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Effect of Treatment with Intranasal Corticosteroid and Oral Antihistamine on Cytokine Profiles of Peripheral Blood Mononuclear Cells of Patients with Allergic Rhinitis Sensitive to *Chenopodium album*

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ABSTRACT

Patients with allergic rhinitis (AR) show increased production of the Th2-related cytokines. Almost always, intranasal corticosteroid (INC) and antihistamine are used as routine therapy of AR. This study was performed to determine the *in vitro* secretion of cytokines profiles of PBMCs in patients with AR sensitive to *Chenopodium album* (Ch.a) pollens before and after treatment with INC (Fluticasone propionate) and oral antihistamine (Loratadine).

PBMCs of 20 patients with AR, were tested *in vitro* for cytokine production. These cells were stimulated with natural or recombinant Ch.a. The levels of IL-4, IL-13 and IFN- γ , were measured in supernatants of cultured cell 96h after stimulation using ELISA. The PBMCs of 20 normal individuals were also similarly treated for comparison of results. The production of IL-4 by the patients' cells stimulated with either Ch.a or rCh.a was significantly higher than normal levels before therapy ($p=0.04$ and $p=0.02$, respectively). After therapy, a significant decrease in production of IL-4 and a significant increase in production of IL-10 were found in PBMCs stimulated with natural Ch.a, in comparison to the results before stimulation ($p=0.03$ for IL-4; $p=0.04$ for IL-10). Similarly, these results were seen in the production of IL-4 and IL-10 stimulated with rCh.a allergen after therapy in comparison to the results before stimulation ($p=0.01$ for IL-4; $p=0.03$ for IL-10).

This study suggests INC (Fluticasone propionate) and oral antihistamine (Loratadine) have the capacity to inhibit the production of IL-4 and shift Th2/Th1 responses, probably due to increase the level of immunoregulatory IL-10. Therefore, it could be concluded that therapy with INC and antihistamine has pharmacologic and immunologic therapeutic effects on AR patients.

Key words: Allergic Rhinitis; Antihistamine; Cytokines; ELISA; Intranasal Corticosteroid; PBMCs

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INTRODUCTION

Allergic rhinitis (AR) is a chronic IgE-mediated inflammatory disease, which is triggered by allergens, including seasonal allergens such as weeds, pollen. The prevalence of AR is growing up worldwide and it is estimated that up to 20% of general population are affected by AR.¹ *Chenopodium album* (Ch.a) is one of the most allergenic plants with high abundance growth in most parts of Iran.² At present, Ch.a natural extracts are used for diagnosis and immunotherapy of AR patients. The natural homemade extract of Ch.a has been prepared and examined in skin prick test in a previous study in Iran.³

Also, Mousavi et al induced asthma in animal model using homemade natural extract of Ch.a which resulted in increasing the levels of IL-4 and IL-5 as well as specific Ch.a IgE in mice.⁴ It has been suggested that increased activity of T-helper (Th)2 cells, which produce IL-4, IL-13 and IL-5 cytokines contribute to allergic inflammation.^{5,6} Intranasal corticosteroid (INC) and antihistamine are introduced as the first line of therapy for AR by the "Allergic Rhinitis and its Impact on Asthma (ARIA)" global guidelines.⁷

Several studies showed that INC reduced the level of cytokines secreted by Th2 cells and inhibited levels of other cytokines such as IL-1 β , IL-8, GM-CSF and TNF- α in AR.⁸⁻¹² These reports indicate that maintenance treatment with INC can have anti-inflammatory nasal effects.

The main goal of this project was, without intervention in the usual treatment of the patients with AR, to study the effects of intranasal corticosteroid and oral antihistamine on cytokine production of PBMCs of patients with AR.

Moreover, we studied cytokine profiles of Th1, Th2 and immunoregulatory cytokines after therapy of AR patients without *in vivo* intervention.

For this, we explored the effects of combination use of INC (Fluticasone propionate) and antihistamine (Loratadine) on the Th1 cytokine [with assay of Interferon (IFN)- γ], Th2 cytokines (with assay of IL-4 and IL-13) and immunoregulatory cytokine (with assay of IL-10) profiles secreted by peripheral blood mononuclear cells (PBMCs) isolated from patients with AR and from normal individuals exposed to natural or recombinant Ch.a (rCh.a) allergens *in vitro*.

MATERIALS AND METHODS

Subjects

Twenty patients with AR were enrolled in this study. Demographic data of all subjects are shown in table 1. The inclusion criteria were subjects with age range of ≥ 18 years, presence of rhinitis symptoms, history of rhinitis ≥ 1 year, and positive SPT to Ch.a allergen (Stallergen, France).¹³ We excluded subjects with more than 50 years who did not participate in second time sampling, and had high titer of total IgE due to nonallergic causes and 20 normal individuals with no history of AR and a negative skin prick test (SPT) to the Ch.a allergen. The patients were classified according to the ARIA classification.⁷ All participants had been free of medication ≥ 3 months prior to the study. INC (Fluticasone propionate, 2 puff, morning, gsk, UK) and antihistamine (Loratadine, 10 mg, orally, daily, Razi co, Iran) had been prescribed for all patients as routine therapy. Blood samples were collected before starting therapy at first visits and 6 weeks later during pollination season of Ch.a (late summer and fall). The study was approved by the local ethics committee. Written informed consents were also obtained from all subjects.

Study Design

Five milliliters (ml) of venipuncture blood from all subjects were collected into falcon tubes containing EDTA (EthyleneDiamineTetraacetic Acid). PBMCs were immediately isolated using Ficoll-Paque (Fresenius Kabi Noge As for Axis-Shield Poc As, Oslo, Norway) and gradient centrifugation. Isolated PBMCs were cultured in a 24-well plate (JET Biofil®, Canada) in RPMI 1640 (Biosera, UK) containing 10% fetal calf serum (FCS) (Biosera, UK), 50 μ g/ml penicillin, 50 μ g/ml streptomycin (Biosera, UK), 0.3 mg/mL L-glutamine (Biosera, UK), at a density of 1×10^6 cells per well and incubated (at 37°C and in 5% CO₂) in the presence of 50 μ g/ml natural Ch.a or 10 μ g/ml rCh.a, separately. Supernatants were collected before and after therapy of patients and aforementioned cytokines were measured.

Natural Ch.a allergen was extracted and purified from whole Ch.a pollen as described before.³ rCh.a allergen was prepared in Immunobiochemistry Lab, Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

Table 1. Subjects' Characteristics

Features	Allergic rhinitis patients	Normal control
Number (n)	20	20
Age (yr)	29±6	29±8
Gender	7 M, 13 F	7 M, 13 F
SPT (mm of Weal)	7.7±3.2	0
Total IgE (IU/ml)	234.7±151.15	47.5±12.6
ARIA classification:		
Mild intermittent	1	-
Moderate- severe intermittent	0	-
Mild persistent	9	-
Moderate- severe persistent	10	-

Skin Prick Test (SPT), Allergic Rhinitis and Its Impact on Asthma (ARIA)

Levels of IFN- γ , IL-4, IL-10, and IL-13 were measured in supernatants 96 h after culture, using ELISA sets from U-CyTech biosciences (Utrecht, The Netherlands) with sensitivity of 2 pg/ml. Total IgE were also measured with ELISA kit in sera of all AR patients with sensitivity 1 IU/l (PADTAN ELM, Iran).

Statistics

Statistical analysis was performed using SPSS 16 software package (San Diego, CA). Comparisons of the results before and after treatments were also done using Wilcoxon Signed Ranks Test. $P < 0.05$ were considered statistically significant.

RESULTS

As shown in table 1, there was no significant difference between these two groups with respect to gender distribution and age. Compared with the patients, normal subjects were negative for allergen skin test and did not show any clinical symptoms of AR at enrolment.

Effect of INC and Antihistamine on IFN- γ Production

The levels of secreted IFN- γ by natural Ch.a or rCh.a-stimulated PBMCs from patients before and after treatments and normal individuals are shown in table 2. There was not any significant difference on IFN- γ production before therapy of patients compared to the

baselines obtained from normal individuals. There was also no significant difference on IFN- γ production in patients before and after therapy.

Effect of INC and Antihistamine on IL-4 and IL-13 Production

PBMCs of AR patients with moderate- severe persistent AR (10 cases) secreted significantly higher IL-4 in comparison with PBMCs of other AR patients (10 cases) ($P=0.04$).

To investigate the inhibitory effects of INC and antihistamine, the cytokine secreted in response to Ch.a allergens by PBMCs, including IL-4 and IL-13 in supernatants were measured. Stimulated PBMCs from patients with AR secreted significantly higher IL-4 in supernatants after exposure to natural Ch.a or rCh.a, compared to normal group ($P=0.04$ and $P=0.02$, respectively), whereas after therapy of AR patients, the level of IL-4 in supernatant of their PBMC decreased significantly. ($P=0.03$ and $P=0.01$, respectively).

Effect of INC and Antihistamine on IL-10 (Immunoregulatory Cytokine) Production

In order to demonstrate whether IL-10 could be an immunoregulatory cytokine to inhibit Th2 responses, we measured IL-10 and found that secreted IL-10 in supernatants after therapy of patients was significantly increased, after exposure to natural Ch.a or rCh.a allergens ($P=0.04$ and $P=0.03$ for both allergens, respectively) (Table 2).

Table 2. Cytokine profiles of natural or recombinant Ch.a (rCh.a) stimulated PBMCs from patients with AR before and after treatments in comparison with stimulated PBMCs from normal control.

Cytokine (pg/ml)	PBMCs exposed to natural Ch.a				PBMCs exposed to rCh.a			
	AR patients		Normal controls	P value	AR patients		Normal controls	P value
	Before treatment	After treatment			Before treatment	After treatment		
IFN- γ	12.7 $\pm$ 5.3	17.7 $\pm$ 3.5	206 $\pm$ 55.3	0.2	12.3 $\pm$ 5.6	17.3 $\pm$ 2	207.3 $\pm$ 56.1	0.3
IL-4	14.6 $\pm$ 4.8	2.7 $\pm$ 0.2	2.2 $\pm$ 0.6	0.03*	15.5 $\pm$ 5.6	2.2 $\pm$ 0.5	2.6 $\pm$ 0.5	0.01*
IL-13	12 $\pm$ 2.2	11.2 $\pm$ 3	2.8 $\pm$ 0.5	0.7	12 $\pm$ 2	11.3 $\pm$ 3.8	2.4 $\pm$ 0.5	0.9
IL-10	13.3 $\pm$ 4.5	26.7 $\pm$ 2.2	14.4 $\pm$ 4	0.04*	14.1 $\pm$ 4	33 $\pm$ 2.9	7.1 $\pm$ 0.7	0.03*

Chenopodium album (Ch.a), Peripheral Blood Mononuclear Cells (PBMCs), Allergic Rhinitis (AR)

Values are mean $\pm$ standard deviation (SD)* $P < 0.05$ compared with normal control group

DISCUSSION

Although INC and antihistamine are commonly used in the treatment of AR, at present the immunomodulatory effects of these drugs on immune responses remain unclear.

Allergic reaction inducing AR symptoms can occur in early and late phases. The late phase reaction occurs 6 to 24 hrs after exposure to an allergen, which is associated with cellular infiltration of the nasal mucosa with activated CD4⁺ Th2 cells and eosinophils.¹⁴ Recent studies showed that cytokines IL-4, IL-5, IL-9 and IL-13 play a crucial role in this inflammatory reaction,^{15,16} while IFN- γ and IL-10 have been concerned as cytokines which counter and regulate the Th2 response by induction of tolerance and protect from the development of allergic disorders.^{17,18} Rudin et al.¹⁹ indicated that *in vitro* stimulation of PBMCs with allergen showed an increased expression of the Th2-associated cytokines (IL-4, IL-5, IL-9, and IL-13), whereas recent study by Heaton et al.²⁰ showed that PBMCs from both normal and allergic patients to house dust mites secreted comparable IFN- γ production after exposure to a mite extract. Our data are in agreement with both studies indicating that PBMCs from patients with AR produced higher IL-4 production.

Previous studies have shown that INC treatment results in ablation of the late phase allergic reaction, possibly due to the selective inhibition of cytokines such as IL-4 and IL-5 expression.²¹⁻²³ Ghaffar et al.²⁴ found that 6-weeks topical glucocorticosteroid treatment in subjects with allergic rhinitis induced a significant reduction in the number of IL-13-expressing

cells. Similarly, our data indicated that the production of IL-4 by PBMCs of AR patients was significantly decreased. But IL-10 in supernatants was increased after therapy with INC and antihistamines. According to Wolk et al.²⁵ who indicate that IL-10 is required for antigen presentation to regulate the nature and extent of the T helper response, we concluded that increase production of IL-10 induced by INC and antihistamine may could regulate the expression of IL-4 by PBMCs. Sim et al.²⁶ also showed that treatment of patients with topical decongestants, as vasoconstrictor, selectively induced decrease of IL-5. This study confirms synergistically effect of combination therapy of INC and antihistamine in patients with AR which enhanced the production of IL-10. We suggest that these findings were the effects of both INC and antihistamine on cytokine profiles of PBMCs of AR patients, because of baseline effects of them and our assessment of patients that did not use other drugs.

Altogether, it could be suggested that both natural Ch.a and rCh.a induce IL-4 as a main Th2-associated cytokine. Also, INC and antihistamine due to their anti-inflammatory effects can significantly inhibit the production of IL-4 which is mediated by downregulatory effect of IL-10.

One goal of INCs and antihistamine drugs prescribed are to control allergic symptoms. In any case, the immunomodulatory effects of these drugs are important. Previous studies and our study have shown that these drugs are able to modulate allergic reaction; therefore INCs and antihistamine as first-line for treatment of patients are suggested. The assessment of cytokine profiles of PBMCs of AR patients several

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times during the course of prescribed drugs is limitation of this study. The results obtained in this experimental study could be used for further investigations to explore the involvement of cytokines effects and potency of INC and antihistamine compounds in protection of allergic reactions.

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