

ORIGINAL ARTICLE

Immunological changes after ASIT in AD allergen-specific immunotherapy and their potential correlation with clinical response in patients with atopic dermatitis patients sensitized to house dust mite

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Abstract

Background Allergen-specific immunotherapy (ASIT) is the main treatment for inducing long-term immunological and clinical tolerance in patients with IgE-mediated allergic diseases. Recent open-label and controlled studies on the efficacy of ASIT in patients with atopic dermatitis (AD) have provided promising results. However, data about possible relationship between the improvement of clinical symptoms and changes of serum cytokines are limited.

Methods Seventy-nine patients with moderate to severe AD sensitized to house dust mite (HDM) were enrolled. Fifty-eight patients were treated with ASIT and 11 controls received only symptomatic treatment. The disease activity in AD patients was evaluated by using the patient-oriented eczema measure (POEM) system. Serum interleukin (IL)-4, IL-10, interferon (IFN)- γ , transforming growth factor (TGF) β 1, total IgE, HDM-specific IgE (s-IgE) and HDM-specific IgG4 (s-IgG4) were measured before and after 2 years of therapy.

Results The mean patient-oriented eczema measure system (POEM) score of AD patients with ASIT significantly decreased after 2 years of treatment, compared to that in patients without ASIT. After ASIT, the serum levels of IL-10, TGF- β 1, IFN- γ and s-IgG4 increased, while the level of IL-4 decreased. The change in the POEM score was negatively correlated with changes of serum concentration of TGF- β 1, s-IgG4 and IFN- γ . Furthermore, s-IgG4 levels were positively correlated with changes in the IL-10 levels. No correlation between POEM score and serum IL-10 or IL-4 was observed.

Conclusion Clinical symptoms and the quality of life of AD with HDM sensitization could be improved after 2 years of ASIT. Changes in serum IL-10, TGF- β 1, s-IgG4 and IFN- γ might be considered as biomarkers to assist clinical evaluation of the therapeutic effects of ASIT in patients with AD.

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Conflicts of interest

None.

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Introduction

Atopic dermatitis (AD) is a common chronic relapsing inflammatory disease, affecting up to 25% of children and 3% of adults worldwide.¹ Complex relationships between skin barrier and immune abnormalities have been implicated in the pathogenesis of AD. AD can be categorized into extrinsic and intrinsic types,

separated on basis of the level of serum IgE and number of eosinophils. Patients with extrinsic AD, often have elevated serum levels of total IgE (t-IgE) and specific IgE (s-IgE) directed to common allergens, mainly aeroallergens and food allergens. The levels of IgE have been shown to be correlated with disease severity.² The involvement of allergens in inducing and promoting allergic inflammation has been well established through the use of atopy patch test and in clinical practice.³ Avoidance of

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related allergens can result in marked improvement of skin lesions.

For more than a century, allergen-specific immunotherapy (ASIT) is the only aetiological therapy that can induce long-term immunological and clinical tolerance of IgE-mediated allergic diseases,^{4–6} as shown in asthma and insect venom allergy.^{7–9} This kind of therapy shows positive results in terms of safety and efficacy. The basic principle of ASIT is to induce immune tolerance to specific allergens by repeated administration of increasing doses of the causative allergen. Although AD, like rhinitis and asthma, belongs to the class of atopic diseases, the clinical significance of application of ASIT for AD patients has been controversial for a long time. Debates may have arisen from the difference in allergen type, immunotherapy schedule, duration, method for evaluation of clinical efficacy and environment control.

Interestingly, several controlled and open-label studies on ASIT have recently been conducted in AD patients with sensitization to dust mite or pollen, and promising results have been obtained^{3,10,11} that the combined treatment with symptomatic medication and ASIT can be considered as a promising therapeutic approach for patients with extrinsic AD. Studies on ASIT for AD that have only confirmed positive clinical outcomes were mainly focused on improvement of clinical severity and safety, a few clinical studies on ASIT have investigated the serum expression levels of cytokines such as IL-4, IL-10 and some chemokines. These studies have reported obvious changes in the expression of these cytokines after therapy.¹² However, reliable parameters for predicting the efficacy of ASIT have not yet been identified.

The patient-oriented eczema measure system (POEM) is a standardized method to quantify the severity of AD and the extent of skin lesions, by using a simple 5-point scale rating the frequency and severity of events during the previous week.^{9,13,14} Compared with the Severity Scoring of Atopic Dermatitis (SCORAD), the POEM scoring system is easy to perform by the patients themselves, and allows patients to be more actively involved in disease management.^{15,16} Thus, it can more accurately reflect the processes involved in development of the disease.

Subjects and methods

Patients

From January 2007 to January 2011, 79 AD patients from the department of dermatology of Southwest Hospital, were prospectively enrolled in the study. All enrolled patients fulfilled the diagnostic criteria of Hanfin and Rajka, with moderate to severe disease severity (POEM value >7), specific IgE to house dust mite (HDM) > 3.5 KU/L (Thermo Fisher, Waltham, MA, USA) and with a positive skin prick test to HDM. Patients with active asthma, severe systemic diseases or under systemic immunosuppressive therapy, pregnant or lactating women were excluded. All patients signed an informed consent form before start of the

study. The study was approved by the Ethics Committee of the Southwest Hospital (SWH2005A020).

Treatment and follow-up

Before the start of clinical study, patients were stratified into the experimental group (58 cases) and the control group (21 cases). Patients in the experimental group received ASIT and symptomatic treatment when needed, including anti-inflammatory therapy based on topical glucocorticosteroids and topical calcineurin inhibitors and oral cetirizine. Such symptomatic treatments were stopped or tapered down once visible lesions were cleared. Patients in the control group were given only symptomatic treatment.

Allergen-specific immunotherapy was performed by repeated subcutaneous injections of dust mite therapeutic vaccines (Allergovit, Reinbek, Germany). This was usually started at the end of spring as during this season patients are less symptomatic. The procedure was subdivided into a build-up phase of 4–6 months followed by a maintenance treatment period of up to 2 years. The initial build-up period consisted of weekly administrations of gradually increasing doses until the recommended maintenance dose was reached. The maintenance dose was administered at 6–8 week intervals for a total therapy time of 24 months. The skin condition and use of drugs were documented by doctors and patients, and patient serum samples were collected for assessment of disease activity before and after treatment.

In the ASIT group, three patients were excluded due to loss in the follow-up, and one patient was withdrawn due to generalized eczema during the initial build-up phase. Serum cytokines could be measured in 34 patients and immunoglobulins in 17 of them. Samples were taken at the start of the study and after 2 years of ASIT (Fig. 1). No systemic anaphylaxis was observed during the study. Local reactions were recorded in five patients. In the control group, one patient was lost.

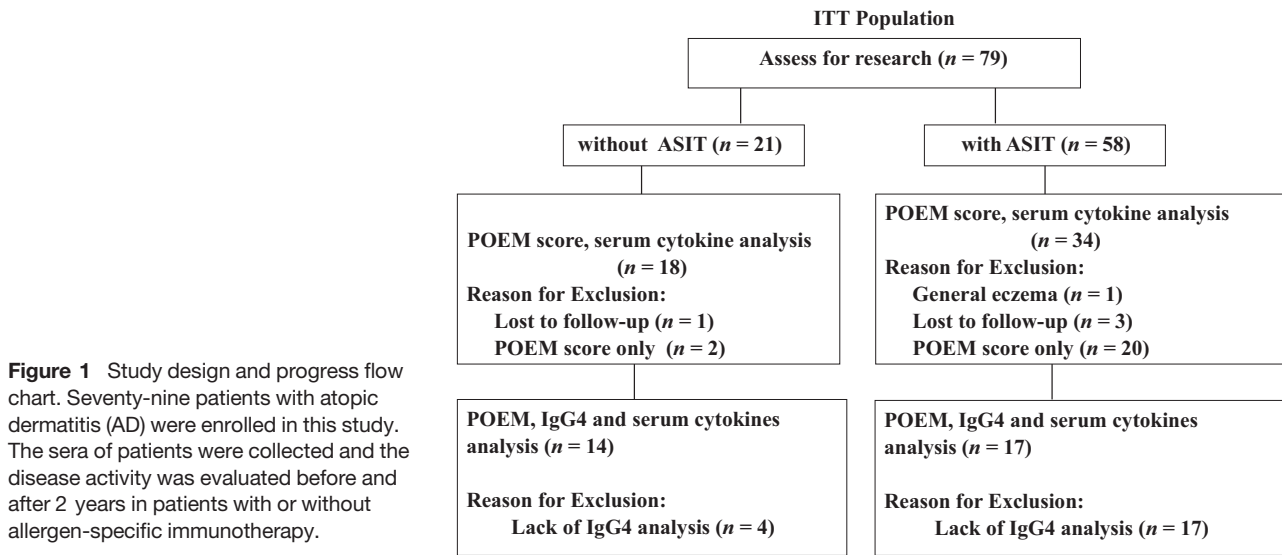
Outcome measures

The main outcome measures include the clinical scores and the levels of serum cytokines. Disease severity was quantified using the POEM system, making use of a questionnaire¹⁷ before the study, and at 1 and 2 years after start of treatment.

Serum t-IgE, HDM s-IgE and allergen-specific immunoglobulin G4 (s-IgG4) were analysed using the ImmunoCAP system (Thermo Fisher). The serum levels of IL-4, IL-10, IFN- γ and TGF- β_1 were detected by ELISA, according to the manufacturer's instructions (Dakewe, Peking, China). The changes in parameter values were calculated in percentages by the formula: (pre-ASIT value – post-ASIT value)/pre-ASIT value $\times$ 100%.

Statistical analysis

Results of the clinical assessment were expressed as mean $\pm$ SD. Data were compared using a paired *t* test. The relationships

**Table 1** Clinical and laboratory characteristics of the patients

Characteristic	With ASIT	Without ASIT
Age (years)	12.52 ± 6.73 (5–35)	10.61 ± 4.96 (6–19)
Gender (F/M)	26/32	8/13
Diagnosis (n, %)		
AD only	43 (79.63)	16 (76.19)
Concurrent rhinitis	5 (9.26)	1 (4.76)*
Concurrent asthma	4 (7.41)	2 (9.52)
Concurrent chronic urticaria	2 (3.7)	0*
HDM s-IgE (kU/L)	75.76 ± 38.12	72.21 ± 32.07
t-IgE (kU/L)	1970.44 ± 1651.73	1736.59 ± 1515.32

**P* < 0.05.

AD, atopic dermatitis; ASIT, allergen-specific immunotherapy; HDM, house dust mite.

between variables were calculated by the Mann–Whitney *U*-test. *P* < 0.05 were considered statistically significant.

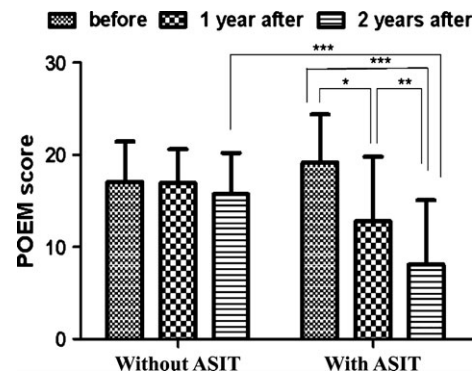
Results

Clinical profiles of patients with AD

A total of 69 patients were enrolled in this study. All patients never received any form of desensitization before this study. In the ASIT group, 54 patients completed the whole survey, and 10 in the control group. No significant differences were found in the demographic characteristics between the two groups, as shown in Table 1.

The mean POEM score decreased significantly after 2 years of ASIT

Compared with the control group, the mean POEM score in patients with SIT decreased after 2 years of ASIT (Fig. 2), which

**Figure 2** The mean patient-oriented eczema measure (POEM) score of atopic dermatitis (AD) patients. The POEM score significantly decreased after 2 years of ASIT from 18.81 ± 5.80 to 8.09 ± 7.39 , as compared with that before ASIT ($n = 54$, $P < 0.0001$). * $P > 0.01$, ≤ 0.05 ; ** $P > 0.001$, ≤ 0.01 ; *** $P \leq 0.001$.

indicated a positive influence of ASIT on the clinical symptoms and the quality of life. After the therapy, episodes of eczema flare-up significantly decreased in the ASIT group, and improvement of symptoms related to rhinitis and asthma were also observed (data not shown).

Changes in serum cytokine levels and the correlation with clinical improvement

Previous studies have shown that the levels of serum cytokines might change with the course of allergic disease,¹⁸ but it is not clear which cytokine was associated with improvement of disease during ASIT. In present study, after 2 years of ASIT, levels of serum TGF- β 1, IFN- γ , IL-10 increased and IL-4 decreased significantly (Fig. 3). Furthermore, correlations between the

change in POEM score and changes in serum cytokine levels were also analysed. As shown in Fig. 4a,b, the changes in TGF- β_1 and IFN- γ were found to be negatively correlated with the change in the POEM score. In contrast, there was no obvious correlation between the POEM score and changes in IL-10 and IL-4 (Fig. 4c,d).

Changes in serum s-IgG4 and IgE and the correlation with clinical improvement

After 2 years of ASIT, the serum levels of s-IgG4 directed to HDM in AD patients significantly increased compared to the group without ASIT (Fig. 5a). The change in s-IgG4 levels was negatively correlated with the change in the POEM score, but positively with IL-10 (Fig. 5c). There was no significant change of the serum t-IgE or s-IgE levels (Fig. 5d,e), neither a change in the POEM score or serum IgE (data not shown) has been observed.

Correlation of serum cytokine expression with degree of clinical improvement

In order to check if the changes in cytokine levels were correlated with different responses to ASIT, we stratified the patients into three subgroups according to the degree of changes in POEM scores. Five patients with change of POEM < 30% were labelled as the group with less effect. Eleven patients with change of POEM > 30% but < 60% were labelled as the improved group. Eighteen patients with change of POEM > 60% were labelled as the markedly improved group. As shown in Fig. 6, s-IgG4, IFN- γ , TGF- β_1 levels increased more in both the markedly improved group and in the improved group. A similar but inverse change was found for IL-4 levels. Meanwhile, there was no difference in the gender and immunologic markers before ASIT between all groups.

Discussion

Atopic dermatitis is a chronic inflammatory disease characterized by the expansion of IL-4-secreting Th2 cells with decreased IFN- γ synthesis. However, current treatments for AD are mostly limited to the control and reduction of the symptoms, and suppression of inflammatory reactions. Although ASIT is the only aetiological treatment that can induce long-term immunological and clinical tolerance in IgE-mediated allergic diseases,^{4,5,19–21} its value in the treatment of AD need further investigation. Up to now, there are more than twenty controlled and uncontrolled studies in the literature addressing the effect of ASIT in AD. Most of them confirmed the effectiveness of ASIT in the relief of AD clinical burden. However, there is only limited information on the immunological changes in AD patients at the cellular and molecular levels,^{3,10,22} or the relationships between the immunological changes and disease activity.²¹

Predictors for a successful response to ASIT, especially in patients with AD, remain incompletely clarified. Many studies have demonstrated the induction of s-IgG4 as the most significant and long-lasting immunoreaction after ASIT.²³ However, the actual role of s-IgG4 in the efficacy of ASIT for AD patients is inconclusive, because its association with clinical improvement could not be confirmed in a number of studies.^{15–17} To check the long-term clinical effects of ASIT and to find possible parameters which may be useful for the prediction of therapeutic efficacy of ASIT, we conducted a prospective study to evaluate the clinical and laboratory changes in moderate to severe AD patients with sensitization to house dust mite (HDM) undergoing 2 years of mite-specific immunotherapy.

Our result is in accordance with previous reports which showed s-IgG4 could be induced during ASIT.^{21,24} Moreover, the change in serum s-IgG4 expression was negatively correlated

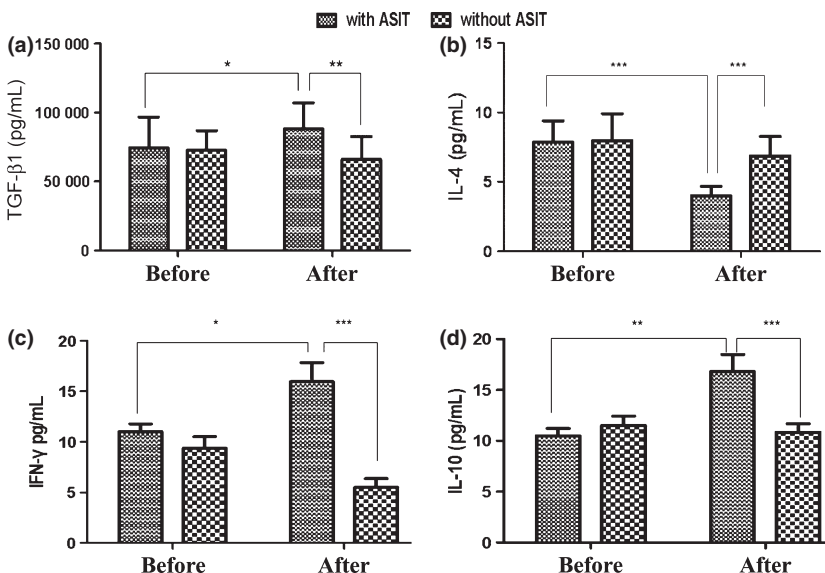


Figure 3 Change in serum cytokines of patients with atopic dermatitis (AD) before and after 2 years of therapy. Serum cytokines of patients in allergen-specific immunotherapy (ASIT) and control groups were measured by ELISA. Changes of TGF- β_1 (75070.05 ± 22338.53 pg/mL to 88475.62 ± 17987.17 pg/mL, $P = 0.0131$, (a), IL-4 (7.85 ± 9.36 pg/mL to 4.01 ± 3.99 pg/mL, $P < 0.0001$, (b), IFN- γ (11.56 ± 3.81 pg/mL to 15.62 ± 8.46 pg/mL, $P = 0.0426$, (c) and IL-10 (10.98 ± 3.77 pg/mL to 16.73 ± 12.35 pg/mL, $P = 0.0076$, (d), before and after ASIT, are shown, respectively. * $P > 0.01$, ≤ 0.05 ; ** $P > 0.001$, ≤ 0.01 ; *** $P \leq 0.001$.

Figure 4 Correlation between POEM scores and serum cytokine levels in atopic dermatitis (AD) patients on allergen-specific immunotherapy (ASIT). Serum levels were determined using ELISA ($n = 34$). Linear regression analysis was performed to determine the correlation between change in POEM score and changes in TGF- β 1 (a) and IFN- γ (b). No obvious correlation was found between the POEM score and changes in IL-10 and IL-4 (c and d).

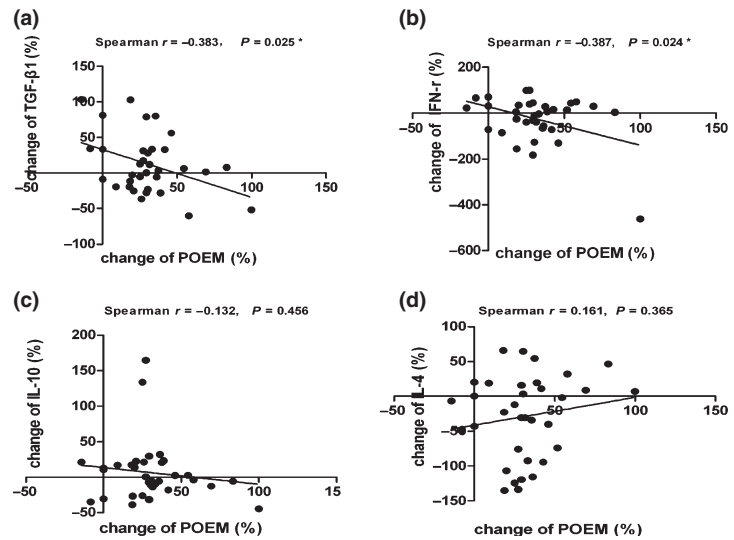
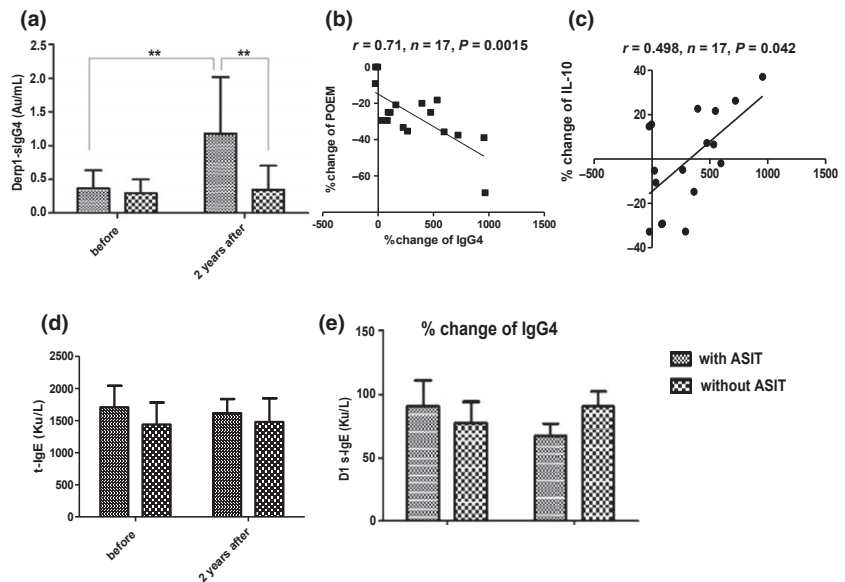


Figure 5 Serum s-IgG4 and IgE levels before and after 2 years of therapy. Serum levels of s-IgG4 against house dust mite (HDM) were significantly increased from 0.33 ± 0.28 Au/mL to 1.80 ± 0.86 Au/mL, $P = 0.0053$ after 2 years of ASIT (a) and positively correlated with the POEM score ($r = 0.710$, $P = 0.0014$) (b) and serum IL-10 ($r = 0.498$, $P = 0.042$) (c). There were no significant changes found in the serum levels of t-IgE and s-IgE (d, e) in both groups. * $P > 0.01$, ≤ 0.05 ; ** $P > 0.001$, ≤ 0.01 ; *** $P \leq 0.001$.



with the POEM score, before and after ASIT, implying that serum s-IgG4 can be taken as an indicator of clinical effect of ASIT, at least in patients with moderate to severe AD.

Interleukin-4 is produced by CD4⁺ T cells specialized in providing help to B cells to proliferate and induce isotype switching to IgE. It also exerts an important effect on eosinophil chemotaxis by up-regulating CCL-26 expression.²⁵ Previous studies focusing on allergic respiratory diseases demonstrated that the immune response during ASIT is characterized by increased serum levels of IL-10 and TGF- β 1, which can suppress proliferation of T cells and secretion of both Th1 and Th2 type cytokines. Although we observed that the level of IL-4 decreased after ASIT,

we were not able to find any relationship between it and the POEM score. On the other hand, it should be noted that the level of serum IFN- γ was not only increased after ASIT, but also showed a slightly inverse correlation with the POEM score. Thus, the increase of IFN- γ , a potent inhibitor of IL-4-induced responses, also reflects the reduction of allergic inflammation in certain patients.

The induction of peripheral T-cell tolerance is mainly characterized by the generation of allergen-specific regulatory T cells (Tregs) during ASIT.^{19,20,26,27} Tolerance for the target allergen can be induced through ASIT by the involvement of Tregs and immunosuppressive cytokines.²⁷ Interleukin-10, a suppressive

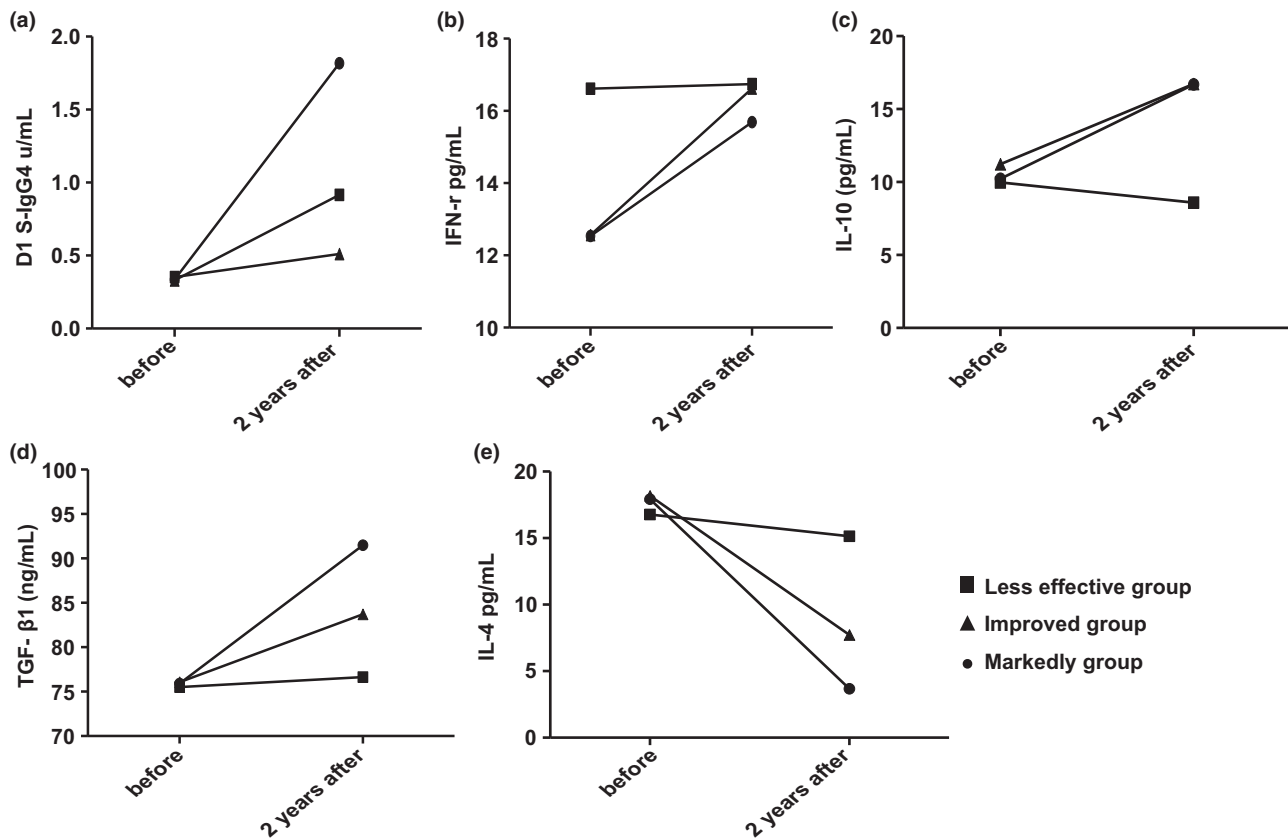


Figure 6 Immunological parameters and relationship with degree of clinical improvement after ASIT. s-IgG4(a), IFN- γ (b) and TGF- β 1(d) were found to increase more in the markedly improved and improved clinical outcome groups, as compared to the less effective group. An inverse effect was found for IL-4(e) expression levels.

chemokine produced by Treg cells, could play an important role in the release of s-IgG4 and may exert potent suppressive effects on the production of s-IgE.²⁸ In this study, we found that serum IL-10 was significantly increased after 2 years of ASIT, which was in line with other studies showing that HDM and grass pollen immunotherapy could not only induce IL-10 production, but also suppress clinical symptoms of asthma and AD.^{27,29} Although no correlation between IL-10 expression and POEM score could be demonstrated, the change of s-IgG4 was positively correlated with serum IL-10 levels after ASIT.

Transforming growth factor- β 1 is another immunosuppressive cytokine affecting the proliferation, differentiation and apoptosis of T cells.^{30–32} Some reports showed that TGF- β 1 and IL-10 can cooperate in the regulation of T-cell responses to mucosal allergens in normal immunity and specific immunotherapy.²⁷ Our data also demonstrated an increase of serum TGF- β 1 after 2 years of ASIT and its change was negatively correlated with changes in the POEM score. All these results indicate that of the expression levels IL-10 and TGF- β 1 can be used as a potential predictor for the effectiveness of ASIT in patients with AD.

In order to reduce the possible influence of multiple sensitizations on clinical effects, only patients with single sensitization to HDM were enrolled in this study. In line with previous reports,^{32,33} we did not find any correlation between the levels of IgE and cytokine concentrations. In addition, there is no relationship between the serum IgE and the POEM score. The exact mechanism for the lack of changes in IgE concentrations after ASIT remains unclear. One of the possible explanations is the very long lifespan of plasma cells residing in the bone marrow that continue to produce IgE.³⁴ Furthermore, as patients with AD often show multiple sensitizations in clinical practice, the identification of a single allergen, even with an elevation of s-IgE, cannot be used to explain the clinical relevance in a single patient.

To our best knowledge, this is the first long-term study on the effect of ASIT in a Chinese population with AD. Our results confirmed that ASIT can result in clinical improvement and changes of serum cytokines with good tolerance in AD patients. Although we present some clues in this manuscript, further examination is necessary to elucidate the exact molecular mechanisms of ASIT in patients with AD.

Conclusions

Our results support the concept that ASIT can be considered as an effective option for patients with AD sensitized to HDM. The changes in serum IL-10 and TGF- β 1, together with s-IgG4 and IFN- γ , may be useful for the prediction of the clinical effectiveness of ASIT.

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