The early effect of specific immunotherapy on lymphocyte response to phytohemagglutinin and allergens in atopic patients with allergic rhinitis

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In the first part of this study, peripheral lymphocyte subpopulations and their proliferative response to phytohemagglutinin (PHA) and allergens were investigated in the 30 patients with allergic rhinitis and 20 healthy non-atopic individuals. Data obtained employing a PHA-induced lymphoproliferative response assay revealed that the allergic rhinitis generated significantly less activity than did the normal control group. Significantly decreased ratio of CD4+/CD8+ T cells was noted in the patients with allergic rhinitis. Mean values of stimulation indices by allergen extracts were higher in the patients sensitive to same antigen than others especially in concentration of 1000 SQU/ml. Stimulation of active lymphocytes revealed no statistically significant group differences between allergens. In the second part of the study, the early effect of immunotherapy on T cell subsets and lymphocyte proliferative response to PHA and allergens were examined in the peripheral blood lymphocytes of patients. A significant increase in PHA-induced and in allergen-induced lymphoproliferatif response were observed in all patients after sixth months of immunotherapy. It is concluded that there may be an association between allergic rhinitis and deficiency of circulating CD4+ cells but further studies are required to substantiate this hypothesis. [Turk J Med Res 1996; 14(2):58-62]

Key Words: Rhinitis, Cellular immunity, Immunotherapy

The allergic state is a condition of altered immunologic activity. There is increased reactivity to specific groups of antigens which are non stimulatory to the majority of individuals. For a number of years, the possible existence of an immune cellular alteration in atopic patients has been under study. It has been suggested that cell-mediated immunity is impaired in patients with atopic disease but the results were controversial (1-4). Some studies have presented evidence for a decreased number of suppressor T-lymphocytes in patients with atopic disease and it has been postulated that the pathogenesis of allergic disease is associated with a disturbance of the balance between suppressor and helper lymphocytes, leading to an excessive production of IgE antibodies against a variety of allergens (3,5,6). Attempts to enumerate helper and suppressor T cell subsets as defined by anti-CD4 and anti-CD8 monoclonal antibodies have yielded conflicting results showing either increased CD4+ cell numbers or decreased CD8+ T cell numbers. Since Noon's report in 1911, hyposensitization has been widely accepted as a specific treatment for allergic disease and has been shown to be clinically effective in numerous controlled trials (7). While the exact mechanism by which immunotherapy causes this reduction in symptoms is unclear, many immunologic changes have been documented to occur in patients receiving this therapy. The immunologic basis for the increased sensitivity to antigens and the effectiveness of immunotherapy is not well explained. Some published reports have shown that allergen immunotherapy could induce an increase in the number of T lymphocytes, enhance the suppressor activity of T cells and generate allergen-specific supressor cells (8-11). These changes associated with immunotherapy could be a part of the immunologic mechanism which accounts for its clinical efficacy. Studies in which lipopolysaccharide (LPS), pokeweed mitogen (PVM) a phytohemagglutinin (PHA) and concanavalin A (Con A) have been used as polyclonal activators of peripheral .blood lymphocytes (PBLs) have greatly advanced our understanding of regulatory mechanisms in immunology (12).

In this study, proliferative response of lymphocytes to PHA and allergen extracts and T helper-inducer/suppresser-cytotoxic (CD4+/CD8+) ratio were investigated in the peripheral blood of newly diagnosed patients with allergic rhinitis at initial and six months of immunotherapy. We compared the results with observations on a group of healthy persons. The aim was to demonstrate the existence of numerical and functional alteration of PBLs patients with allergic rhinitis and change of them at early phase of specific immunotherapy.

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MATERIALS AND METHODS

Subjects: The study population consisted of 30 patients with allergic rhinitis (21 females, 9 males, mean age±SD;

26.2±7.4 years). The diagnosis of allergy was made by the personal and family history, physical examination, positive skin test, conjunctival provocation tests and specific IgE at sera. While thirteen out of 30 patients were sensitive to grass pollens 7 and 10 were to weed pollens and house dust mite respectively. Patients did not receive any systemic or topical medication for seven days before the study and no one had received immunotherapy or long-term steroid treatment.

Age and sex matched twenty healthy non-atopic volunteers were served as controls. The controls were subjected to the same tests as the study group initially. All subjects were screened for allergy by history and skin tests with extracts from grasses, trees, ragweed pollens, molds and house dust mites (ALK. DENMARK). In the atopic group, each subject had a least one positive immediate skin test that correlated with history. None of the control group had positive skin test at comparable extract solution.

Hyposensitization: Hpyosensitization was started with weekly injections and the allergen dose was increased as rapidly as possible until a maximal tolerated dose was reached. The effectiveness of hyposensitization was evaluated six months after treatment by using both symptom scoring and the amount of medications consumed as parameters and compared with those of previous year without treatment.

Cell preparation: Peripheral blood mononuclear cells (PBMCs) were isolated from fresh heparinized venous blood by the standard Ficoll-Hypaque density gradient centhfugation technique under sterile conditions. Cells were washed three times with Hank's balanced solution (Sigma 2513) and resuspended in RPMI-1640 culture medium (GIBCO, Grand Island N.Y) supplemented with 10% heatinactivated fetal calf serum (GIBCO), HEPES 25 mM/liter, penicillin 100 U/mL streptomycin 100 mg/ml (GIBCO) and 2 mM L-glutamine/ml (GIBCO). After studying the viability, the lymphocyte concentration was adjusted with culture medium to a concentration of $1x10^{6}$ cells/ml. Mitogenic assays were set up in triplicate in 96 well round-bottom microtiter plates.

Proliferation assay: PMBCs were stimulated by addition of different concentrations of a T cell mitogen phytohaemagglutinin (PHA) and three different allergen extracts. The allergen extracts (ALK, Denmark), used in the study, were mixed grasses' pollens (MGP), mixed weeds' pollens (MTP) and mites. The dose ranges were 0.5 ug/ml and 1 mg/ml for PHA and 500,1000,10.000 SOU for allergen extracts. One hundred ul of diluted mitogen or medium alone in each well. Each mitogen or allergen was run in duplicate for every dilution. Cultures were incubated for 5 days in a 5% $C0_2$, humidified incubators at 37°C. Cells were pulsed with 1 uCi of [3 H] thymidine for last 24 hours of culture. The cells were harvested (Titertek cell harvester). (3 radioactivity was measured in an automatic liquid scintillation counter (Beckman Instruments Inc. Fullerton, CA). The results from the amount of (3 H) thymidine incorporation was determined as a stimulation index (SI). A SI for each dilution of mitogen was calculated by dividing the counts per minute (cpm) from each sample by the cpm of control well as follows:

$$\frac{\text{Sample well (cpm)}}{\text{Control well (cpm)}} = \text{SI}$$

Determination of lymphocyte subsets: CD4+ and CD8+ cells were enumerated by using monoclonal antibodies in flow cytometer (FACScan) according to standard methods recommended by Becton&Dickinson. Monoclonal antibodies against CD4 (T helper/inducer), CD8 (T supressor/cytotoxic) receptor were purchased from Becton&Dickinson (U.S.A).

Statistical analysis: The Student's t test was used for statistical analysis throught the study. Differences were regarded as significant when probality of p value<0.05. Correlation coefficients and their statistical significance was determined using Pearson's linear regression analysis.

RESULTS

In the first set of experiments, we investigated peripheral blood T lymphocyte subpopulations in patients with allergic rhinitis and normal individuals. The distribution of T cell subsets and T helper/T supressor ratio in peripheral blood samples from patients and healthy controls were presented in Table 1. As shown in Table 1, the mean percentage of CD4+/CD8+ ratio of the untreated patients was found significantly lower than that of normal subjects (1.21±0.46 versus 1.59+0.60; p<0.04). In the present study, we also found a statistically significant difference in mixed lymphocyte response between sensitizing and non sensitizing antigens. The proliferative responses to specific allergen and others and to PHA in patients and

Table 1. Lymphocyte subpopulations in study groups

		Patients	
	Controls	Before treatment	After treatment
CD4+	46.85+8.34	42.73±7.61	43.56±9.32
CD8+	33.42±8.04	38.69±7.44	37.15+8.41
CD4/CD8 ratio	1.59±0.60	1.21±0.46	1.30±0.48

Table 2. Mean stimulation indices for PHA and allergen extracts in study population

	PHA 0.5	PHA 1	MGP	MTP	Mites
Controls	5.79±3.32	8.99±4.35*	1.73±1.15*	2.20±1.94	1.55±0.28*
Patients	4.76±3.15	4.99±2.87			
Sensitive			3.49±2.25	3.30±2.70	4.08±2.71
Non sensitive			1.30±1.80	2.30±2.21	2.04±1.85

normal subjects are presented in Table 2. As shown in Table 2, the means value of Sis by sensitizing allergen extracts as calculated from amount of [3H] Thymidine incorporation were higher in the patients than other allergen extracts especially in concentration of 1000 SQU/ml (for MGP p=0.01, for mites p<0.005). We could not find any difference between control and non sensitive patients according to PMBCs response to allergen extracts. When compared to nonatopic subjects, patients had significantly decreased PBMCs proliferation to PHA in 1 u.g/ml (p<0.02). After hyposensitization, it increased remarkably (p<0.001). Table 3 depicts the changes of lymphoproliferative responses to allergens and PHA by immunotherapy. As shown in Table 1 and 3 the mean percentage of CD4+/CD8+ ratio did not change, but the proliferative response of peripheral blood lymphocytes of patients to PHA and allergens were augmented after immunotherapy. A significant decrease in clinical symptoms has been reported in patients receiving immunotherapy at sixth months but we could not find any correlation between symptom scores and lymphocyte proliferative response to allergens.

DISCUSSION

The immunologic basis for the increased sensitivity to antigens and the effectiveness of immunotherapy is not well explained in atopic patients. The humoral and cellular changes that occur are complex. Some studies showed that the distribution of lymphocyte markers may be altered in atopic patients and it has been suggested that a supressor T cell deficiency, resulting in increased IgE production, may underline the allergic diathesis in man (6,13,14). On the other hand, some investigators found to be normal (1,2,15,16). To date, the status of supressor T cells in atopic patient has been controversial.

At the first part of the present study, peripheral blood lymphocytes from atopic individuals and a healthy nonatopic population were examined and compared to determine whether any variation from the normal distribution existed in the atopic population. Our present data has demonstrated decreased percentage of CD4+/CD8+ ratio in the patients with allergic rhinitis as compared to controls (p<0.04). We could not found diminution in the CD8+ population and the results of our present study didn't support the hypothesis that a relative deficiency of supressor T cells might have a pathogenetic role in the atopic state. In this work, we did not evaluate IgE specific suppressor activity so that it does not exclude the reduced suppressor activity in subjects with allergic rhinitis.

At present, none of the immunologic mechanisms appears to account for clinical success achieved by this form of immunotherapy. Lymphocyte subpopulations were studied over the course of six months to determine whether immunotherapy produced any change in the relative distribution of these markers. Patients with allergic rhinitis has demonstrated no difference in the lymphocyte distribution between at the initial and at sixth months of immunotherapy. The ratio of CD4+/CD8 T cells did not change with immunotherapy and did not correlate with symptom scores. In Rak's study, after 3 years of immunotherapy the percentage of CD8+ cells remained unaltered in the patients with allergic rhinitis or asthma caused by sensitivity to birch-pollen (17).

Considerable evidence has accumulated to suggest that cell-mediated immunity is impaired in patients with atopic patients. The depressed PHA response became normal after a clinical improvement in the patients. In Hsieh's study, the proliferative response of CD4+ T cells to PHA, which was reduced before immunotherapy, increased to normal levels in mite-treated patients. In contrast the CD4+ T cell response to mite antigen was decreased (18,19). On the other hand, some investigators found that PBMC proliferative response to PHA was normal in atopic patients (20). At the present study, the response of PBMCs to a mitogen PHA and antigen stimulation were measured by the incorporation of (3H) thymidine. The mean stimulation indices for allergen extracts had been found significantly higher in the patients with sensitizing antigen except weed pollens sensitive patients. Lymphocyte responses to allergen extracts were same in controls and unsensitized patients. Our present data showed decreased percentage of CD4+/CD8+ ratio and decreased response to PHA in patients with allergic rhinitis. It is possible that the observed abnormalities in atopic subjects may reflect an invivo deficiency in antigen specific and/or nonspecific T cell activity in atopic patients. Several studies have examined the effect of conventional immunotherapy on the invitro response of T cell isolated from atopic patients. Previous work showed that mononuclear cells from patients undergoing immunotherapy became less responsive in vitro to the allergen in that lymphocyte proliferation and lymphokine production was reduced (16,19). The most striking observation in this study has been the increased lympho-

Table 3. The effect of immunotherapy on mean stimulation indices for PHA and allergen extracts in the patients with allergic rhinitis

	PHA 0.5	PHA 1	MGP	MTP	Mites
Patients					
Before treatment	4.76±3.15	4.99±2.87			
Sensitive			3.49±2.25*	3.30±2.70	4.08±2.71*
Non sensitive			1.30±1.80	2.30±2.21	2.04±1.85
After treatment	14.42±8.25*	14.61±8.80*			
Sensitive			4.23±2.02	4.34±3.42	3.94±2.80
Non sensitive			6.77±3.74*	4.33±3.1	3.06±3.19

cyte response to the allergen extracts in pollen sensitized patients.

The immunologic mechanism behind this observation is not known, but several explanations have been postulated. First, aggravation of lymphocyte response to house dust mites was not seen in the patients sensitized or unsensitized. There may be different immunologic mechanism between in pollen and in house dust-mite sensitivity. Second, there may be cross-reactions between pollen extracts. Third, increase at specific IgE levels at the beginning of immunotherapy had been shown previously (8-11). Augmentation of immune response may be at the beginning and it may be reducing after years. Further studies by long-term immunotherapy may clarify this question. A significant decrease in clinical symptoms has been reported in patients receiving immunotherapy at sixth months (next pollen season) but we could not find any correlation between symptom scores and proliferative response of PBMCs of patients to allergen. The immunologic mechanism behind this observation is not known; PHA and allergen response of PBMCs seem like IgG blocking antibodies, although titers do not necessarily predict clinical success in individual patients (8-11).

The results of this study do not support the concept of suppressor T cell decrease in atopic patients, they may be in circulation but are functionally deficient. This observation suggests an association between allergic rhinitis and deficiency of circulating CD4+ cells but further studies are required to support this hypothesis. We found that PHA response was reduced before immunotherapy in allergic patients but it increased after immunotherapy without change in CD4+/CD8+ ratio. Dysfunction of helper cell contributes to the pathogenesis of atopic disease states. At present there is no absolute way to determine which patient will respond to immunotherapy and which will not. Even in treated patients who derive clinical benefit there is no best immunological parameter to follow. In this study, we tried to determine the effect of immunotherapy on lymphocyte response to allergen whether it could be used to determine the effect of immunotherapy in early phase of the treatment but we could not find any correlation with symptom scores. We have demonstrated increased lymphocyte response to PHA and allergen extracts at sixth months of immunotherapy. The effect of immunotherapy on lymphocyte response may not only depend on specific allergen and it may effect of immunotherapy on lymphcyte response to allergen whether it could be used to determine the effect of immunotherapy in early phase of the treatment but we could not find any correlation with symptom scores. We have demonstrated increased lymphocyte response to PHA and allergen extracts at sixth months of immunotherapy. The effect of immunotheray on lymphocyte response may not only depend on specific allergen and it may effect the response of lymphocyte to other allergen and mitogen and it may have immunomodulatory activities. All these questions need to be investigated further. Our results only shows the short-term effect of immunotherapy and it is necessary to follow these results 3-5 years after immunotherapy.

As a result, the immune system dysregulation in atopic patients may be due to the dysfunction's of T cells. Further study of T cell function is required to explain the immunopathology of atopic diseases.

Allerjik rinitli atopik hastalarda lenfositlerin PHA ve allerjenlere karşı cevabı üzerinde spesifik immünoterapinin erken etkileri

Çalışmanın birinci kısmında, allerjik rinitli 30 hasta ve sağlıklı non-atopik 20 gönüllüde periferik lenfosit alt grupları ve bunların PHA ve allerjenlere karşı cevapları araştırıldı. Allerjik rinitli grupta PHA ile uyarılan lenfosit cevabı aktivitesinin kontrol grubuna göre daha az olduğu bulundu. Yine allerjik rinitli hastalarda T hücrelerinde CT4+/CD8+ oranı anlamlı olarak daha düşük idi. Allerjen ekstreleri ile elde edilen uyarı indeksleri ortalamaları aynı antijene 1000 SQU/ml konsantrasyonunda olmak üzere duyarlı hastalarda diğerlerine göre daha yüksek bulundu. Aktif lenfositlerin uyarılması ile allerjenler arasında anlamlı farklılık elde edilemedi. Çalışmanın ikinci kısmında, hasta grubunun periferik kan lenfositlerinde immünoterapinin T hücre alt grupları ve PHA ve allerjenlere karşı proliferatif cevabı üzerindeki erken dönemdeki etkileri araştırıldı. İmmünoterapinin altıncı ayından sonra tüm hastalarda PHA ve allerjen ile uyarılan lenfosit cevaplarında anlamlı artış izlendi. Bu bulgular ışığında, allerjik rinit ile dolaşımdaki CD4+ ve bu hipotezin benzer çalışmalar ile desteklenmesi gerektiği sonucuna varıldı. [Türk J Med Res 1996, 14(2):58-62]

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