



Decreased level of osteopontin in children with allergic rhinitis during sublingual immunotherapy



Chunrui Wang^{a,1}, Kai Wang^{b,1}, Shaoting Liu^a, Xiaowei Qin^a, Kewa Chen^a, Tianhong Zhang^{a,*}

^a Department of Otolaryngology, First Affiliated Hospital to Harbin Medical University, Harbin, China

^b Department of Otolaryngology, Ningbo Medical Treatment Center Lihuili Hospital, Ningbo, China

ARTICLE INFO

Article history:

Received 1 November 2015

Received in revised form 30 November 2015

Accepted 3 December 2015

Available online 13 December 2015

Keywords:

Allergic rhinitis

Osteopontin

Sublingual immunotherapy

Th2 cytokines

ABSTRACT

Objective: Sublingual immunotherapy (SLIT) is proven to be very effective in the treatment of allergic rhinitis (AR), but its regulatory mechanism and biomarkers for predicting efficacy are still unknown. Osteopontin (OPN), as a recently described Th2 inflammation related protein, plays key role in the pathogenesis of AR. The aim of this study was to identify the expression and role of OPN during SLIT in children.

Methods: Fifty house dust mite (HDM)-sensitized children with AR were enrolled in this study. AR children received HDM allergen extract or placebo for SLIT. Serum of different time points during treatment was collected and used for enzyme-linked immuno sorbent assay (ELISA) of OPN and related cytokines, respectively. Peripheral blood mononuclear cells from children after SLIT or placebo treatment were collected and stimulated with HDM with or without OPN/anti-OPN after one year's treatment.

Results: Our results showed that expression of OPN protein was decreased after one year's therapy. The decreased OPN expression was positively related to decreased Th2 cytokines and negatively related to enhanced IL-10 and TGF- β expression. *In vitro* experiments confirmed that children received SLIT treatment showed decreased production of Th2 cytokines by PBMCs after HDM stimulation.

Conclusion: During SLIT, decreased OPN expression was related to low Th2 cytokine expression and enhanced IL-10 and TGF- β expression. High serum OPN expression predicts poor treatment efficacy. OPN may be used as a biomarker for SLIT treatment.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Allergic rhinitis (AR) is one the most common chronic diseases in children with increased incidence [1,2]. Allergen-specific sublingual immunotherapy (SLIT) is the only treatment so far that intervene the natural course of allergic disease and it can prevent the development of asthma in AR children [3]. In the past, immunotherapy was believed to change the immune response from a Th2-predominant response dominated by cytokines IL-4, IL-5, and IL-13, eosinophils, and IgE, toward a Th1 response characterized by the absence of eosinophils and an

increased IgG response, with raised IgG4 levels. However, recent studies showed that regulatory T cells (Treg) may be key regulators of immunological processes in peripheral tolerance to allergens during immunotherapy. Skewing of allergen-specific effector T cells to a regulatory phenotype appears as a key event in the development of healthy immune response to allergens and successful outcome in SLIT [4]. However, the underlying mechanism in the process and potential biomarkers is still not fully characterized.

Osteopontin (OPN) is a phosphorylated acidic glycoprotein that can bind with certain CD44 variants and integrin receptors and mediate cell-matrix interactions and cellular signaling [5]. Previously, OPN was described as a Th1 cytokine, and elevated levels of OPN were observed in several Th1-associated diseases, including sarcoidosis, tuberculosis, rheumatoid arthritis, multiple sclerosis, and Crohn's disease [6–10]. Recent studies have showed that OPN is

* Corresponding author. Tel.: +86 451 8555564; fax: +86 451 8555564.

E-mail address: crystalwang_18@163.com (T. Zhang).

¹ Contribute equally to this study.

also involved in Th2-mediated diseases [11]. Increased OPN level was found in the tissues of nasal polyps in patients with chronic rhinosinusitis plus nasal polyps [12]. All these studies suggested that OPN may play different roles in different disease background due to its complicated structure and several functional motifs.

As the role of OPN in AR and asthma becomes more and more clear, we postulated that OPN may be a potential candidate marker for SLIT treatment. The aim of this study was to evaluate OPN expression in the process of SLIT in children and the direct effect of OPN on peripheral blood mononuclear cells (PBMCs) during SLIT.

2. Materials and methods

2.1. Patients

Fifty children aged 6–16 years with a clinical history of mono-allergen dust mite (HDM) induced AR for at least 2 years were included. Skin prick test (SPT) or specific immunoglobulin IgE was performed to screen children only allergic to HDM. The cut-off value of specific is 3.5 IU/mL and positive SPT was defined as mean wheal diameter ≥ 2 mm. Those children with chronic diseases (e.g. asthma, malnutrition, anatomic malformation of the respiratory system, chronic lung disease, heart disease, gastro-oesophageal reflux disease, cystic fibrosis) were excluded from the study. All these patients were unresponsive to nasal steroids treatment. Patients with a history of chronic drug use (e.g. oral or nasal corticosteroids, antiepileptics, immune suppressives, antihistamines, etc.) were excluded from the study. The study was performed with the approval of the local ethics committee and with the parent's written informed consent.

2.2. Sublingual immunotherapy

Fifty AR children were divided into SLIT and placebo group randomly with 25 cases in each group. The drugs were labeled with patient code numbers, and the investigator assigned patients in a sequential randomized fashion to a study code number. Individual drug bottles were identity masked such that both patients and researchers were blind to treatment assignment. Study blinding was preserved at the study sites until all subjects completed the study. The HDM allergen extract (CHANLLERNGEN, Dermatophagoides Farinae Drops) for SLIT was manufactured by Wolwopharma Biotechnology Company (Zhejiang, China) and used in the form of drops (no. 1, 1 mg/mL; no. 2, 10 mg/mL; no. 3, 100 mg/mL and no. 4, 333 mg/mL). According to the manufacture's instruction, the patients were asked to take increasing doses (from no. 1 to 3) during the first three weeks up-dosing phase, and then were instructed to have 3 drops of no. 4 solution once daily during the maintenance phase. Children in the placebo group received a diluent containing 50% glycerol and 50% saline buffer and the usage was similar to that of SLIT group with increasing doses given first three weeks and maintenance dose after that. Drops were instructed to be kept under the tongue for 2–3 min before swallowed.

2.3. Clinical evaluation

A 7-day run-in period for all patients with diary symptom and medication monitoring of AR symptoms, frequency and amount of rescue medications (antihistamines and corticosteroids, etc.) was finished before study. Following the run-in period, patients were re-evaluated for eligibility in an enrolment visit before treatment with HDM extract SLIT. The symptoms and medications were recorded using a Patient Diary as described earlier [13]. All of the patients recorded their daily symptom score and drug requirement before and throughout the 12-month SLIT study.

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 maximal cumulated clinical score per patient was 12. Although some patients had ocular symptoms associated with rhinitis, only nasal symptoms were evaluated for analysis of efficacy.

Patients were allowed to use the following anti-allergic drugs: oral antihistamine, intranasal corticosteroid, and betamethasone 0.5-mg tablets. Other medications, such as long-lasting antihistamines, cromones, and parenteral corticosteroids, were not permitted during the study. The patients had to record on the same diary card whenever they used medications, and a specific weekly score (AMS) was assigned (1 point: one puff of nasal corticosteroid per nostril, 2 points: one oral antihistamine tablet, 3 points: one betamethasone tablet).

2.4. Blood and nasal lavage samples preparation and analysis

Venous blood samples were collected into Vacuette tubes and centrifuged at $3000 \times g$ for 15 min at 4 °C. Serum samples were stored at -80 °C. These samples were used for Enzyme-linked immunosorbent assay (ELISA) measurement. The whole blood cell counts were measured by LH-785 system (Beckman Coulter, Mervue, Galway, Ireland). Total serum IgE was measured by ECLIA (Electrochemiluminescence) method using an ELX-800 system. Unicap system was used for measuring ECP levels, according to the manufacturer's protocols.

Nasal lavage was performed using saline warmed to 37 °C. The process was performed according to method described elsewhere [14]. The samples were centrifuged to remove cellular debris and aliquots of the supernatants were stored at -20 °C in eppendorf tubes until analysis. Total protein concentrations were determined with Bio-Rad protein assays according to Bradford.

Both the blood and nasal samples were taken at 0, 6, and 12 months for determination.

2.5. ELISA for protein expression

ELISA kits were used for measuring serum OPN, IL-4, IL-13, IL-5, IL-12, IFN- α , IL-10, TGF- β (R&D systems, USA) according to the manufacturer's protocols. The detection limits of the assays were as follows: OPN, 0.312 ng/mL, IL-4, 1.56 pg/mL, IL-13, 93.8 pg/mL, IL-5, 7.8 pg/mL, IL-12, 2.5 pg/mL, IFN- α , 12.5 pg/mL, IL-10, 3.9 pg/mL, TGF- β , 15.4 pg/mL.

2.6. Peripheral blood mononuclear cells (PBMCs) preparation

PBMCs were isolated by Lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway) density-gradient centrifugation from heparinized leucocyte-enriched buffy coats, which were obtained from 14 children (7 received SLIT and 7 received placebo) one year after the treatment. PBMCs were isolated by means of density gradient centrifugation and cultured at 2×10^6 /mL in 24-well plates in culture medium: RPMI 1640 supplemented with 5% human AB serum, 5 mmol/L glutamine, and 13 penicillin and streptomycin solution (all from Invitrogen, except serum from Sigma-Aldrich). Stimulation was through addition of allergen (100 ng/mL HDM extract) + PHA (1 μ g/mL), with or without anti-OPN (100 ng/mL) or rhOPN (100 ng/mL) for 24 h.

2.7. Statistical analysis

Statistical analysis was performed using Mann–Whitney or Wilcoxon signed rank test. The pre-test Friedman test was done for

Table 1
Clinical data of 50 AR children.

	Treatment	
	SLIT	Placebo
Number of patients	25	25
Sex(M/F)	11/14	12/13
Age (years)	6–14	6–16
Duration of rhinitis	4.3 ± 2.1	3.6 ± 1.8

multiple comparisons. The Spearman rank correlation test was used to analyze the correlation among the expression of biomarkers. $p < 0.05$ was considered as significant difference.

3. Results

3.1. Demographic characteristics of study population and clinical outcome

This study was conducted with 50 children with ages ranging between 73 and 177 months (mean age: 108.6 ± 39.0 months, 32 males). The baseline data of patients were summarized in Table 1. After one year's SLIT treatment, all symptom and medication scores significantly decreased when compared with the baseline values (Table 2).

3.2. Decreased serum and nasal OPN protein levels during SLIT treatment

The serum and nasal OPN protein expression decreased significantly after one year's SLIT compared with baseline level (3.2 ± 1.2 vs 9.4 ± 3.1 ng/mL; 1.2 ± 0.2 vs 6.0 ± 2.1 ng/mL, Fig. 1A and B). Our results also showed decreased protein expression of ECP and IgE one year after SLIT treatment (11.0 ± 4.2 vs 37.9 ± 9.1 ng/mL; 44.0 ± 8.2 vs 84.3 ± 18.1 IU/mL, Fig. 1C and D). Correlation analysis showed that decreased OPN expression was positively related to IgE and ECP expression, respectively (Fig. 2A and B).

Table 2
Clinical outcome of 50 AR children after SLIT or placebo treatment.

	SLIT		Placebo	
	Baseline	1 year	Baseline	1 year
Sneezing	2.1 ± 0.5	$0.9 \pm 0.3^*$	2.3 ± 0.4	$2.2 \pm 0.6^{**}$
Rhinorrhea	1.7 ± 0.4	$1.0 \pm 0.4^*$	1.5 ± 0.2	$1.6 \pm 0.3^{**}$
Nasal itching	1.8 ± 0.2	$1.1 \pm 0.5^*$	1.9 ± 0.3	$1.9 \pm 0.5^{**}$
Congestion	1.6 ± 0.3	$1.0 \pm 0.2^*$	1.3 ± 0.1	$1.9 \pm 0.4^{**}$
TNNS	6.7 ± 2.3	$3.1 \pm 0.8^*$	6.2 ± 2.3	$7.1 \pm 3.3^{**}$
AMS	9.1 ± 4.3	$5.2 \pm 1.4^*$	9.3 ± 3.9	$8.3 \pm 3.1^{**}$

* Compared with baseline level, $p < 0.05$.

** Compared with SLIT group level, $p < 0.05$.

3.3. Th1/Th2/Treg cytokines expression during SLIT

One year after SLIT treatment, serum IL-12 and IFN- γ protein expression increased significantly compared with baseline level (46.0 ± 14 vs 24.9 ± 9.3 pg/mL; 104.0 ± 27 vs 53.5 ± 18.2 pg/mL), while serum IL-4, IL-5 and IL-13 decreased significantly (1.8 ± 0.4 vs 3.3 ± 0.5 pg/mL; 8.1 ± 2.6 vs 24.0 ± 9.1 pg/mL; 97.0 ± 24 vs 288.9 ± 58.1 pg/mL, Fig. 3A–E). We also found that serum OPN expression was positively related to Th2 cytokines (Fig. 3H–J) and had no relation with Th1 cytokines (data not shown). Our results showed that serum IL-10 and TGF- β expression increased significantly one year after SLIT (55.0 ± 17.2 vs 29.1 ± 12.6 pg/mL; 580 ± 123 vs 320 ± 67 pg/mL, Fig. 3F and G). No relationship was found between serum OPN and IL-10 as well as TGF- β expression (data not shown).

3.4. Anti-OPN alleviates Th2 inflammation of PBMCs induced by HDM

After stimulated with HDM + PHA combined with rhOPN, we found significant up-regulation of Th2 cytokines (IL-4, IL-5, IL-13) level in PBMCs from both SLIT and placebo and this effect can be blocked by anti-OPN antibody (Fig. 4A–C). Besides, both combinations of PBS + anti-OPN and PBS + rhOPN had no effect on production of Th2 cytokines (data not shown). When compared

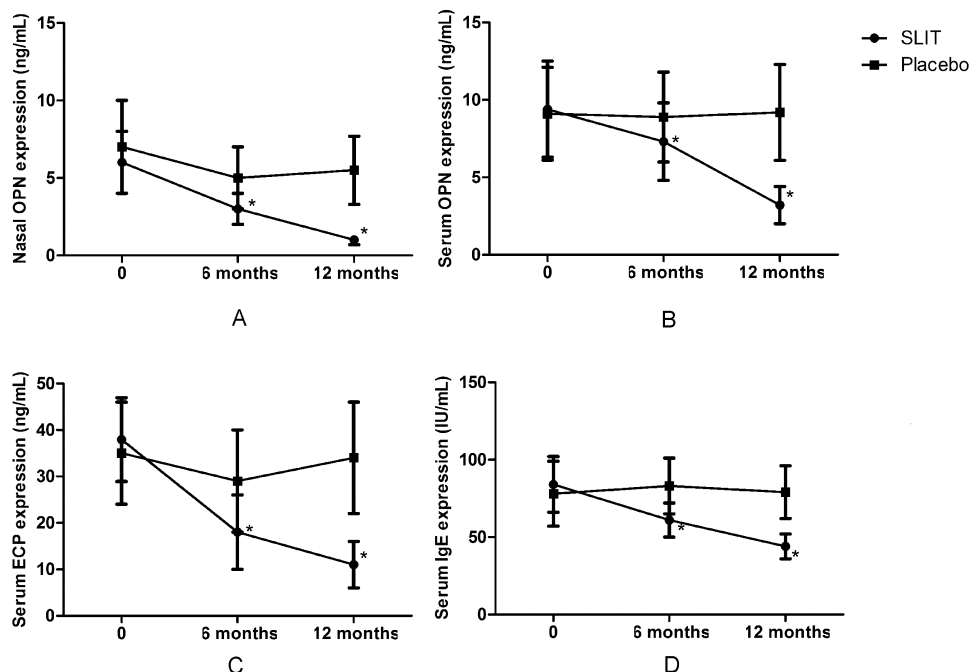


Fig. 1. Serum and nasal OPN protein expression decreased significantly after one year's SLIT compared with baseline level (A and B). Decreased protein expression of ECP and IgE also decreased significantly after one year's SLIT compared with baseline level (C and D). * Compared with baseline level, $p < 0.05$.

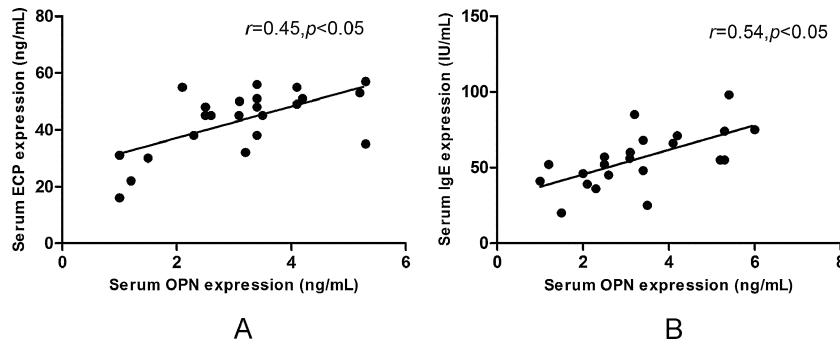


Fig. 2. Serum OPN expression was positively related to IgE and ECP expression after one year's SLIT (A and B).

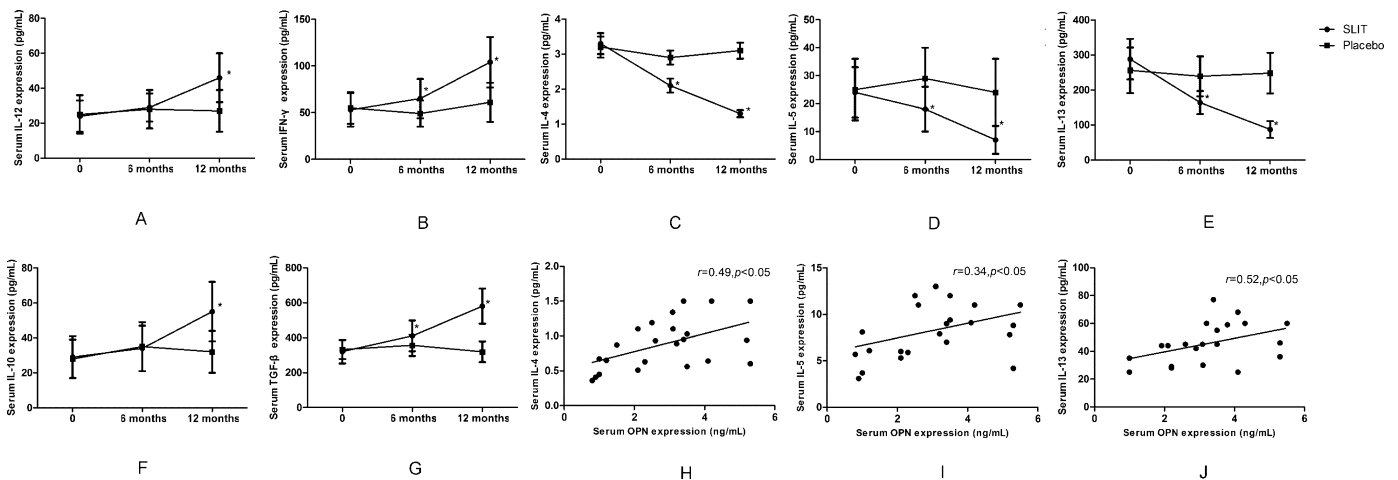


Fig. 3. Increased serum Th1 cytokines (IL-12 and IFN- α) and decreased Th2 cytokines (IL-4, IL-5 and IL-13) after one year's SLIT (A–E). Increased serum IL-10 and TGF- β after one year's SLIT (F and G). Serum OPN expression was positively related to Th2 cytokines after one year's SLIT (H–J). * Compared with baseline level, $p < 0.05$.

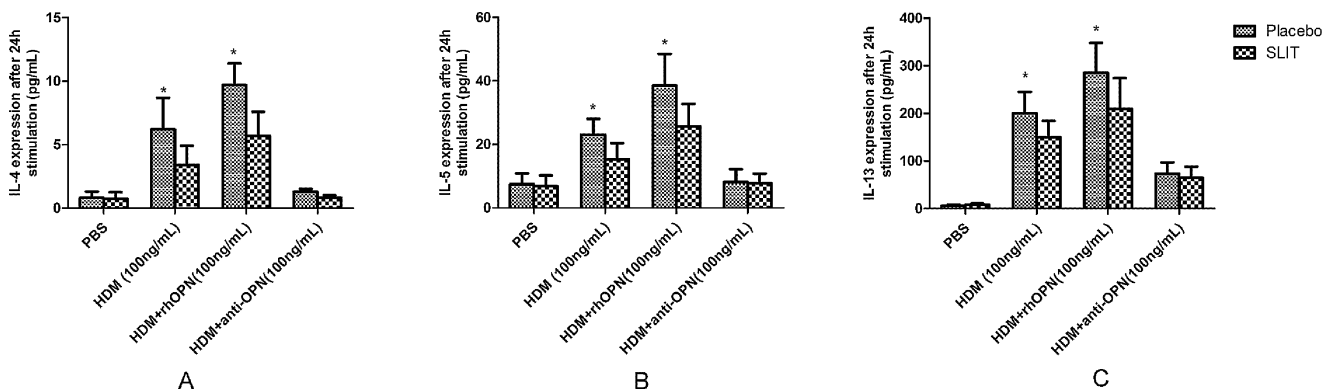


Fig. 4. After stimulated with HDM extract combined with anti-OPN, significant down-regulation of Th2 cytokines (IL-4, IL-5, IL-13) level in PBMCs (A–C). * $p < 0.05$, comparison between two groups.

between groups, children received SLIT treatment showed decreased production of Th2 cytokines (IL-4, IL-5, IL-13) level by PBMCs after HDM stimulation combined with or without rhOPN compared with placebo group. Besides, IL-10 and TGF- β as well as Th1 cytokines was not changed (data not shown).

3.5. Baseline serum OPN expression predicts SLIT treatment efficacy

We subdivided AR children underwent SLIT into two groups according to baseline serum OPN level and compared the

improvement of TNSS and AMS score. Our results showed that children with low baseline serum OPN level acquired better improvement of symptom and medication score compared with high baseline serum OPN level (2.73 ± 1.25 vs 1.62 ± 0.72 ; 3.56 ± 1.11 vs 1.97 ± 0.64 , Fig. 5A and B).

4. Discussion

Our present study demonstrates for the first time that decreased OPN expression during SLIT treatment was related to

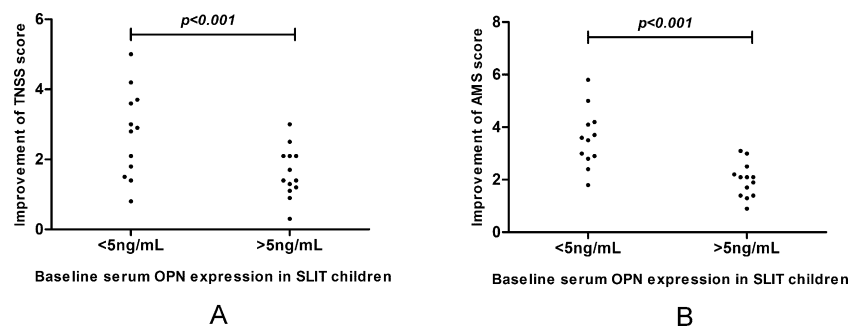


Fig. 5. Children with low baseline serum OPN level acquired better improvement of symptom and medication score compared with high baseline serum OPN level (A and B).

reduced Th2 cytokines and enhanced protective cytokines such as IL-10 and TGF- β . *In vitro* study showed that anti-OPN antibody alleviated Th2 inflammation induced by HDM.

SLIT is the only way of reducing sensitivity to a given allergen by repeated administration of a dose of that allergen. The main mechanism of SLIT includes reducing Th2 inflammation, enhancing Th1 inflammation and inducing allergen specific T cells regulatory cells [4,15].

OPN, also known as early T lymphocyte-activating gene-1, is a phosphorylated glycoprotein generated or secreted by osteoblasts, osteoclasts, macrophages, T-cells, hematopoietic cells, vascular smooth muscle cells, fibroblasts, myocardial cells, and other types of cells [16,17]. It has been found that OPN is chiefly involved in cellular migration or infiltration, restoration of tissues, and inflammatory diseases [18]. OPN as a multifunctional protein has been investigated in allergy and asthma only recently. Several studies have recently addressed the role of OPN in promoting chemotaxis inflammatory cells such as eosinophils and mast cells in asthma [19,20]. OPN was also found to be positively related to expression of Th2 cytokines. However, the relation was not very strong (IL-4, $r = 0.49$; IL-5, $r = 0.34$, IL-13, $r = 0.52$). These results suggested that OPN only plays partial role in Th1/2 regulation and other factors also contributed to Th1/2 regulation since allergic diseases are complex process. Previous studies also showed that OPN was involved in Th1 type inflammation. Thus, we supposed that OPN may be deeply involved in SLIT treatment. We first examined OPN protein level during SLIT treatment and found that SLIT treatment caused significant OPN decreasing from about one year after treatment. Interestingly, the changing trend of TNSS scores was almost the same with OPN, suggesting that OPN may play an important role in SLIT. To prove our postulation, we further investigated Th1/Th2/Treg cytokines changes during the treatment, as we expected, SLIT treatment caused a shift from Th2 to Th1 inflammation and this change occurred at least six months after SLIT treatment. However, our *in vitro* study suggested that OPN can affect Th2 cytokines rather than Th1 expression directly. Inconsistent with our study, both Konno and Yavuz's study [21,22] showed that increased expression of OPN was found in patients who completed one or 5 to 6 years of venom allergen immunotherapy. This complete opposite results may be attributed to methodological differences or even ethnical differences. For example, different methods/measurements in studies may liberate different amount of intracellular protein. Two studies performed by Liu's group [23,24] showed that OPN expression was enhanced in Th2 disease of Chinese population (nasal polypsis and allergic rhinitis), suggesting that successful SLIT should also inhibit OPN expression. In summary, the mechanisms of OPN in different immunotherapies need further study and exploration.

IL-10 and TGF- β were important protective cytokines as described in literature. IL-10 inhibits synthesis of both Th1 and Th2 inflammatory cytokines. TGF- β is believed to be important in

regulation of the immune system by Foxp3 + Regulatory T cell and the differentiation of both Foxp3 + Regulatory T cells, which can suppress immune reactions. However, our data showed that decreased OPN was not related to IL-10 and TGF- β expression. Furthermore, we stimulated PBMCs with allergen combined with anti-OPN and found significant down-regulation of Th2 cytokines level in PBMCs. Our data suggested that children received SLIT treatment showed decreased capacity of Th2 cytokines (IL-4, IL-5, IL-13) production by PBMCs after HDM stimulation. On the other hand, IL-10 was not changed significantly. These results suggested that OPN had a direct regulatory effect on Th2 cytokines rather than Th1 or Treg cytokines during SLIT.

5. Conclusion

Collectively, our finding showed that during SLIT, OPN expression was decreased one year after treatment and this down-regulation of OPN was related to low Th2 cytokine production. We also found that children with low baseline serum OPN level acquired better improvement of symptom and medication score. Although the improvement between the two groups was significant, the variance within groups suggested that further studies with large sample size were needed to prove our results. Moreover, the exact mechanism of OPN expression during SLIT is still largely unknown and needs further exploration.

Conflict of interest statement

None.

Acknowledgements

This study is funded by foundation of Heilongjiang Education Department Project (no. 12541277). The authors have no other funding, financial relationships, or conflicts of interest to disclose.

References

- [1] D. Strachan, B. Sibbald, S. Weiland, N. Ait-Khaled, G. Anabwani, H.R. Anderson, et al., Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the International Study of Asthma and Allergies in Childhood (ISAAC), *Pediatr. Allergy Immunol.* 8 (4) (1997) 161–176.
- [2] B. Björkstén, T. Clayton, P. Ellwood, A. Stewart, D. Strachan, ISAAC Phase III Study Group, Worldwide time trends for symptoms of rhinitis and conjunctivitis: phase III of the International Study of Asthma and Allergies in Childhood, *Pediatr. Allergy Immunol.* 19 (2) (2008) 110–124.
- [3] A.J. Frew, Sublingual immunotherapy, *N. Engl. J. Med.* 358 (21) (2008) 2259–2264.
- [4] C.A. Akdis, M. Akdis, Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens, *World. Allergy Organ. J.* 8 (1) (2015) 17.
- [5] S. Ashkar, G.F. Weber, V. Panoutsakopoulou, M.E. Sanchirico, M. Jansson, S. Zawaideh, et al., Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity, *Science* 287 (5454) (2000) 860–864.

- [6] A.W. O'Regan, G.L. Chupp, J.A. Lowry, M. Goetschkes, N. Mulligan, J.S. Berman, Osteopontin is associated with T cells in sarcoid granulomas and has T cell adhesive and cytokine-like properties in vitro, *J. Immunol.* 162 (2) (2001) 1024–1031.
- [7] Y. Koguchi, K. Kawakami, K. Uezu, K. Fukushima, S. Kon, M. Maeda, et al., High plasma osteopontin level and its relationship with interleukin-12-mediated type 1T helper cell response in tuberculosis, *Am. J. Respir. Crit. Care Med.* 167 (10) (2003) 1355–1359.
- [8] G. Xu, H. Nie, N. Li, W. Zheng, D. Zhang, G. Feng, et al., Role of osteopontin in amplification and perpetuation of rheumatoid synovitis, *J. Clin. Invest.* 115 (4) (2005) 1060–1067.
- [9] T. Sato, T. Nakai, N. Tamura, S. Okamoto, K. Matsuoka, A. Sakuraba, et al., Osteopontin/Eta-1 upregulated in Crohn's disease regulates the Th1 immune response, *Gut* 54 (9) (2005) 254–262.
- [10] M. Comabella, I. Pericot, R. Goertsches, C. Nos, M. Castillo, J. Blas Navarro, et al., Plasma osteopontin levels in multiple sclerosis, *J. Neuroimmunol.* 158 (1–2) (2005) 231–239.
- [11] D.C. Simoes, G. Xanthou, K. Petrochilou, V. Panoutsakopoulou, C. Roussos, C. Gratzou, Osteopontin deficiency protects against airway remodeling and hyper-responsiveness in chronic asthma, *Am. J. Respir. Crit. Care Med.* 179 (10) (2009) 894–902.
- [12] X. Lu, X.H. Zhang, H. Wang, X.B. Long, X.J. You, Q.X. Gao, et al., Expression of osteopontin in chronic rhinosinusitis with and without nasal polyps, *Allergy* 64 (1) (2009) 104–111.
- [13] E. Valovirta, L. Jacobsen, C. Ljorring, A. Koivikko, J. Savolainen, Clinical efficacy and safety of sublingual immunotherapy with tree pollen extract in children, *Allergy* 61 (10) (2006) 1177–1183.
- [14] K. Irander, J.P. Palm, B.P. Borres, B. Ghafouri, Clara cell protein in nasal lavage fluid and nasal nitric oxide-biomarkers with anti-inflammatory properties in allergic rhinitis, *Clin. Mol. Allergy* 6 (10) (2012) 4.
- [15] S.M. Nasser, S. Ying, Q. Meng, A.B. Kay, P.W. Ewan, IL-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy, *Eur. J. Immunol.* 31 (12) (2001) 3704–3713.
- [16] A. Koh, A.P. da Silva, A.K. Bansal, M. Bansal, C. Sun, H. Lee, et al., Role of osteopontin in neutrophil function, *Immunology* 122 (4) (2007) 466–475.
- [17] R. Patarca, G.J. Freeman, R.P. Singh, F.Y. Wei, T. Durfee, F. Blattner, et al., Structural and functional studies of the early T lymphocyte activation 1 (Eta-1) gene. Definition of a novel T cell-dependent response associated with genetic resistance to bacterial infection, *J. Exp. Med.* 170 (1) (1989) 145–146.
- [18] D.T. Denhardt, X. Guo, Osteopontin: a protein with diverse functions, *FASEB. J.* 7 (15) 1475–1482.
- [19] I. Puxeddu, N. Berkman, D. Ribatti, R. Bader, H.M. Haitchi, D.E. Davies, et al., Osteopontin is expressed and functional in human eosinophils, *Allergy* 65 (2) (2010) 168–174.
- [20] A. Takahashi, M. Kurokawa, S. Konno, K. Ito, S. Kon, S. Ashino, et al., Osteopontin is involved in migration of eosinophils in asthma, *Clin. Exp. Allergy* 39 (8) (2009) 1152–1159.
- [21] S. Konno, D.B. Golden, J. Schroeder, R.G. Hamilton, L.M. Lichtenstein, S.K. Huang, Increased expression of osteopontin is associated with long-term bee venom immunotherapy, *J. Allergy Clin. Immunol.* 115 (5) (2005) 1063–1067.
- [22] S.T. Yavuz, O.U. Soyer, B.E. Sekerel, B. Buyuktiyaki, O. Cavkaytar, U.M. Sahiner, et al., Increased osteopontin levels in children undergoing venom immunotherapy may serve as a marker of clinical efficacy, *Int. Arch. Allergy Immunol.* 165 (3) (2014) 206–213.
- [23] W.L. Liu, H. Zhang, Y. Zheng, H.T. Wang, F.H. Chen, L. Xu, et al., Expression and regulation of osteopontin in chronic rhinosinusitis with nasal polyps, *Clin. Exp. Allergy* 45 (2) (2015) 414–422.
- [24] W. Liu, W. Xia, Y. Fan, H. Wang, K. Zuo, Y. Lai, et al., Elevated serum osteopontin level is associated with blood eosinophilia and asthma comorbidity in patients with allergic rhinitis, *J. Allergy Clin. Immunol.* 130 (6) (2012) 1416–1418.