Int J Clin Exp Med 2017;10(1):853-862
www.ijcem.com /ISSN:1940-5901/IJCEM0038191

Original Article

Associations between FOXP3/CTLA4, and regulatory T cells in patients with obstructive sleep apnea-hypopnea symptoms

Hairui Xie[1], Jingxuan Shi[2], Qiyi Zeng[1], Dabo Liu[1,2]

[1]Department of Pediatrics, Zhujiang Hospital of Southern Medical University, 253 Gongyedadao, Guangzhou 510280, Guangdong, People's Republic of China; [2]Department of Otorhinolaryngology, Guangzhou Women and Children's Medical Center, No. 318, People's Road, Guangzhou 510120, Guangdong, People's Republic of China

Received April 7, 2016; Accepted October 21, 2016; Epub January 15, 2017; Published January 30, 2017

Abstract: Obstructive sleep apnea-hypopnea syndrome (OSAHS) is a common chronic respiratory disease in children, and chronic inflammationplaysan important role in its pathology. We investigated FOXP3 and CTLA-4 expression in OSAHS patients, the numbers of Treg cells in sections of tonsil tissue from OSAHS patients, and the possible association of FOXP3 and CTLA4 expression with the clinicopathological characteristics of OSAHS. We also examined the levels of TGF-β, IL-10, and IL-35 expression in the OSAHS patients. Tonsils were obtained from 40 patients with OSAHS and 19 control patients undergoing a tonsillectomy after the subjects had provided their informed consent. The levels of FOXP3 and CTLA-4 expression were detected by qRT-PCR, and their correlation with the clinicopathological characteristics of OSAHS was examined. The numbers of Treg cells and their correlation with FOXP3/CTLA4 expression were evaluated by flow cytometric analyses. TGF-β, IL-10, and IL-35 levels were quantified by ELISA. Our results showed that the levels of FOXP3 and CTLA4 expression in sections of tonsil tissue obtained from the OSAHS patients were significantly higher than those in sections of tonsil tissue obtained from the control patients. Furthermore, those expression levels were significantly correlated with allergic rhinitis, chronic tonsillitis, the apnea-hypopnea index (AHI), the obstructive apnea index (OAI), arterial oxygen saturation (SpO_2), and immunoglobulin E (IgE) levels. The 40 OSAHS patients had significantly higher numbers of Treg cells than did the 19 non-apneiccontrol patients, and the numbers of Treg cells were significantly correlated with the levels of FOXP3 and CTLA4 expression. Additionally, the OSAHS patients showed significantly higher levels of TGF-β and IL-10 expression when compared with those in the control patients, whereas the two groups of patients similar levels of IL-35. Our data clearly indicate that an inflammatory process contributes to the pathology of OSAHS, and suggest that activated T cells play a role in the pathophysiological aspects of OSAHS. Therefore, immunomodulatory therapies may guide the future clinical treatment of obstructive sleep apnea-hypopnea syndrome.

Keywords: FOXP3, CTLA-4, Treg, obstructive sleep apnea-hypopnea syndrome, cytokine

Introduction

Obstructive sleep apnea-hypopnea syndrome (OSAHS) is a common chronic respiratory disorder in children [1-4], and occurs in 1.75%~2.25% of children aged 4-5 years in England [5]. Moreover, epidemiological surveys estimate that OSAHS affects 5.5% of children aged 2-6 years in China [4].

OSAHS is a syndrome characterized by a repetitive, complete or partial collapse of the pharyngeal airway during sleep, and results in disruption of normal ventilation, hypoxemia, and sleep fragmentation [6]. While complete airway obstruction produces apnea, a partial occlusion may produce only snoring and hypopnea [6]. Patients affected by OSAHS have tonsils and adenoids that are disproportionately larger than predicted by their associated skeletal structures. They therefore experience greater than normal airway resistance, and have a pharyngeal airway that is more susceptible to physiological collapse. Furthermore, the diagnosis of tonsillectomies is profoundly affected by obstructive sleep apnea-hypopnea [7, 8]. Children with OSAHS complain of loud snoring, stoppage of breathing, daytime somnolence, and exces-

sive chronic fatigue. Enuresis, hyperactivity, aggression, anxiety, depression, and somatization occur in 30%-40% of children with sleep-disordered breathing. Obstructive sleep apnea-hypopnea is associated with poor school performance and quality of life [7, 8], as well as a wide range of morbidities. Repetitive apnea-hypopnea produces intermittent hypoxia and re-oxygenation, which are known to promote oxidative stress in the presence of elevated levels of reactive oxygen species (ROS) that can induce systemic inflammation and atherosclerosis [9, 10]. However, a patient's apnea-hypopnea index (AHI) as determined by polysomnography, and their postoperative behavior and quality of life can be improved by a tonsillectomy [7, 8].

Tonsillectomies currently account for ~16% of all surgeries performed on children less than age 15 years [7, 8]. While a strong association between tonsillar hypertrophy and OSAHS has been demonstrated in children [11, 12], the pathogenesis of tonsillar hypertrophy in children affected by OSAHS remains poorly understood. Furthermore, OSAHS is multifactorial in origin, and its symptoms depend on various anatomical features, as well as the presence or absence of chronic inflammation [13-15].

Chronic inflammation plays an important role in OSAHS.A previous study [16] showed that tonsillar lymphoid tissues collected from children with recurrent tonsillitis contained increased levels of proinflammatory cytokines [16]. A microarray analysis of RNA derived from the peripheral leukocytes of children with obstructive sleep apnea revealed the recruitment of functionally relevant gene clusters, and prominent involvement of their associated inflammatory pathways [17]. Other studies have shown that an induction of cytokine secretion promotes the proliferation of smooth muscle cells in blood vessel walls, endothelial dysfunction, and atherogenesis; all of which can contribute to development of OSAHS [18, 19]. Patients with OSAHS often display increased levels of interleukin-6 (IL-6), TNF-α, and C-reactive protein [20]; all of which become significantly decreased following the application of continuous positive airway pressure [21-24]. Reduced systemic levels of IL-10 are thought to underlie the pathogenesis and severity of OSAHS [21, 25]. While a one study found no significant differences between the mean values for IL-1α and TGF-β levels inOSAHS patients and control subjects [21], another recent study reported that TGF-β levels were lower in subjects with OSAHS when compared with their levels control subjects. Furthermore, the TGF-β levels were inversely related to a patient's apnea-hypopnea index (AHI), percentage of time spent at an arterial blood O_2 saturation (SpO_2) <90%, and the oxygen desaturation index [26]. After the use of continuous positive airway pressure therapy, the TGF-β levels were significantly increased [26].

Recurrent stimulation either by infections or other processes such as an increase in T cell numbers may lead to tonsillar hypertrophy [27]. Regulatory T cells are critical for the maintenance of immune cell homeostasis. FOXP3 is a regulatory T cell specific transcription factor that controls the differentiation of lymphocytes into regulatory T cells [28]. Alterations in the differentiation of regulatory T cells may cause a secondary immunologic cascade that results in aberrant inflammatory responses [29].

Cytotoxic T lymphocyte antigen 4 (CTLA-4) is required for the suppression of immune responses. A specific deficiency of CTLA-4 in regulatory T cells results in the spontaneous development of systemic lymphoproliferation, fatal T cell-mediated autoimmune diseases, the hyperproduction of immunoglobulin E in mice, as well as a potent tumor immune response [30]. CTLA-4 was found to be expressed in tonsillitis tissue obtained from patients with obstructive sleep apnea syndrome [31]. Various associations between FOXP3, CTLA4, and regulatory T cells, as well as changes in inflammatory cytokines, might be responsible for the inflammatory responses seen in patients affected by OSAHS.

Although previous studies have suggested that an inflammatory process contributes to tonsillar hypertrophy in OSAHS patients, the association between anti-inflammatory responses in the tonsils and the clinicopathological characteristics of OSAHS has remained unclear.

This study was conducted to examine the levels of FOXP3 and CTLA-4 expression in OSAHS patients, the numbers of Treg cells in sections of tonsil tissue obtained from OSAHS patients, and then analyze the association between those parameters and the clinicopathological characteristics of OSAHS. We also examined the levels of TGF-β, IL-10, and IL-35 in OSAHS patients.

Table 1. Clinicopathological characteristics of the 40 obstructive sleep apnea-hypopnea syndrome (OSAHS) patients

Clinical parameter	Number of cases (%)
Age (years)	
<5	30 (75.00)
>5	10 (25.00)
Sex	
Male	34 (85.00)
Female	6 (15.00)
Rhinosinusitis	
Yes	2 (5.00)
No	38 (95.0)
Allergic Rhinitis (AR)	
Yes	14 (35.00)
No	26 (65.00)
Chronic Tonsillitis	
Yes	12 (30.00)
No	28 (70.00)
Sleep Apnea Hypopnea Syndrome (AHI)	
<5	7 (17.50)
>5	33 (82.50)
Obstructive Apnea Index (OAI)	
<1.5	16 (40.00)
>1.5	24 (60.00)
SpO_2	
<90%	35 (87.50)
>90%	5 (12.50)
IgE	
<1	9 (22.50)
>1	31 (77.50)

Materials and methods

Patient selection and specimens

Between March 2014 and February 2016, we conducted an outpatient-based prospective study of individuals diagnosed as moderate or severe OSAHS in the Department of Respiratory, Sleep, Allergy, and Critical Care Medicine of Zhujiang Hospital of Southern Medical University, China. The study protocol was approved by the hospital's Institutional Ethics Committee, and the study subjects provided their written Informed Consent to use of their tissue specimens for research purposes.

During the study period, a total of 40 subjects referred to our Sleep Laboratory for symptoms of loud snoring, daytime somnolence, and fatigue were clinically evaluated for OSAHS. Patients with thyroid disease, an abnormal chest radiograph image or with clinical evidence of neurological, hepatic, renal or peripheral vascular disease were excluded from enrollment. Subjects with a history of alcohol or substance abuse, as well as current smokers were also excluded. Nineteen subjects whose diagnosis was not OSAHS but agreed to remain under observation for the subsequent five weeks served as a control group. Tonsils were obtained from patients undergoing a tonsillectomy, and who provided their Informed Consent. All tonsillectomies were performed under general anesthesia in an operating room, and using either a Coblator or electrocautery.

FOXP3 mRNA and CTLA4 mRNA expression

The levels of FOXP3 and CTLA4 mRNA expression in sections of tonsil tissue obtained from OSAHS patients and control patients were evaluated by the quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR). Samples of total isolated RNA were reverse transcribed into cDNA; after which, qRT-PCR was performed using aPTC-200 automated thermocycler (MJ Research; Watertown, MA, USA) in conjunction with a SYBR Supermix kit (Thermo Fisher Scientific; Waltham, MA, USA), aRT2 PCR Primer Set for FOXP3/CTLA4 (SuperArray Biosciences; Frederick, MD, USA), and β-actin as the reference gene (RT2 PCR Primer Set; Super Array Biosciences). RT-PCR amplification of β-actin mRNA was performed using 20 μmoles of the forward primer (5'-GGCATCGTGATGGACTCCG-3') and 20 μmoles of the reverse primer (3'-GCTGGAAGGTGGACAGCGA-5'). The RT2 PCR Primer Set was used to amplify FOXP3/CTLA4 (SuperArray Biosciences) mRNA in a total reaction volume of 25 μL.

Flow cytometricanalysis of Treg cells and FOXP3/CTLA4 expression

Tregs from 40 OSAHS patients and 19 non-apneiccontrol subjects, and FOXP3/CTLA4 expressing Tregs obtained from OSAHS patients were examined by a flow cytometric method which detected both surface and intra-cellular staining. Cells isolated from the tonsil tissue sections of OSAHS patients and control subjects were prepared by Ficollgradient centrifugation (Histopaque, Sigma; St. Louis, MO, USA); after which, the trypan blue exclusion method showed their viability to be 95%. Intracellular staining for FOXP3 was performed by adding 2

Table 2. Chi-square test results for various prognostic factors in 40 obstructive sleep apnea-hypopnea syndrome (OSAHS) patients and 19 patients without OSAHS

Model		OSAHS		χ^2	*P*-value
		Without (%)	With (%)		
Sex	Female	4 (40%)	6 (60%)	0.335	0.563
	Male	15 (30.6%)	34 (69.4%)		
Rhinosinusitis	No	15 (28.3%)	38 (71.7%)	3.634	0.057
	Yes	4 (66.7%)	2 (33.3%)		
Allergic Rhinitis	No	17 (39.5%)	26 (60.5%)	3.904	0.048
	Yes	2 (12.5%)	14 (87.5%)		
Chronic Tonsillitis	No	7 (20%)	28 (80%)	5.869	0.015
	Yes	12 (50%)	12 (50%)		

Table 3. Student's T-test analysis of various prognostic factors in 40 obstructive sleep apnea-hypopnea syndrome (OSAHS) patients and 19 patients without OSAHS

Model	OSAHS		t	*P* value
	Without (Mean ± SD)	With (Mean ± SD)		
Age	4.53 ± 0.37	4.58 ± 0.30	0.097	0.9230
Sleep Apnea Hypopnea Syndrome	4.44 ± 0.82	10.96 ± 1.09	3.851	0.0003
Obstructive Apnea Index	1.43 ± 0.22	2.65 ± 0.31	2.521	0.0145
SpO_2	0.88 ± 0.01	0.81 ± 0.01	3.384	0.0010
IgE	0.89 ± 0.22	1.90 ± 0.22	2.857	0.0060
FOXP3	1.56 ± 0.27	4.52 ± 0.59	3.345	0.0015
CTLA4	0.50 ± 0.09	1.40 ± 0.16	3.715	0.0005

mL of cold 1× permeabilization buffer to the isolated cells, followed by a 5 minute incubation at 4°C. Next, anti-FOXP3 antibody (10 μL) or CTLA4 antibody (10 μL) (eBiosciences; San Diego, CA, USA) was added, thoroughly mixed with the cells, and then incubated for 30-60 minutes in the dark at 4°C. The cell pellet was then re-suspended in a suitable amount of flow cytometry staining buffer for analysis. After staining, the cell pellet was re-suspended in 500 μL of flow cytometry staining buffer, and the cells were analyzed using a FACSC alibur flow cytometer (Becton Dickinson; Franklin Lakes, NJ, USA) equipped with Cell Quest Software (Becton Dickinson).

Cytokine analysis

The amounts of TGF-β, IL-10, and IL-35 in the tonsil tissue sections of 40 OSAHS patients and 19 non-apneic subjects were quantified by ELISA as per the manufacturer's recommendations (Biolegend; San Diego, CA, USA), and expressed in units of ng/mL.

Statistical analysis

All statistical analyses were performed using IBM SPSS 19.0 Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp. The distribution of experimental data was evaluated using the Shapiro-Wilk test. Continuous variables are expressed as the mean ± standard deviation and categorical variables are expressed as a frequency and percent. Continuous variables were compared using thet test for independent samples or the Mann-Whitney U test when comparing two groups. Categorical variables were compared using either Pearson's χ^2 test or Fisher's exact χ^2 test. The association of various factors with OSAHS was analyzed by multivariate logistic regression analyses, and the results are presented as odds ratios (ORs) with 95% confidence intervals (95% CIs). *P*-values <0.05 were considered statistically significant.

Results

Clinicopathological characteristics of the 40 OSAHS patients

Among the 40 OSAHS patients (**Table 1**), 12 were diagnosed as chronic tonsillitis, 14 asallergic rhinitis, and 2 asrhinosinusitis. Allergic rhinitis (*P*<0.05) and chronic tonsillitis (*P*= 0.015) were closed associated with OSAHS (**Table 2**). Other factors, including sleep apnea hypopnea syndrome, SpO_2, IgE, FOXP3, and CTLA4 were also closed associated with OSAHS (all *P*-values <0.05) (**Table 3**).

Correlation between clinicopathological characteristics and expression of FOXP3 and CTLA4

The 40 OSAHS patients and 19 non-apneic subjects were not significantly differentin terms

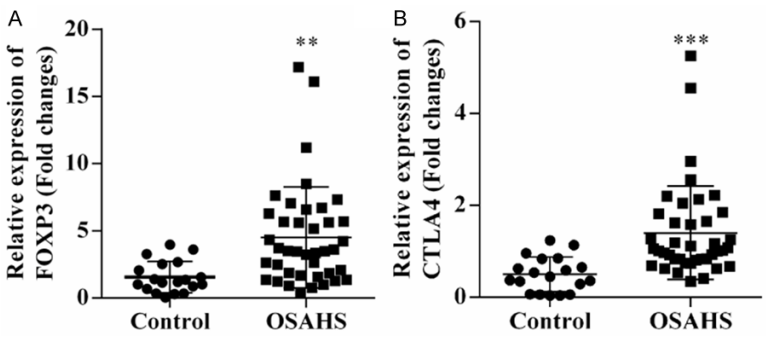

Figure 1. The levels of FOXP3 and CTLA4 mRNA expression in sections of tonsil tissue were detected by qRT-PCR. The levels of FOXP3 (A) and CTLA4 (B) mRNA expression were significantly higher insections of tonsil tissue obtained from 40 OSAHS patients than from 19 control subjects. ***P*<0.01; ****P*<0.001.

Table 4. Clinicopathologic associations of FOXP3 expression in 40 obstructive sleep apnea-hypopnea syndrome (OSAHS) patients

Clinical parameter	Number of cases (%)	Relative FOXP3 expression	t-value	*P*-value
Age (years)				
<5	30 (75.00)	4.73 ± 0.74	0.6307	0.5320
>5	10 (25.00)	3.86 ± 0.86		
Sex				
Male	34 (85.00)	4.44 ± 0.68	0.2816	0.7798
Female	6 (15.00)	4.92 ± 1.06		
Rhinosinusitis				
Yes	2 (5.00)	4.59 ± 1.09	0.0266	0.9789
No	38 (95.0)	4.51 ± 0.62		
Allergic Rhinitis (AR)				
Yes	14 (35.00)	6.11 ± 1.33	2.044	0.0479
No	26 (65.00)	3.66 ± 0.52		
Chronic Tonsillitis				
Yes	12 (30.00)	6.42 ± 1.54	2.198	0.0341
No	28 (70.00)	3.70 ± 0.48		
Sleep Apnea Hypopnea Syndrome (AHI)				
<5	7 (17.50)	1.54 ± 0.36	2.309	0.0265
>5	33 (82.50)	4.97 ± 0.67		
Obstructive Apnea Index (OAI)				
<1.5	16 (40.00)	2.78 ± 0.44	2.542	0.0152
>1.5	24 (60.00)	5.67 ± 0.88		
SpO_2				
<90%	35 (87.50)	4.99 ± 0.64	2.258	0.0298
>90%	5 (12.50)	1.14 ± 0.26		
IgE				
<1	9 (22.50)	2.01 ± 0.46	2.411	0.0209
>1	31 (77.50)	5.24 ± 0.71		

of age, sex, BMI, smoking status or hypertension status (Data not shown). The levels of FOXP3 and CTLA4 mRNA expression in the tonsil tissue sections were detected by qRT-PCR (**Figure 1**). The mean level of FOXP3 mRNA expression in the OSAHS group was significantly higher than that in the control group (4.52 ± 0.59 vs. 1.56 ± 0.27, respectively, *P*<0.01) (**Figure 1A**). Likewise, the mean level of CTLA4 mRNA expression in the OSAHS group was also higher than that in the control group (1.40 ± 0.16 vs. 0.50 ± 0.09, respectively, *P*<0.001) (**Figure 1B**).

The FOXP3 levels in OSAHS patients were independently associated with age, sex, and rhinosinusitis (**Table 4**). Moreover, the FOXP3 levels were significantly correlated with allergic rhinitis, chronic tonsillitis, AHI, OAI (obstructive apnea index), SpO_2, and IgE levels (**Table 4**). Similarly, the CTLA4 levels in OSAHS patients were independently associated with age, sex, and rhinosinusitis (**Table 5**), and significantly correlated with allergic rhinitis, chronic tonsillitis, AHI, OAI, SpO_2, and IgE levels (**Table 5**).

A multiple regression analysis revealed that allergic rhinitis, chronic tonsillitis, sleep apnea hypopnea syndrome, obstructive Apnea Index, SpO_2, and IgE were all independent risk factors for OSAHS (*P*<0.05) (**Table 6**).

Correlation between Treg cells and expression of FOXP3 and CTLA4

The mean percentage of Treg cells in the 40 OSAHS patients was significantly greater than that in the 19 non-apneiccontrol subjects (5.31 ± 0.38% vs. 3.98 ± 0.41%, respectively, *P*<

Table 5. Clinicopathologic associations of CTLA4 expression in 40 obstructive sleep apnea-hypopnea syndrome (OSAHS) patients

Clinical parameter	Number of cases (%)	Relative FOXP3 expression	t value	*P* value
Age (years)				
<5	30 (75.00)	1.54 ± 0.20	1.513	0.1386
>5	10 (25.00)	0.99 ± 0.14		
Gender				
Male	34 (85.00)	1.41 ± 0.18	0.939	0.9257
Female	6 (15.00)	1.36 ± 0.29		
Rhinosinusitis				
Yes	2 (5.00)	0.64 ± 0.29	1.093	0.2811
No	38 (95.0)	1.44 ± 0.17		
Allergic Rhinitis (AR)				
Yes	14 (35.00)	1.86 ± 0.39	2.216	0.0328
No	26 (65.00)	1.15 ± 0.12		
Chronic Tonsillitis				
Yes	12 (30.00)	2.02 ± 0.43	2.711	0.0100
No	28 (70.00)	1.14 ± 0.11		
Sleep Apnea Hypopnea Syndrome (AHI)				
<5	7 (17.50)	0.69 ± 0.10	2.093	0.0431
>5	33 (82.50)	1.55 ± 0.18		
Obstructive Apnea Index (OAI)				
<1.5	16 (40.00)	0.98 ± 0.13	2.226	0.0320
>1.5	24 (60.00)	1.68 ± 0.24		
SpO_2				
<90%	35 (87.50)	1.58 ± 0.16	2.105	0.0414
>90%	5 (12.50)	0.63 ± 0.06		
IgE				
<1	9 (22.50)	0.76 ± 0.13	2.252	0.0302
>1	31 (77.50)	1.59 ± 0.19		

0.05) (**Figure 2A**). Furthermore, the number of Treg cells was significantly correlated with the levels of FOXP3 (*P*=0.0025, **Figure 2B**) and CTLA4 (*P*=0.0028, **Figure 2C**).

Cytokine levels in OSAHS patients

IgE levels were positively correlated with the levels of FOXP3 and CTLA4 expression (**Tables 2** and **3**). We next measured the levels of TGF-β, IL-10, and IL-35 expression in tonsil tissue sections of the 40 OSAHS patients and 19 non-apneic control subjects to determine whether there was a relationship between different cytokines involved in the generation of Treg cells or their effector functions (**Figure 3**).

We found increased levels of TGF-β and IL-10 expression in the tonsil tissues of OSAHS patients when compared those levels in the control group (*P*<0.01 and *P*<0.01, respectively); however, the two groups displayed similar levels of IL-35 expression. These results clearly indicate a higher production of pro-inflammatory cytokines by T cells in the tonsil tissues of OSAHS patients, and strongly suggest that activated T cells play a role in the pathophysiological aspects of OSAHS.

Discussion

We found significantly higher levels of FOXP3 and CTLA4 expression in sections of tonsil tissue obtained from a group of OSAHS patients when compared with sections obtained from a group of control patients. Furthermore, the higher levels of FOXP3 and CTLA4 expression were significantly correlated with allergic rhinitis, chronic tonsillitis, AHI, OAI, SpO_2, and IgE levels. The mean number of Treg cells in the 40 OSAHS patients was significantly higher than that in 19 non-apneic subjects, and the Treg cells numbers were significantly correlated with the levels of FOXP3 and CTLA4 expression. Finally, we found increased levels of TGF-β and IL-10 in the OSAHS patients when compared with those levels in the control group. However, the two groups displayed similar levels of IL-35 expression. These data clearly reflect a higher production of pro-inflammatory cytokines by T cells in the tonsils tissues of OSAHS patients, and suggest that activated T cells play a role in the pathophysiological aspects of OSAHS.

OSAHS is multifactorial in origin, and the extent of its pathological effects depends on anatomical features and the presence or absence of chronic inflammation [13-15]. Chronic inflammation plays an important role in patients affected by OSAHS, as these patients display increased levels of IL-6, TNF-α, and C-reactive protein [20]; however, these levels become significantly reduced following surgical treatment of OSAHS or application of continuous positive airway pressure [21-24]. Tonsillectomies cur-

Table 6. Multivariate analysis of various prognostic factors in 40 obstructive sleep apnea-hypopnea syndrome (OSAHS) patients

Model	B	S.E.	Wals	df	p	OR	OR 95% CI
Age	0.094	0.306	0.093	1	0.760	1.098	0.602~2.002
Sex	0.204	0.794	0.066	1	0.798	1.226	0.259~5.807
Rhinosinusitis	-1.298	1.128	1.325	1	0.250	0.273	0.030~2.489
Allergic Rhinitis	1.305	0.869	2.254	1	0.133	3.688	0.671~20.244
Chronic Tonsillitis	-1.568	0.653	5.766	1	0.016	0.208	0.058~0.750
Sleep Apnea Hypopnea Syndrome	0.513	0.205	6.242	1	0.012	1.671	1.117~2.499
Obstructive Apnea Index	-1.208	0.608	3.951	1	0.047	0.299	0.091~0.983
SpO_2	-28.562	13.402	4.542	1	0.033	0.000	0.000~0.101
IgE	1.034	0.444	5.436	1	0.020	2.814	1.179~6.713

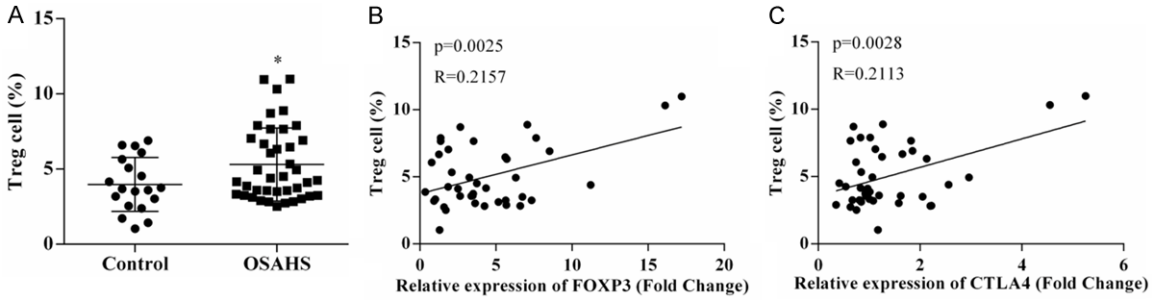

Figure 2. Correlations between Treg cell numbers and levels of FOXP3 and CTLA4 expression. A. Number of Treg cells, *P<0.05; B. The correlation between Treg cells and FOXP3 levels; C. The correlation between Treg cells and CTLA4 levels.

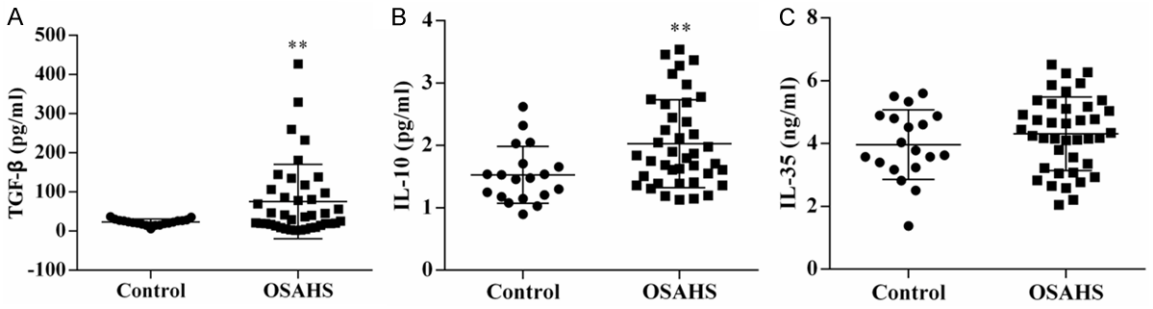

Figure 3. Cytokineprofiles insections of tonsil tissue from OSAHS patients. (A) TGF-β, (B) IL-10, and (C) IL-35 concentrations in sections of tonsil tissue from 40 OSAHS patients and 19 non-apneic control subjects were quantified by ELISA. **P<0.01.

rently account for 16% of all surgeries performed on children younger than age 15 years [7, 8], and a strong association has been reported between tonsillar hypertrophy and OSAHS in children [11, 12]. Reduced levels of IL-10 in plasma are thought to account for the pathogenesis and severity of OSAHS [21, 25]. While one study found no significant differences between the mean levels of IL-1α and TGF-β in OSAHS patients and control subjects [21], a more recent study [26] reported lower levels of TGF-β in OSAHS patients than in control subjects. Furthermore, the TGF-β levels were inversely related to a patient's apnea-hypopnea index (AHI), percentage of time spent at an O_2 saturation <90% (SpO_2), and oxygen desaturation index. The use of continuous positive airway pressure therapy significantly increased the levels of TGF-β. Here, we found increased levels of TGF-β and IL-10 in OSAHS patients

when compared with those levels in a control group, whereas the two groups displayed similar levels of IL-35. The different degrees of change in IL-10 and TGF-β levels in the circulating blood and tonsil tissues of OSAHS patients may reflect the contributions of inflammation and tonsillar hypertrophy to the severity of OSAHS.

It is well known that a relative balance of activity between effector T cells and regulatory T cells is an important factor for maintaining immune homeostasis [32]. The recurrent stimulation of T cells either by infections or other processes may lead to tonsillar hypertrophy [27]. When naive T cells are activated by antigen-presenting cells, they become effector T cells which are selectively activated when exposed to a specific type of antigen [33]. Moreover, the effector T cells produce proinflammatory cytokines that promote B-cell maturation [33]. The proinflammatory function of effector T cells is countered by the activity of regulatory T cells (Treg cells), which are especially important for immune tolerance to self and foreign antigens [33]. Activated T-cell populations in patients with obstructive sleep apnea have been previously studied [34-36]. Alterations in the differentiation of regulatory T cells may produce a secondary immunologic cascade resulting in aberrant inflammatory responses [29]. An induction of cytokine secretion promotes the proliferation of vessel wall smooth muscle cells, endothelial dysfunction, atherogenesis, and contributes to the development of OSAHS [18, 19]. In our current study, we examined the associations between FOXP3, CTLA4, and regulatory T cells.

Regulatory T cells can be identified by their constituent intracellular transcription factor, FOXP3. A loss of FoxP3 function leads to the development of fatal autoimmune diseases in mice (Scurfy) and humans (IPEX) [37-40]. Regulatory T cells also produce cytokines (e.g., L-10) and cell surface proteins (e.g., CTLA-4) that suppress inflammation [37-40]. It has demonstrated that the *FOXP3* gene is hypermethylated in the peripheral blood of patients with OSAHS [41]. Consistent with that finding, we found that the number of Treg cells was significantly correlated with FOXP3 and CTLA4 levels in the tonsil tissue sections of OSAHS patients.

In the present study, we found that an inflammatory process contributed to the development of OSAHS, and was associated with its clinicopathological characteristics. Therefore, itis extremely important to take into consideration the potential contribution of an inflammatory/anti-inflammatory response when treating OSAHS patients. Future studies might identify a serologic factor that correlates with OSAHS; if so, immunomodulatory therapies may guide the clinical treatment of obstructive sleep apnea-hypopnea syndrome.

Disclosure of conflict of interest

None.

Address correspondence to: Dabo Liu, Department of Pediatrics, Zhujiang Hospital of Southern Medical University, 253 Gongyedadao, Guangzhou 510280, Guangdong, People's Republic of China; Department of Otorhinolaryngology, Guangzhou Women and Children's Medical Center, No. 318, People's Road, Guangzhou 510120, Guangdong, People's Republic of China. E-mail: 1126961549@qq.com; daboliu@126.com

References

[1] Fernandes FR, Apolinario D and Esquinas AM. Obstructive sleep apnea hypopnea syndrome and pulmonary embolism: warfarin versus positive airway pressure as determinant. Chin Med J (Engl) 2016; 129: 251-252.

[2] Li F, Huang H, Song L, Hao H and Ying M. Effects of obstructive sleep apnea hypopnea syndrome on blood pressure and C-reactive protein in male hypertension patients. J Clin Med Res 2016; 8: 220-224.

[3] Wang Y, Yang Q, Feng J, Cao J and Chen B. The prevalence and clinical features of hypertension in patients with obstructive sleep apnea hypopnea syndrome and related nursing strategies. J Nurs Res 2016; 24: 41-47.

[4] Xie J, Liu S and Wei YX. Obstructive sleep apnea hypopnea syndrome: an incognito player contributing to repeated pulmonary embolism? Chin Med J (Engl) 2016; 129: 252.

[5] Gislason T and Benediktsdottir B. Snoring, apneic episodes, and nocturnal hypoxemia among children 6 months to 6 years old. An epidemiologic study of lower limit of prevalence. Chest 1995; 107: 963-966.

[6] Goel AK, Talwar D and Jain SK. Evaluation of short-term use of nocturnal nasal continuous positive airway pressure for a clinical profile and exercise capacity in adult patients with ob-

structive sleep apnea-hypopnea syndrome. Lung India 2015; 32: 225-232.
[7] Roland PS, Rosenfeld RM, Brooks LJ, Friedman NR, Jones J, Kim TW, Kuhar S, Mitchell RB, Seidman MD, Sheldon SH, Jones S, Robertson P; American Academy of Otolaryngology-Head and Neck Surgery Foundation. Clinical practice guideline: polysomnography for sleep-disordered breathing prior to tonsillectomy in children. Otolaryngol Head Neck Surg 2011; 145: S1-15.
[8] Baugh RF, Archer SM, Mitchell RB, Rosenfeld RM, Amin R, Burns JJ, Darrow DH, Giordano T, Litman RS, Li KK, Mannix ME, Schwartz RH, Setzen G, Wald ER, Wall E, Sandberg G, Patel MM; American Academy of Otolaryngology-Head and Neck Surgery Foundation. Clinical practice guideline: tonsillectomy in children. Otolaryngol Head Neck Surg 2011; 144: S1-30.
[9] Lavie L. Obstructive sleep apnoea syndrome-an oxidative stress disorder. Sleep Med Rev 2003; 7: 35-51.
[10] Ryan S, Taylor CT and McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. Circulation 2005; 112: 2660-2667.
[11] Friedman M, Ibrahim H and Bass L. Clinical staging for sleep-disordered breathing. Otolaryngol Head Neck Surg 2002; 127: 13-21.
[12] Kuno K, Ohi M, Chin K and Mishima M. Symposium on clinical aspects of sleep-disordered breathing and circulation. Diagnosis; guideline for diagnosis, and multimodal staging system. Intern Med 1995; 34: 310-314.
[13] Sunbul M, Sunbul EA, Kanar B, Yanartas O, Aydin S, Bacak A, Gulec H and Sari I. The association of neutrophil to lymphocyte ratio with presence and severity of obstructive sleep apnea. Bratisl Lek Listy 2015; 116: 654-658.
[14] Weinstock LB, Myers TL, Walters AS, Schwartz OA, Younger JW, Chopra PJ and Guarino AH. Identification and treatment of new inflammatory triggers for complex regional pain syndrome: small intestinal bacterial overgrowth and obstructive sleep apnea. A A Case Rep 2016; 6: 272-276.
[15] Ryan S, Taylor CT and McNicholas WT. Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnoea syndrome? Thorax 2009; 64: 631-636.
[16] Kim J, Bhattacharjee R, Dayyat E, Snow AB, Kheirandish-Gozal L, Goldman JL, Li RC, Serpero LD, Clair HB and Gozal D. Increased cellular proliferation and inflammatory cytokines in tonsils derived from children with obstructive sleep apnea. Pediatr Res 2009; 66: 423-428.
[17] Khalyfa A, Capdevila OS, Buazza MO, Serpero LD, Kheirandish-Gozal L and Gozal D. Genome-wide gene expression profiling in children with non-obese obstructive sleep apnea. Sleep Med 2009; 10: 75-86.
[18] Mor A, Planer D, Luboshits G, Afek A, Metzger S, Chajek-Shaul T, Keren G and George J. Role of naturally occurring CD4+ CD25+ regulatory T cells in experimental atherosclerosis. Arterioscler Thromb Vasc Biol 2007; 27: 893-900.
[19] Tan HL, Gozal D, Wang Y, Bandla HP, Bhattacharjee R, Kulkarni R and Kheirandish-Gozal L. Alterations in circulating T-cell lymphocyte populations in children with obstructive sleep apnea. Sleep 2013; 36: 913-922.
[20] Dyugovskaya L, Lavie P, Hirsh M and Lavie L. Activated CD8+ T-lymphocytes in obstructive sleep apnoea. Eur Respir J 2005; 25: 820-828.
[21] Alberti A, Sarchielli P, Gallinella E, Floridi A, Floridi A, Mazzotta G and Gallai V. Plasma cytokine levels in patients with obstructive sleep apnea syndrome: a preliminary study. J Sleep Res 2003; 12: 305-311.
[22] Kataoka T, Enomoto F, Kim R, Yokoi H, Fujimori M, Sakai Y, Ando I, Ichikawa G and Ikeda K. The effect of surgical treatment of obstructive sleep apnea syndrome on the plasma TNF-alpha levels. Tohoku J Exp Med 2004; 204: 267-272.
[23] Bravo Mde L, Serpero LD, Barcelo A, Barbe F, Agusti A and Gozal D. Inflammatory proteins in patients with obstructive sleep apnea with and without daytime sleepiness. Sleep Breath 2007; 11: 177-185.
[24] Liu H, Liu J, Xiong S, Shen G, Zhang Z and Xu Y. The change of interleukin-6 and tumor necrosis factor in patients with obstructive sleep apnea syndrome. J Tongji Med Univ 2000; 20: 200-202.
[25] Leon-Cabrera S, Arana-Lechuga Y, Esqueda-Leon E, Teran-Perez G, Gonzalez-Chavez A, Escobedo G and Velazquez Moctezuma J. Reduced systemic levels of IL-10 are associated with the severity of obstructive sleep apnea and insulin resistance in morbidly obese humans. Mediators Inflamm 2015; 2015: 493409.
[26] Steffanina A, Proietti L, Antonaglia C, Palange P, Angelici E and Canipari R. The plasminogen system and transforming growth factor-beta in subjects With obstructive sleep apnea syndrome: effects of CPAP treatment. Respir Care 2015; 60: 1643-1651.
[27] Steiniger B, Trabandt M and Barth PJ. The follicular dendritic cell network in secondary follicles of human palatine tonsils and spleens. Histochem Cell Biol 2011; 135: 327-336.

[28] Sakaguchi S, Miyara M, Costantino CM and Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol 2010; 10: 490-500.
[29] Sakaguchi S, Wing K and Miyara M. Regulatory T cells - a brief history and perspective. Eur J Immunol 2007; 37 Suppl 1: S116-123.
[30] Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T and Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008; 322: 271-275.
[31] Takahara M, Kishibe K, Nozawa H and Harabuchi Y. Increase of activated T-cells and up-regulation of Smad7 without elevation of TGF-beta expression in tonsils from patients with pustulosis palmaris et plantaris. Clin Immunol 2005; 115: 192-199.
[32] Buchwald ZS and Aurora R. Osteoclasts and CD8 T cells form a negative feedback loop that contributes to homeostasis of both the skeletal and immune systems. Clin Dev Immunol 2013; 2013: 429373.
[33] Anderson ME, 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.
[34] 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.
[35] Ye J, Liu H, Zhang G, Li P, Wang Z, Huang S, Yang Q and Li Y. The treg/th17 imbalance in patients with obstructive sleep apnoea syndrome. Mediators Inflamm 2012; 2012: 815308.
[36] Tan HL, Gozal D, Samiei A, Bhattacharjee R, Wang Y, Ramirez HM, Bandla HP, Kulkarni R and Kheirandish-Gozal L. T regulatory lymphocytes and endothelial function in pediatric obstructive sleep apnea. PLoS One 2013; 8: e69710.
[37] Kasprowicz DJ, Smallwood PS, Tyznik AJ and Ziegler SF. Scurfin (FoxP3) controls T-dependent immune responses in vivo through regulation of CD4+ T cell effector function. J Immunol 2003; 171: 1216-1223.
[38] Passerini L, Di Nunzio S, Gregori S, Gambineri E, Cecconi M, Seidel MG, Cazzola G, Perroni L, Tommasini A, Vignola S, Guidi L, Roncarolo MG and Bacchetta R. Functional type 1 regulatory T cells develop regardless of FOXP3 mutations in patients with IPEX syndrome. Eur J Immunol 2011; 41: 1120-1131.
[39] d'Hennezel E, Ben-Shoshan M, Ochs HD, Torgerson TR, Russell LJ, Lejtenyi C, Noya FJ, Jabado N, Mazer B and Piccirillo CA. FOXP3 forkhead domain mutation and regulatory T cells in the IPEX syndrome. N Engl J Med 2009; 361: 1710-1713.
[40] Ochs HD, Gambineri E and Torgerson TR. IPEX, FOXP3 and regulatory T-cells: a model for autoimmunity. Immunol Res 2007; 38: 112-121.
[41] Kim J, Bhattacharjee R, Khalyfa A, Kheirandish-Gozal L, Capdevila OS, Wang Y and Gozal D. DNA methylation in inflammatory genes among children with obstructive sleep apnea. Am J Respir Crit Care Med 2012; 185: 330-338.