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ORIGINAL ARTICLE

Response to polysaccharide antigens in patients with ataxia-telangiectasia

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Abstract

Objective: To analyze the production of antibodies to polysaccharide antigens in patients with ataxia-telangiectasia.

Patients and methods: We used the ELISA technique to measure the levels of IgG antibodies to serotypes 1, 3, 5, 6B, 9V and 14 of *Streptococcus pneumoniae* in 14 patients with ataxia-telangiectasia before and after immunization with 23-valent polysaccharide vaccine. Adequate response to individual polysaccharide can be defined as a postimmunization antibody titer equal to or greater than 1.3 µg/ml or as a minimum fourfold increase over the baseline (preimmunization) value.

Results: Six (43%) patients showed an absent response to all serotypes analyzed. Four patients showed adequate response to only one serotype, one patient to two serotypes, two patients to three serotypes and only one patient to four out of six serotypes analyzed. No patient had adequate response to all serotypes tested. Postimmunization pneumococcus IgG levels were higher than preimmunization levels to all serotypes analyzed, except for serotype 3. In spite of this, the mean postimmunization levels were lower than 1.3 µg/ml in all serotypes, except for serotype 14. Mean increment was less than four in all serotypes analyzed.

Conclusion: Our results suggest that patients with ataxia-telangiectasia are at a high risk of having an impaired response to pneumococcus, which may be one of the causes of recurrent sinopulmonary infections in these patients.

J Pediatr (Rio J). 2006;82(2):132-6: Ataxia-telangiectasia, *Streptococcus pneumoniae*, pneumococcus, polysaccharide, immunodeficiency, humoral immunity, antibody.

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Introduction

Ataxia-telangiectasia (AT) is an autosomal recessive disease that coexists with progressive cerebellar ataxia, immunodeficiency, sinopulmonary infections, skin disorders, including telangiectasia, cancer risk, radiosensitivity and early aging.[1,2] It is caused by mutations of the ATM gene located on chromosome 11q22-23, which contains 66 exons.[3] Patients with AT have an undetectable intracellular ATM level or absence of catalytic activity.[2]

Although AT patients are susceptible to recurrent infections and to immunological disorders (both humoral and cellular), a specific immunological disorder has not yet been identified.[4,5] Despite normal IgG levels, 80% of AT patients have IgG2 deficiency; the remaining 20% do not have a defined pattern.[6-8] The production of viral and bacterial antibodies and antigens may be deficient. The

immunopathogenic mechanism linking ATM dysfunction, immunodeficiency, and infection is yet to be established.

The aim of this study was to assess the production of antibodies to polysaccharide antigens in Brazilian AT patients after immunization with 23-valent polysaccharide vaccine.

Patients and methods

Fourteen AT patients (nine males aged between 4 and 17 years) were assessed regardless of whether they had a clinical history of recurrent infections. All of them were diagnosed with AT, based on criteria established by the European Society for Immunodeficiencies (ESID) and by the Pan-American Group for Immunodeficiency (PAGID).[9] Total levels of IgG, IgM and IgA were monitored in all patients and in seven patients, IgG subclasses (radial immunodiffusion) were also determined. Results were compared with age-matched controls.[10] The production of antibodies to protein antigens was evaluated in all patients for at least two antibodies to vaccine antigens: tetanus, diphtheria, rubella, or measles.

The production of antibodies to polysaccharide antigens was analyzed by measuring serum IgG antibodies levels to pneumococcal serotypes 1, 3, 5, 6B, 9V, and 14 by using a modified ELISA protocol.[11] Serum samples were collected from the patients before and after immunization with 23-valent polysaccharide vaccine (Pneumo23®-Pasteur-Mérieux). Appropriate immune response to a specific serotype was defined as the presence of serum IgG levels equal to or higher than 1.3 µg/ml or as a fourfold increase over the baseline values.[11] The variables were analyzed by non-parametric Wilcoxon tests, and an alpha equal to or less than 5% was regarded as statistically significant. The present study was approved by the local Research Ethics Committee and an informed consent form was signed by all parents or surrogates.

Results

All patients initially presented with symptoms of ataxia in the first two years of life, and 11 (78%) of them had oculocutaneous telangiectasia at that time. However the age of diagnosis ranged from 2 to 11 years. Eight (57%) patients had recurrent sinopulmonary infections, and the alpha-fetoprotein serum levels ranged from 61.7 to 857 ng/ml (normal: < 5-10 ng/ml).

Serum IgA levels equal to or less than 7 mg/dl were observed in seven of 14 (50%) patients; IgA levels were normal in only four (28%) patients (Figure 1). Unlike IgA levels, serum IgM levels were elevated in 11 (78%), normal in 2/14 and low in 1/14 patients (Figure 2). IgG was the immunoglobulin with the highest frequency of normal levels (78%). Only one patient had IgG levels below the 3rd percentile for age (Figure 3). As to IgG subclass levels of seven patients, IgG1 was normal in all, and IgG3 in six of them. IgG2 level below the 3rd percentile for age was observed in only one patient, and IgG4, in three.

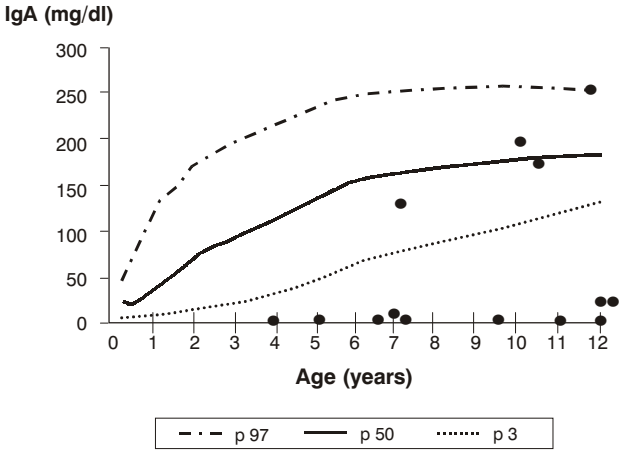

Figure 1 - IgA levels in ataxia-telangiectasia patients compared to the normal values for the Brazilian population[10]

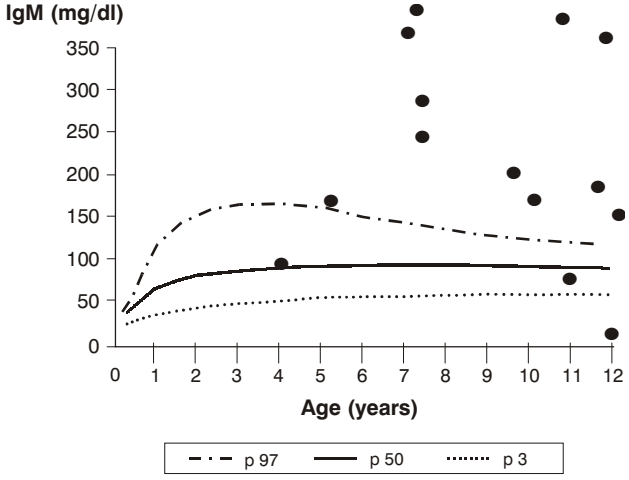

Figure 2 - IgM levels in ataxia-telangiectasia patients compared to the normal values for the Brazilian population[10]

The production of antibodies to at least two protein antigens was normal in all patients.

With regard to the production of antibodies to polysaccharide antigens, six patients showed no response to any of the six serotypes analyzed, 4/14 responded to only one, 1/14 to two, 2/14 to three and only 1/14 showed an appropriate response to four serotypes (patient who had low IgG2 level). The mean levels of

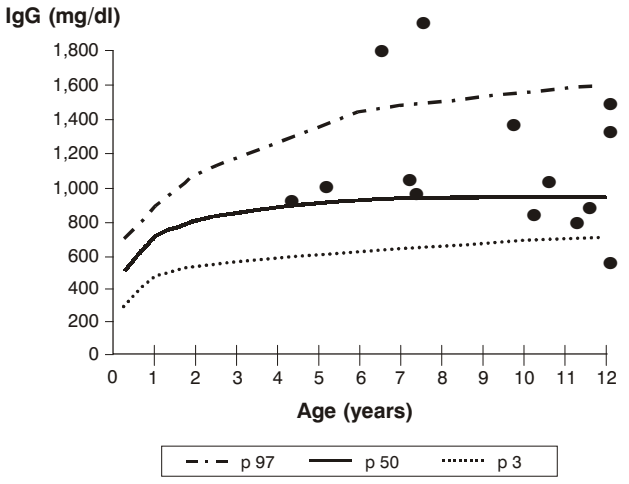

Figure 3 - IgG levels in ataxia-telangiectasia patients compared to the normal values for the Brazilian population[10]

antibodies to different serotypes, before and after immunization, are shown in Table 1. The mean level of antibodies after immunization was less than 1.3 µg/ml for all serotypes analyzed, except for serotype 14 (Table 1). By comparing pre and postimmunization levels, we noted that, except for serotype 3, all other serotypes had higher post values. Although postimmunization levels were higher, the mean increment was lower than four for all serotypes (Table 1). Serotypes with a higher percentage of positive response in decreasing order were 5 and 14 (5/14 and 4/14 respectively) followed by 1 and 9B (2/14) and 6 (1/14). None of the patients had an adequate response to serotypes 3.

Of the six patients who presented absence of response to any serotypes, two had normal levels for the three immunoglobulin classes, three showed low IgA levels with high IgM titers and one patient had low IgM levels. IgG2 levels, which were normal, were assessed in only two of these six patients.

Discussion

Ataxia was observed in all patients and telangiectasia in 11/14 patients before the age of two. However, some patients were not diagnosed till the age of 11 years reflecting unawareness of this disease. The earliest diagnoses occurred in patients with already affected siblings. Lack of information about the disease resulted in unnecessary and excessive radiological examinations, and this is contraindicated in this syndrome due to the radiosensitivity presented by the patients. None of the patients were on any type of treatment when they arrived at our department. All patients were immunized according to the official immunization schedule, including BCG, and no side effects were reported. All patients showed high levels of alpha-fetoprotein, which were the most characteristic and consistent findings in these patients.[12]

Lung infections are frequent in AT patients and they may develop into bronchiectasis and pulmonary fibrosis. Only eight of our patients had a past history of sinopulmonary infections. Some patients do not develop

Table 1 - Mean levels of antibodies to pneumococcal serotypes (µg/ml) before and after immunization with 23-valent polysaccharide vaccine in ataxia-telangiectasia patients (n = 14)

Serotypes	Pre (Range)	Post (Range)	Mean increment	Pre x post (Wilcoxon)
1	0.34 (0.12-0.87)	0.57 (0.14-1.5)	1.73	p = 0.018
3	0.29 (0.13-0.49)	0.33 (0.07-0.95)	0.32	p > 0.05
5	0.56 (0.15-0.72)	0.91 (0.07-4.0)	1.67	p = 0.027
6B	0.32 (0.14-1.58)	0.45 (0.08-2.3)	1.13	p = 0.027
9V	0.24 (0.03-0.45)	0.48 (0.06-1.5)	3.47	p = 0.032
14	0.48 (0.24-0.85)	3.70 (0.01-37)	3.61	p < 0.05

Pre = preimmunization; Post = postimmunization.

respiratory infections until the later stages of the disease, and opportunistic infections are extremely rare.[2] In addition to immunodeficiency, aspiration of saliva due to poorly coordinated swallowing triggered by neurological problems is an important factor for the development of pneumonia in these patients.[2,4]

AT was initially associated with IgA deficiency, observed in 60 to 70% of patients.[6,8] IgA levels less than 7 mg/dl were detected in 50% of our patients, more often associated with an increase in IgG and/or IgM levels and with a small number of CD4 cells (data not shown). Elevated IgM levels have been observed in AT patients,[8] sometimes mimicking the hyperviscosity syndrome, suggesting a disorder in the maturation and differentiation of B lymphocytes. The association between IgA deficiency and unresponsiveness to polysaccharide antigens has been described.[13] Of seven patients with IgA less than 7 mg/dl, three did not respond to the pneumococcal vaccine, two showed an appropriate response to one serotype, one of them to three serotypes and another one to four serotypes. The latter patient was the one who had the best response after immunization.

Normal IgG levels have been observed in AT patients,[5,6,8] in agreement with our findings. Controversial results have been described for IgG subclasses in these patients.[6-8] However, IgG2 in subnormal concentration does not necessarily reflect immunodeficiency.[14] Stray-Pedersen et al. found a positive relationship between antibody levels to pneumococcus and IgG2.[1] We could not find this relationship in our patients, since only two unresponsive patients had their IgG2 levels assessed and were normal. The only patient with low IgG2 levels was the one who showed an appropriate response to the largest number of serotypes.

Polysaccharide antigens are referred to as thymus-independent and complement receptors in B cells (BCR) appear to be of crucial importance in the response to such antigens.[15,16] All analyzed patients showed normal levels of total hemolytic complement (CH50) (data not shown).

In humans, IgG2 is the prevailing antibody class induced by pneumococcal capsular polysaccharides.[17] Pneumococcal immunization and evaluation of the result of the IgG class antibody response to pneumococcal polysaccharide serotypes included in the vaccine are an accepted method to identify deficiencies in the development of antibodies against polysaccharide antigens.[11] In this study, we included the serotypes that largely account for pneumococcal invasive disease in Brazil[18] and we noted that serotypes 5 and 14 turned out to be the most immunogenic in this group of patients. Response intensity increases significantly with age, and is negligible or absent in the first two years of life.[11] As all of our patients were over 4 years old at the time of immunization, we can rule out immaturity of the immune system as causing an inadequate response. Recently, Sanal et al. have reported absence of polysaccharide response in 22/31 AT patients. Of the remaining nine, five patients responded to only one serotype, one patient to two serotypes, and three patients to more than three serotypes.[19] Those authors did not find any correlation between the production of these antibodies and the presence or absence of intracellular ATM protein. All patients, but one, presented with homozygous truncating mutations and there was no correlation between this distal or proximal mutation with pneumococcus antibody levels.[19] These patients did not show an appropriate response even after immunization with the conjugate vaccine.[20] Inappropriate response to pneumococcus and to *Haemophilus influenzae* type b after immunization has also been described by other authors.[1] Inadequate response to polysaccharide antigens is frequent amongst these patients and occurs regardless of the presence of recurrent infections. Also important is the fact that our patients have different types of mutation,[21] which means that impaired production of antibodies to polysaccharide antigens does not seem to be related to these mutations.

The ability of B lymphocytes to express surface immunoglobulins with identical antigen specificity, but with different effector functions, results from the cells' capacity to undergo class switch recombination (CSR). The ATM protein may be required for the signal transduction in B lymphocytes.[1] A-T cells are defective in signaling through the B cell receptor (BCR), with a likely involvement of tyrosine kinase dysfunction.[22]

Our results suggest that AT patients are at a greater risk of showing impaired response to pneumococcus, which may be one of the causes of recurrent sinopulmonary infections. Early treatment must be initiated when such an infection is suspected.

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