Int J Clin Exp Med 2016;9(9):17761-17771
www.ijcem.com /ISSN:1940-5901/IJCEM0031166

Original Article

Association of transcription factor T-Bet and transforming growth Factor-B_1 expression with allergic rhinitis in children with obstructive sleep apnea hypopnea syndrome

Fang Chen, Hongming Xu, Kun Ni, Limin Zhao, Xiaoyan Li

Department of Otorhinolaryngology and Head & Neck Surgery, Shanghai Children's Hospital , Shanghai Jiaotong University, Shanghai, China

Received April 25, 2016; Accepted July 30, 2016; Epub September 15, 2016; Published September 30, 2016

Abstract: Objective: This study aimed to investigate the expression of transcription factor T-bet and transforming growth factor beta 1 (TGF-β_1) in allergic rhinitis (AR) children with obstructive sleep apnea hypopnea syndrome (OSAHS) as well as their roles in pathological of allergic rhinitis. Methods: 140 children with OSAHS were recruited. Patients were divided into AR group and non-AR group. Results: T-bet expression in AR group was significantly lower than in non-AR group ($P < 0.05$). T-bet expression was related to the severity of adenoid hypertrophy, while it in III and IV degree was significantly lower than in I and II degree ($P < 0.05$). TGF-β_1 in AR group was markedly higher than in non-AR group ($P < 0.01$). The serum TGF-β_1 in AR group was significantly higher than in NAR group before surgery, but it was comparable between two groups at 3 months after surgery ($P > 0.05$). Moreover, serum TGF-β_1 reduced dramatically after surgery in in AR group and there was correlation between TGF-β_1 expression in peripheral blood and adenoid tissues ($P < 0.05$). There was negative correlation between T-bet expression and TGF-β_1 expression in adenoid tissues ($P < 0.01$). Conclusion: The T-bet expression in adenoid tissues and TGF-β_1 expression in adenoid tissues and blood are significant different between OSAHS children with and without AR. Significant correlation is observed between T-bet expression and TGF-β_1 expression in adenoid tissues, indicating potential roles of T-bet and TGF-β_1 in the pathogenesis of AR in OSAHS children.

Keywords: Obstructive sleep apnea hypopnea syndrome, allergic rhinitis, T-bet, TGF-β_1, adenoid tissue

Introduction

The obstructive sleep apnea hypopnea syndrome (OSAHS) is a common respiratory disease in children and caused by partial or complete collapse on the upper airway during sleep [1], resulting in intermittent hypoxemia and hypercapnia, frequent awaking and sleep pattern breaks [2-7], with a dramatic impact on the systemic health and development [8-10]. It has been reported that the incidence of OSAHS in children is approximately 2% depending on the diagnostic criteria [5, 7]. If left untreated, OSAHS may cause behavioral and nervous system-related damages [9, 11, 12], or even a series of complications including learning disabilities, hyperactivity, cognitive decline, Cor pulmonale and delayed growth and development [5, 11, 13, 14]. Currently, there are no ideal treatments available, and OSAHS children are commonly managed by tonsillectomy and adenoidectomy (T&A), which, however, have not been demonstrated to fully abolish apnea in all patients, and/or ventilation with positive airway pressure, which has a very poor compliance and is not appropriate for all children [8, 15, 16].

OSHAS is always complicated by other diseases such as the otitis media with effusion and allergic rhinitis (AR) [17-19]. Of them, AR, a common disease in children, is an important cause of nasal obstruction and often associated with adenoidal hypertrophy in children [5, 14, 19, 20]. It has also been recognized as a risk factor for pediatric OSHAS in various epidemiologic studies [14, 21]. Therefore, AR treatment is usually an integral part in the management of OSHAS children with AR.

Anderson et al found that CD4+ T cells are increased locally in the tonsils, whereas CD8+ T cells reduced. T cell population imbalances, such as Th1/Th2 imbalance, have been observed in OSHAS patients [22, 23]. AR is a nasal allergic response to environmental allergens characterized by increased production of antigen-specific immunoglobulin E (IgE) in the nasal mucosa, T helper (Th) 2 cells-skewed, and eosinophil infiltration [24-28]. Studies have revealed that the differentiation imbalance of two T-helper cell (Th) subsets, Th1/Th2, also plays an important role in the pathogenesis of AR, as a shift is observed in the Th1/Th2 cytokine balance towards increased Th2 activity and allergic symptoms can be alleviated by maintenance of immune balance [22, 29, 30].

Naive T cells may differentiate toward different T-cell subtypes depending on the expression of certain transcription factors [31]. T-box express in T cells (T-bet), a member of the T-box family of transcription factors, is expressed in multiple immune cells, such as CD4+ cells, CD8+ T cells, B cells, regulatory T (Treg) cells, natural killer (NK) cells and NK T cells, and it may regulate the development and/or effector functions of above immune cells [32-34]. It was initially identified as master regulator of Th1 differentiation [33-35], playing an important roles in the differentiation of Th0 cells into Thl and Th2 cells [29, 36-39] and thus the regulation of Th1/Th2 cytokine balance.

Several cytokines have been identified to be involved in the differentiation of Th1/Th2 cells [33]. Among these cytokines, transforming growth factor β1 (TGF-β_1), which is recently identified as a key anti-inflammatory cytokine [22], may down-regulate IgE synthesis, increase circulating TGF-β_1-producing T cells [40-43], negatively regulate Th1 cell development and plays an important role in the immune tolerance because Treg cells have the ability to directly inhibit activation of allergen-specific TH2 cells and suppress allergic inflammation in a TGF-β_1 dependent manner [44].

This study was undertaken to detect the expression of T-bet and TGF-β_1 in the adenoid tissues, the serum TGF-β_1 before and 3 months after surgery and the relationship among T-bet, TGF-β_1 and severity of disease in OSAHS children with AR, which will provide new targets for the therapy of AR in OSAHS children.

Materials and methods

Subjects

From December 2013 to July 2014, a total of 140 OSAHS children (71 males and 69 females) weighing 6.5 ± 1.2 years (range: 4-11 years) were recruited from the Department of Otorhinolaryngology Head and Neck Surgery, Affiliated Children's Hospital of Shanghai Jiaotong University. According to the Clinical Practice Guideline for the Diagnosis and Management of Childhood Obstructive Sleep Apnea Syndrome (draft) developed by the Chinese Association of Otorhinolaryngology Head and Neck and Otolaryngology in Urumqi in 2007, all the children underwent overnight polysomnography and were proven to have OSAHS according to the diagnostic criteria in above guideline. The medical history was reviewed, fiber nasopharyngoscopy was performed to assess the adenoid size, and skin prick tests were performed to identify allergens prior to endoscopic adenotonsillectomy. This study was approved by the Institutional Ethics Committee of Affiliated Children's Hospital of Shanghai Jiaotong University. The parents or guardians of all participants signed the informed consent before study.

Inclusion criteria

OSAHS was diagnosed according to following criteria: 1) Patients had clinical signs and symptoms: restless sleep, including snoring, mouth breathing, apnea, night crying, night terrors, enuresis, sweating, hyperactivity, daytime sleepiness, craniofacial abnormalities or adenoid face leading by long-term mouth breathing, and even cognitive deficits, memory deterioration, abnormal behavior and growth retardation; 2) Polysomnography was used for nocturnal sleep monitoring, and OSAHS was diagnosed if obstructive apnea index (OAI) > 1 time/h and/or apnea hypopnea index (AHI) > 5 times/h, with the lowest oxygen saturation ($LAaO_2$) < 92% were present; 3) Children with chronic respiratory/cardiac conditions, central sleep apnea/hypopnea syndrome (CSAHS), narcolepsy or other diseases were excluded.

Grouping

According to whether there was concomitant AR, OSAHS children were divided into AR group

(n=77; 35 males and 42 females; mean age: 5.8 ± 1.6 years [range: 3-10 years]) and non-AR group (n=63; 27 males and 36 females; mean age of 6.3 ± 1.1 years [range: 4-11 years).

Surgical methods

140 OSAHS children underwent surgery after tracheal intubation and intravenous anesthesia, and SERFAS Energy Probes (ArthroCare Corp, US) was used to remove the tonsil. ReFlex70 knife was used to remove the internal portion of the tonsil capsules under a nasal endoscope and the remaining part was handled by ablation. Postoperatively, the patients received anti-histamines and antibiotics treatment and intranasal administration of angiotonics and hormone.

Immunohistochemistry

Adenoid tissues were collected, fixed in 10% paraformaldehyde and cut into 4-μm sections which were then treated in xylene for 10 min twice and rehydrated in graded alcohols. Antigen retrieval was performed in citrate buffer (PH=6.0) for 12 min and then the sections were allowed to cool to room temperature. Endogenous peroxidase was inactivated with 3% hydrogen peroxide for 30 min at room temperature. After washing in PBS thrice (5 min for each), sections were incubated with protein blocking buffer for 30 min and then with primary antibodies (T-bet 1:50 and TGF-β_1 1:30) at 4°C overnight. In negative control group, sections were incubated with PBS instead of primary antibodies. On the next day, sections were incubated for 30 min-45 min at room temperature and washed thrice (10 min for each) with PBS, followed by incubation with biotin conjugated anti-mouse secondary antibody (Jackson, Anti-mouse IgG, 1:100) for 20 min. Visualization was done with diamino-benzidine diaminobenizidine (DAB); were for 3-10 min at room temperature. After rinsing in PBS, the sections were counterstained with hematoxylin, dehydrated, and finally mounted. Images were photographed with the OLYMPUS CX-40 Microscope (Olympus, Japan) at a magnification of × 400.

The pathological scoring was performed as described previously [17]. Nuclear expression of T-bet was graded from 0 to 4 according to the proportion of positive cells (0, ≤ 5%; 1+, 1%-25%; 2+, 6%-25%; 3+, 26%-75%; 4+, > 75%). Cytoplasmic expression of TGF-β_1 was graded from 1 to 3 according to the staining intensity (1+, light yellow; 2+, yellow; 3+, brown yellow). The product of both scores was graded from - to +++ (-, 0; +, 1-3; ++, 4-5; +++, > 5) [17]. All sections were blindly evaluated.

Immunofluorescence staining

Immunofluorescence staining was also performed for the detection of T-bet and TGF-β_1 expression in the adenoid tissues. Tissues were cut into 4-μm sections and fixed in acetone at 4°C for 10 min. After washing in PBS thrice (15 min for each), sections were permeabilized with 0.3% Triton X-100 and then blocked for 30 min with 3% BSA, followed by incubation at 4°C overnight with primary antibodies (T-bet 1:50 and TGF-β_1 1:30) or PBS in negative control. The sections were washed thrice in PBS (15 min for each) and then incubated with Cy3-conjugated anti-mouse secondary antibody (1:100) for 30 min in dark. Counterstaining of the sections was performed using DAPY (1:1000) for 5-10 min. Fluorescent images were photographed using OLYMPUS CX-40 Microscope (Olympus, Japan).

The pathological scoring was performed as described previously [18]. The fluorescence intensity was graded from 0 to 3+ (-, no or weakly visible fluorescence; +, visible fluorescence; ++, bright fluorescence; +++, dazzling fluorescence). All sections were blindly evaluated.

TGF-β_1 expression in peripheral blood

Blood was collected from OSAHS children during surgery and serum was separated. TGF-β_1 content of the peripheral blood was measured by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions.

Blood detections

In brief, 4 mL peripheral blood was collected from OSAHS children and anti-coagulated with EDTA before and at 1 and 3 months after surgery. An automatic biochemical analyzer (OlympusAU5400) was used to detect the levels of IgA, IgM and IgG in the peripheral blood by turbidimetric analysis. A flow cytometer (BD FACS can flow cytometry, Becton Dickinson, USA)

Table 1. T-bet expression in AR group and non-AR group (n=140)

Groups	T-bet expression	χ^2	P
AR (n=77)	37 (48.1%)	4.072	0.044
Non-AR (n=63)	41 (65.1%)		

Table 2. T-bet expression of different intensities in AR group and non-AR group

Group	T-bet positive patients (n)	T-bet			χ^2	P
		(+)	(++)	(+++)		
AR	37	19	11	7	9.757	0.008
Non-AR	41	8	15	18		

Table 3. T-bet expression in AR patients with different degree of adenoid hypertrophy

Group	n	T-bet		χ^2	P
		-	+/++/+++		
AR	77				
I		9	10	5.758	0.016
II		6	14		
III		14	5		
IV		11	8		

Table 4. TGF-β_1 expression in AR group and non-AR group (n=140)

Groups	TGF-β_1 expression	χ^2	P
AR (n=77)	50 (64.9%)	7.820	0.005
Non-AR (n=63)	26 (41.3%)		

Table 5. TGF-β_1 expression of different intensities in AR group and non-AR group

Group	TGF-β_1 positive patients (n)	TGF-β_1			χ^2	P
		(I)	(II)	(III)		
AR	50	9	14	27	11.007	0.004
Non-AR	26	13	8	5		

was used to determine the CD16+ 56+/CD45+ T-lymphocyte, CD19+ B-lymphocyte, CD3+, CD4+ and CD8+ T-lymphocytes, and then the CD4+/CD8+ ratio was calculated. During the hospitalization, children did not receive treatment affecting the immune function except for antibiotics used in perioperative period.

Statistical analysis

All statistical analyses were conducted using the SPSS version 16.0. Categorical variables were compared with chi-square test. The relationships between variables were determined using the Spearman's correlation analysis. The paired data was assessed according to the paired t-test. The measurement data were evaluated using one way analysis of variance. Data are presented as means ± standard deviation (SD) and a value of $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics

A total of 140 patients were recruited into present study. There were 62 males and 78 females with a mean age of 6.7 ± 1.4 years. In addition, age of onset was 3-6 years in 50 patients, 6-9 years in 63, and 9-11 years in 27. There was no significant difference in age between males and females ($P > 0.05$). These patients received sleep test and divided into four groups according to the severity of OSAHS determined on the basis of clinical manifestations and AHI score determined by PSG: mild in 16.4% OSAHS ($1 \leq OAI \leq 5$ and/or $5 \leq AHI \leq 10$, with $0.85 \leq LAaO_2 \leq 0.91$; 10 males and 13 females), moderate in 52.1% ($5 < OAI \leq 10$ and/or $10 < AHI \leq 20$, with $0.75 \leq LAaO_2 \leq 0.84$; 34 males and 39 females) and severe in 31.5% (OAI > 10 times/h and/or AHI > 20 times/h, with $LAaO_2 < 0.75$; 21 males and 23 females =.

There were 77 patients in AR group (35 males and 42 females) with a mean age of 5.8 ± 1.6 years (range: 3-10 years) and 63 patients in non-AR group (27 males and 36 females) with a mean age of 6.3 ± 1.1 years (range: 4-11 years). There were no significant differences in age and gender between two groups.

Among 140 patients, 103 children had clinical signs and symptoms of snoring together with mouth breathing, 71 had additional apnea, and 45 had additional night terrors needing the change of body position for fall asleep. Of 140 patients, 34 had serious sleep apnea and 20 had craniofacial abnormalities or adenoid face leading to long-term mouth breathing.

3 children had complications of circulating system due to long term hypoxia, such as the pulmonary artery and right cardiac hypertrophy. In addition, rhinosinusitis was observed in 24 patients, AR in 77, chronic tonsilitis in 89 and secretory otitis media in 97.

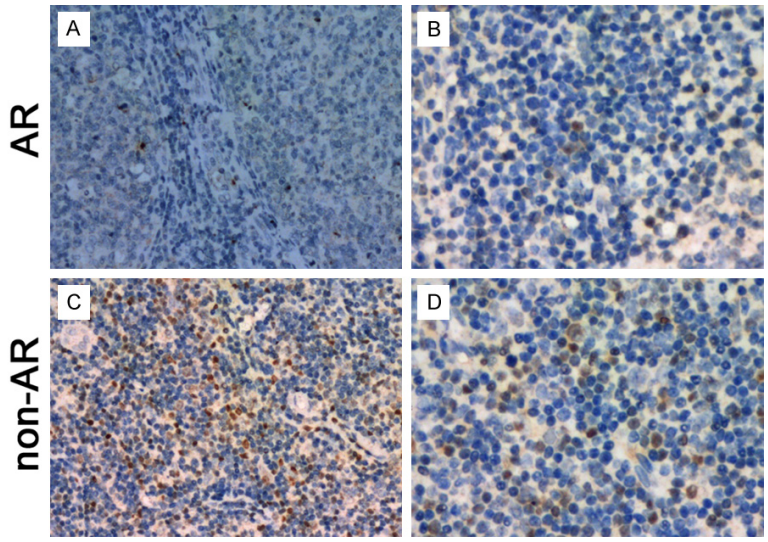

Figure 1. Immunohistochemistry for T-bet in adenoid tissues from AR patients and non-AR patients. left: 200 ×; right: 400 ×.

Fiber nasopharyngoscopy

All the patients enrolled received fiber nasopharyngoscopy by an experienced endoscopist. Based on the relation with narium choana, the severity of adenoid hypertrophy was divided into I degree (0-25% nostril blockage), II degree (26-50% blockage), III degree (51-75% blockage), and IV level (76-100% blockage) [22, 45]. In our study, I and II degree was found in 27 and 34 patients, respectively, in whom mild hypertrophy was present and obstruction of the airway was not observed. III degree was found in 36 patients in whom moderate hypertrophy was present, and IV degree was noted in 43 patients in whom were severe hypertrophy was observed (**Table 5**) [22].

Skin prick tests (SPTs)

Patients did not receive antihistamine treatment at least 7 days before SPTs for dermatophagoides alone. The wheal equal to or larger than 3 mm in diameter (++) was suggestive of positive reaction (AR) [46]. SPT reactivity to dermatophagoides farinae alone was found in 8 patients (+++) and 6 patients (++++), to dermatophagoides pteronyssinus alone in 9 patients (+++) and 7 patients (++++), to both dermatophagoides farinae and dermatophagoides pteronyssinus in 47 patients: dermatophagoides farinae (+++) and dermatophagoides pteronyssinus (+++) in 10, dermatophagoides farinae (++++) and dermatophagoides pteronyssinus (++++) in 11 patients, dermatophagoides farinae (+++) and dermatophagoides pteronyssinus (++++) in 14 and dermatophagoides farinae (++++) and dermatophagoides pteronyssinus (+++) in 12.

Acoustic immittance measurement was performed before surgery. Tympanometry curve of type A was found in 66 ears, type A in 36 ears, type C in 81ears, and type B in 97 ears. For patients with type B, conventional pure-tone audiometry was conducted before tympanostomy or indwelling of ventilation tube. The mean age of children receiving tympanostomy was 7.1 ± 1.3 years range: 5-9 years), the median course of disease was 1.6 years (range: 6 months to 2 years) and the mean pure tone auditory threshold was 27.5 ± 3.8 dB nHL before surgery. In children receiving indwelling of ventilation tube, the mean age was 6.7 ± 1.5 years (range: 4-9 years), the median course of disease was 1.8 years (range: 8 months to 3 years), and the mean pure tone auditory threshold was 26.7 ± 2.6 dB nHL before surgery.

T-bet expression in adenoid tissues

Adenoid tissues were collected for the detection of T-bet expression in AR group and non-AR group by immunohistochemistry (**Figures 1** and **2**). 37 (48.1%) patients in AR group and 41 (65.1%) patients in non-AR group were positive for T-bet expression, showing significant difference between two groups in the proportion of patients positive for T-bet expression (χ^2=4.072, P=0.044 < 0.05) (**Table 1**). The intensity of T-bet expression in non-AR group (43.90% for "+++") was stronger than in AR group (51.35% for "+") (χ^2=9.757, P=0.008 < 0.05) (**Table 2**). There was correlation between T-bet expression and severity of adenoid hypertrophy: T-bet expression in III and IV degree was significantly lower than in I and II degree (χ^2=5.758, P=0.016 < 0.05; **Table 3**).

TGF-β_1 expression in adenoid tissues

TGF-β_1 expression was detectable in adenoid tissues of both AR group and non-AR group and mainly found in the cytoplasm (**Figures 3** and

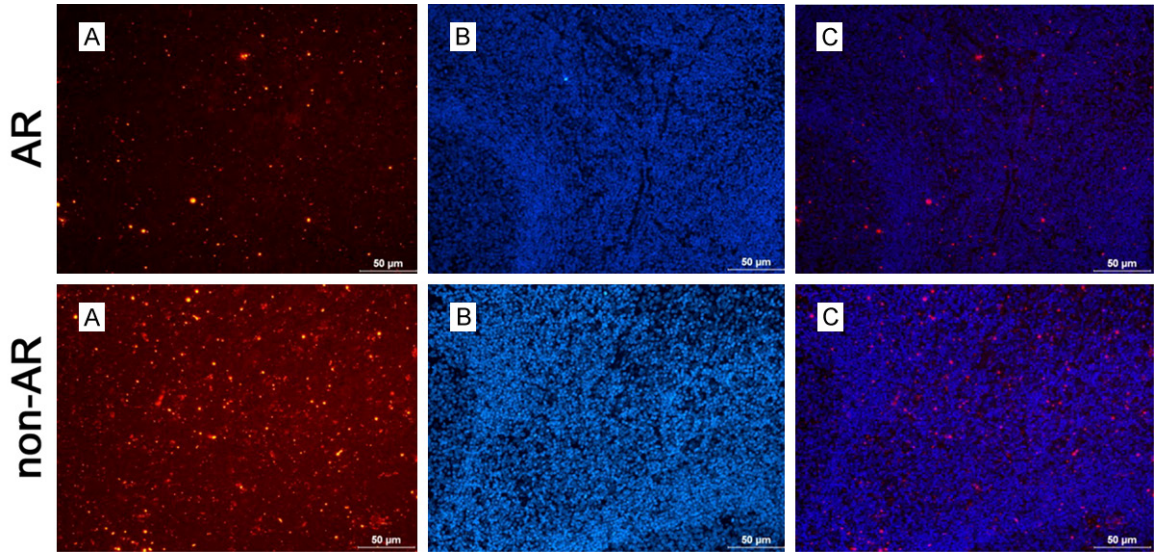

Figure 2. T-bet + cells in the human adenoid tissues. T-bet (red) positive cells (A) and DAPI (blue) positive nuclei (B) were observed under a fluorescence microscope. (C) mixed (A and B). Scale bar: 50 μm. DAPI, 4'-6-Diamidino-2-phenylindole, dihydrochloride.

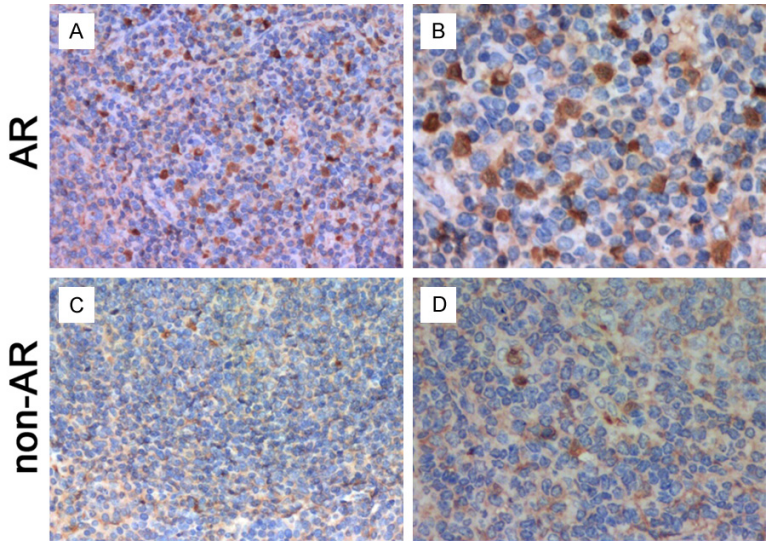

Figure 3. Immunohistochemistry for TGF-β_1 in adenoid tissues from AR patients and non-AR patients. left: 200 ×; right: 400 ×.

4). In addition, 50 patients in AR group (64.9%) and 26 patients in non-AR group (41.3%) were positive for TGF-β_1 expression, showing significant difference between two groups (χ^2=7.820, P=0.005 < 0.01) (**Table 4**). The intensity of TGF-β_1 expression in AR group (54.0% for "+++") was significantly stronger than in non-AR group (50.00% for "+") (χ^2=11.007, P=0.004 < 0.01) (**Table 5**). There was no correlation between TGF-β_1 expression and severity of adenoid hypertrophy (χ^2=3.228, P=0.072 > 0.05) (**Table 6**).

There was negative correlation between T-bet expression and TGF-β_1 expression in adenoid tissues of AR group and non-AR group (r^2=-0.697, P < 0.01).

Discussion

OSAHS is a common respiratory disease in children and most commonly caused by adenotonsillar hypertrophy. OSAHS is closely associated with AR [17]. T cell population imbalances, including Th1/Th2 imbalance, have been observed in patients with either OSHAS or AR [22-24]. T-bet, a protein mainly expressed in CD4+ T cells and NK cells, is a T-box transcription factor critical for the differentiation of Th1 consigning T cells into Th1 subset [32, 35]. T-bet also antagonizes Gata3, therefore down-regulating Th2 cytokines and inducing the differentiation of Th2 cells to Th1 cells [46, 47]. Szabo et al [48] found retrovirus mediated transduction of T-bet gene could induce the differentiation of Th2 cells into Th1 cells. T-bet knockout mice may develop airway inflammation without allergen stimulation because T-bet deficiency reduces the differentiation of Th1 cells and expression of cytokines

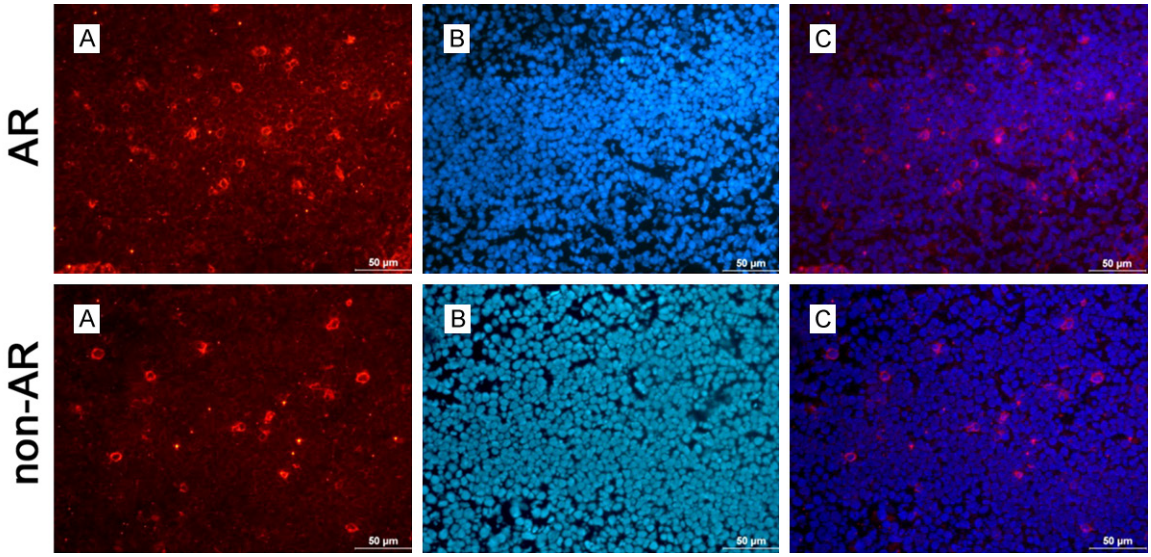

Figure 4. TGF-β_1+ cells in human adenoids. TGF-β_1 (red) positive cells (A) and DAPI (blue) positive nuclei (B) were observed under a fluorescence microscope. (C) mixed (A and B) Scale bar: 50 μm. DAPI, 4'-6-Diamidino-2-phenylindole, dihydrochloride.

Table 6. TGF-β_1 expression in AR patients with adenoid hypertrophy of different degrees

Group	n	TGF-β_1		χ^2	P
		-	+/++/+++		
AR	77				
I		7	11	3.228	0.072
II		5	12		
III		10	9		
IV		13	10		

in Th1 cells. In the absence of T-bet induced inhibition, there would be significant increase in Th2 cells and Th2 cytokines. Our results showed that the T-bet expression in adenoid tissues increased in OSAHS patients, and it in OSAHS + AR patients was significantly lower than in OSAHS + non-AR patients. This may be related to the lower local Th1 cytokines in the respiratory tract and thus Th2 cytokines increase in the local mucosa of AR and asthmatic patients [49] due to absence of inhibition on Th1 cells and related cytokines. T-bet as a Th1 related transcription factor shows a reduced expression in the adenoid tissues is an important cause of Th1/Th2 imbalance. Thus, to correct the Th1/Th2 imbalance by targeting transcription factor T-bet will be helpful for clinical inoculation of immunoadjuvants in the lymphoid tissue of nasopharynx and for the down-regulation of local immune response because T-bet is able to induce the inhibition of Th2 shift, leading to Th1 shift.

Adenoid tissues are an important component of the pharyngeal lymphoid ring and also an immune organ. There are a lot of lymphocytes at different developmental stages including B lymphocytes, T lymphocytes, T cells and dendritic cells. They have immune regulatory function as in central and peripheral lymphoid organs. In our study, the severity of adenoid hypertrophy had a negative correlation with the T-bet expression in OSAHS + AR group, while the T-bet expression was similar in patients with different degrees of adenoid hypertrophy in OSAHS + non-AR group. The primary cause of adenoidal hypertrophy in children is non-specific chronic inflammation except for physiological enlargement. Subepithelial and intraepithelial lymphocytes in the adenoid tissues play an important role in both local and systemic immune response. CD4+ T cells in the adenoid tissues increase significantly and becomes activated when there is presence of repeated inflammatory stimulation in the adenoid tissues. The T-bet expression is dependent on CD4+ T cell, and thus T-bet expression was higher in the adenoid tissues of OSAHS + non-AR group and it had no relationship with the severity of adenoid hypertrophy but was related to the local inflammation. Allergic inflammation in local stimulation is stronger in the OSAHS +

AR group than the non-allergic inflammation when the repeated stimulation is present. The low T-bet expression is mainly ascribed to the Th2 dominance and the reduced production of IFN-γ and IL-12 by CD4+ T and the increased production of IL-4 and IL-5/IL-13 (Th2 cytokines), leading to the attenuation of focal inflammation due to cell infiltration [35]. This indicates that the stronger local allergic reaction may cause more severe adenoidal hypertrophy, and the enhanced Th2 immunity may reduce low T-bet expression, which was consistent with our findings. Marek et al [51] confirmed that hormone and antihistamine were able to attenuate adenoidal hypertrophy and nasal airway obstruction syndrome in children with allergy. Thus, early prevention of allergy is helpful for the attenuation of adenoidal hypertrophy.

TGF-β_1, a member of TGF-β superfamily, is a polyphenic cytokine with multiple regulatory functions and can regulate immune homeostasis by suppressing the differentiation of naive T cells toward Th1/Th2 subtypes [50]. TGF-β_1 can enhance the recruitment of nasal epithelial mast cells, which is a major characteristic of AR, and the activated mast cells may secret a variety of inflammatory mediators leading to the deterioration of local allergic reaction. Moreover, TGF-β_1 can also promote the differentiation of mast cells. TGF-β_1 may act on mast cells and neutrophils to cause their degranulation, which plays an important role in local inflammation. Our results showed TGF-β_1 expression was detectable in the adenoid tissues of OSAHS children with and without AR, but the TGF-β_1 expression in AR children was significantly higher than in non-AR children, which may be related to the local recruitment and accumulation of mast cells [51].

In addition, TGF-β_1 has both pro-inflammatory and anti-inflammatory activities in the pathophysiology of asthma. TGF-β_1 at a high concentration is involved in the repair process of the airway with inflammatory injury and inhibit the inflammation. However, repeated stimulation may cause chronic inflammation, leading to the excess production of TGF-β_1, which may cause tissue fibrosis, airway remodeling and chronic obstruction [50]. On the other hand, excess production of TGF-β_1 has been found in local lymphoid tissues of AR patients. In the development of AR, TGF-β_1 is excessively produced in local lymphoid tissues and long lasting allergen stimulation may result in damage to the mucosal epithelial cells, goblet cells metaplasia and accumulation of extracellular matrix [33]. Thus, TGF-β_1 is possibly an important factor participating in the remodeling of mucous epithelium [34].

It has been reported that TGF-β_1 from the peripheral blood may increase circulating Treg T cells which then further release TGF-β, leading to the down-regulation of Th2 immune response and the attenuation of allergic reaction [52]. In the present study, the TGF-β_1 content of the peripheral blood was also measured in OSAHS children. Results showed serum TGF-β_1 in AR group was significantly higher than in non-AR group, consistent with its expression in adenoid tissues. This may be closely related to the immunoregulatory effect of TGF-β_1 on the proliferation of T-cells and B-cells, leading to the inhibition of Th2 cells and the Th2 cytokine production [55]. The TGF-β_1 expression in blood has similar trend as in adenoid tissues, which indicates it plays an important role in local and systemic immune responses.

At 1 month after radiofrequency ablation of tonsil adenoidal hypertrophy, the CD4+ T lymphocytes markedly increased, whereas CD8+ T lymphocytes reduced significantly, accompanied by increased CD4+/CD8+ ratio in OSAHS children, but the CD3T and CD19B remained unchanged. At 3 months after surgery, these returned to x baseline level. The IgA, IgG and IgM at 1 month after surgery slightly decreased as compared to those before surgery, but were in normal range of children and returned to preoperative levels at 3 months after surgery. This may be explained as follows: a low dose antigen is presented to dendritic cells in the germinal center of lymphoid nodules via M cells and reticular cells of APC cells, leading to B cell response, the generation of immunoglobulin and the memory clonal amplification [53]. This indicates that the immune function returns to normal at 3 months after surgery without impairment of host immunity. As compared to the serum TGF-β_1 before surgery in AR group, it decreased significantly when the immune function returned to normal after adenotonsillectomy and was comparable to that in non-AR group. This may be related to the reduction in antigen stimulation due to the removal of adenoids and tonsils and then the local allergic

reaction is attenuated and its progression delayed. It is clear that TGF-β_1 is not only able to activate epithelial cells in AR and enhance the chemotaxis of mast cells, but plays an important role in the local and systemic immune responses by inhibiting the proliferation of T cells and the inflammatory response [38].

Our results show that the TGF-β_1 in peripheral blood and adenoid tissues increased in OSAHS group and was significantly higher in OSAHS children with AR. The TGF-β_1 expression was associated with the severity of adenoid hypertrophy, which further illustrates this point. The differential expression of TGF-β_1 in AR group and non-AR group in local tissue, and consistency in TGF-β_1 expression between peripheral blood and adenoid tissues indicated that TGF-β_1 plays an important role in the local and systemic immune responses and allergic diseases.

T-bet is a T-box transcription factor that can inhibit T lymphocyte immune, and mainly induce the differentiation of naive T cells into Th1 subset, while another anti-inflammatory cytokine TGF-β_1 is involved in the inhibition effect to both Th1 and Th2, which plays an important role in the immunoregulation. In this study, the correlation between T-bet expression and TGF-β_1 expression in adenoid tissues was evaluated in AR group and non-AR group. A negative correlation between T-bet expression and TGF-β_1 expression was observed. This indicates that the TGF-β_1 induced inhibition of Th1 shift is related to the T-bet. TGF-β_1 may inhibit the Itk expression, Ca^{2+} signal transduction and NFATcl activation, or protein phosphotyrosine phosphatase SHP-1, exerting its inhibitory effect. The retrovirus mediated transduction of T-bet gene has been found to reverse the TGF-β_1 induced inhibition of Th1 shift, which also confirms that T-bet is a key factor in the TGF-β_1 induced inhibition of Th1 shift. On the basis of available findings, T-bet and TGF-β_1 may interact with each other to maintain the Th1/Th2 balance. Thus, to correct the Th1/Th2 imbalance by targeting T-bet and TGF-β_1 may be helpful for the reversal of abnormal shift, which also provides targets for the therapy of allergic diseases including AR.

Disclosure of conflict of interest

None.

Address correspondence to: Xiaoyan Li, Department of Otorhinolaryngology and Head & Neck Surgery, Shanghai Children's Hospital , Shanghai Jiaotong University, Shanghai, China. Tel: +86-13918299182; E-mail: lixiaoyandoc@sina.com

References

[1] Gonzalez Mangado N, Troncoso Acevedo MF and Gomez Garcia T. Home ventilation therapy in obstructive sleep apnea-hypopnea syndrome. Arch Bronconeumol 2014; 50: 528-534.

[2] Kaditis A, Kheirandish-Gozal L and Gozal D. Pediatric OSAS: Oximetry can provide answers when polysomnography is not available. Sleep Med Rev 2015; 27: 96-105.

[3] Marcus CL, Moore RH, Rosen CL, Giordani B, Garetz SL, Taylor HG, Mitchell RB, Amin R, Katz ES, Arens R, Paruthi S, Muzumdar H, Gozal D, Thomas NH, Ware J, Beebe D, Snyder K, Elden L, Sprecher RC, Willging P, Jones D, Bent JP, Hoban T, Chervin RD, Ellenberg SS, Redline S; Childhood Adenotonsillectomy Trial (CHAT). A randomized trial of adenotonsillectomy for childhood sleep apnea. N Engl J Med 2013; 368: 2366-2376.

[4] Lee CH, Kang KT, Weng WC, Lee PL and Hsu WC. Quality of life after adenotonsillectomy for children with sleep-disordered breathing: a linear mixed model analysis. Int J Pediatr Otorhinolaryngol 2014; 78: 1374-1380.

[5] Ramos RT, da Cunha Daltro CH, Gregorio PB, de Freitas Souza LS, de Andrade NA, de Souza Andrade Filho A, de Souza Machado Junior A. OSAS in children: clinical and polysomnographic respiratory profile. Braz J Otorhinolaryngol 2006; 72: 355-361.

[6] Goldstein NA, Pugazhendhi V, Rao SM, Weedon J, Campbell TF, Goldman AC, Post JC and Rao M. Clinical assessment of pediatric obstructive sleep apnea. Pediatrics 2004; 114: 33-43.

[7] Kotagal S. Sleep disorders in childhood. Neurol Clin 2003; 21: 961-981.

[8] Huynh NT, Desplats E and Almeida FR. Orthodontics treatments for managing obstructive sleep apnea syndrome in children: A systematic review and meta-analysis. Sleep Med Rev 2016; 25: 84-94.

[9] Lal C, Strange C and Bachman D. Neurocognitive impairment in obstructive sleep apnea. Chest 2012; 141: 1601-1610.

[10] Capua M, Ahmadi N and Shapiro C. Overview of obstructive sleep apnea in children: exploring the role of dentists in diagnosis and treatment. J Can Dent Assoc 2009; 75: 285-289.

[11] Dai ZY, Huang DY and Zhou CY. Effects of partial tonsillectomy on the immune functions of children with obstructive sleep apnea-hypopnea syndrome at early stage. Genet Mol Res 2014; 13: 3895-3902.

[12] Landau YE, Bar-Yishay O, Greenberg-Dotan S, Goldbart AD, Tarasiuk A and Tal A. Impaired behavioral and neurocognitive function in preschool children with obstructive sleep apnea. Pediatr Pulmonol 2012; 47: 180-188.

[13] Hill CM, Hogan AM, Onugha N, Harrison D, Cooper S, McGrigor VJ, Datta A and Kirkham FJ. Increased cerebral blood flow velocity in children with mild sleep-disordered breathing: a possible association with abnormal neuropsychological function. Pediatrics 2006; 118: e1100-1108.

[14] Cheng PW, Fang KM, Su HW and Huang TW. Improved objective outcomes and quality of life after adenotonsillectomy with inferior turbinate reduction in pediatric obstructive sleep apnea with inferior turbinate hypertrophy. Laryngoscope 2012; 122: 2850-2854.

[15] Friedman M, Wilson M, Lin HC and Chang HW. Updated systematic review of tonsillectomy and adenoidectomy for treatment of pediatric obstructive sleep apnea/hypopnea syndrome. Otolaryngol Head Neck Surg 2009; 140: 800-808.

[16] Bhattacharjee R, Kheirandish-Gozal L, Spruyt K, Mitchell RB, Promchiarak J, Simakajornboon N, Kaditis AG, Splaingard D, Splaingard M, Brooks LJ, Marcus CL, Sin S, Arens R, Verhulst SL and Gozal D. Adenotonsillectomy outcomes in treatment of obstructive sleep apnea in children: a multicenter retrospective study. Am J Respir Crit Care Med 2010; 182: 676-683.

[17] Szalmas A, Papp Z, Csomor P, Konya J, Sziklai I, Szekanecz Z and Karosi T. Microbiological profile of adenoid hypertrophy correlates to clinical diagnosis in children. Biomed Res Int 2013; 2013: 629607.

[18] Braun T, Dreher A, Dirr F, Reichel O and Patscheider M. [Pediatric OSAS and otitis media with effusion]. HNO 2012; 60: 216-219.

[19] Sih T and Mion O. Allergic rhinitis in the child and associated comorbidities. Pediatr Allergy Immunol 2010; 21: e107-113.

[20] Rondon C, Fernandez J, Canto G and Blanca M. Local allergic rhinitis: concept, clinical manifestations, and diagnostic approach. J Investig Allergol Clin Immunol 2010; 20: 364-371; quiz 362 p following 371.

[21] Ng DK, Chan CH, Hwang GY, Chow PY and Kwok KL. A review of the roles of allergic rhinitis in childhood obstructive sleep apnea syndrome. Allergy Asthma Proc 2006; 27: 240-242.

[22] Ni K, Zhao L, Wu J, Chen W, HongyaYang and Li X. Th17/Treg balance in children with obstructive sleep apnea syndrome and the relationship with allergic rhinitis. Int J Pediatr Otorhinolaryngol 2015; 79: 1448-1454.

[23] Anderson ME Jr, Buchwald ZS, Ko J, Aurora R and Sanford T. Patients with pediatric obstructive sleep apnea show altered T-cell populations with a dominant TH17 profile. Otolaryngol Head Neck Surg 2014; 150: 880-886.

[24] Stelmaszczyk-Emmel A, Zawadzka-Krajewska A, Glodkowska-Mrowka E and Demkow U. FoxP3 Tregs Response to Sublingual Allergen Specific Immunotherapy in Children Depends on the Manifestation of Allergy. J Immunol Res 2015; 2015: 731381.

[25] Stelmaszczyk-Emmel A. Regulatory T cells in children with allergy and asthma: it is time to act. Respir Physiol Neurobiol 2015; 209: 59-63.

[26] Ring J, Akdis C, Behrendt H, Lauener RP, Schappi G, Akdis M, Ammann W, de Beaumont O, Bieber T, Bienenstock J, Blaser K, Bochner B, Bousquet J, Crameri R, Custovic A, Czerkinsky C, Darsow U, Denburg J, Drazen J, de Villiers EM, Fire A, Galli S, Haahtela T, zur Hausen H, Hildemann S, Holgate S, Holt P, Jakob T, Jung A, Kemeny M, Koren H, Leung D, Lockey R, Marone G, Mempel M, Menne B, Menz G, Mueller U, von Mutius E, Ollert M, O'Mahony L, Pawankar R, Renz H, Platts-Mills T, Roduit C, Schmidt-Weber C, Traidl-Hoffmann C, Wahn U and Rietschel E. Davos declaration: allergy as a global problem. Allergy 2012; 67: 141-143.

[27] Tang H, Jiang H, Zheng J, Li J, Wei Y, Xu G and Li H. MicroRNA-106b regulates pro-allergic properties of dendritic cells and Th2 polarisation by targeting early growth response-2 in vitro. Int Immunopharmacol 2015; 28: 866-874.

[28] Greiner AN, Hellings PW, Rotiroti G and Scadding GK. Allergic rhinitis. Lancet 2011; 378: 2112-2122.

[29] Luo Y, Deng Y, Tao Z, Chen S, Xiao B, Ren J, Chen Z, Han J, Kong Y, Xu Y and Deng M. Regulatory effect of microRNA-135a on the Th1/Th2 imbalance in a murine model of allergic rhinitis. Exp Ther Med 2014; 8: 1105-1110.

[30] Yamamoto K, Kawamura I, Tominaga T, Nomura T, Kohda C, Ito J and Mitsuyama M. Listeriolysin O, a cytolysin derived from Listeria monocytogenes, inhibits generation of ovalbumin-specific Th2 immune response by skewing maturation of antigen-specific T cells into Th1 cells. Clin Exp Immunol 2005; 142: 268-274.

[31] Van Bruaene N, Perez-Novo CA, Basinski TM, Van Zele T, Holtappels G, De Ruyck N, Schmidt-Weber C, Akdis C, Van Cauwenberge P, Bachert C and Gevaert P. T-cell regulation in chronic paranasal sinus disease. J Allergy Clin Immunol 2008; 121: 1435-1441, 1441, e1431-1433.

[32] Mulligan AM, Pinnaduwage D, Tchatchou S, Bull SB and Andrulis IL. Validation of Intra-

tumoral T-bet-positive Lymphoid Cells as Predictors of Disease Free Survival in Breast Cancer. Cancer Immunol Res 2016; 4: 41-8.
[33] Yang B, Zhai F, Jiang J, Wang X, Cao Z and Cheng X. Elevated expression of T-bet in mycobacterial antigen-specific CD4(+) T cells from patients with tuberculosis. Cell Immunol 2015; 298: 1-8.
[34] Lazarevic V, Glimcher LH and Lord GM. T-bet: a bridge between innate and adaptive immunity. Nat Rev Immunol 2013; 13: 777-789.
[35] Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG and Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 2000; 100: 655-669.
[36] Yagi R, Zhu J and Paul WE. An updated view on transcription factor GATA3-mediated regulation of Th1 and Th2 cell differentiation. Int Immunol 2011; 23: 415-420.
[37] Naito T, Tanaka H, Naoe Y and Taniuchi I. Transcriptional control of T-cell development. Int Immunol 2011; 23: 661-668.
[38] Paul WE. What determines Th2 differentiation, in vitro and in vivo? Immunol Cell Biol 2010; 88: 236-239.
[39] Zhu J. Transcriptional regulation of Th2 cell differentiation. Immunol Cell Biol 2010; 88: 244-249.
[40] Urra JM, Carrasco P, Feo-Brito F, De La Roca F, Guerra F and Cabrera CM. Immunotherapy reduces CD40L expression and modifies cytokine production in the CD4 cells of pollen allergy patients. J Investig Allergol Clin Immunol 2014; 24: 98-105.
[41] Akdis CA and Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. J Allergy Clin Immunol 2009; 123: 735-746; quiz 747-738.
[42] Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, Francis JN and Durham SR. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. J Immunol 2007; 178: 4658-4666.
[43] Ajduk J, Marinic I, Aberle N, Rabatic S and Gagro A. Effect of house dust mite immunotherapy on transforming growth factor beta1-producing T cells in asthmatic children. Ann Allergy Asthma Immunol 2008; 100: 314-322.
[44] Monteleone G, Del Vecchio Blanco G, Palmieri G, Vavassori P, Monteleone I, Colantoni A, Battista S, Spagnoli LG, Romano M, Borrelli M, MacDonald TT and Pallone F. Induction and regulation of Smad7 in the gastric mucosa of patients with Helicobacter pylori infection. Gastroenterology 2004; 126: 674-682.
[45] Ganz FD. Sleep and immune function. Crit Care Nurse 2012; 32: e19-25.
[46] Fields PE, Kim ST and Flavell RA. Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. J Immunol 2002; 169: 647-650.
[47] Loke WS, Freeman A, Garthwaite L, Prazakova S, Park M, Hsu K, Thomas PS and Herbert C. T-bet and interleukin-27: possible TH1 immunomodulators of sarcoidosis. Inflammopharmacology 2015; 23: 283-290.
[48] Mullen AC, High FA, Hutchins AS, Lee HW, Villarino AV, Livingston DM, Kung AL, Cereb N, Yao TP, Yang SY and Reiner SL. Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. Science 2001; 292: 1907-1910.
[49] Nakamura Y, Ghaffar O, Olivenstein R, Taha RA, Soussi-Gounni A, Zhang DH, Ray A and Hamid Q. Gene expression of the GATA-3 transcription factor is increased in atopic asthma. J Allergy Clin Immunol 1999; 103: 215-222.
[50] Nakao A. Is TGF-beta1 the key to suppression of human asthma? Trends Immunol 2001; 22: 115-118.
[51] Salib RJ, Kumar S, Wilson SJ and Howarth PH. Nasal mucosal immunoexpression of the mast cell chemoattractants TGF-beta, eotaxin, and stem cell factor and their receptors in allergic rhinitis. J Allergy Clin Immunol 2004; 114: 799-806.
[52] Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Ruckert B, Mantel PY, Menz G, Akdis CA, Blaser K and Schmidt-Weber CB. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol 2004; 114: 1425-1433.
[53] Quiding-Jarbrink M, Granstrom G, Nordstrom I, Holmgren J and Czerkinsky C. Induction of compartmentalized B-cell responses in human tonsils. Infect Immun 1995; 63: 853-857.