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Effect of immunotherapy on autoimmune parameters in children with atopic asthma

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There is an increased rate of reported autoantibody production in patients with atopic and nonatopic asthma. The possibility of generating autoantibodies after the induction of immunotherapy can be explained by several mechanisms. One of these is immune deviation from TH2 to TH1 response by the effect of immunotherapy in favor of unregulated response to self-antigens. The other theory is a possible antigenic mimicry enabling autoantibody formation in these patients, Sixty-three atopic asthmatic children were included in the study. The patients were divided into three groups: Group I: patients with atopic bronchial asthma without immunotherapy: Group II: patients receiving immunotherapy for a maximum of 3 years; Group III: patients receiving immunotherapy for 4-5 years. The autoantibodies examined in the study population were anti-nuclear antibody, anti-double stranded DNA, rheumatoid factor, liver-kidney microsomal antibody, anti-mitochondrial antibody, anti-thyroglobulin and anti-microsomal antibody, anti-Smith antibody and lupus anticoagulant. An overall incidence of 17.5% autoantibody positivity was observed in patients, with no statistical significance between the treatment groups. IgG levels were significantly elevated in Group III when compared with Group I. Based on these findings it is suggested, in accordance with other studies, that long-term immunotherapy in the pediatric age group does not cause a significant autoantibody formation other than the overall increased incidence that occurs in asthmatic patients.

Key words: atopic, bronchial asthma, immunotherapy, autoantibodies.

Development of bronchial asthma usually involves a complex cascade of events depending upon the bronchial hyperreactivity and immunological features of the host. T cell derived cytokines are considered to play a key role in the pathogenesis of both humoral and cell-mediated aspects of allergic inflammation[1,2]. There is also a well established association between TH2 type cytokine production and allergic pathology and TH1 type cytokine production with non-atopic responses[3-6]. It is suggested that in intrinsic (non-atopic) asthma, viral airway infection and chronic eosinophilic inflammation of the airways may affect optimal lymphocyte regulation, making the individual more prone to develop autoantibodies[7]. On the other hand, the presence of antigens from microorganisms that share determinants with self-antigens creates the opportunity for antigenic mimicry[1,4,8]. In previous studies, organ and non-organ specific autoantibodies have been observed to be between 41-71% in intrinsic and 21-39% in extrinsic asthma (atopic) in comparison to 11-16% of healthy controls[4,9,10].

The efficacy of specific allergen immunotherapy in selected patients with IgE mediated disease has led to considerable interest in the mechanisms underlying this treatment. One way in which immunotherapy may act is by modifying the T lymphocyte response to subsequent natural allergen exposure. Studies in peripheral blood and target organ exhibit a shift in the balance of T cell subsets away from TH2 (production of IL-4 and IL-5) to TH1 (preferential production of IFN-gamma)[3]. Enhanced TH1 response and autoimmune

activation are more frequently observed in intrinsic asthma rather than in atopic asthma which has TH2 type cytokine profiles. Thus, it can be assumed that immunotherapy which is known to change TH2 T cell profile to TH1 might have profound effects in developing autoantibodies. To our knowledge, in children with atopic asthma receiving immunotherapy studies about developing autoimmunity are insufficient. We therefore planned to examine the effect of immunotherapy on autoantibody generation in atopic asthmatic children.

Material and Methods

Sixty-three children with the diagnosis of atopic bronchial asthma (ABA) were included in the study. The diagnosis of ABA was based upon the history, clinical findings and routine laboratory tests in addition to high total and specific IgE, total eosinophil count and skin test positivity. All the patients had moderate asthma (exacerbation of wheezing more frequent than one a week, low grade degree of wheezing between acute episodes, diminished exercise tolerance, clinically and/or radiographically evident hyperinflation, and evident signs of airway obstruction on pulmonary function tests) on admission.

The study population consisted of 21 female (33.3%) and 42 male (66.7) patients ranging in age from 5 to 20 (12.8±3.4) years. History for rheumatologic diseases, local or systemic infection or treatment with immunosuppressants were reasons for exclusion.

The patients were classified into the following groups: I. Patients with ABA who had not received immunotherapy (n=21) II. Patients with ABA who had received conventional immunotherapy for a period equal to or less than 3 years (n=22) III. Patients with ABA who had received conventional immunotherapy for a period of 4-5 years (n=20). The immunotherapy given to the patients included the allergens to which they were sensitive. The more common ones were house dust mites, and tree and weed pollens for the majority of the patients.

Routine and specific laboratory analyses including autoantibodies performed for all study subjects were as follows: erythrocyte sedimentation rate (ESR) (Westerngreen), >13 mm/h was accepted as high; C-reactive protein (CRP); rheumatoid factor (RF); total immunoglobulins (IgG, IgM, IgA); C3 and C4 (by Beckman, Nephelometer 100 Analyzer); liver-kidney microsomal antibodies (LKM) and anti-mitochondrial antibodies (AMA): indirect immunofluorescence, values above 1/100 were considered as positive (Euroimmune, Germany); anti-thyroglobulin antibody (anti-T) and anti-microsomal antibody (anti-M): values above 1/400 were considered as positive (Thymune M, Murex, U.K.); antinuclear antibody (ANA): indirect immunofluorescence, Hep-2 cells as substrate, values above 1/40 were considered as positive (Meridian Diagnostics Milano, Ital); anti-Smith (anti Sm): ELISA, values above 25 U/ml were considered as positive (The Binding Site, Birmingham, U.K.); anti double stranded DNA (anti ds DNA): ELISA, values above 60 IU/ml were considered as positive (The Binding Site, Birmingham, U.K.); and lupus anticoagulant (LA): qualitative assay, (Staclot LA, France). Mann-Whitney U and chi-square tests were used for statistical analysis.

Results

The mean ages of the patients with respect to their distribution within study groups were 10.6±3.9 years (5-7 years), 118±3.9 years (6-19 years), 13.3±3.5 years (8-20 years) in Group I (ABA patients without immunotherapy), Group II (ABA patients with immunotherapy for maximum of 3 years) and Group III (ABA patients with immunotherapy for 4-5 years), respectively. The ratio of female patients to male patients was 6/15 in Group I, 8/14 in Group II and 7/13 in Group III.

Erythrocyte sedimentation rate (ESR) was found to be mildly elevated (>13 mm/h) in 82.5% (52/63) of the patients, the mean value being 16.3±6.2 mm/h. There were only three children (4.7%) having ESR values exceeding 20 mm/h. However, no significant difference was found in ESR values between the three groups ($p>0.05$).

Serum levels of CRP, C3, C4, IgA and IM were normal in all of the subjects. The difference of age, CRP, C3, C4, IgA and IgM between the three study groups was not statistically significant (Group I vs Group II, Group I vs Group III, Group II vs Group III) ($p>0.05$).

The IgG levels were higher than age-related normal values in nine patients (40%) in Group I, in 14 (63.6%) of Group II and in 18 (81%)

of Group III. IgG values were 1255±410 mg/dl, 1422±411 mg/dl, and 1740±674 mg/dl in these three groups, respectively. These levels were found to be elevated in 41 patients (65%) in the whole study population. Only the difference in IgG levels between Group I and Group III was found to be statistically significant (p=0.044).

The immunotherapy given to the patients with respect to their sensitivity to specific allergens include house dust mites, of which Dermatophagoides farinae constituted 46% (n=29) and Dermatophagoides pteronyssinus 41.2 (n=26) and both were applied to 26.9% (n=17) of the patients. The hypersensitivity to house dust mites was most prevalent when compared with tree pollens given to 28.5% (n=18) of patients and weed pollens given to 28.5% (n=18) of patients.

The percentage of all patients with one or more autoantibody positivity was 17.5% (n=11). These were outlined as: positive reactions for ANA in 3 (4.7%), anti ds DNA in 2 (3.1%), ANA+anti ds DNA in 2 (3.1%), LKM in 1 (1.6%), AMA in 2 (3.1%) and LA in 4 (9.5%) patients (Table I). RF was positive in 3 patients (4.7%). Anti-T/anti-M and anti-Smith were negative in all of the subjects. None of the patients with antibody positivity nor their relatives presented evidence of autoimmune pathology.

Discussion

Significant IgG elevation between the asthmatic children who did not receive immunotherapy (Group I) and those who received immunotherapy for 4-5 years (Group III) was observed. Serum IgG level in Group II (patients receiving immunotherapy for maximum of 3 years) was also higher than in Group I, but the difference was not statistically significant. One of the distinctive features of immunotherapy is its ability to inhibit late-phase responses. During immunotherapy, allergen specific IgG (blocking antibody) increases, but this increase has not been found to be correlated with clinical response[3,11]. In Egeskjold et al's study[12], 87% of atopic patients showed positive reactions for IgG anti-IgG antibodies, which increased to 100% after more than 13 months of hyposensitization program; 7% of the controls exhibited IgG anti-IgG antibodies. Djurup and Maling[13] reported large increases in G1 and G4 antibodies specific for the allergen used during imunotherapy. Although we did not have the chance to measure anti-IgG antibodies and IgG subgroups specific for the allergen, our findings support the data of other studies that have observed elevated IgG values in direct relation with the period of immunotherapy.

Erythrocyte sedimentation rate (ESR) was found to be mildly elevated in 82.5% of the

Table I. Patients with Autoantibody Positivity and the Allergens They Received During Immunotherapy

	Autoantibodies						Allergens received by immunotherapy			
Patient group	ANA	Anti dsDNA	AMA	LKM	LA	RF	DP	DF	Weed	Tree
I	+	–	–	–	–	–	–	–	+	–
I	–	–	–	–	+	–	+	+	–	–
I	–	–	–	–	+	–	–	–	–	+
II	–	–	–	–	+	–	–	–	+	–
II	–	–	–	–	–	+	–	–	+	+
II	+	+	–	–	–	–	+	+	–	+
III	–	–	+	–	+	–	+	+	+	–
III	–	–	–	–	–	+	+	+	+	+
III	–	–	+	+	–	–	+	+	–	–
III	–	–	–	–	–	+	+	+	–	–
III	+	+	–	–	–	–	–	–	–	+

ANA : antinuclear antibody.
anti ds DNA : anti double stranded DNA.
AMA : antimitochondrial antibody.
LKM : liver-kidney microsomal antigen.
LA : lupus anticoagulant.
RF : rheumatoid factor.
DP : Dermatophagoides pteronyssinus.
DF : Dermatophagoides farinae.

Group I : Atopic bronchial asthma (ABA) patients without immunotherapy.
Group II : ABA patients with immunotherapy for maximum of 3 years.
Group III : ABA patients with immunotherapy for 4-5 years.

patients. However, no significant difference was found between the three groups, suggesting that the elevation is related with the inflammatory process in atopic bronchial asthma and not with immunotherapy or its duration. As in our study, some authors have reported mild ESR elevations in atopic asthmatic patients[1,14].

The results of our study yielded a 17.5% autoantibody positivity for all patients but we did not encounter a statistically significant difference between the study groups. In a study designed with three treatment groups similar to ours, elevated incidence of ANA (23.3%) in the whole study group was found, but no statistical difference was found between the groups[15]. In Fujimori's study[7], the presence of ANA was evaluated in the sera of patients with atopic asthma, non-atopic asthma, lung cancer and control subjects, and the incidence of positive ANA was found to be 20%, 53%, 30% and 11%, respectively. In Menon's study[4], an incidence rate of 20% of anticytoplasmic antibody, which was statistically significant in relation to controls, was observed in atopic asthmatic adults. LA positivity observed in four of our subjects did not lead to any clinical relevance, although these patients are still being closely followed for possible consequences of LA positivity. Our ANA incidence rate for the study group is in accordance with the ratios attained in the literature, supporting no enhanced risk in respect to immunotherapy, its content or its duration.

Proliferation assays in grass pollen and bee-venom sensitive patients have shown induction of peripheral T cell unresponsiveness to allergen after immunotherapy[3]. Atopic individuals receiving five or more years of hyposensitization with allergenic extracts showed no increased autoimmune, collagen, vascular, or lymphoproliferative disease[16]. Besides these findings, it seems that neither dose nor duration of anti-asthma medication and immunotherapy influences the presence of antinuclear or anticytoplasmic antibodies[4].

In conclusion, immunotherapy still has its promising place in the treatment of atopic asthma of childhood, with almost no specific effect on autoantibody generation other than the well known increased incidence of autoantibody occurrence due to the disease process itself.

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