

## Defective anti-polysaccharide antibody response in patients with ataxia-telangiectasia

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**SUMMARY:** Sanal Ö, Özbaş-Gerçeker F, Yel L, Ersoy F, Tezcan İ, Berkel Aİ, Metin A, Gatti RA. Defective anti-polysaccharide antibody response in patients with ataxia-telangiectasia. Turk J Pediatr 2004; 46: 208-213.

The immunodeficiency in ataxia-telangiectasia (A-T) patients involves both cellular and humoral immunity; however, the specific antibody response is not well defined. Frequent respiratory infections are a prominent feature in A-T. *Streptococcus pneumoniae* is a common pathogen responsible for these infections. Defective B cell membrane signaling has been reported in A-T cells. These observations prompted us to investigate the B cell response to six frequently encountered pneumococcal serotypes in A-T patients. We found defective IgG antibody production to all studied serotypes (3, 6B, 7F, 14, 19F, and 23F) in 22 of 31 A-T patients (71%) who were immunized with a polyvalent pneumococcal vaccine. The impaired antibody responses did not correlate with either history of infection or serum immunoglobulin isotype levels. In addition, we did not observe any correlation between the pneumococcal antibody production and a specific mutation or level of intracellular ATM (ataxia-telangiectasia mutated) protein in lysates of lymphoblastoid cell lines from these patients. Our results suggest that the extent and severity of the recurrent sinopulmonary infections may depend not only on the immunological defects but also on other ATM-dependent physiological responses.

**Key words:** ataxia-telangiectasia, pneumococcal vaccine, antibody response, Atm mutation.

Ataxia-telangiectasia (AT) is an autosomal recessive multisystem disease characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, increased radiosensitivity, predisposition to lymphoid malignancies, frequent sinopulmonary infections and variable cellular and humoral immune deficiencies<sup>1-3</sup>. The latter include selective IgA and IgE deficiency and IgG subclass deficiency. Defective B cell signaling has been reported in A-T cells<sup>4</sup>. Pulmonary infections may progress to bronchiectasis and pulmonary fibrosis, severe enough to cause respiratory insufficiency and death. Although the severity of sinopulmonary infections tends to be associated with low levels of serum and salivary IgA, often in association with low levels of IgG2, some patients who lack serum and salivary IgA are free of recurrent infections while others

suffer from severe progressive infection with a normal immunoglobulin pattern. Aspirations of saliva due to neurological impairment of swallowing can be another contributing factor to pulmonary infections in these patients.

*Streptococcus pneumoniae* is a common pathogen responsible for pulmonary infections. We measured the B cell function, as assessed by IgG antibody production to six pneumococcal serotypes, in 31 A-T patients after immunization with a polyvalent pneumococcal vaccine. We had found defective antibody production in the majority of these patients in our previous study<sup>5</sup>. In this study intracellular ataxia-telangiectasia mutated (ATM) protein and specific mutations in the ATM gene were also studied and their correlations with polysaccharide antibody production and susceptibility to infection were sought.

## Material and Methods

### Patients

This study included 31 patients (age range 4-20 years) with a classical phenotype of A-T, diagnosed and followed up at the Immunology Unit, Hacettepe University Children's Hospital, Ankara, using a research protocol approved by the institutional Ethical Committee. "Excess recurrent infections" was defined as more than eight upper respiratory tract infections or at least four episodes of sinusitis per year with or without lower respiratory tract infections and bronchiectasis. All patients had normal serum IgG levels. They were immunized with a polyvalent pneumococcal vaccine (Pneumo 23, Pasteur Merieux) containing 25 µg of purified type-specific capsular polysaccharide antigen for each of 23 pneumococcal serotypes. Blood was drawn before and four weeks after immunization. All serum samples were stored at -80°C until used.

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

### Anti-pneumococcal polysaccharide antibody (Ab) determination

IgG antibody levels to six common pneumococcal serotypes [(3,7F, strong immunogenic), (14,19F, intermediate immunogenic), (6B, 23F, weak immunogenic)] were measured by ELISA in pre- and postimmunization serum samples. 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 to cell wall polysaccharides. Antibody concentrations were expressed as the percentage of reference serum, the hyperimmune plasma pool, (AS Pneumococcal Reference serum FDA7 CBER, Bethesda, MD) in units per ml where the reference plasma pool represents 100 U/ml for each serotype. Based on the results obtained from 40 age-matched, healthy controls from the same ethnic group, a post vaccination IgG antibody titer

of 10-20 U/ml to each serotype was considered to be weakly positive and of  $\geq 20$  U/ml to be positive antibody response.

### ATM Mutation and protein analyses

ATM mutation analyses were performed in the Department of Pathology, UCLA School of Medicine, Los Angeles CA, USA, and were identified by either protein truncation testing or single strand conformational polymorphism screening, followed by DNA sequencing<sup>6-9</sup>. Intracellular ATM protein levels were determined by Western blotting of lysates from lymphoblastoid cell lines<sup>7</sup>.

Statistical analyses were performed by  $\chi^2$  and Fisher exact  $\chi^2$  tests.

## Results

Of 31 patients immunized with a polyvalent pneumococcal vaccine, 22 showed an impaired polysaccharide antibody response with either no detectable antibody or with antibody titers below the cut-off range for a positive response. Nine patients (29%) had a positive antibody response to at least one serotype: 5 patients (16%) responded to one serotype; 1 patient (3%) responded to two serotypes; 2 patients (6%) responded to four serotypes; and 1 patient (3%) responded to 6 serotypes (Table I). Patients were categorized into three groups according to their antibody response profile: Group I: Patients with a positive antibody response at least to one serotype; Group II: Patients with a negative, yet detectable, antibody response to at least one serotype; and Group III: Patients with no detectable antibody. Correlations were sought between antibody production and the following parameters: 1) infection susceptibility, 2) ATM mutations, 3) ATM protein, 4) disease progression, and 5) cancer susceptibility.

### 1. Infection susceptibility

Recurrent infections were common in all three groups: 33% in Group I, 44% in Group II, and 100% in Group III. Although the frequency in Group III appeared to be higher, the total number of patients was low. One patient from Group I (Pt 3), two from Group II (Pts 10,17), and one from Group III (Pt 4) experienced recurrent severe pulmonary infections at an early age which resulted in bronchiectasis. Antibody production did not correlate with serum IgA or IgG2 deficiency.

Table I. Continued (Group II. Patients with Negative, yet Detectable Polysaccharide Ab Response)

Patient	Present age	AFP	Rec. sino-pulmonary infection	Ca. in family members	IgA	IgG2	Mutation (homozygous)	ATM protein
1	16	168	+	Negative	Low	N	ND	ND
2	9	177	+	Negative	Low	Low	ND	Negative
3	14	148	-	Pulmonary Ca. (mat. grand aunt grandfather)	N	N	ND	ND
4	13	ND	-	Gastric Ca. (pat. grandmother)	N	N	ND	ND
5	12	100	+	Ca. (mat. grand aunt grandfather)	Low	Low	ND	ND
6	8	35	±	Pulmonary Ca. (pat. grand uncle, mat. grand uncle) Lymphoma (grand uncle's son)	N	N	ND	ND
7	7	29	-	Lymphoma (sibling with AT)	N	Low	ND	Negative
8	12	126	-	Ca. cases in multiple family members	N	N	IVS44-1G>T	ND
9	5	51	±	Larynx Ca. (mat. grand father) Leukemia (sibling with AT)	Low	N	ND	Positive
10	7	108	+	(severe) Negative	Low	ND	5610delC	Positive
11	12	170	±	Negative	Low	Low	IVS60+del27	Negative
12	24*	698	+	Patient died of lymphoma	N	Low	5554InsC	Negative
13	22	138	-	Negative	Low	N	IVS60+del27	ND
14	16	168	-	Renal Ca. (mat. grandmother)	N	UD	1564delA>G	ND
15	21	29	+	Negative	Low	ND	2503InsA	Negative
16	10	ND	+	Ca cases in multiple family members	Low	Low	5623C>T	Positive
17	13	369	+	(severe) Gastric Ca (pat. grandfather) Renal Ca (mat. grandfather)	Low	N	IVS21+3InsT	Negative
18	14	29	-	Negative	N	N	6188G>A	Positive

  

(Group III: Patients with no Polysaccharide Ab Production)								
Patient	Present age	AFP	Rec. sino-pulmonary infection	Ca. in family members	IgA	IgG2	Mutation (homozygous)	ATM protein
1	13	ND	+	Not known	Low	UD	ND	ND
2	11	91	+	Not known	Low	UD	ND	ND
3	18*	265	+	Skin Ca. (pat. grandfather) Gastric Ca. (uncle, mat. great-grand-mother leukemia (mat. grand uncle)	Low	Low	ND	ND
4	7	130	+	(severe) Negative	Low	Low	ND	ND

\* Age of death.

UD: undetectable.

ND: not done.

## 2. ATM mutations

ATM mutations were identified in three patients in Group I and in 10 patients in Group II. All of them were homozygous truncating mutations except for the patient (Pt 18) who had a homozygous missense mutation. Since it has been reported that patients with truncating mutations before residue 1125 had significantly worse survival and growth than those with truncations after that point<sup>10</sup>, we sought correlation between antibody production and proximal and distal truncations; however, no such correlation could be found. Two patients who responded to more than half of the serotypes were homozygous for 3576G>A, a mutation which terminates the protein at residue 1192. However, other patients with distal mutations 5610delC and 5554 InsC had negative antipolysaccharide antibody responses.

## 3. ATM protein

No correlation was found between antibody production and the presence or absence of intracellular ATM protein. Intracellular ATM protein was not present in three patients studied in Group I, while four patients out of 10 studied had ATM protein in Group II. ATM protein was not measured in any of the patients in Group III.

## 4. Disease progression

Those patients with 3576G>A mutation who produced antipolysaccharide antibody tended to have a slower progression of the disease with a longer life span. Three affected siblings in one of those families (Pt 9, Group I) are still alive at the age of 22, 24 and 30 years, and another patient (Pt 7, Group I) is doing much better clinically at age 12 years than most of the patients with classical phenotype. Another two patients (Pts 13 and 18, Group II) also showed marked difference from the rest of the patients with slow progression of the disease. These latter patients had 6188G>A (missense) and IVS60del27 mutations.

## 5. Cancer susceptibility

A family history of one or more malignancies (22% in Group I, 50% in Group II, and 50% in Group III) did not correlate with antibody response. Although the frequency in Group I appeared to be lower, it was not statistically significant.

## Discussion

The underlying mechanism of defective antibody responses in patients with A-T is unknown. The possibility of defective signaling through T cell and B cell membrane receptors has been investigated<sup>4,11</sup>. Despite the recent report of ATM-independent transmembrane signaling in transformed T cell lines from A-T patients<sup>11</sup>, transformed B cell lines from A-T patients are reported to have impaired calcium mobilization and impaired responses to cross-linking of the B cell receptor<sup>4</sup>, suggesting a role for the ATM protein in intracellular signaling. Since cross-linking of the surface immunoglobulin molecules can be considered as an equivalent of polyclonal B cell activation by T-independent type 2 antigens, our finding of a defective anti-polysaccharide antibody response in A-T may be a result of such faulty signaling in B cells. There are some reports on antibody response to various antigens in A-T; however, anti-polysaccharide antibody production is not a well-known aspect of the disease<sup>12-15</sup>. In one report the mean rise in antibody titers to blood group substance, E. coli, Vi antigen, and tularemia antigen was significantly less than in controls, but there was considerable variation in the group<sup>12</sup>. The primary IgG, IgM and IgA antibody responses to HPH (helix pommatia hemocyanin), a T cell dependent antigen, were found to be defective in patients with A-T, while secondary responses to diphtheria, tetanus and polio vaccine were normal<sup>13</sup>. In another report weak antibody response to tetanus and polio antigen was reported<sup>14</sup>. In five A-T patients studied, various anti-viral antibodies were found to be low or absent<sup>12</sup>. Lymphocytes from A-T patients produced (in vitro) less anti-influenza antibody than lymphocytes from normal controls<sup>15</sup>. In our previous study all of the 12 A-T patients studied produced normal anti-tetanus toxoid antibody after booster immunization<sup>5</sup>. Although the immunological abnormalities observed in A-T patients are usually considered to be quite variable, we report impaired antibody production against pneumococcal polysaccharide in the majority of 31 patients with classical A-T. However, we found no correlation between the degree of antibody production and frequent infections. Eighteen of 31 patients had poor antibody production although very low titers could be detected technically. Four patients did not have any detectable antibodies. Although these four patients experienced recurrent infections, the total number was too low to imply a cause-effect relationship; susceptibility to sinopulmonary infections was present in all three groups.

Pneumococcal capsular polysaccharide antibody production has been reported previously to correlate with serum levels of IgG2<sup>16</sup>. Furthermore, an impaired response to polysaccharide antigens has been reported in some patients with IgA deficiency<sup>17</sup>. Despite this, we observed no correlation between anti-polysaccharide antibody response and serum immunoglobulin isotype levels.

The ATM protein is a phosphatidylinositol-3 (PI-3) kinase that senses ds DNA breaks and signals to cellular regulatory systems, through the phosphorylation of various substrates<sup>18,19</sup>. Over 400 unique mutations distributed across the full length of the ATM gene have been described in A-T patients to date. Approximately 70% of mutations truncate the ATM protein. Despite some variability in several features of the disease, clinical subsets have not been described. Occasionally, however, milder cases of the diseases have been reported. Such patients may show either later age at onset, more moderate severity of the ataxia, intermediate cellular radiosensitivity, or longer life span<sup>10,20</sup>. There are several reports on possible genotype-phenotype correlations in A-T patients<sup>7,10,20-22</sup>. One mutation (IVS+1126A>G, 5762 ins 137) in the United Kingdom population has been associated with milder phenotypes, i.e., slower progression of cerebellar ataxia, intermediate radiosensitivity and reduced cancer susceptibility<sup>20</sup>. In a recent study by Li and Swift<sup>10</sup>, mean survival and height distribution of 134 A-T patients appeared to correlate with specific mutations, although the data were not very convincing. 3576G>A is the most common ATM mutation in Turkey, affecting 12.5% of patients. Our patients with the 3576G>A mutation tended to have a rather longer life span considering the natural course of the disease as described also by Gilad et al.<sup>21</sup>. Three affected siblings in one of our 3576G>A families are still alive at the ages of 22, 24 and 30 years, and all of them had positive anti-polysaccharide antibody response. Three affected siblings with the same homozygous mutation in another family died at the ages of 24, 29 and 32 years, relatively late considering the natural course of the disease.

At the molecular level, most genetic diseases exhibit a loss or reduction of protein secondary to a decrease in mRNA levels. In contrast, ATM mRNA has been found in every A-T patient tested to date. Despite this, a majority of A-T

patients (83%) lack detectable protein<sup>8</sup>. It has been suggested that patients whose cells are capable of producing even a modest amount of ATM protein may have distinct phenotypic features when compared with those cells that produce no detectable protein<sup>21</sup>. Alternatively it is possible that small but intracellular ATM protein are present in many A-T patients. One mutation (8494C>T) has been associated with detectable intracellular ATM protein<sup>7</sup>. However, in our study, four patients with detectable ATM protein showed no correlation with antibody production or with any other parameters.

Gatti et al.<sup>18,23</sup> proposed a model for the phenotypic effects of ATM mutations in which truncation mutations, as are found in most A-T patients, would predispose to classical A-T with full neurological features, whereas most missense mutations would predispose to malignancy but not to the full neurological aspects of the classical A-T syndrome. We have two families with a homozygous missense mutation (6188G>A resulting in gly>glu). Both have had a milder form of AT. One patient showed a very weak pneumococcal antibody response (7 u/ml) to a single serotype. Neither family had a family history of malignancy. However, in another family with the missense mutation 6047A>G (asp>gly), multiple cancers were noted in relatives. It may be that the extent and severity of the disease depends not only on the type or site of the ATM mutations, but on the other genes as well.

Further investigations are needed to elucidate the role of the ATM protein in immunological abnormalities, sinopulmonary infections, and disease pathogenesis.

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