TITLE: EFFECTS OF IMMUNOTHERAPY ON T-LYMPHOCYTE AND CYTOKINE COMPOSITION
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Objective: In this study, we aimed to compare serum IFN-g and T4 and T8 lymphocyte levels before and after immunotherapy in patients treated for allergic rhinitis. Thus, the effects of immunotherapy on these pathways were investigated.
Methods: Five study groups including 40 patients with allergic rhinitis and 10 healthy individuals were included. Group I (n=10): Patients whose IFN-g levels were measured before implementation of IT. Group II (n=10): Patients whose IFN-g levels were measured after implementation of IT. Group III (n = 10): Patients whose IFN-g levels were measured in the 3rd control year after implementation of IT Group IV (n=10): Patients whose levels of T4 and T8 lymphocytes were measured before and after implementation of IT. Group V (n=10): Control individuals.
Results: In our study, IFN-g levels before and after the implementation of IT, and in the 3rd control year after the implementation of IT were compared with the control group. IFN-g levels immediately after and three years after IT were found to be significantly higher compared to pre-IT measured values. According to our findings, T4 lymphocyte counts decreased and T8 cell levels decreased after the IT. According to these results, T4/T8 ratio also decreased.
Conclusion: There was a decrease in T4 level, an increase in T8 and decrease in the rate of T4/T8 after the implementation of IT.
Key Words: immunotherapy, interferon-gamma, T4 and T8 lymphocyte.

INTRODUCTION
The aim of immunotherapy for more than a century is to train the immune system and teach the body that allergens are not enemies. Allergic response substances are given to patients in increasing doses and over a long period of time to change the immune response of the patient and to prevent the development of allergies in the next encounter with the allergen. Most drugs used in the medical treatment of allergy successfully suppress symptoms over the long term, but do not cure the disease. Immunotherapy is the only successful treatment modality to eliminate the disease. Immunotherapy can be applied to: i) allergies that cannot be controlled despite the use of allergen prevention methods and medication, ii) inability to continue the drug due to side effects of allergy drugs, and iii) to prevent new allergies and asthma development [1].

Allergic rhinitis is an inflammatory disease of nasal mucosa, that occurs by reacting against a normally harmless antigen. It is characterized by troubled local nasal symptoms and general fatigue and adversely affects quality of life [2,3]. Allergen-specific IT is an important treatment method for patients with allergic rhinitis and is the only curative intervention today that potentially balances the immune system and thus affects the natural course of the allergic disease. The long-term efficacy and safety of allergen-specific IT has been confirmed by many clinical studies [4]. There are significant changes in cellular and humoral immunity composition as a result of the Implementation of IT. Primary changes occur in the ratio of T4 (T-helper, CD4) and T8 (T-suppressor, CD8) and the types of cytokines they secrete (interferon and interleukin) [5,6].

The way to contact with an allergen and its concentration determine the type and amount of cytokine (interferon and interleukin) secreted by allergen-specific T-lymphocytes. Normally, a contact with the antigen results from inhalation. However, there is a change in the relationship between antigen-presenting cells and T-lymphocytes because there will be a contact in intense concentrations and in a short time through implementation of IT.
subcutaneously. Thus, instead of production of interleukin-4 (IL-4) by T-cells, interferon-gamma (IFN-g) is produced. IL-4 is a cytokine in the form of polypeptide that is synthesized by T8 lymphocytes. IL-4 is an important growth and differentiation factor for B-lymphocytes. Furthermore, it is the activator for mast cells and macrophages [7]. IFN-g is also called as immune or type II interferon. IFN-g is a cytokine in the form of glycoprotein produced by T4 and T8 lymphocytes. T-helper lymphocytes are divided into two main groups based on the cytokines they secrete. Although Th1 lymphocytes secrete IFN-g and lymphotoxin, Th2 lymphocytes secrete IL-4 and IL-5 [8]. As well as functioning as a co-factor in the proliferation of B-lymphocytes, IL-4 which is synthesized from Th2 lymphocytes, provokes the secretion of Ig-G and Ig-E, and B-lymphocytes stimulated by lipopolysaccharide (LPS). IFN-g is secreted by Th1 cells and inhibits B-lymphocyte functions through IL-4 [9-11]. In many cases, IL-4 antagonizes INF-g activity [12,13].

In this hospital-based case-control study, we aimed to compare serum IFN-g and T4 and T8 lymphocyte levels before and after immunotherapy in patients treated for allergic rhinitis. Thus, the effects of immunotherapy on these pathways were investigated.

METHOD

Ten control individuals and 40 patients with allergic rhinitis admitted to the allergic outpatient clinic (Hospital of Cerrahpaşa Medical Faculty, Istanbul University) due to complaints of nasal congestion, nasal itching, sneezing, red eye and watering of the eyes were included in the study. As the results of anamnesis, ear, nose and throat examination, prick test, and laboratory findings, patients were found to be sensitive to dermatophagoides pteronyssinus (Dp) and dermatophagoides farinea (Df). The main distinctions between the patient groups were based on IFN-g and T4-T8 lymphocyte levels before and after implementation of IT. Thus, five groups were formed along with the control group.

Group I (n=10): Patients whose IFN-g levels were measured before implementation of IT.
Group II (n=10): Patients whose IFN-g levels were measured after implementation of IT.
Group III (n=10): Patients whose IFN-g levels were measured in the 3rd control year after implementation of IT.
Group IV (n=10): Patients whose levels of T4 and T8 lymphocytes were measured before and after implementation of IT.
Group V (n=10): Control individuals.

The participants who were included in the study were informed about the study and the Informed Consent Form was signed from the consent of the guardians and the results of the study were used for scientific purposes. The principles of the Declaration of Helsinki and Guidelines for Good Clinical Practices were followed during the study. The ethical approval of study supplied from Istanbul University-Cerrahpasa Medical Faculty.

Immunotherapy was administered subcutaneously Dp (50%) and Df (50%) (HAL Allergy, Haarlem, The Netherlands) at doses of 10 AU, 100 AU, 1000 AU and 10,000 AU each week for the first six months. Subsequently, the classical IT plan (AU 10,000) was implemented once a month for two years. Patients were invited to check for routine follow-up for three years after the IT application.

IFN-g activities were determined by commercially available the radioimmunoassay (RAI) test kits (Medenix Diagnostics SA, Fleurus, Belgium) according to the manufacturer's instructions. T lymphocyte subgroups were counted using Ventrex Research Lab. monoclonal antibodies. In all patients, after a significant improvement in clinical allergy symptoms following implementation of IT, the degree to which serum IFN-g and IT change was changed in allergic patients, 1- IFN-g levels before implementation of IT in Group I, 2- IFN-g levels after implementation of IT, 3- IFN-g levels in the 3rd year after implementation of IT in Group III, and 4- IFN-g levels in the control group were investigated. In addition, the changes between IT and cellular immune composition (T4, T8 lymphocyte count and ratio before and after IT) in allergic patients were examined.

The data were analyzed using Mann-Whitney U - Wilcoxon Rank Sum W test, Wilcoxon Matched - Paris Signed - Ranks test and Person Correlation analysis. The variables were examined at 95% confidence level and p value was accepted as less than 0.05.

Figure 1. Comparison of Interferon gamma (IFN-g) levels of groups. Values were expressed as mean ± SD.
RESULTS

The participants (40 patients and 10 healthy individuals) were 33 females and 17 males. The age of participants were between 15 and 56 years and the mean age was 25.4 years. The mean and standard deviations of IFN-g levels of the groups are shown in Figure 1. According to this graph, a statistically significant increase was observed in Group II and Group III when compared with Group I (p values 0.047 and 0.007, respectively). Differences observed in other comparisons were not significant.

Before and after implementation of IT, T4 and T8 levels in Group IV are shown in Figure 2. Accordingly, T4 levels decreased significantly (p = 0.014), whereas T8 values increased (p = 0.007).

The rates of T4 and T8 levels in Group IV before and after IT application are shown in Figure 3. Accordingly, T4/T8 ratio decreased significantly after IT application (p=0.028).

DISCUSSION

T-lymphocytes, B-lymphocytes and their products -cytokines which are activated by allergens play a role directly and indirectly in the formation of immunological mechanisms of allergic diseases and emergence of their clinical symptoms. In this study, after the efficacy of the IT was clinically shown, serum IFN-g levels that were measured at specific times before and after the implementation of IT were compared with the non-allergic control group. Also, changes in T-lymphocyte composition (T4, T8) were determined. According to HanGlass et al., IT mainly affects the T-lymphocyte composition and its subgroups. This effect occurs in two ways: 1) an increase in allergen-specific T8 lymphocytes and 2) a change in allergen-specific T4 lymphocyte phenotype [14].

A study showed an increase in IFN-g producing lymphocytes and a decrease in T8 lymphocytes in atopic individuals compared with the normal population. Based on the impact of IT implementation on the T4/T8 ratio, there was a change in favor of T8 [15] and an increase in T8 level from conventional IT management [16]. In addition, a change in the T4/T8 ratio was found after administration of IFN-g in subjects with hyper-Ig syndrome [17]. A study by Gideon et al. also showed significant changes in the T cell composition resulting from IT administration [18].

In another study, it was found that there were allergic reactions and that the changes caused by implementation of IT were indirectly mediated by cytokines released from T-lymphocytes [19]. IL-4 stimulates allergen-specific Ig E production of B-lymphocytes, allowing eosinophils differentiation and maturation in bone marrow in IL-5. T-helper 2 lymphocyte subgroup synthesizes IL-4 and IL-5 which are responsible for allergic reactions. But, T-helper 1 lymphocytes produce IFN-g which causes the suppression of allergic reactions [20-23].

In their study, Gajewski et al. found a significant increase in the level of IFN-g resulting from the IT. They showed that while this process inhibits proliferation of the allergen-specific T-helper 2 lymphocyte clones selectively, it activates the proliferation of T-helper 1 lymphocyte clones [24]. Jutel et al. found a decrease in antigen-specific IL-4 production in mononuclear cell cultures taken from the peripheral blood of patients undergoing implementation of IT and an increase in IFN-g synthesis [25].

In our study, IFN-g levels before and after the implementation of IT, and in the 3rd control year after the implementation of IT were compared with the control group. IFN-g levels immediately after and three years after IT were found to be significantly higher compared to pre-IT measured values. That is, IT raises the IFN-g level. These findings
were consistent with the literature. According to our findings, T4 lymphocyte counts decreased and T8 cell levels decreased after the IT. According to these results, T4/T8 ratio also decreased.

**Conclusion**

There was no significant difference in the serum IFN-g levels between the allergic patients whom IT was not implemented and that of the control group. There was a significant increase in the serum IFN-g levels of the patients' IT was administered compared to the levels before the implementation of IT and levels of the control group. There was a decrease in T4 lymphocytes in the peripheral blood, an increase in T8 lymphocytes and a decrease in the rate of T4/T8.

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