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Effect of Allergen Specific Immunotherapy on Serum levels of IL-17 and IL-35 in Allergic Rhinitis Patients

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Allergic rhinitis (AR) is mediated by many proinflammatory and anti-inflammatory cytokines as interleukin-17 (IL-17) and interleukin-35 (IL-35) respectively. We assessed the effect of allergen specific immunotherapy (IT) on the serum levels of IL-17 and IL-35 in patients with AR sensitive to house dust mites (HDMs). Twenty patients with AR sensitive to HDMs and ten healthy control subjects were included in the study. Sensitivity to HDMs was diagnosed by positive skin prick test. Patients were treated by cluster IT. They were assessed by medical history, skin prick test, nasal symptoms scores, and measurement of serum IL-17 and IL-35 levels by ELISA technique before and after IT. All patients showed significant improvement in the skin test reactions and nasal symptoms scores after IT except for the postnasal drip which showed non-significant improvement. Serum IL-17 levels were higher in patients before IT than in control subjects and showed a significant decrease after IT. Serum IL-35 levels were lower in patients before IT than in control subjects and showed a significant increase after IT. No significant difference in the serum levels of IL-17 and IL-35 was observed between the patients and control subjects after IT. It was concluded that allergen specific IT decreases IL-17 and increases IL-35 in patients with AR sensitive to HDMs.

Allergic rhinitis (AR) is one of the most prevalent airway diseases worldwide. The immunopathogenesis of AR is mediated by many pro-inflammatory and anti-inflammatory cytokines as interleukin-17 (IL-17) and interleukin-35 (IL-35) respectively. IL-17 is a proinflammatory cytokine produced by Th17 cells, mast cells and basophils. It has a crucial role in allergic airway inflammation [1]. It induces the production of many other cytokines (such as IL-6, G-CSF, GM-CSF, IL-1 β , TGF- β , TNF- α), chemokines, and prostaglandins from many cell types as fibroblasts, endothelial cells, epithelial cells, and macrophages. The release of these cytokines causes many functions such as airway remodeling which is a characteristic of IL-17 responses [2].

Interleukin-35 (IL-35) is a suppressive and anti-inflammatory cytokine belonging to IL-12 family and is produced by regulatory, but not effector, T cells [3]. It is a dimeric

protein composed of IL-12 α and IL-27 β chains, which are encoded by two separate genes called IL12A and Epstein-Barr virus induced 3 (EBI3) genes, respectively [4]. IL-35 can suppress a variety of T cells including proinflammatory Th1 and Th17 cells and probably dendritic cells. It also supports proliferation of regulatory T cells (Tregs), increases their inhibitory function, and induces a new set of Tregs called inducible IL-35-producing Tregs (iT_h35 cells). In addition, this cytokine promotes production of anti-inflammatory cytokines such as IL-10 and down-regulates expression of proinflammatory cytokines such as IL-17 [5].

Cluster immunotherapy (IT) is a safe treatment method which is more effective and faster than conventional immunotherapy for treatment of AR caused by dust mites [6]. In addition, the safety profile is comparable between conventional and cluster IT [7]. In this study, we assessed

the effect of allergen specific cluster immunotherapy on the serum levels of IL-17 and IL-35 in patients with AR sensitive to house dust mites.

Subjects and Methods

Patients

Twenty patients with AR sensitive to HDMs *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* were included in the study. The patients were attending the allergy and immunology clinic of Suez Canal University Hospitals, Egypt. They were 13 males and 7 females with age ranging from 16-45 years. Sensitivity to HDMs was diagnosed by positive skin prick test (Omega, Montereal, Canada). Written consent was taken from each patient to participate in the study. Ten matching apparently healthy non-atopic subjects were also included in the study as a control group. Patients receiving antihistaminics or corticosteroids were asked to stop these medications two days before starting skin testing and blood sampling. Patients having nasal diseases other than PAR were excluded from the study.

Evaluation of patients before and after immunotherapy

Before immunotherapy, the condition of each patient was evaluated by medical history, skin prick test,

nasal symptom score and measurement of serum IL-17 and IL-35 levels. Nasal symptoms were scored according to Spector *et al.* [8]. Serum IL-17 and IL-35 were measured by ELISA kit (MyBioSource, San Diego, California, USA) according to the manufacturer's instructions. The same measures of assessment were repeated after completing the course of immunotherapy.

Immunotherapy

Patients were treated by allergen-specific cluster immunotherapy (Omega, Montereal, Canada) according to the protocol of American Academy of Asthma Allergy and Immunology (AAAAI), 2011 [9]. The treatment schedule consists of 8 weeks with three injections in each visit for the first three weeks, two injections in each visit for the next 4 weeks and only one injection in the last week. The injections were given subcutaneously with an interval between each two injections 30 minutes. The schedule of immunotherapy is shown in table (1).

Statistical Analysis

Statistical analyses were performed using SPSS (Version 17.0). All data were expressed as mean $\pm$ SD. The paired t-test was used to compare the change of parameters. All tests were two tailed, and the level of significance was set at 0.05.

Table 1. Cluster immunotherapy schedule with the dose in milliliter (ml) and concentration as dilution of maintenance vial (AAAAI, 2011).

Visit	1 st injection		2 nd injection		3 rd injection	
	Dose	Conc.	Dose	Conc.	Dose	Conc.
Visit (1)	0.10	1:1000	0.40	1:1000	0.10	1:100
Visit (2)	0.20	1:100	0.40	1:100	0.07	1:10
Visit (3)	0.10	1:10	0.15	1:10	0.20	1:10
Visit (4)	0.35	1:10	0.50	1:10	-	-
Visit (5)	0.07	1:1	0.10	1:1	-	-
Visit (6)	0.15	1:1	0.20	1:1	-	-
Visit (7)	0.30	1:1	0.40	1:1	-	-
Visit (8)	0.50	1:1	-	-	-	-

Results

Before treatment, most of the patients (60%) showed grade 3 skin test reaction while only 10% showed grade 4 reaction. No patients showed grade 1 reaction. After IT, most of

the patients showed grade 2 (55%) reactions and none of them showed grade 4 reaction. Table 2 shows skin test scoring of the studied patients before and after IT.

Table 2. Skin test scoring of the studied patients before and after cluster IT.

Grade	Before IT		After IT		P value
	No.	%	No.	%	
Grade 1	0	0	1	5	0.002*
Grade 2	6	30	11	55	
Grade 3	12	60	8	40	
Grade 4	2	10	0	0	

* P value <0.05 is significant

Assessment of AR symptoms by the nasal symptom scoring system revealed that all symptoms of AR showed highly significant

improvement after cluster IT except for the postnasal drip that showed non-significant improvement (Table 3).

Table 3. Mean scoring of nasal symptoms before and after cluster IT:

Symptoms	Before IT	After IT	P value
	Mean±SD	Mean±SD	
Rhinorrhea	5.4±1.05	3.6±1.12	< 0.001*
Sneezing	4.4±1.38	2.2±0.41	< 0.001*
Nasal congestion	4.7±2.01	2.6±1.39	< 0.001*
Nasal itching	4.5±2.30	2.4±1.05	< 0.001*
Postnasal drip	3.6±1.67	3.1±0.93	NS
Total nasal symptoms	22.6±0.65	13.9±0.57	< 0.001*

*P value > 0.05 is not significant (NS)

Serum IL-17 levels were higher in patients before IT than those in healthy controls and showed a significant decrease to 129 ± 56 pg/ml after IT ($P < 0.001$). By completing

the course of IT, there was no significant difference in serum IL-17 level between the patients and control subjects ($P > 0.05$).

Serum IL-35 levels were lower in patients before IT than those in control subjects and showed a significant increase to 379 ± 114 pg/ml after IT ($P < 0.001$). By completing the course of IT, there was no significant

difference in serum IL-35 level between the patients and control subjects ($P > 0.05$). Table (4) shows serum levels of IL-17 and IL-35 in control subjects and in patients before and after IT.

Table 4. Serum levels of IL-17 and IL-35 in control subjects and in patients before and after immunotherapy (Mean $\pm$ SD).

Studied groups		Serum level in pg/ml	
		Serum IL-17	Serum IL-35
Control		106 ± 28	396 ± 127
Patients before IT		312 ± 79	225 ± 64
Patients after IT		129 ± 56	378 ± 114
P value	Patients before and after IT	$< 0.001^*$	$< 0.001^*$
	Control and patients after IT	NS	NS

*P value > 0.05 is not significant (NS)

Discussion

Allergic diseases display various pathways of T lymphocyte inflammation and largely comprise Th2-associated processes. Additionally, novel T-cell subtypes, each with its own distinct cytokine profile, contribute to the heterogeneous presentations of allergic diseases [10]. Interleukin 17-producing T helper cells have confronted the classic Th1/Th2 paradigm and have been implicated in a growing number of inflammatory conditions [11]. Secreted by regulatory T-cells, IL-35 suppresses inflammatory responses of immune cells. It is also able to increase regulatory responses by inducing a potent population of IL-35 expressing Tregs [12]. In this study, we assessed the effect of allergen specific cluster immunotherapy on the serum levels of IL-17 and IL-35 in patients with AR to house dust mites.

Skin prick testing is used for assessment of allergic diseases because of its sensitivity and high specificity. It is also simple to use, less expensive and without any complications [13]. In addition, it has a high predictive value for atopic eczema, asthma or any allergy-related disease [14]. Before IT, most of our patients showed grade 3 skin test reaction and 10% of them showed grade 4 reaction. After IT, skin test scoring was significantly improved as most of the patients showed grade 2 reaction and none of them showed grade 4 reaction. In the study of song *et al.*, they concluded that the standardized dust mite allergen specific IT is efficacious and safe and can significantly reduce dust mite skin sensitivity [15].

After cluster IT, patients showed a significant improvement in all symptoms of AR except the postnasal drip that showed non-significant improvement. This agrees with the study of Song *et al.* who reported

that clinical symptom scores improve significantly after IT [15]. Luo *et al.* found that both the nasal symptom scores and medicine scores were significantly decreased in AR patients after cluster immunotherapy [6]. On the other hand, the study of Lal *et al.* 2011 reported different degrees of improvement in AR symptoms after IT as 59% of their patients showed very good control of symptoms, 24% showed fairly good control of symptoms and 16% showed only poor control of symptoms [16].

Serum IL-17 levels were higher in patients before IT than those in the healthy controls and showed a significant decrease after IT. This agrees with Ciprandi *et al.* who confirmed that allergic patients had higher levels of IL-17 producing T cells than normal subjects [17]. Also, Cai *et al.* reported that IL-17 is significantly increased in patient with AR in comparison to those who are non-allergic [18]. This can be explained by the study of Tsvetkova-Vicheva *et al.* who proved that genetically predisposed individuals who are continually exposed to allergens and environmental factors may produce a Th-17 cell-dependent immune response, factor IL-17, which could be used as a biomarker of polysensitization in AR patients [19]. Lu *et al.* measured the serum levels of IL-17 in AR patients before and after specific IT and they found that the level of IL-17 constantly declined in the whole course of IT and that the level change of IL-17 correlates with patients' symptom only two years after IT and so the level change of IL-17 can be used as an index for evaluating the treatment effectiveness of IT at that time [20].

Measuring the serum levels of IL-35 revealed that these levels were lower in the patients before IT than those in healthy controls and showed a significant increase after IT. Sawant *et al.* 2015 reported that the

suppression of IL-35 in allergic patients is due to inhibition of proliferation and induction of an IL-35-producing regulatory T cell population referred to as iTr35 [21]. In a study by Wan *et al.* they found that not only IL-35 level in AR group was significantly lower compared with that in the normal control group, but also the mRNA expression level of EBI3 in peripheral blood mononuclear cells in patients with AR was significantly lower than that in the normal control group [22].

Ding *et al.* reported that IT is associated with suppression of IL-17 and promotion of IL-35 expression in children with AR [23]. Whitehead *et al.*, proved that the increase in IL-35 levels is always associated with a decline in the levels of IL-17 because IL-35 can suppress Th17-dependent adaptive immune response [24]. This can be explained by the study of Yin *et al.*, which concluded that the lower expression of IL-35 may inhibit the propagation of Treg cells, lead to hyperfunction of Th17 cells, expand production of IL-17 and result in unbalance of Treg/Th17 cells; these may be the main mechanism in the pathogenesis of AR, therefore, regulation of IL-35 may become a new target for the immunological therapy of AR [25].

In conclusion, allergen specific immunotherapy is associated with improvement in allergy skin test reactions and nasal symptom scores in patients with AR sensitive to HDM. It also significantly decreases the serum levels of IL-17 and increases IL-35 levels with the possibility of using IL-17 and IL-35 as markers for assessment of the efficiency of immunotherapy in AR patients. Further studies are required to study the possibility of using IL-35 as a therapeutic modality for control of allergic rhinitis.

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