

CC chemokines as potential immunologic markers correlated with clinical improvement of atopic dermatitis patients by immunotherapy

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Abstract: Although immunotherapy is not accepted as a curative treatment for atopic dermatitis (AD), most studies have shown positive effects of immunotherapy on AD patients. The serum levels of CC chemokine ligand 17 (CCL17), CCL22 and CCL18 have been reported to be highly correlated with disease severity, which suggests important roles for CC chemokines in the pathogenesis of AD.

Objective: The purpose of this study was to investigate the changes in clinical and immunologic markers before and after immunotherapy and to find which CC chemokines correlate with clinical improvement after immunotherapy with house dust mite (HDM) allergens in AD patients.

Methods and Results: A total of 20 AD patients who were sensitized to HDM allergens through a skin-prick test and Pharmacia CAP system were treated with subcutaneous immunotherapy using HDM allergens (treatment duration 12–

60 months). Eczema area and severity index scores in 20 patients with AD decreased significantly after immunotherapy ($P < 0.001$). Serum total immunoglobulin E (IgE) and *Dermatophagoides pteronyssinus*-specific IgE levels tended to decrease after treatment although this was not statistically significant, and the *D. farinae*-specific IgE level showed no change. Serum CCL17, CCL22 and CCL18 levels decreased significantly from baseline after treatment ($P = 0.043$, 0.017 and <0.001 , respectively). The percentage reductions in serum CCL17 and CCL22 level were significantly correlated with reductions in disease severity ($P = 0.007$, $R^2 = 0.301$ and $P = 0.037$, $R^2 = 0.177$, respectively).

Conclusion: We suggest that CCL17 and CCL22 are good immunological marker candidates that can be used to assess clinical improvement after immunotherapy in AD patients.

Key words: atopic dermatitis – CCL17 – CCL18 – CCL22 – immunotherapy with house dust mites

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by severe pruritus and relapsing eczematous skin lesions (1). Of several pathogenesises of AD, immunoglobulin E (IgE)-mediated hypersensitivity is thought to be one of the most important pathogenesis (2). Elevated total and allergen-specific serum IgE levels and positive skin-prick tests to common inhalant and food allergens are found in over 80% of AD patients (3).

House dust mite (HDM) allergens are the most important indoor allergens as evidenced by their high rate of sensitization and perennial prevalence (4). Immunotherapy

through subcutaneous administration of HDM allergens has been used to treat IgE-mediated allergic diseases such as asthma (5). The main mechanism of immunotherapy is thought to be a transition from a Th2 to a Th1 immune response and the suppression of allergen-specific IgE production and induction of allergen-specific IgG production (6,7). Because exacerbation of active skin lesions is often observed after immunotherapy, immunotherapy with HDM allergens is not a primary treatment option for AD. However, clinical trials in AD using immunotherapy have shown good outcomes (8). Although many studies have been performed to determine which immunologic parameters are correlated with therapeutic effects, levels of

immunologic markers including IgE, IgG4, eosinophil cationic protein, serum-soluble Fas antigen, vascular cell adhesion molecule-1, interleukin-4 (IL-4), IL-8, tumor necrosis factor- α and transforming growth factor- β do not correlate well with clinical outcome (9–12).

Recently, serum CC chemokine ligands (CCL) including CCL17, CCL22 and CCL18 have been reported to be associated with the pathogenesis of AD (13–15), and CCL17 and CCL22 levels have been reported to decrease after immunotherapy in AD patients (10).

The objective of this study was to investigate changes in clinical and immunologic markers before and after immunotherapy and to evaluate which immunological parameters correlate with clinical improvement in AD after immunotherapy.

Patients and methods

Patients

Twenty AD patients (8 men and 12 women) who showed sensitization to *Dermatophagoides pteronyssinus* and *D. farinae* through skin-prick tests (wheal diameter ≥ 3 mm) or the Pharmacia CAP system (Phadia, Uppsala, Sweden) ($\geq 3+$) have been treated with immunotherapy using HDM allergens for more than 1 year. AD was diagnosed according to the criteria of Hanifin and Rajka (16), and the patients had moderate to severe AD (investigator's global assessment score of 3 or 4). Patients aged 5 years or older with actual AD, but without evidence of persistent bronchial asthma or food allergy were enrolled for this study. Patients with bronchial asthma requiring inhaled steroids and severe systemic disease or malignancy were excluded. Only oral antihistamines and mid-potency topical steroids were used concomitantly with immunotherapy. This study was approved by the institutional review board of our hospital, and informed consent was obtained from each patient and his/her parent in the case of paediatric patients.

Treatment

A mixture of HDM allergens (three different concentrations) consisting of *D. pteronyssinus* and *D. farinae* (Alavac[®]; Ben-card, Brenford, UK) was administered subcutaneously. Vial 1 and vial 2 were 1/100 and 1/10 dilutions, respectively, of vial 3. During the induction period, patients were treated with increasing doses from 0.2 ml of vial 1 to 0.6 ml of vial 3 at weekly intervals for 10 weeks. Once induction was attained, 0.6 ml of vial 3 was injected subcutaneously as a monthly maintenance dose.

Assessment of clinical severity

Clinical severity before and 4 weeks after last immunotherapy was evaluated using the eczema area and severity index (EASI). The EASI is a composite score that includes an

assessment of the disease extent and percentage of the area involved, and is assigned as a proportional score from 0 to 6 (0 = no eruption, 1 = <10%, 2 = 10–29%, 3 = 30–49%, 4 = 50–69%, 5 = 70–89% and 6 = 90–100%). The EASI score ranges from 0 to 72.

Two horizontal visual analogue scales (VASs) that include subjective measures of pruritus and loss of sleep (LOS) were also used to assess AD patients (none: 0, most severe: 10).

Assay of total serum IgE, *Dermatophagoides pteronyssinus*-specific IgE, and *Dermatophagoides farinae*-specific IgE levels

Serum total IgE and *D. pteronyssinus* and *D. farinae*-specific IgE levels were measured before and 4 weeks after last immunotherapy using a Pharmacia CAP system (Phadia). Results are expressed as international units per milliliter (IU/ml) and CAP classes, respectively.

Assay of serum CCL17, CCL22 and CCL18 levels

Peripheral venous blood samples for determination of serum CCL17, CCL22 and CCL18 levels were drawn into vacutainer SST tubes (Becton Dickinson, Mountain View, CA, USA) and allowed to clot at room temperature (20–24°C) for 30 min. The tubes were centrifuged at $1000 \times g$ for 10 min. The serum was aliquoted and stored at -70°C until testing for chemokine levels. Serum CCL17, CCL22 and CCL18 levels in the study were measured using CCL17, CCL22 and CCL18 enzyme linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). These assays were performed according to the manufacturer's instructions. Optical density was measured at 405 nm.

Statistical analysis

Statistical analyses were performed using the Statistical Product and Service Solutions program (SPSS Inc., Chicago, IL, USA) for windows (version 12). The results are expressed as mean $\pm$ standard deviation. The statistical significance of differences in EASI scores and laboratory findings before and after immunotherapy were analysed with paired *t*-tests. The relationships between the percentage reduction of CC chemokines such as CCL17, CCL22 and CCL18 and EASI scores were examined by linear regression analyses. Statistical significance was defined as a *P*-value of less than 0.05.

Results

Patients

The ages of enrolled patients ranged from 6 to 33 years, with a mean of 17.3 ± 7.3 years. The duration of treatment ranged from 12 to 60 months with a mean of 26.9 ± 16.6 months. Demographic and clinical characteristics of 20 enrolled patients at baseline are shown in

Table 1. Two patients (10%) had positive history for asthma and five patients (25%) had positive history for allergic rhinitis. Enrolled 20 patients completed this study and there were no dropped-out patients during the study. Two patients showed generalized itching and/or mild flare-ups after vaccine administration in the build-up phase, but oral antihistamines and topical steroid subsided their flare-ups and made the patients continue immunotherapy.

Changes in clinical severity after immunotherapy

Eczema area and severity index scores decreased significantly after immunotherapy with HDM allergens compared with baseline ($P < 0.001$). The mean EASI scores before and after treatment were 21.6 ± 8.9 and 5.4 ± 4.3 , respectively. All patients showed over 50% improvement in their EASI score (Fig. 1). All of enrolled patients showed clinical response and there were no patients without response through immunotherapy.

Two VASs including subjective scores of pruritus and LOS also decreased significantly after treatment ($P < 0.001$ and $P < 0.001$). The mean pruritus scores were 6.6 ± 2.0 before treatment and 1.7 ± 1.1 after treatment (Fig. 2a). The mean LOS scores were 5.7 ± 2.3 before treatment and 1.3 ± 1.6 after treatment (Fig. 2b).

Changes in serum total IgE levels and *Dermatophagoides Pteronyssinus*- and *Dermatophagoides farinae*-specific IgE levels after immunotherapy

The serum total IgE level decreased from 1655.1 ± 1648.0 to 1346.2 ± 1330.4 IU/ml after treatment; this difference is

not statistically significant ($P = 0.087$). *Dermatophagoides Pteronyssinus*- and *D. farinae*-specific IgE levels were evaluated in 14 AD patients. The *D. pteronyssinus*-specific IgE level decreased slightly from baseline after treatment although this was not significant ($P = 0.263$), and the *D. farinae*-specific IgE level showed no change (Table 2).

Changes in serum CCL17, CCL22 and CCL18 levels after immunotherapy

Serum CCL17 levels decreased significantly from 906.1 ± 651.8 to 599.1 ± 416.5 pg/ml after treatment ($P = 0.043$). Nevertheless, six patients showed an increase in the level of CCL17 after treatment (Fig. 3a). Serum CCL22 levels were also significantly reduced after treatment ($P = 0.017$); however, serum CCL22 levels increased in seven AD patients (Fig. 3b). Serum CCL18 levels showed statistically significant reduction after treatment ($P < 0.001$), with only two patients showing a slight increase in CCL18 after treatment (Fig. 3c).

Correlation between reduction of clinical severity and serum CCL17, CCL22 and CCL18 levels after immunotherapy

The reduced EASI scores were significantly correlated with percentage reduction of serum CCL17 ($R^2 = 0.301$, $P = 0.007$) and serum CCL22 ($R^2 = 0.177$, $P = 0.037$) after immunotherapy (Table 3). However, reduction of serum CCL18 demonstrated no significant correlation with clinical severity scores. Age and treatment duration were not associated with changes in clinical scores.

Table 1. Baseline characteristics of 20 patients with atopic dermatitis

Case	Sex	Age (years)	Total IgE (IU/ml)	Duration of treatment (months)	<i>Dermatophagoides pteronyssinus</i> -specific IgE	<i>Dermatophagoides farinae</i> -specific IgE	Skin-prick test (wheal diameter, mm)		Medical history
							<i>D. pteronyssinus</i>	<i>D. farinae</i>	
1	F	26	3525	35	6	6			
2	M	29	5000	14	3	3			Asthma
3	M	11	213	15			5	7	Allergic rhinitis
4	M	15	5000	13	6	6			
5	F	22	1127	59	4	4			
6	F	13	61.8	14			12.5	8	
7	F	13	4440	18	6	6			
8	F	11	214	57	4	5			
9	F	6	226	47	4	5			
10	F	15	1106	12	6	6			
11	M	12	237	18			6.5	4	Allergic rhinitis, asthma
12	F	26	483	23			14.5	9	Allergic rhinitis
13	F	11	1501	27	5	6			
14	F	12	1969	19			7.5	10.5	
15	F	21	350	22			8.5	10.5	
16	M	18	1827	16	6	6			Allergic rhinitis
17	M	10	1901	12	6	6			
18	M	20	780	18	3	4			Allergic rhinitis
19	F	33	437	39	3	4			
20	M	22	2705	60	4	5			

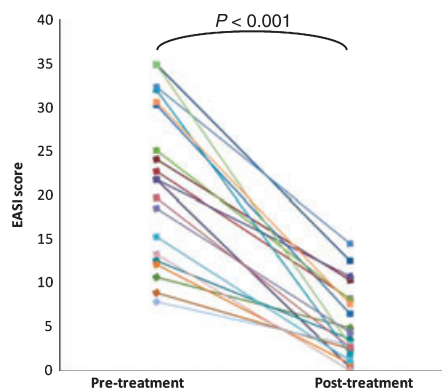


Figure 1. The EASI scores of 20 atopic dermatitis patients were evaluated before and after immunotherapy. EASI scores decreased significantly after immunotherapy (* $P < 0.001$).

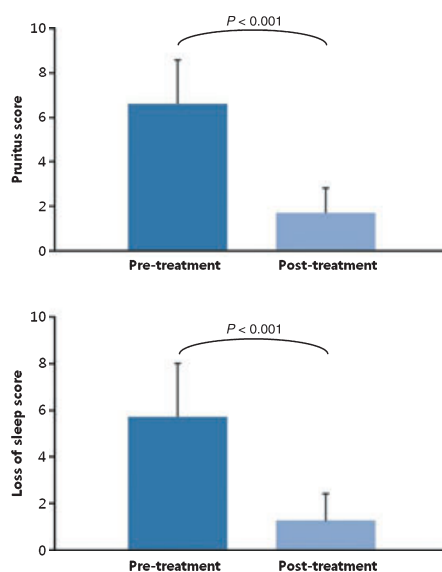


Figure 2. (a) The pruritus scores of 20 atopic dermatitis patients were evaluated before and after immunotherapy. The pruritus scores decreased significantly after immunotherapy (* $P < 0.001$). (b) The LOS scores of 20 atopic dermatitis patients were evaluated before and after immunotherapy. The LOS scores decreased significantly after immunotherapy (* $P < 0.001$).

Discussion

The current management of AD is mostly limited to the control and reduction of symptoms (17). Allergen-specific immunotherapy may be the only curative and specific treatment for allergic disease. Although the clinical efficacy of allergen-specific immunotherapy for the treatment of allergic rhinitis and asthma has been demonstrated, immunotherapy in AD is controversial (18).

We investigated the efficacy of immunotherapy with HDM allergens in AD patients and tried to identify immunologic parameters correlated with clinical improvement after immunotherapy. EASI scores and VAS scores were examined before and after treatment. Laboratory tests including serum total IgE, *D. farinae* and *D. pteronyssinus*-specific IgE, CCL17, CCL22 and CCL18 levels were evaluated. In our study, the EASI and pruritus/LOS scores decreased significantly after treatment. Therefore, this study shows that immunotherapy with HDM allergens is a very effective treatment modality for AD.

The reason why there were differences in the treatment duration among patients in this study is that in case with rapid clinical improvements after immunotherapy, the treatment was discontinued early, and in patients with more than 50% improvement of the EASI score who complained of the inconvenience of subcutaneous injection and about coming to the hospital, the treatment was also discontinued. These differences in treatment duration might induce a bias in comparing the relationship between chemokine levels and clinical manifestations. Nevertheless, there was no statistical correlation between the treatment duration and various immunologic markers and EASI score.

Combined statistical analysis of seven observational studies revealed a 70% improvement in AD after immunotherapy (18). Serum total IgE, *D. farinae*- and *D. pteronyssinus*-specific IgE levels showed no significant changes after immunotherapy in our study. Three recent studies monitoring the level of total and allergen-specific IgE in AD patients after immunotherapy reported no changes in serum total and allergen-specific IgE (9,12,19), while one

Table 2. Comparison of serum total IgE and *Dermatophagoides Pteronyssinus*- and *D. farinae*-specific IgE levels before and after immunotherapy with HDM allergens in atopic dermatitis patients

	Before treatment	Four weeks after last treatment	P-value
Total IgE, IU/ml (n = 20)	1655.1 ± 1648.0 (61.8–5000)	1346.2 ± 1330.4 (65.4–5000)	0.087
Log total IgE (n = 20)	3.0 ± 0.5	2.9 ± 0.5	
<i>D. pteronyssinus</i> -specific IgE (n = 14)	4.7 ± 1.3 (3–6)	4.4 ± 0.9 (2–6)	0.263
Log <i>D. pteronyssinus</i> -specific IgE (n = 14)	0.7 ± 0.1	0.6 ± 0.1	
<i>D. farinae</i> -specific IgE (n = 14)	5.1 ± 1.0 (3–6)	5.1 ± 0.9 (2–6)	0.671
Log <i>D. farinae</i> -specific IgE (n = 14)	0.7 ± 0.1	0.7 ± 0.1	

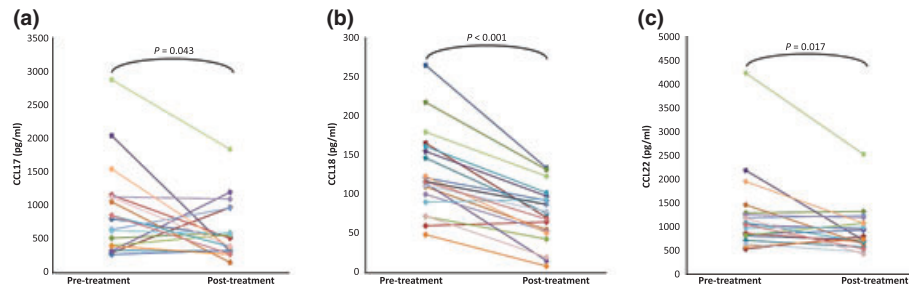


Figure 3. (a) Serum CCL17 levels decreased significantly after immunotherapy (* $P = 0.043$). (b) Serum CCL22 levels decreased significantly after immunotherapy (** $P = 0.017$). (c) Serum CCL18 levels decreased significantly after immunotherapy (** $P < 0.001$).

Table 3. Correlation between the changes in clinical scores and the changes in serum CCL17, CCL22 and CCL18 levels after immunotherapy

	EASI scores		
	Standardized beta	P-value	R^2
CCL17	0.581	0.007	0.301
CCL22	0.470	0.037	0.177

study reported a decrease of allergen-specific IgE only (10). Because of these conflicting results, the association between immunotherapy efficacy and the reduction of allergen-specific IgE remains controversial. The formation of IgG blocking antibody has been suggested as one mechanism of immunotherapy. The blocking activity of IgG against IgE has been reported to increase significantly after immunotherapy (20). This result suggests that the mechanism of immunotherapy in AD might involve an increase in the blocking activity of IgG, and not solely an increase in the quantity of the blocking antibody.

CC chemokine ligand 17 is usually secreted by keratinocytes and dermal endothelial cells, whereas CCL22 is produced mainly by epidermal Langerhans cells and dermal dendritic cells (13,14). At the initial step of T-cell recruitment, CCL17 induces integrin-dependent adhesion and transendothelial migration of T cells. CCL22, a ligand of CC chemokine receptor 4, is a potent chemoattractant for monocytes, monocyte-derived dendritic cells and natural killer cells, and also serves to recruit a fraction of CD4 + CD45RO+ T cells to produce Th2 cytokines. CCL18, which is highly expressed in AD, is produced by epidermal Langerhans cells and dermal dendritic cells (21). It is also an important chemotactic factor that induces the migration of T-cells in AD (15). CCL18 has been reported to be highly induced by allergens, especially HDM allergens, supporting a crucial role for CCL18 in allergen-induced atopic skin inflammation (21–23).

Several studies have reported that serum CCL17, CCL22 and CCL18 levels were well correlated with EASI scores (13–15). A 6-month immunotherapy study in eight patients with AD reported a significant decrease in serum CCL17 levels with a slight decrease in the serum CCL22 levels (10). Although some AD patients in our study showed an increase in the levels of CCL17 and CCL22 after treatment, the overall changes in CCL17 and CCL22 levels correlated well with the reductions in the EASI scores. Based on this finding, the levels of CCL17 and CCL22 might be useful immunologic parameters for monitoring disease severity and evaluating the efficacy of immunotherapy. These chemokines may be associated with the mechanism of immunotherapy.

Previous trials with immunotherapy in AD have not been well controlled with respect to the types of applied allergens, treatment schedules, treatment duration, inclusion criteria and the read-out criteria. In addition, most immunotherapy studies have been performed without a placebo group and appropriate immunologic parameters. Because a high placebo effect is expected in an uncontrolled study and AD shows spontaneous variation in time and with age, the results of study without placebo control might be less reliable. However, a double-blind, placebo-controlled design is neither ethical nor feasible for this kind of disease (8). Although our study did not address all of these weaknesses, our results suggest that immunotherapy can significantly improve certain immunologic parameters that may provide an objective clinical measure of the subjective symptoms of AD patients.

In summary, clinical improvement was evident in AD patients treated with immunotherapy using HDM allergens for more than 1 year. Serum total IgE and *D. pteronyssinus*- and *D. farinae*-specific IgE levels showed no significant changes after immunotherapy. Serum CCL17, CCL22 and CCL18 levels decreased significantly after treatment. Among these chemokines, the changes in CCL17 and CCL22 levels correlated well with the reductions in the EASI scores. We suggest that CCL17 and CCL22 can be used as objective immunologic parameters to monitor the efficacy of immu-

notherapy. Further double-blind, placebo-controlled trials on larger groups of patients with longer treatment durations are needed to confirm these results.

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