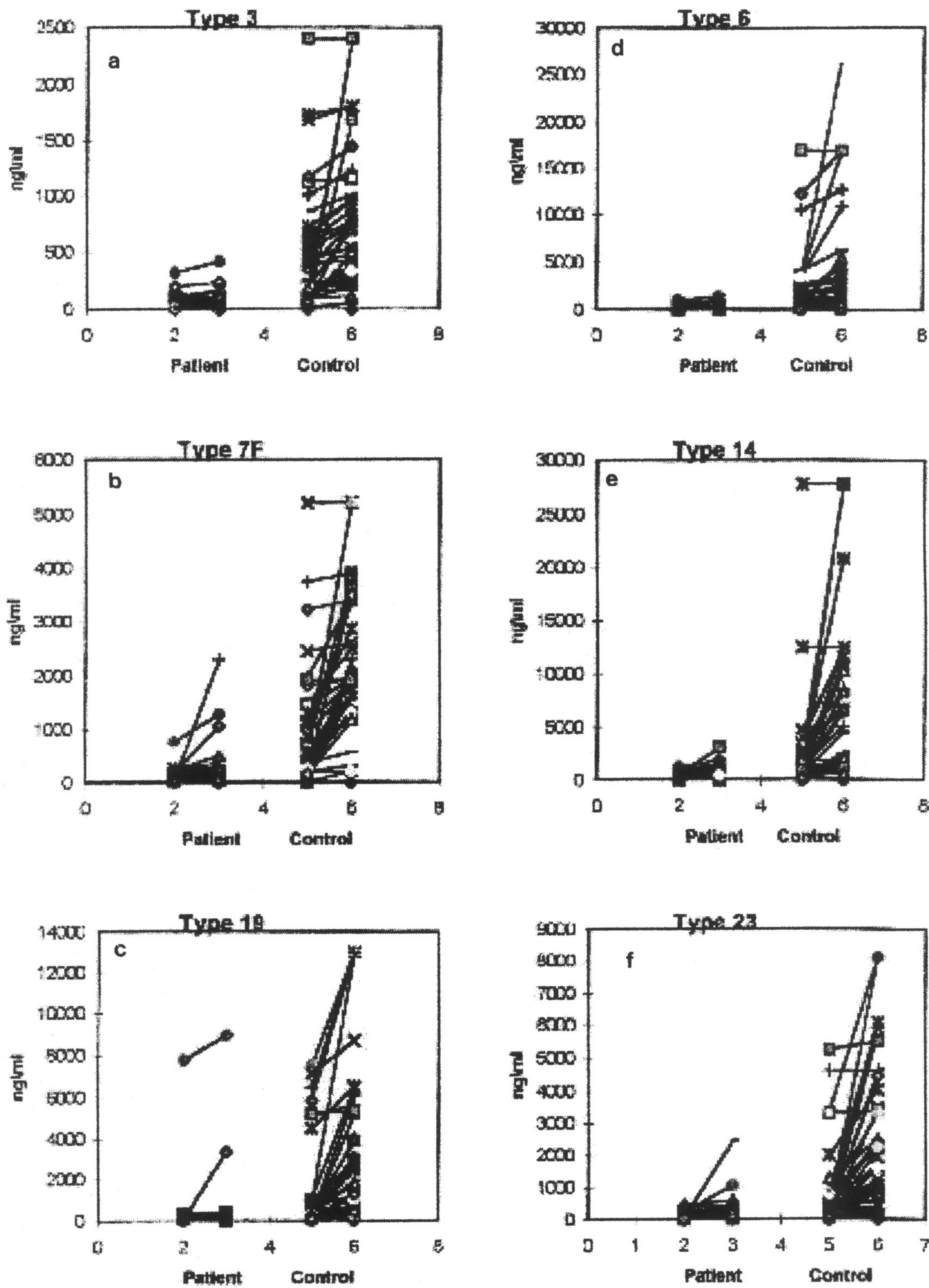

Fig 2. Comparison of pre- and postimmunization antibody titers against 6 pneumococcal serotypes (ng/ml; ELISA) in A-T versus healthy Turkish children (control group). Some lines represent more than one individual.

Table II. Geometric Mean Titers of Antibodies to Six Pneumococcal Serotypes in 29 A-T Patients

	Preimmunization (n = 29)		Postimmunization (n = 29)		
Serotype	U/ml	ng/ml	U/ml	ng/ml	-Fold increase
3	2.5 (12)[a]	61 (287)	2.7 (23)	65 (560)	1.1 (1.9)
6B	1.6 (5)	282 (919)	1.7 (9)	294 (1717)	1.0 (1.8)
7F	1.6 (9)	81 (451)	2.8 (37)	147 (1923)	1.8 (4.1)
14	1.2 (4)	316 (1111)	1.4 (17)	394 (4748)	1.2 (4.3)
19F	1.4 (4)	186 (524)	1.6 (15)	204 (1787)	1.1 (3.7)
23F	1.3 (5)	101 (391)	1.9 (19)	151 (1571)	1.5 (3.8)

[a] Numbers in parantheses are the values obtained from healthy Turkish children (n = 40).

serotype, taking into account that the RIA results were considered positive at >300 ng Ab N/ml (27, 28). The -fold increase was calculated for each patient by dividing the postvaccination titer by the preimmunization titer.

In 13 A-T patients, pneumococcal polysaccharide antibodies were also determined by one of the authors by RIA.

Twelve patients were given a booster immunization with tetanus toxoid and antibody titers were determined before and 10 days after immunization by ELISA (Bindazyme anti-tetanus toxoid IgG; The Binding Site, Birmingham, England).

Serum immunoglobulin (Ig) isotypes were measured by nephelometry, and IgG subclasses by radial immunodiffusion (The Binding Site). Serum Ig levels of patients were compared with age-matched values of Turkish children (±2 SD for IgG, IgM, and IgA and ±3 SD values for IgG subclasses were taken as the normal range) (30).

The geometric mean titers of antipolysaccharide antibodies and -fold increase between pre- and postvaccination antibody titers were given for each serotype. The correlations between immunoglobulin deficiency and susceptibility to infection and between immunoglobulin deficiencies and antibody production were analyzed by Fischer exact χ^2 test.

RESULTS

The results of the pneumococcal antibody responses (U/ml) after vaccination with a 23-valent pneumococcal vaccine showed that 22 of 29 patients (76%) did not respond to any of the six pneumococcal serotypes studied. Five of 29 patients (17%) showed positive responses to only one serotype (the response was weak in three of these patients), 1 patient (3.4%) showed a positive (weak) response to two serotypes, and 1 patient (3.4%) showed a positive response to four serotypes (the responses to two of the serotypes were weak). In four patients, polysaccharide antibody to all serotypes was undetectable. The rest of the patients (18 patients) had detectable but very low antibody titers (Table I, Figs. 1a–f). Among 40 healthy age-matched controls, 2 children (5%) responded to one, 1 (2.5%) responded to two, 9 (22.5%) responded to three, and 28 (70%) responded to four or more serotypes.

Four patients (13.8%) had >300 ng Ab N/ml (>1800 ng/ml) serotype-specific antibody to one serotype, and one patient (3.4%) to two serotypes. Twenty patients had <300 ng Ab N/ml antibody levels to all serotypes. Six controls (15%) had >300 ng Ab N/ml serotype specific antibody to one serotype, 9 (22.5%) to two serotypes, 12 (30%) to three serotypes, and 13 (32.5%) to four or more serotypes (Table I, Figs. 2a–f).

In A-T patients, geometric means of antibody titers (GMT) were below 3.0 U/ml for all serotypes both before and after immunization, and most of them were even below 2 U/ml (Table II). In contrast, the preim-

Table III. Geometric Mean Titers of Antibodies to 12 Pneumococcal Serotypes in 13 A-T Patients, Determined by RIA (ng Ab N/ml)

Pneumococcal serotype	Prevaccination	Postvaccination	-Fold increase	n
1	324	482	1.5	12
3	305	733	2.4	12
4	375	379	1.0	6
6	333	446	1.3	12
7	254	929	3.7	11
8	654	1080	1.7	12
9	216	345	1.6	11
12	230	302	1.3	8
14	470	721	1.5	9
18	157	184	1.2	8
19	129	134	1.0	6
23	164	224	1.4	6

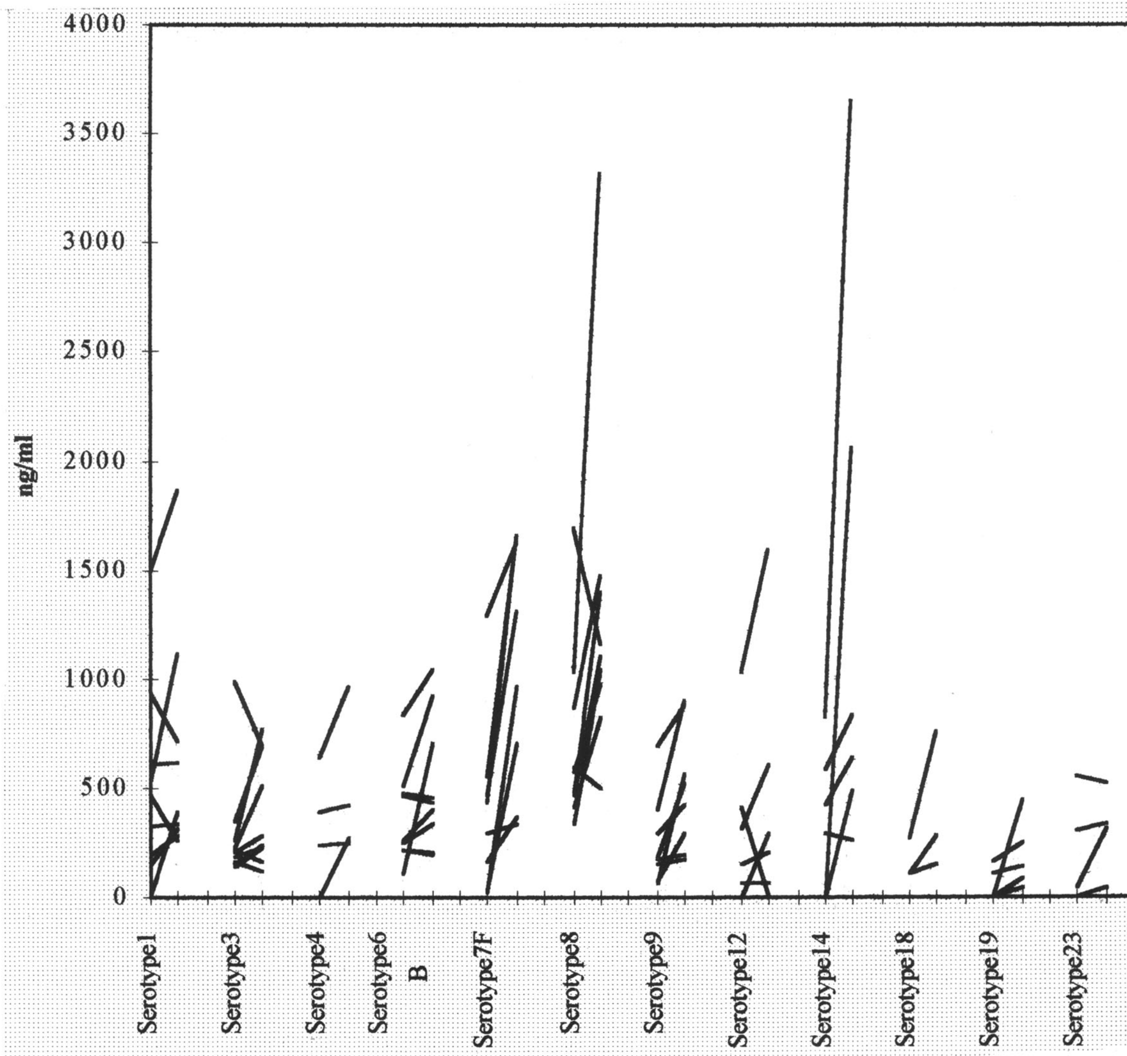

Fig 3. Pre- and postimmunization antibody titers against 12 pneumococcal serotypes (ng Ab N/ml; RIA) in A-T patients.

munization GMTs for healthy children were between 4 and 12 U/ml. The -fold increase in GMT between pre- and postimmunization for six serotypes in A-T patients varied between 1.0 and 1.8, thus all of them were ≤1.8. These values for healthy children were between 1.8 and 4.3.

Anti-pneumococcal polysaccharide antibodies in 13 of 29 A-T patients were also determined by RIA (Table III, Fig. 3). Using this assay, 10 (77%) patients produced >300 ng Ab N/ml to more than 6 of the 12 pneumococcal serotypes studied (in addition to 6 serotypes shared by ELISA and RIA, specific antibody concentrations were determined by RIA against another 7 serotypes).

In the healthy control group, the highest response rate (U/ml) was found for the most immunogenic serotypes, i.e., 82.5% for serotype 3 and 92.5% for serotype 7; for serotypes 6B, 14, 19F, and 23F, the response rates were 45, 60, 60, and 75%, respectively. In the A-T patient group these differences were not apparent: the antibody response to serotypes 3 and 7 was as low as to the other pneumococcal serotypes studied.

Tetanus Antibody Production

All 12 patients produced antibody to tetanus toxoid after booster immunization. All patients had a preimmunization level >0.15 IU/ml (range, 0.16–4.8 IU/ml; median, 0.7 IU/ml), which has been cited as the protective level. Upon vaccination a 2- to 28-fold increase in

antibody titers was obtained (range, 1.9–10 IU/ml; median, 9.8 IU/ml) (data not shown).

Serum Immunoglobulin Isotype Levels

Sixteen of 28 patients (60.7%) had normal, 4 (14.2%) had lower limit of normal, and 7 (25%) had high IgG values. Sixteen of 27 patients (59.3%) had low IgG2 levels (3 being undetectable) and 4 patients (14.8 %) had low IgG3 levels. IgG1 levels were normal or increased in all patients. Eighteen of 29 patients (62.1%) had low serum IgA values. There was no correlation between IgA or IgG subclass deficiency and antibody response against pneumococcal polysaccharides. Susceptibility to sinopulmonary infection did not correlate with antibody production to pneumococcal polysaccharides and IgG subclass deficiency either. A correlation existed between IgA deficiency and frequent sinopulmonary infections ($P<0.05$), although two patients of seven had a low serum IgA level without frequent infections and seven patients had a normal serum IgA with frequent infections.

DISCUSSION

Sinopulmonary infection is one of the prominent clinical features of A-T. In general, *S. pneumoniae* is a common pathogen responsible for these infections. Although in A-T patients, there are various predisposing factors to infections, antibody production to pneumococcal polysaccharides is not a well-investigated aspect of A-T. In addition, there are no well-established criteria for interpretation of antibody responses to pneumococcal polysaccharides (31). It is also not well known whether a normal individual should respond to all serotypes and by what criteria an individual is considered a responder. The -fold increase (two-, three-, or fourfold) and/or certain minimal postvaccination antibody level (20 U/ml or 200–300 ng Ab N/ml, depending on the laboratory) for more than half of the serotypes studied has been proposed as the response criteria to pneumococcal vaccination in previous reports (23, 28, 31). We chose a minimal concentration of 10 U/ml as a positive postvaccination antibody titer but considered it a weak response when the level was between 10 and 20 U/ml for three or more of the six pneumococcal serotypes, taking into account the results in healthy age-matched Turkish children. Seventy-six percent of the A-T patients did not show an ELISA IgG response to any of the pneumococcal serotypes studied, while all these patients produced antitetanus antibody. However, antibody production evaluated by RIA led to different results. Ten of thirteen (77%) patients produced >300 ng Ab N/ml to more than 6 of the 12 serotypes studied. The fact that the antibody titers determined by RIA were total immunoglobulins and sera were not preadsorbed with cell wall polysaccharide may explain the difference between the ELISA and the RIA results (18, 32). However, when the antibody response is expressed as the -fold increase in titer, both ELISA and RIA show a deficient antipolysaccharide response in A-T patients. While bacterial polysaccharides are thymus-independent antigens (TI-2), T cells seem to contribute to TI-2 responses (33–37). However, how they contribute is not clear.

An impaired antibody response after infection with encapsulated bacteria or vaccination with polysaccharide vaccines is seen in patients with an immunoglobulin (Ig) isotype deficiency such as IgA deficiency or IgG2 subclass deficiency, common variable immunodeficiency, and Wiskott Aldrich syndrome (WAS) (19–21, 38). Impaired antipolysaccharide antibody formation has also been found in patients with recurrent respiratory tract infections, without any sign of an underlying immunodeficiency (20, 22, 28). The cellular and molecular basis of a defective response to polysaccharide antigens is unknown and is probably not the same for all patients. Several mechanisms related to T or B lymphocytes could be responsible: disturbed T cell regulation of the TI-2 response, inability of lymphoid cells to process stimulation signals when challenged with large inflexible polymeric structures, and capsular polysaccharides, as suggested for WAS, have all been put forward as probable causes of a defective polysaccharide antibody response (37, 38).

B cell abnormalities such as immaturity of B cells and deficient proliferation of committed B lymphocytes may also contribute to defective antibody responses to polysaccharides. Mice deficient in B-1 B cells have been shown to be susceptible to infection with *S. pneumoniae* because they fail to produce specific antibody (37).

A-T is caused by mutations in the ATM gene (AT, mutated), encoded on chromosome 11q22-23 (39). The proposed function of the ATM protein is sensing of DNA damage and signaling (through protein phosphorylation) to cellular regulatory systems. Recently, it has been demonstrated that signaling through the B cell antigen receptor, induced by antiimmunoglobulin cross-linking, is impaired in Epstein–Barr virus-transformed A-T B lymphocytes (40), suggesting that ATM may also function in this signaling pathway. Because cross-linking of surface immunoglobulin can be considered a polyclonal equivalent of B cell activation by TI-2 antigens (36, 40), our finding of a

defective antipolysaccharide antibody response in A-T is compatible with such a mechanism.

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