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Suppression of TIM-1 predicates clinical efficacy of sublingual immunotherapy for allergic rhinitis in children

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ABSTRACT

Objective: To evaluate the clinical efficacy of sublingual immunotherapy (SLIT) with house-dust mite (HDM) extract and to examine the change of biomarkers (TIM-1, IL-5 and IL-10) after 6-month SLIT in children with allergic rhinitis (AR).
Methods: One hundred and sixteen HDM-sensitized children with persistent AR were enrolled to assess the clinical efficacy of SLIT by determining the individual nasal symptom score (INSS) and total nasal symptom scores (TNSS) after 6-month SLIT. Moreover, the mRNA expression of TIM-1, IL-5 and IL-10 in peripheral blood mononuclear cells (PBMCs) was examined in 16 well-controlled and 12 uncontrolled AR patients using quantitative reverse transcription polymerase chain reaction (qRT-PCR).
Results: After 6-month SLIT, both TNSS and INSS scores were significantly decreased compared with the baseline value ($p < 0.01$). The rates for well-controlled, partly controlled and uncontrolled children were 43.1%, 32.8% and 24.1%, respectively. Accordingly, the mRNA levels of TIM-1 and IL-5 decreased significantly and IL-10 mRNA level increased significantly compared with the baseline value in well-controlled children ($p < 0.05$).
Conclusion: Our findings suggest SLIT with HDM extract is effective and safe for AR children and TIM-1 may be considered as an indicator for evaluating the clinical efficacy of SLIT.

1. Introduction

Allergic rhinitis (AR), as well as bronchial asthma, is characterized by excessive eosinophil infiltration, Th2 cytokine responses, elevated levels of allergen-specific IgE, mucus secretion, and airway hyperresponsiveness. With the development of socio-economic environment, the AR prevalence is increasing worldwide, and its burden on sleep and learning is substantial in children. Moreover, AR is considered to be the risk factor of subsequent asthma comorbidity [1,2]. At present, allergen-specific immunotherapy has been shown to be highly effective by reducing nasal symptoms and rescue medication use and markedly improves the quality of life. In contrast to the symptom-relief pharmacotherapy, immunotherapy is the only available therapy that might modify the diseases and thus potentially prevent the progression from AR to asthma [3].

Since its introduction a century ago, allergen-specific immunotherapy has been administered subcutaneously (subcutaneous immunotherapy [SCIT]). There is a growing body of evidence regarding the clinical efficacy of SCIT in AR and asthma [4]. However, SCIT in children is hampered by the inconvenience of injection and the risk of severe adverse events including anaphylaxis and, rarely, death. As an alternative, sublingual immunotherapy (SLIT) was gradually introduced in clinical practice during the 2 last decades, with the primary aim of improving safety and convenience [5]. The efficacy of SLIT has been demonstrated in numerous clinical trials and confirmed by several meta-analyses [5,6]. Despite some aspects still needing clarification, SLIT is regarded as a suitable therapeutic option for AR and asthma through a TGF-β mediated immunological suppression in clinically effective SLIT in addition to an increase in regulatory T cells and IL-10 level with suppressor function [7,8]. Up to now, there has been limited literature concerning the clinical efficacy of SLIT in Chinese AR children by using domestic product. Moreover, no specific biomarker was determined to predict the clinical efficacy of SLIT with domestic product is available.

TIM-1, originally identified as hepatitis A virus cellular receptor 1, has been identified as a gene associated with AR, asthma and other allergic diseases in multiple human genetic studies [9–11].

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Accumulating data in the murine system support the essential role of TIM-1 in allergic diseases by regulating DC function and thus shifting the balance between effector and regulatory T cells toward Th2-dependent inflammation [12,13]. These findings raise the possibility that suppression of TIM-1 might act as an indicator for the clinical efficacy of SLIT. To address this issue, we investigated the clinical efficacy of SLIT on AR children after 6-month treatment. Moreover, laboratory parameters including TIM-1 and IL-10 were analyzed to assess the clinical efficacy of SLIT.

2. Materials and methods

2.1. Subjects

One hundred and sixteen pediatric AR patients were enrolled from 2 independent medical centers in Guangzhou of southern China. Mandatory inclusion criteria were as follows: (1) clinical criteria of moderate to severe persistent AR in the past 2 years; (2) sensitization to house dust mites (HDM) including *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae* with positive skin prick test (wheal diameter > 6 mm) and/or CAP-Pharmacia score > class 2 (Phadia, Uppsala, Sweden); (3) age between 5 and 14 years; and (4) FEV1 within normal limits (>79% of predicted value). The diagnosis of moderate to severe persistent AR was made on the basis of clinical criteria, including sneezing, itching, rhinorrhea, and nasal congestion, etc. Asthma was diagnosed by a physician. Exclusion criteria included children who have moderate persistent asthma, or with anatomic abnormalities of the upper respiratory tract, or undergoing chronic treatment with systemic steroids or having systemic immunologic disorders, and intercurrent treatment with β-blockers or oral corticosteroid treatment in the previous 6 months. Treatment with other symptomatic medications (antihistamines, β2-agonists and/or topical corticosteroids) for AR and/or asthma was permitted during the study period. This study was supported by the local ethical committee, and all children had informed consent from their parents.

A 7-day run-in period followed with diary symptom and medication monitoring of AR symptoms, frequency and amount of rescue medications (antihistamines and corticosteroids, etc.). Following the run-in period, patients were re-evaluated for eligibility in an enrolment visit before treatment with HDM extract SLIT.

2.2. SLIT with HDM extract

The HDM allergen extract (CHANLLERNGEN, Dermatophagoides Farinae Drops) for SLIT was domestically manufactured by Wolwopharma Biotechnology Company, Zhejiang, China. The biologically standardized extracts were labeled in concentration of total protein and used in the form of drops (No. 1, 1 μg/mL; No. 2, 10 μg/mL; No. 3, 100 μg/mL and No. 4, 333 μg/mL) which was determined by BCA protein assay, and the biological activity of allergen extracts was determined by indirect ELISA assay, as approved by China Food and Drug Administration. As described elsewhere [14], the patients were asked to take daily increasing doses (from No. 1 to No. 3) according to the manufacture's instruction during the 3-week up-dosing phase, then were instructed to had 3 drops of No. 4 solution once daily during the maintenance phase. Drops were instructed to be kept under the tongue for 2–3 min before being swallowed.

2.3. Clinical evaluation

All of the patients recorded their daily symptom score and drug requirement throughout the 6-month SLIT study. The questionnaires covered symptoms, medication use, etc. The severity of individual nasal symptom score (INSS), including nasal rhinorrhea, sneezing, itching and congestion, was assessed on a scale of 0 to 3 (0 = no symptom, 1 = mild, 2 = moderate, 3 = severe). Total nasal symptom score (TNSS) was defined as the sum of the scores of nasal rhinorrhea, sneezing, itching and congestion.

The following symptomatic rescue medications were allowed to use: oral or intranasal antihistamine, and/or intranasal corticosteroid. The rescue medication scores were arbitrarily assigned on a scale of 0–3 (0 = no medication, 1 = rare and mild medication, 2 = moderate medication, 3 = frequent and much medication). In this study, the efficacy of 6-month SLIT was determined by assessing the children's condition which was defined as follows: group A, well controlled: no symptom, or mild symptom which can be completely controlled by mild medication; group B, partially controlled: mild to moderate symptom in case of mild or moderate medication; group C, uncontrolled: moderate to severe symptom in case of much medication.

To evaluate adverse effects related to SLIT, the participants were asked to record pertinent adverse events including mouth discomfort, gastrointestinal trouble and breathing discomfort on diary cards during the whole period.

2.4. Laboratory parameter

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples collected from the specially enrolled children (group A, well controlled, $n = 16$; group C, uncontrolled, $n = 12$) before and after 6-month SLIT by Ficoll-Hypaque density gradient centrifugation (TBD, Tianjin, China). The total RNA was extracted from PBMCs using the TRIzol reagent (Life Technologies, Carlsbad, CA, USA). The procedure for qRT-PCR was performed as described previously [10]. Briefly, the mRNA expression was measured using an ABI PRISM 7500 Detection System (Applied Biosystems, Foster City, CA, USA) and SYBR Premix Taq™ (TAKARA). The sequences of the primers were as follows: TIM-1 forward, 5′-CTA CTG ACG GCC AAT ACC AC-3′; TIM-1 reverse, 5′-AGA GCA AGA AGC ACC AAG AC-3′; IL-5 forward, 5′-TGC TGA TAG CCA ATG AGA CTC TG-3′; IL-5 reverse, 5′-TTT CCA CAG TAC CCC CTT GC-3′; IL-10 forward, 5′-CCT CCA CC ATG CCA AGT GGT-3′; IL-10 reverse, 5′-AGC TGC GCT GAT AGA CAT CC-3′; β-actin forward, 5′-AAG ATG ACC CAG ATC ATG TTT GAG ACC-3′; and β-actin reverse, 5′-AGC CAG GTC CAG ACG CAG GAT-3′. The PRISM samples contained 1× SYBR Green Master Mix, 1.5 μL of 5 μM primers, and 25 ng of synthesized cDNA in a 25-μL reaction volume. The mean value of the replicates for each sample was calculated and expressed as a cycle threshold (C_t) value. The relative expression of target gene in one sample was determined as $2^{-\Delta C_t}$ (ΔC_t, the difference between the C_t value of the target gene and the C_t value of β-actin). Fold changes in the target gene mRNA in different samples before and after SLIT treatment were determined as $2^{-\Delta\Delta C_t}$.

2.5. Statistical analysis

The statistical significance of each difference between baseline and treatment value was determined using the Student's *t*-test or the non-parametric Mann–Whitney *U*-test or Wilcoxon signed-rank test. The Spearman rank correlation test was used to analyze the correlation between different biomarkers. A p value less than 0.05 was considered significant.

3. Results

3.1. AR patients

In this study, a total of 116 children with HDM-sensitized AR were enrolled after a 7-day run-in period. They were from 2

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Table 1
The demographic and clinical characteristics of 116 AR patients.

Character	
Case no.	116
Gender (male/female)	79/37
Age (years)	8.4
Duration (years)	3.4
Monosensitized subjects (%)	101 (87.1%)
Asthma comorbidity (%)	8 (6.90%)
INSS score	
Nasal rhinorrhea	1.66 ± 0.08
Itching	1.22 ± 0.08
Sneezing	1.74 ± 0.09
Congestion	1.78 ± 0.09
TNSS score	6.45 ± 0.25

independent medical centers in Guangzhou of Southern China. The overall demographic and clinical characteristics are presented in Table 1. The majority of subjects were Han nationality (94.0%), and the mean age and duration was 8.4 and 3.4 years. The coexisting rate of asthma is much low (6.90%) and the monosensitized subjects shared a population of 87.1%.

3.2. Change of symptom scores

After 6-month SLIT treatment, all INSS (nasal rhinorrhea, itching, sneezing and congestion), TNSS and medication score significantly decreased when compared with the baseline values. As illustrated in Fig. 1A–E, rhinorrhea score changed from 1.66 ± 0.08 to 0.58 ± 0.06; itching score changed from 1.22 ± 0.08 to 0.27 ± 0.04; sneezing score changed from 1.74 ± 0.08 to 0.59 ± 0.07; nasal congestion score changed from 1.78 ± 0.09 to 0.49 ± 0.06; all with significant difference. Consequently, TNSS and medication score significantly decreased when compared with the baseline value (TNSS, 6.45 ± 0.25 vs 1.93 ± 0.16, $p < 0.01$; medication score, 2.61 ± 0.06 vs 1.23 ± 0.08, $p < 0.01$), suggesting SLIT is able to improve the nasal symptoms of AR children and decrease medication consumption (Fig. 1E and F). By establishing an arbitrary classification system which combined nasal symptom with medication consumption, we evaluated the ratio of improvement for all subjects after 6-month SLIT treatment. As illustrated in Fig. 1G, we found 43.1% of 116 children belonged to well controlled group (group A); 32.8% of 116 children belonged to partially controlled group (group B); only 24.1% of 116 children were allocated to the uncontrolled group (group C), suggesting SLIT is generally effective (75.5%) for AR children with drug-sparing effect.

3.3. Compliance and adverse event

All patients finished the 6-month SLIT treatment, and the drop-out rate was 0. Twenty-four children (20.7%) reported adverse effects including itching sensation in the oral cavity or of the lip in 11 (14.8%), gastrointestinal trouble in 7 (6.0%) and skin itching or rash in 6 (5.2%). There were no life-threatening adverse effects. These results indicated SLIT treatment is safe for AR children.

3.4. Change of TIM-1, IL-5 and IL-10 mRNA in PBMCs

Twenty-eight AR children (group A, $n = 16$; group C, $n = 12$,) were specially enrolled to examine the change of TIM-1, IL-5 and IL-10 mRNA levels in PBMCs after 6-month SLIT. As illustrated in Fig. 2A–F, TIM-1 and IL-5 mRNA level was significantly reduced compared to the baseline value, and a positive association of TIM-1 and IL-5 mRNA levels was observed ($r = 0.59$, $p < 0.01$). However, IL-10 mRNA level was significantly increased compared to the baseline value after 6-month SLIT ($p < 0.01$), and an inverse association of TIM-1 and IL-10 mRNA levels was observed

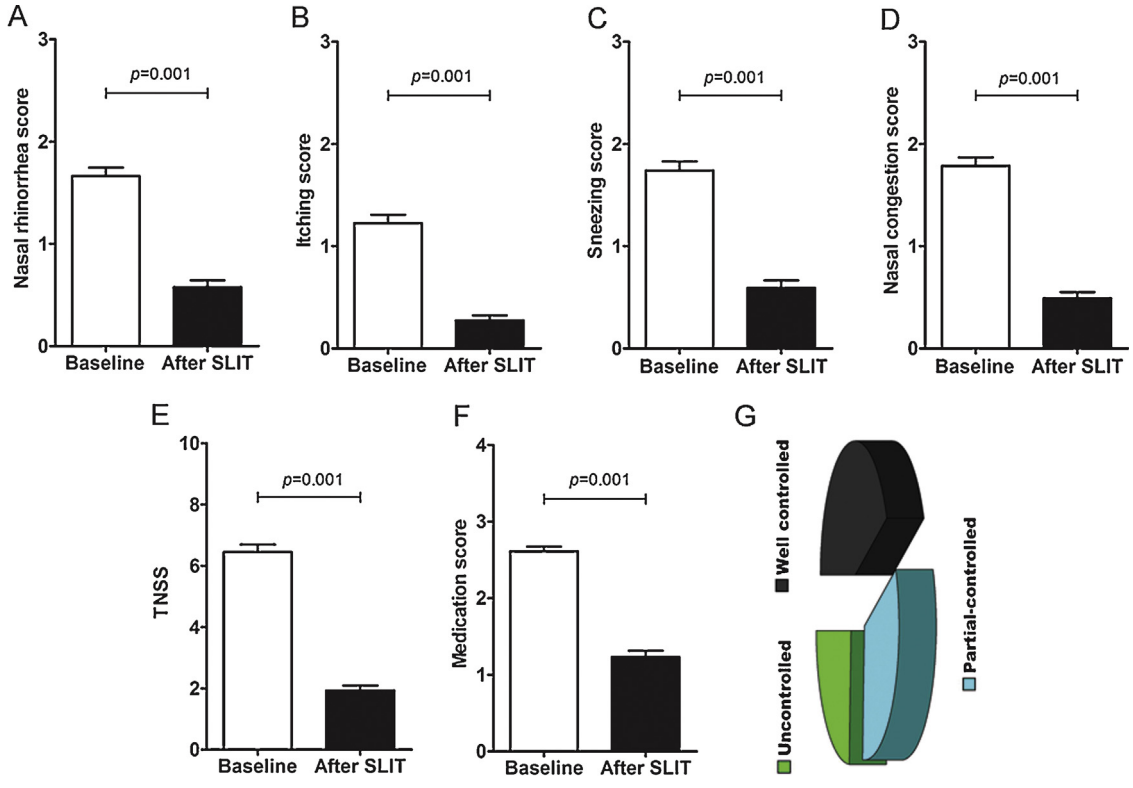

Fig. 1. Change of nasal symptom scores after 6-month SLIT treatment. After 6-month SLIT treatment, nasal rhinorrhea (A), itching (B), sneezing (C) and congestion (D), TNSS (E) and medication score (F) significantly decreased when compared with the baseline values. After SLIT treatment, 43.1% of 116 children belonged to well controlled group (group A); 32.8% of 116 children belonged to partially controlled group (group B); only 24.1% of 116 children were allocated to the uncontrolled group (group C) (G).

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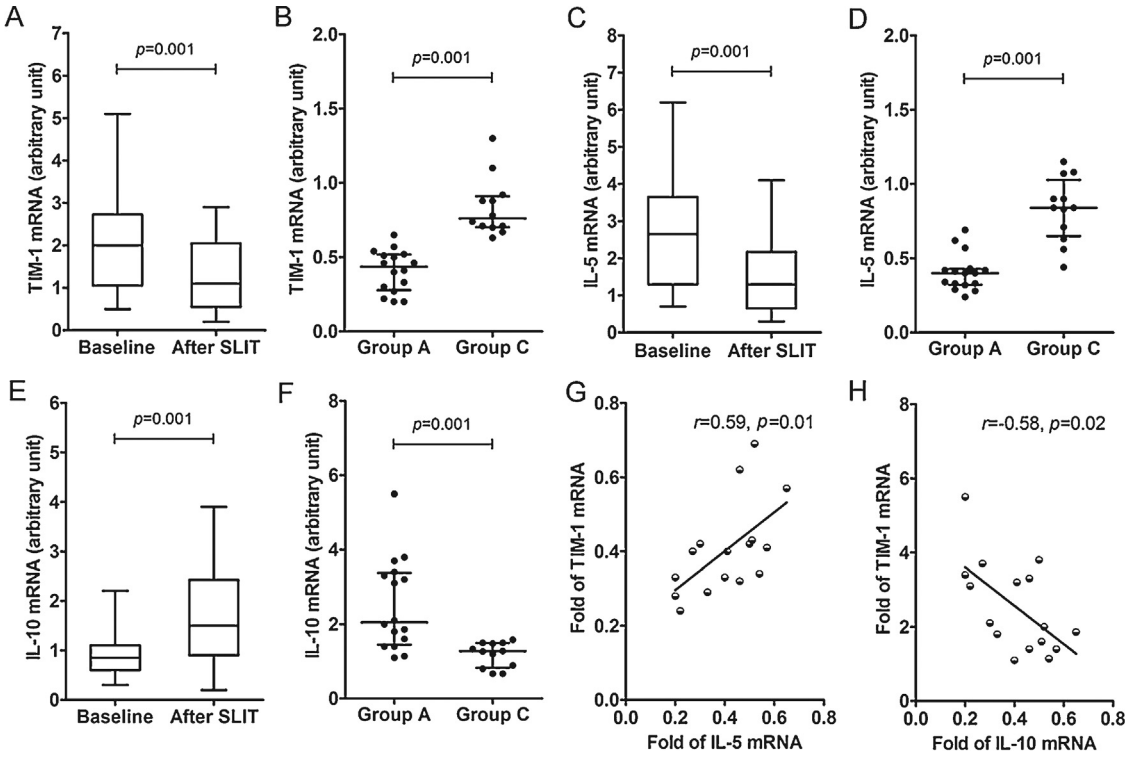

Fig. 2. The levels of TIM-1, IL-5 and IL-10 mRNA in PBMCs before and after 6-month SLIT treatment. After 6-month SLIT treatment, TIM-1 (A) and IL-5 mRNA (C) level was significantly reduced compared to the baseline value, and a positive association of TIM-1 and IL-5 mRNA levels was observed (G). However, IL-10 mRNA level was significantly increased compared to the baseline value after 6-month SLIT (E), and an inverse association of TIM-1 and IL-10 mRNA levels was observed (H). After 6-month SLIT, the change fold of TIM-1 and IL-5 mRNA level was significantly reduced in group A compared with that in group C (B and D), but change fold of IL-10 mRNA was significantly increased in group A compared with that in group C (F). Group A: well controlled group; group C: uncontrolled group.

($r = -0.58$, $p < 0.01$). However, the change of TIM-1 and IL-10 mRNA levels in the two groups is different. After 6-month SLIT, the fold of TIM-1 and IL-5 mRNA level was significantly reduced in group A compared with that in group C ($p < 0.01$) (Fig. 2B and D). As a correspondence, change of IL-10 mRNA was significantly increased in group A compared with that in group C ($p < 0.01$) (Fig. 2E). These findings suggesting TIM1 has the potential to predicate the clinical efficacy of SLIT in AR children.

4. Discussion

Although there is increasing use of SLIT in Europe, SLIT as a treatment for respiratory allergy has not yet approved by some countries, and there have been limited studies in AR patients in Asian area [14–16]. In the present study, we provided the evidence that SLIT is efficacious and safe for HDM-sensitized AR children in China. We found the efficacy of SLIT may be associated with TIM-1 suppression. Hopefully, our findings will help us to further understand the molecular process of SLIT and to improve its clinical efficacy.

SLIT is currently recommended as a suitable alternative to SCIT, which requires multiple injections and frequent visits to the physician's office. As to AR children, SLIT is particularly recommendable for its easiness and painlessness and improved safety with little risk of systemic adverse effects [6]. In 2006, Valovirta et al. reported the clinical efficacy and safety of SLIT with pollen in AR children and showed a significant reduction of symptom and medication use [17]. In 2010, Yonekura et al. demonstrated that SLIT with house-dust extract administered for more than 30 weeks is effective and safe to HDM-sensitized AR in Japanese children [16]. Similarly, Park et al. recently reported that all nasal and non-nasal symptoms and quality of life were significantly improved after SLIT treatment and very few minor adverse effects were observed in Korean AR children [15]. The efficacy of SLIT is not ubiquitous and a recent report documented that some AR patients with SLIT showed an ineffective clinical response [18]. Given all studies concerning SLIT were conducted in western and other Asian area, it is unknown whether SLIT with HDM is effective and safe to HDM-sensitized AR children in China.

In the present study, we thus evaluated the change of nasal symptom in AR children after 6-month SLIT treatment. As a result, we found the INSS (nasal rhinorrhea, itching, sneezing and nasal congestion) and TNSS were significantly decreased after 6-month SLIT treatment, which was consistent with the previous studies. These results indicated that SLIT is globally effective for AR children in China. Moreover, we observed low medication consumption and significantly decreased medication scores in most AR children after treatment. When arbitrarily defined the clinical control of AR by means of combined symptom improvement and medication consumption to evaluate the efficacy of SLIT, we found the controlled rate (including the well and partially controlled AR children) was 75.5% after 6-month treatment. As to the safety of SLIT, in agreement with the previous studies, we found very few minor adverse effects were observed in these AR children during 6-month SLIT. Taken together, our findings showed that SLIT is effective and safe to AR children in China.

Although SLIT is widely used in different countries, the immunological mechanisms underlying successful SLIT have not been completely understood. Similar to SCIT, reported immunological mechanisms of effective SLIT include down-regulation of the adverse Th2 immune response to allergen, immune deviation, blocking antibody production, and regulatory T cell (Treg) induction [19]. Of them, change in specific IgE is not the immunological indicator for successful SLIT since many studies of SLIT reported that there were no changes in specific IgE after treatment [20,21]. However, the induction of Treg cells, which is

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initiated by the cytokines IL-10 and transforming growth factor-β (TGF-β), has been considered as an essential step in allergen-specific SLIT. For example, Bohle et al. reported SLIT induces Treg suppression through IL-10 during the early phase and specific nonreactivity [7]. Piconi et al. demonstrated SLIT resulted in increased IL-10 production, programmed cell death ligand 1 expression, and concentration of allergen-specific IgG4, as well as in the reduction of CD80 and CD86 expression and IL-4 production [22]. SLIT, thus, is associated with modulation of Treg expression and IL-10 synthesis and favors the production of allergen-specific IgG4. In agreement with these studies, we found significant changes in mRNA levels of IL-5 (suppression) and IL-10 (induction), which were associated with the clinical control of AR in this study. These findings reflected a shift to balance of Treg/Th2 response after 6-month SLIT treatment.

In addition, we found the costimulatory molecule TIM-1 mRNA was also significantly changed after 6-month SLIT treatment. TIM-1 is constitutively expressed in CD4+ T cells, and crosslinking of TIM-1 with an agonistic anti-TIM-1 mAb or with its ligand, TIM-4, costimulated T-cell proliferation and drive them to develop into Th2 cells [23]. We previous reported increased TIM-1 expression is associated with GATA-3 expression in asthma mouse model. More recently, Xiao et al. reported that TIM-1 signaling upregulated costimulatory molecule expression and proinflammatory cytokine production, thereby promoting effector T cell responses, while inhibiting Treg response [12]. These findings suggested TIM-1 plays an important role in the progress of allergic response. Moreover, the levels of TIM-1 in Th2 cells of AR patients were shown to be significantly reduced after 3-month SCIT [24]. In this study, we found TIM-1 mRNA was significantly decreased in well controlled AR patients after 6-month SLIT. Moreover, change of TIM-1 mRNA was associated with the IL-5 mRNA suppression and IL-10 mRNA induction, suggesting TIM-1 suppression may contribute to modulating the balance of Treg/Th2 response in AR children. Taken together, our finding thus suggested TIM-1 may act as the therapeutic target of SLIT, and TIM-1 suppression may be used as an immunological indicator for successful SLIT in AR children.

We acknowledge that this study is not without flaws. First, no placebo group was included for efficacy measures for the preparation of placebo is unavailable. Second, the duration of follow-up was shorter than is usually prescribed in practice and the biomarkers were examined only in partial HDM-sensitized AR subjects with small-size. Therefore, to further validate the efficacy of SLIT in Chinese AR children of larger size in RCT clinical trial is necessary.

5. Conclusion

We have demonstrated the efficacy and safety of SLIT with HDM extract in HDM-sensitized AR children in China. Moreover, we found TIM-1 mRNA was significantly decreased in well controlled AR patients after 6-month SLIT, which was associated with the IL-5 mRNA suppression and IL-10 mRNA induction. Our finding thus suggested TIM-1 suppression may be used as an immunological indicator for successful SLIT in AR children.

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