

Specific Immunotherapy Effect on Interleukin-18 and CD30 Serum Levels in Monosensitized Patients with Rhinitis

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ABSTRACT

To clarify the immunologic changes associated with specific immunotherapy (SIT), we analyzed interleukin (IL)-18 and CD30 serum levels in a group of patients with allergic rhinitis before and after SIT. IL-18 is a proinflammatory cytokine that plays an important role in the Th1 response. CD30 is a marker of Th2 lymphocytes. We selected 16 healthy donors (HDs) and 16 patients affected by allergic rhinitis, matched for sex and age. Serum IL-18 and CD30 levels were assayed by an immunoenzymatic method. IL-18 serum levels in the patients were lower than in the HDs before SIT (200.69 ± 93.48 pg/mL versus 296.50 ± 66.29 pg/mL; $p < 0.05$). After SIT, patients showed an increase of serum IL-18 levels (288.69 ± 146.69 pg/mL versus 200.69 ± 93.48 pg/mL; $p < 0.05$). On the contrary, serum CD30 levels were higher in patients before SIT with respect to HDs (14.78 ± 8.30 IU/mL versus <1 IU/mL; $p < 0.05$). SIT caused a decrease of serum CD30 levels in patients who were allergic (5.95 ± 5.70 IU/mL versus 14.78 ± 8.30 IU/mL; $p < 0.05$). In conclusion, in this study we showed for the first time the shift of IL-18 and CD30 production after SIT. (Allergy and Asthma Proc 24:179–183, 2003)

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Allergen-specific immunotherapy (SIT) was first used in medical practice at the beginning of the last century for the treatment of patients suffering from respiratory allergic diseases. In 1998, the World Health Organization Position Paper,¹ based on a careful revision of the available literature, discussed the safety and efficacy of parenteral and local SIT when administered under adequate conditions. Since then, additional studies supporting the efficacy and safety of both injection and local SIT have been published.^{2,3} A long-term effect of SIT after 3-years of treatment, consisting in a marked improvement of symptom score, accompanied by a persistent alteration in immunologic reactivity (reduction in CD3⁺ T-cell infiltration and interleukin [IL]-4 messenger RNA [mRNA] expression), has also been reported.⁴

The study of the mechanisms of SIT has served as a model to gain insight into the immunologic changes associated with allergic disease and is a prerequisite for improving the efficacy and safety of SIT. For many years, attention has been focused on the change in the specific immunoglobulin E (IgE) and IgG during SIT, but the role of changes in T-cell responses and cytokines has become increasingly more important. In fact, there is a consensus that SIT is associated with a modification in the cytokine network in patients with allergies. Several different mechanisms seem to lead to the shift in cytokine production. The hypothesis that anergy, *i.e.*, an induced antigen-specific unresponsiveness by Th2/Th0 cells, is a possible mechanism that is supported by numerous studies.⁵

Another possible mechanism is immune deviation, *i.e.*, a shift in the Th1/Th2 balance from the predominantly Th2-like T-cell population, usually found in patients with aller-

gies, to the more Th1-like T-cell population, seen in patients who are nonatopic.⁶ Probably, both anergy and immune deviation are mechanisms of SIT.

IL-18, formerly called interferon (IFN) γ -inducing factor, is a pleiotropic proinflammatory cytokine that plays an important role in the Th1 response.⁷ The two major sources of IL-18 are monocytes and macrophages, but it also is expressed by various cell types including phagocytes, osteoblasts, keratinocytes, adrenal cortex cells, microglia, astrocytes, and epithelial cells.⁸ IL-18 contributes to inflammation and fever because it is a potent inducer of gene expression and synthesis not only of IFN- γ , but also of tumor necrosis factor (TNF), IL-1, Fas ligand, and several chemokines in immunocompetent cells; it is able to activate killing by lymphocytes and up-regulate the expression of certain chemokine receptors.⁸ IL-18 is related to the IL-1 family in terms of structure, processing, receptor, signal transduction pathway, and proinflammatory capacities.

CD30 is a member of the TNF-nerve growth factor receptor superfamily present as a membrane glycoprotein of 105 and 120 kDa, derived from a 90-kDa precursor and a 57-kDa intracellular form. CD30 is a marker of Th2 lymphocytes.⁹ There is also an 88-kDa soluble form of CD30 (sCD30), which originated from the proteolytic cleavage of the extracellular portion of CD30, released by CD30⁺ cells. It is well known that serum sCD30 levels are high in patients with allergies.¹⁰

To clarify if the immunologic changes associated with SIT can involve a Th1 inducing factor such as IL-18 and a Th2 marker such as CD30, we analyzed serum levels of these two molecules in a group of patients with allergic rhinitis before and after SIT. Moreover, to evaluate the efficacy of SIT, we observed the shift in symptom score during the same period of treatment.

MATERIALS AND METHODS

Study Population

Two groups of subjects were included in the study. Group A consisted of 16 healthy donors (HDs; 9 men and 7 women; mean age, 35.2 years; range, 14–52 years). Group B consisted of 16 patients (6 men and 10 women; mean age, 37.3 years; range, 15–60 years), affected by allergic rhinitis, monosensitized to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (6 patients) or *Parietaria judaica* (10 patients). The sensitization was evaluated by skin-prick test (+++ or more) and/or *in vitro*-specific IgE (class IV or greater).

Exclusion criteria in both groups were the following: a low or high respiratory tract infection within 4–6 weeks of the visit and immunologic diseases or other serious diseases for which the patients have taken drugs likely to interfere with the study.

Venous blood was collected from patients (within 1 week for the beginning of SIT and again within 1 week after the end of SIT) and subjects after signing an informed consent

TABLE I

Symptom Score Definition	
Ocular Symptoms	Nasal Symptoms
Tear	Sneezing
Conjunctival itching	Nasal obstruction
Hyperemia	Nasal itching
Swelling	Rhinorrhea
<i>Score of ocular and nasal symptoms: 0 = absence of symptoms; 1 = slight symptoms not interfering with the patient's daily activity and/or his sleep; 2 = moderate symptoms not interfering with the patient's daily activity and/or his sleep; 3 = severe symptoms not interfering with the patient's daily activity and/or his sleep.</i>	

form. In addition, patients (or in the case of minors, their parent/guardian) signed an informed consent form before SIT and again after SIT.

All patients interrupted every antiallergic and anti-inflammatory therapy during a period of 4 weeks before the beginning of SIT.

Global clinical assessment of each patient was based on the nasal and ocular score with a range between 0 and 24 (Table I), recorded on a clinical diary, either 7 days before SIT or 7 days after SIT.

Patients were treated with SIT for 4 years, with native allergens pharmaceutically prepared as injection depot vaccines, absorbed on aluminum or calcium phosphate, to make immunotherapy more effective and reduce side effects (position paper) and were commercially available from different manufacturers (Alk-Abello, Milan, Italy; Lofarma, Milan, Italy). The induction phase was performed according to the manufacturer's recommendations and was followed by a perennial schedule with a maintenance injection at 4-week intervals according to a standard procedure that consists of the administration of a dose of 0.7 mL throughout the year for perennial allergenic extracts and for seasonal allergenic extracts, the dose was reduced to 0.3 mL during the pollen season.

Sera

Sera were obtained from peripheral blood allowed to clot at room temperature for 2 hours, separated by centrifugation at $200 \times g$ for 15 minutes in a 4235 A (ALC Int. S.r.L., Milan, Italy) centrifuge and stored at -80°C until use.

Cytokine Detection

Serum IL-18 levels were assayed by immunoenzymatic methods (Human IL-18 ELISA Kit; MBL, Naka-ku Nagoya, Japan). Detection limit for IL-18 was <12.5 pg/mL.

Serum CD30 levels were determined by the commercial sandwich ELISA Dako CD30 (Ki-1 antigen; Dako, Milan, Italy). The limit of detection of the assay was 1 IU/mL.

TABLE II

IL-18 and CD30 Levels in Sera Obtained from Either HDs (Group A) or Patients with Rhinitis (Group B) Before (T0) and After (T1) SIT

Subjects	Group A IL-18 (pg/mL)	Group A CD30 (IU/mL)	Group B IL-18 (T0) (pg/mL)	Group B IL-18 (T1) (pg/mL)	Group B CD30 (T0) (IU/mL)	Group B CD30 (T1) (IU/mL)
1	309	<1	102	266	13.44	1.46
2	337	<1	169	201	4.17	5.59
3	340	<1	371	628	7.14	<1
4	331	<1	187	343	23.60	6.12
5	212	<1	280	409	6.00	8.50
6	355	<1	394	448	14.87	<1
7	457	<1	112	147	6.93	<1
8	236	<1	145	281	15.13	13.34
9	247	<1	263	317	10.42	2.20
10	250	<1	110	78	22.92	6.31
11	287	<1	191	465	6.10	<1
12	338	<1	131	173	33.16	12.46
13	184	<1	163	353	20.77	16.08
14	276	<1	108	160	22.58	2.40
15	320	<1	297	163	9.67	5.18
16	265	<1	188	187	19.64	15.53
Mean	296.50	<1	200.69	288.69	14.78	5.95
SD	66.3	0	93.5	146.7	8.3	5.7
IL-18	Group B (T0) versus Group A $p < 0.05$			Group B (T1) versus Group B (T0) $p < 0.05$		
CD30	Group B (T0) versus Group A $p < 0.05$			Group B (T1) versus Group B (T0) $p < 0.05$		

Statistical Analysis

Differences in serum levels were assessed by one-way analysis of variance and the Student-Newman-Keuls test. Data were expressed as mean $\pm$ SD. A value of $p < 0.05$ was considered significant.

Correlation coefficients were calculated with the Spearman rank correlation coefficient test. All data were analyzed using the Stat View SE program (Abacus, Berkeley, CA).

RESULTS

As shown in Table II, group B had lower serum levels of IL-18 before beginning SIT (T0) with respect to group A (200.69 ± 93.5 pg/mL versus 296.50 ± 66.3 pg/mL; $p < 0.05$). After SIT for 4 years (T1), in group B a significant increase in serum IL-18 levels was observed (288.69 ± 146.7 pg/mL versus 200.7 ± 93.5 pg/mL; $p < 0.05$; Fig. 1). No significant difference was found between IL-18 serum levels in the group B-treated patients and in HDs. We also found a correlation between serum IL-18 levels at T0 and T1 in group B ($\rho = 0.650$; $p = 0.0118$).

On the contrary, serum CD30 levels were significantly higher in group B at T0 than in group A (14.78 ± 8.3 IU/mL versus <1 IU/mL; $p < 0.05$). SIT decreased significantly

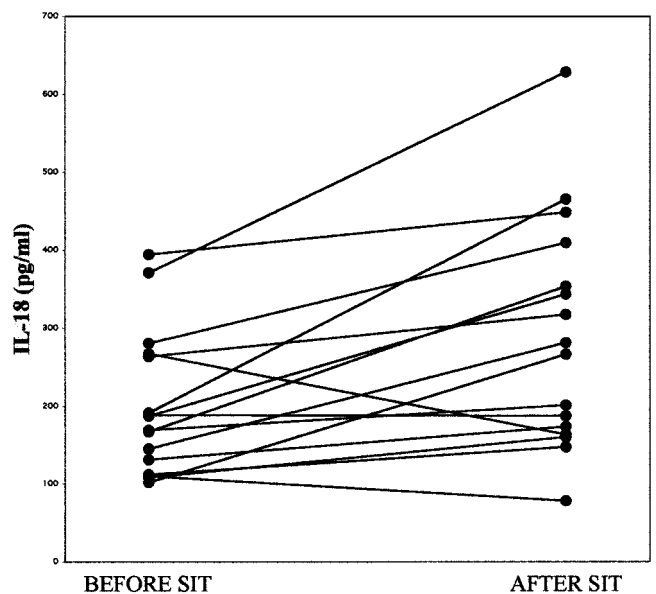


Figure 1. effect of SIT on IL-18 serum levels in patients with rhinitis. Data points are from duplicate determination performed either before or after 4 years of treatment.

the serum CD30 levels in patients with allergies (5.95 ± 5.7 IU/mL versus 14.78 ± 8.3 IU/mL; $p < 0.05$; Table II).

No correlation was found between serum IL-18 levels and serum CD30 levels.

After SIT treatment, patients significantly improved the symptom score (shifted from a total initial symptom score of 13.43 ± 3.09 to 4.37 ± 3.22).

DISCUSSION

At present, specific immunotherapy is the only treatment known that can affect the natural course of respiratory allergic diseases by modifying the immune response. There is evidence from investigation in peripheral blood and tissues that immunotherapy may decrease production of Th2-type cytokines, increase production of Th1-type cytokines, or induce a state of T-cell unresponsiveness to allergens. More recent studies have suggested that processes may occur concomitantly.¹¹

We evaluated IL-18 levels in the serum of patients affected by allergic rhinitis before and after SIT. Our findings show that IL-18 levels at T0 are significantly lower in the serum of patients with rhinitis with respect to HDs.

It is known that IL-18, in combination with IL-12, is potent in inducing IFN- γ production and augmenting Th1 responses both *in vitro* and *in vivo*. This activity was revealed showing that injection of a mixture of IL-18 and IL-12 completely inhibited IgE production in mice inoculated with helminths, a prototype pathogen for the Th2 response *in vivo*. This inhibitory effect of IL-18 was completely IFN- γ dependent.¹² Moreover, Hofstra *et al.* have shown that treatment with IL-12/IL-18 can inhibit the development of Th2 cells, airway hyperresponsiveness, and eosinophilic infiltration in a mouse model of allergic asthma.¹³

However, recently, IL-18 has been shown to have distinct roles in atopic asthma, depending on its immunologic environment. Surprisingly, IL-18 alone has the capacity to induce IgE accumulation *in vivo*; daily administration of IL-18 caused a striking, dose-dependent increase in serum IgE.¹⁴ Other studies showed that serum levels of IL-18 in patients with allergic asthma were significantly higher than in normal control subjects and that serum IL-18 levels during acute asthma exacerbations were significantly higher compared with remission days.¹⁵

Probably, serum IL-18 levels in respiratory allergy depends on the major involvement of upper and lower airways, by clinical phase of the pathology (exacerbation or stability), by patient age, and duration of disease. This is the first time that serum IL-18 levels after SIT have been evaluated; we found an increase of levels observed before SIT. These values were similar to those found in HDs. Furthermore, a correlation between serum IL-18 at T0 and T1 was found.

Studies of allergen-induced late responses in the skin and nose¹⁶ indicate that immunotherapy results in a decrease in CD4⁺ cell recruitment and a reduction in local eosinophilia.

These changes are accompanied by increases in a subpopulation of CD4⁺ cells expressing IFN- γ transcripts after allergen provocation, whereas the number of cells expressing mRNA for IL-4 and IL-5 remained unchanged. Studies of late cutaneous biopsy specimens suggest that these responses may be amplified/sustained by local production of IL-12, a potent inducer of Th1 responses. There is a reciprocal association between IL-12⁺ cells and IL-4⁺ cells and a positive association with IFN- γ cells, which supports the fact that IFN- γ responses may be IL-12 driven.¹⁷ In light of these findings, our data confirm the important involvement of Th1 cell-inducing cytokines in SIT.

We wanted to analyze the Th1 lymphocyte changes after SIT and the role of Th2 molecules. Our results show higher serum CD30 levels in patients with rhinitis with respect to HDs. Because it is known that serum sCD30 levels are high in patients with allergies,¹⁰ the presence of elevated amounts of sCD30 in patients who are atopic seems to confirm the role of this molecule as an activation marker, useful for *in vivo* evaluation of a Th2 immune response.

Our results show a decrease of serum sCD30 levels in patients with allergies after SIT. These data are in agreement with the results of other authors who noted a significant reduction of sCD30 levels after SIT in patients affected by allergic asthma caused by Hop Japanese pollen.¹⁸

These changes in the Th1/Th2 balance produce a well-known reduction of symptoms caused by exposure to etiologic allergens, for which SIT was begun, through the reduction of the symptom score, as well as a reduction in the production in specific IgE against new allergens and a consequent decrease of new sensitizations, shown for the first time in our recent study.¹⁹

In conclusion, in this study we showed possibly for the first time the changes in IL-18 and CD30 production before and after SIT.

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