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Immunotherapy in Allergic Rhinitis: It's Effect on the Immune System and Clinical Symptoms

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Abstract

BACKGROUND: Allergic rhinitis is one of the most common allergic diseases and characterised by sneezing, rhinorrhea, nasal congestion and nasopharyngeal itching. Subcutaneous immunotherapy (SCIT) for specific allergens is an effective treatment and induces the inhibitory effect of T regulatory lymphocytes and decreases clinical symptoms in allergic rhinitis.

AIM: In this study effect of subcutaneous immunotherapy with specific allergens on clinical symptoms and T regulatory and T Helper cells cytokines, in patients with allergic rhinitis are evaluated.

METHODS: In this study, 30 patients with moderate to severe allergic rhinitis according to clinical criteria and positive skin prick test for aeroallergens were selected and treated by SCIT. Clinical symptoms and T cells cytokines IL4, IL17, IFN gamma, TGF beta, GITR, FOXP3 and IL-10 (by RT-PCR) were evaluated before and one year after initiation of treatment.

RESULTS: Thirty (30) patients with allergic rhinitis at age range 15-45 years old were treated by SCIT, and 23 (14 female, 9 male) patients continued the study, and 7 patients did not continue treatment. After immunotherapy, clinical symptoms decreased significantly. The specific cytokines TGF beta and IL10 levels increased and changes were statistically significant. (Respectively P = 0.013 and P = 0.05) The IL17 level was also increased, but not statistically significant. (P = 0.8) IFN gamma, IL4, GITR, FOXP3, all decreased, but the changes were not statistically significant (P > 0.05).

CONCLUSION: Subcutaneous Immunotherapy for specific allergens decreases clinical symptoms in patients with allergic rhinitis and induces tolerance in T lymphocytes, especially by increasing T regulatory cells cytokines, TGF beta and IL10.

Introduction

Allergic rhinitis (AR) is one of the most common types of allergy worldwide. Recent studies have shown an increase in its prevalence during recent decades ranging from 1.4 to 45%. Allergic rhinitis has direct and indirect effects on the quality of life and is accompanied by a group of another disease including asthma, middle ear inflammation, nasal polyps, sinusitis and lower respiratory tract infections [1] [2].

The diagnosis of AR is made based on clinical symptoms such as sneezing, rhinorrhea, itchy nose and nasal congestion when there is no sign of lower respiratory tract infections or anatomic abnormalities of the nose. Also when lab findings including a positive prick test and IgE specific antibody are in

favour of allergy, regarding the patients' history and clinical examination [3] [4].

The treatment of allergic rhinitis initially includes the avoidance of allergens especially common inhaled allergens. Indoor allergens such as house dust mites (HDM) especially in bed and house fungi which grow in damp places besides pets, indoor plants, grass, trees and grass pollen and other allergenic plants should also be avoided [1] [3] [4] [5]. Also, certain medications such as oral antihistamines, topical decongestants, inhaled corticosteroids and in certain cases oral corticosteroids are prescribed [1] [3] [5]. Immunotherapy with allergens is a therapeutic method in which the allergen is gradually and with an incremental dose administered resulting in the alleviation of clinical symptoms and reduced disease severity while preventing disease progression [1] [5] [6].

Immunotherapy with allergens has proven efficacy in the treatment of allergic rhinitis/ asthma and allergy to insect sting [1]. Today, subcutaneous injection of an allergen (SCIT) is the most common type of immunotherapy. Several studies have shown desirable clinical efficacy in the single and combined administration of allergens [1] [7] [8]. In different studies immunotherapy has resulted in a decrease in the number of principal cells in allergic responses (eosinophils/basophils and mast cells) and an increase in IgG4; moreover changes in lymphocytes including a rise in CD8+ and Treg cells and a decrease in IL4 and IL5 levels has been observed [1] [4] [6].

It seems that immunotherapy has a major role in the induction of specific Treg cells and that the induction of tolerance in T lymphocytes is the base of immunotherapy. The tolerance of peripheral T lymphocytes is recognised by the production of allergen-specific Treg cells. In addition to tolerance induction, immunotherapy prevents sensitisation towards new allergens and allergy progression [3][9][10]. CD4+ Treg-cells are divided into two categories: Natural Treg (nTreg) and Inducible Treg (iTreg) cells. Each of these subsets has a specific marker and express their specific receptors which for nTreg cells include CCR4/GITR/CTLA4/CD62L and for iTreg cells include IL10/TGF-beta. Natural Treg cells react to autoantibodies which are expressed in the thymus whereas Inducable Treg cells react towards peripheral antigens which are expressed by dendritic cells [1] [11] [12].

The nTreg cells originating from the thymus express an intracellular marker named FOXP3. It has been proved that following immunotherapy, the presence of FOXP3+ cells is increased in the nasal mucosa and this increase is consistent with the improvement of allergic rhinitis [4] [12].

The present study aimed at evaluating the effect of immunotherapy on the clinical symptoms and cytokine changes related to T lymphocytes in moderate to severe AR patients.

Methods

The present study is an experimental interventional trial which investigates the effect of immunotherapy on the clinical symptoms and the immune system of patients with moderate to severe perennial AR in Ghaem Hospital, Mashhad, IRAN during October 2008 to October 2009. A full medical history was initially taken from patients with perennial AR, and a thorough physical examination was performed. Prick test with the standard method and by using the common regional aeroallergens was performed with 6 extracts (Hollister, USA) to confirm the basis of allergy. In the mentioned test more than 3mm induration from the negative control was regarded as a positive test result.

The used tools were the following:

1. A structured questionnaire according to the AR scoring system

2. Skin scratch testing according to the European Academy of Allergy and Clinical Immunology

3. Allergenic extracts used for cluster immunotherapy

The inclusion criteria were typical signs of AR in exposure with inhaled allergens, positive results in the standard questionnaire, and a positive skin prick test with at least 3 standard inhaled allergen extracts. Patients were excluded in case of consuming beta blockers, accompanying uncontrolled asthma, and concomitant conditions including autoimmune disease, psychotic disorders, pregnancy and other forms of rhinitis. For lab studies 5-7 cc blood was taken from the brachial vein and lymphocyte markers regarding the phenotype of TH1, TH2 TH17 and Treg and specific markers of GITR, FOXP3, IFN-γ, IL17, IL10, IL4, TGFβ were evaluated with RNA extraction, cDNA production and eventually RT-PCR with the standard method (TaqMan for FOXP3 and cyber green for other cases).

For patients with allergic rhinitis who received a minimum of immunotherapy to treat pollen allergies using Allergovit (composition: 015 grass/cereals-100%; or composition: 108 Birch-35%, 115 Alder-30%, 129 Hazel-35%, Allergopharma, Reinbek, Germany).

The sample size was calculated as 25 cases concerning similar studies. Immunotherapy based on a planned schedule was conducted by the subcutaneous injection of mixed inhaled allergens extracts (Hollister, USA) with a concentration of

1:1000 of the original vial each week for 10 sessions, 1:100 every two weeks for 10 sessions and finally 1:10 monthly for one year. After a year after immunotherapy initiation, clinical and laboratory findings were once again evaluated. Wilcoxon test was used for IFN-γ, IL17 and IL10 measurements analysis whereas Paired T-test and the SPSS software version 11 were used for FOXP3, GITR, TGFβ, and IL4 measurements.

The study protocol was approved by the Research Council of Mashhad University of Medical Sciences and written informed consent was obtained from each participant before study entry.

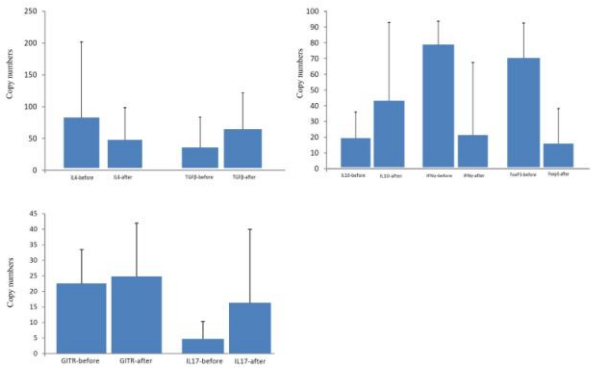

Figure 2: Expression of Cytokines before and after treatment

Results

Thirty patients with the diagnosis of allergic rhinitis underwent immunotherapy for one year. Twenty three (23) individuals, 9 males and 14 females, completed the study. Seven patients did not consent to continue treatment. The participants' age ranged from 15 to 45 years, and most patients experienced significant improvement in their clinical symptoms after the treatment period. Based on the clinical indices of TNS and TSS, disease severity was highest in the 30 to 39-year age group whereas the best therapeutic response was achieved in the 30 to 34-year age group (Figure 1).

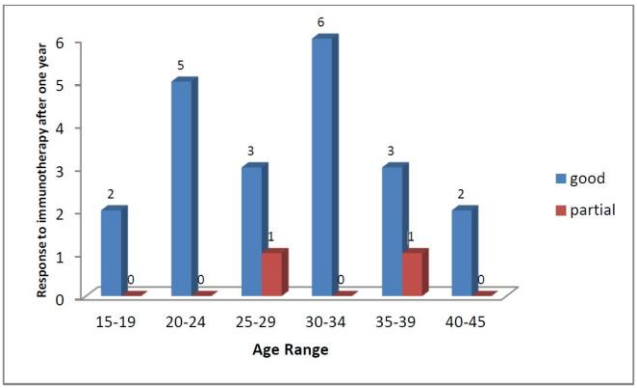

Figure 1: Response to immunotherapy after one year according to age

Regarding sex and response to immunotherapy; no meaningful response was obtained (P = 0.7). Considering the studied variables a year after treatment, an increase in the IL17 level was detected which was not statistically significant (P = 0.81). IFN-γ levels showed a reduction but with no meaningful significance (P = 0.21). However, the rise in TGB and IL10 levels was statistically significant, (P = 0.013, P = 0.05). Finally, a decrease in the level of IL4, GITR, FOXP3 was obtained, none showing a statistical significance (P > 0.05) (Figure 2).

Discussion

Immunotherapy if performed in an appropriate way in the right patient will be a safe and effective treatment that not only prevents the symptoms but will also result in reduced disease severity beside preventing disease progression [3] [6] [13]. Immunotherapy with allergens is a slow-process treatment which alters the immunologic mechanisms of disease. Meta-analysis of the performed studies comparing the therapeutic effects of subcutaneous immunotherapy with medical therapy has shown that immunotherapy has equal effects on medical therapy and can even be considered at treatment initiation [14]. The induction of tolerance in peripheral T lymphocytes resulting in a change in the immune system pathway from TH2 to Treg cells is the main goal in immunotherapy which is accompanied by elevated IL10 and TGF beta levels [4]. Eventually, the production of IL10 will lead to the inhibition of allergen-specific IgE production and on the other hand will result in IgG4 production [15]. Although at the start of immunotherapy a transient rise in the allergen-specific IgE level is observed, subsequently a gradual decrease takes place in the specific IgE level over years which prevents the rise of IgE in the mating season of plants in such patients [4].

In recent studies, it has been reported that the blocking effects of IgG in immunotherapy are FCR IIB receptor-mediated [16] [17] [18]. Attention should be paid to the fact that IgG4 immunoglobulin levels decrease the following immunotherapy, but their protective and biologic effects remain constant [15].

Nevertheless, IL10 can reduce the release of pro-inflammatory cytokines from mastocytes. Also, IL10 can reduce the function and activity of basophils and suppress IL5 production by TH0 and TH2 lymphocytes [9] [16] [18].

Cezmi et al. studied the therapeutic mechanisms of Treg cells in immunotherapy and published their results in 2009 in JACI. They stated that an increase in the number of Treg, CD25+ and CD4+ cells in allergen-specific immunotherapy (SIT) has a significant role and that immunotherapy with the grass group will result in increased IL10 and TGF-β expression in T cells and the mucosa.

One of the main characteristics of Treg cells is the expression of FOXP3 in them. In case of receiving signals from pro-inflammatory cytokines such as IL6, the FOXP3 function is inhibited, and the TH17 pathway is activated. For the induction of both the Treg and TH17 cells, TGF-β is required; but the function of these two cell groups are at odds with each other [19]. Although FOXP3 is expressed to a lesser degree by T-effector cells, the amount is very small, and it is expressed transiently; therefore FOXP3 is considered as a specific marker for Treg cells only [13] [15].

Bacchetta reported the principle role of Treg expressed FOXP3 as tolerance induction and prevention of effector T cell responses. In the mentioned study those Treg cells originating from the thymus were named as nT-reg cells, and those forming outside the thymus were called aT-reg (adaptive) cells [15] [17].

nT-reg cells react toward self-antigens expressed in the thymus whereas nT-reg or iT-reg cells including Trl (producing IL10) and TH3 (producing TGFB) result from the differentiation of naive T cells following antigenic stimulation in the environment. In several studies, it has been proved that the number of CD4+CD25+FOXP3+ cells increases in the nasal mucosa following immunotherapy, inconsistent with the improvement of the symptoms of allergic rhinitis [10].

The deviation of allergen-specific effector cells towards the Treg phenotype is the key to successful immunotherapy even in healthy individuals' immune responses. The inhibitory effect of IL10, known as an inhibitory cytokine for T cells, has been well proved in inducing tolerance towards allergens, autoantigens, bonding antigens and tumoral antigens. In 2009 Ciprandi et al., from Italy evaluated the TGF-β and IL17 levels in 23 allergic rhinitis patients before and after immunotherapy. TGF-β was 12.503 ± 23.354 ng/ml and 43.305 ± 31.861 ng/ml before and after immunotherapy, respectively, (P = 0.0016). This study showed a remarkable rise in TGF-β levels one year after immunotherapy. It also confirmed a rise in IL10 following immunotherapy [5].

Recently SLIT (sublingual immunotherapy) has found its place in immunotherapy. In a study by L. Cosmi et al., on the effects of SLIT in allergic rhinitis patients and mite-sensitive asthma, immunologic changes included reduced allergen-specific IgE and increased TGF-β, IL10 and IFN-γ levels [20]. In a study by Jutel et al., in 2003 in Switzerland, the mechanism of immunotherapy was studied in HDM-sensitive patients after 70 days of immunotherapy. In a part of this study, the IL10 and TGF-β receptors were blocked and re-evaluated. Eventually, it was observed that the inhibitory responses of T-cells by IL10 and TGF-β are the key mechanism of the mucosal immune response to allergens. Also, the inhibitory effects of IL10 on T-lymphocytes were due to changes in the CD28 mediated signalling pathway while the inhibitory effects of TGF-β were due to its inhibitory effect on TCR/CD3 and the inhibition of the CD28 pathway. Moreover, IL15 which is one of the main factors in the survival and growth of T-cells is inhibited by IL10 and TGF-β [7].

In our study, a significant decrease was observed in the clinical symptoms of allergic rhinitis patients after immunotherapy. In lab studies similar to Cezmi and Ciprandi studies, a rise in TGF-β and IL10 was detected in our patients. However, in contrast to previous studies, the FOXP3 was reduced although not to a significant level. Moreover, an increase in IL17 was detected following immunotherapy which could be suggestive of TH17 induction by TGF-β following the increase in this inhibitory factor.

Regarding the obtained results from this study, it seems that the immunologic mechanism leading to the alleviation and improvement of symptoms in AR patients mainly includes a rise in the number of inducible Treg cells accompanied by increased TGF-β and IL10 levels.

Limitation of the study: The limitation of the current study was small sample size because only 30 patients had inclusion criteria.

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References

1. Corren J, Baroody F, Pawankar R. Allergic and non allergic rhinitis. In: Adkinson Jr NF, Bochner BS, Burks AW, Busse WW, Holgate ST, Lemanske Jr RF, et al., editors. Middleton's allergy: principles and practice. 1.8 ed. USA: Elsevier Health Sciences,

2014:664-85.

2. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol. 2003; 33(5):1205-14. https://doi.org/10.1002/eji.200322919 PMid:12731045

3. Ciprandi G, De Amici M, Negrini S, Marseglia G, Tosca MA. TGF-β and IL-17 serum levels and specific immunotherapy. International immunopharmacology. 2009; 9(10):1247-9. https://doi.org/10.1016/j.intimp.2009.07.004 PMid:19622397

4. Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. Journal of Allergy and Clinical Immunology. 2009; 123(4):735-46. https://doi.org/10.1016/j.jaci.2009.02.030 PMid:19348912

5. Till SJ, Francis JN, Nouri-Aria K, Durham SR. Mechanisms of immunotherapy. Journal of Allergy and Clinical Immunology. 2004; 113(6):1025-34. https://doi.org/10.1016/j.jaci.2004.03.024 PMid:15208578

6. Ziegler SF, Buckner JH. FOXP3 and the regulation of Treg/Th17 differentiation. Microbes and Infection. 2009; 11(5):594-8. https://doi.org/10.1016/j.micinf.2009.04.002 PMid:19371792 PMCid:PMC2728495

7. Farid R, Ghasemi R, Baradaran-Rahimi M, Jabbari F, Ghaffari J, Rafatpanah H. Evaluation of six years allergen immunotherapy in allergic rhinitis and allergic asthma. Iranian Journal of Allergy, Asthma and Immunology. 2006; 5(1):29-31. PMid:17242501

8. Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK. Type 1 T regulatory cells. Immunological reviews. 2001; 182(1):68-79. https://doi.org/10.1034/j.1600-065X.2001.1820105.x PMid:11722624

9. Blaiss MS, editor Allergic rhinitis: Direct and indirect costs. Allergy and Asthma Proceedings; OceanSide Publications, Inc., 2010. PMCid:PMC2824441

10. Calderon M, Larenas D, Kleine-Tebbe J, Jacobsen L, Passalacqua G, Eng P, et al. European Academy of Allergy and Clinical Immunology task force report on 'dose–response relationship in allergen-specific immunotherapy'. Allergy. 2011; 66(10):1345-59. https://doi.org/10.1111/j.1398-9995.2011.02669.x PMid:21707645

11. Lee JH, Yu HH, Wang LC, Yang YH, Lin YT, Chiang BL. The levels of CD4+ CD25+ regulatory T cells in paediatric patients with allergic rhinitis and bronchial asthma. Clinical & Experimental Immunology. 2007; 148(1):53-63. https://doi.org/10.1111/j.1365-2249.2007.03329.x PMid:17349011 PMCid:PMC1868849

12. Maggi L, Santarlasci V, Liotta F, Frosali F, Angeli R, Cosmi L, et al. Demonstration of circulating allergen-specific CD4+ CD25highFoxp3+ T-regulatory cells in both nonatopic and atopic individuals. The Journal of allergy and clinical immunology. 2007; 120(2):429-36. https://doi.org/10.1016/j.jaci.2007.05.002 PMid:17604089

13. Sabin BR, Saltoun CA, Avila PC. Advances in upper airway diseases and allergen immunotherapy. Journal of Allergy and Clinical Immunology. 2011; 127(2):342-50. https://doi.org/10.1016/j.jaci.2010.11.049 PMid:21281864

14. Matricardi PM, Kuna P, Panetta V, Wahn U, Narkus A. Subcutaneous immunotherapy and pharmacotherapy in seasonal allergic rhinitis: a comparison based on meta-analyses. Journal of Allergy and Clinical Immunology. 2011; 128(4):791-9. e6.

15. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. Journal of Allergy and Clinical Immunology. 2008; 121(6):1467-72. https://doi.org/10.1016/j.jaci.2008.03.013 PMid:18423565

16. Allan SE, Passerini L, Bacchetta R, Crellin N, Dai M, Orban PC, et al. The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. Journal of Clinical Investigation. 2005; 115(11):3276-84. https://doi.org/10.1172/JCI24685 PMid:16211090 PMCid:PMC1242190

17. Bacchetta R, Gregori S, Roncarolo M-G. CD4+ regulatory T cells: Mechanisms of induction and effector function. Autoimmunity reviews. 2005; 4(8):491-6. https://doi.org/10.1016/j.autrev.2005.04.005 PMid:16214084

18. Cantillo JF, Puerta L. [New approaches for allergen-specific immunotherapy]. Biomedica: revista del Instituto Nacional de Salud. 2009; 30(3):440-53.

19. Chatila TA. Role of regulatory T cells in human diseases. Journal of allergy and clinical immunology. 2005; 116(5):949-59. https://doi.org/10.1016/j.jaci.2005.08.047 PMid:16275360

20. Crellin NK, Garcia RV, Levings MK. Flow cytometry-based methods for studying signaling in human CD4+ CD25+ FOXP3+ T regulatory cells. Journal of immunological methods. 2007; 324(1):92-104. https://doi.org/10.1016/j.jim.2007.05.008 PMid:17582431