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CD$_4^+$CD$_{25}^+$Treg cells and IgA nephropathy patients with tonsillectomy: a clinical and pathological study

Hongdong Huang · Weiming Sun · Yumei Liang · Youming Peng · Xi-Dai Long · Zhihua Liu · Xiaojun Wen · Meng Jia · Ru Tian · Chengli Bai · Cui Li

Abstract

Background The relationship between tonsillar autoimmune response and the pathogenesis of IgA nephropathy (IgAN) has been previously demonstrated. However, the role of CD$_4^+$CD$_{25}^+$Treg cells, which play critical roles in maintaining peripheral tolerance and preventing autoimmunity, has not yet been defined in IgAN.

Methods Thirty-five patients with IgAN and 35 patients without renal disease were studied. The CD$_4^+$CD$_{25}^+$Treg cells were examined by flow cytometry. Clinical and laboratory data, such as serum creatinine and urinary samples, were obtained from each patient. Glomerular injury was assessed by histopathology. Serum IgA, C3, IL-2, IL-4 and IL-6 were analyzed by ELISA.

Results CD$_4^+$CD$_{25}^+$Treg cells significantly decreased in IgAN patients compared with the controls before tonsillectomy ($p < 0.05$). CD$_4^+$CD$_{25}^+$Treg cells were negatively correlated with blood urea nitrogen, supernatant IL-4 and proteinuria in IgAN patients, and positively with estimated glomerular filtration rate. CD$_4^+$CD$_{25}^+$Treg cells gradually decreased as the severity of renal histology increased. In addition, serum IgA, IL-2, IL-6 and supernatant IL-4 elevated while CD$_4^+$CD$_{25}^+$Treg cells decreased in IgAN patients. CD$_4^+$CD$_{25}^+$Treg cells were significantly increased when serum IgA, IL-2, IL-6 and supernatant IL-4, urine protein and urine erythrocytes were decreased after tonsillectomy in patients with IgAN, but were still lower than those of the controls ($p < 0.05$).

Conclusions CD$_4^+$CD$_{25}^+$Treg cells were associated with IgAN, and tonsillectomy may increase CD$_4^+$CD$_{25}^+$Treg cells in IgAN patients, leading to clinical improvement.

Keywords CD$_4^+$CD$_{25}^+$Treg cells · IgA nephropathy · Tonsillectomy

H. Huang (✉) · W. Sun (✉) · Z. Liu · X. Wen · M. Jia · R. Tian · C. Bai · C. Li
Division of Nephrology, Beijing Shijitan Hospital, Capital Medical University, 10 Yangfangdian Tieyi Road, Haidian District, Beijing 100038, People's Republic of China
e-mail: huanghd1126@126.com

W. Sun
e-mail: sunweiming301@126.com

H. Huang · Y. Liang
Division of Nephrology, Hunan Normal University, Hunan Provincial People's Hospital of China, Changsha, People's Republic of China

Y. Peng
Hunan Key Laboratory of Nephrology and Hemoperfusion, Division of Nephrology, Second Xiangya Hospital of Central South University, Changsha, Hunan Province, People's Republic of China

X.-D. Long
Department of Liver Surgery, the Affiliated Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

Introduction

Immunoglobulin A (IgA) nephropathy (IgAN) is the most common glomerulonephritis in the world [1]. Although this disease was once considered to have a good prognosis, it has more recently been observed that 30–40 % of IgAN patients progress to end-stage renal disease (ESRD) within 20 years. Thus, analysis of the mechanism of occurrence or progression of IgAN is important to reduce or prevent the number of patients with ESRD. One type of IgAN pathogenesis is associated with polymeric IgA of the

IgA1 subclass, which can react to the glomerular mesangium. Much evidence has shown that the tonsils are closely related to IgAN [2]. Tonsillectomy can improve the urinary findings, keep stable renal function, and have a favorable effect on long-term renal survival in some IgAN patients [2]. Recent studies have shown that $CD_4^+CD_{25}^+$Treg cells are of critical importance to the maintenance of tolerance by inhibiting the activation and proliferation of autoreactive T cells [3]. $CD_4^+CD_{25}^+$Treg cells are regulators in nearly all of the animal models of human organ-specific diseases, transplant rejection and allergic diseases [4]. Our experiments have shown [5] that $CD_4^+CD_{25}^+$Treg cells were significantly decreased and dimeric IgA-producing cells were dramatically increased in tonsils in IgA nephropathy patients compared with the controls before and after stimulation with HS-IgAN [α-hemolytic streptococcus (HS) isolated from the tonsillar crypts of IgAN patients] or HS-controls [α-hemolytic streptococcus (HS) isolated from the tonsillar crypts of controls]. To date, there is no other study of $CD_4^+CD_{25}^+$Treg cells in human IgAN. Some patients with recurrent chronic tonsillitis do not suffer from renal disease, implying that it is possible to find a balance between immunity and tolerance. However, other patients do suffer from IgAN along with recurrent chronic tonsillitis. We therefore hypothesize that a numerical and/or functional deficit of $CD_4^+CD_{25}^+$ Treg cells in IgAN patients might trigger the development of disease. The purpose of the present study was to observe the number of $CD_4^+CD_{25}^+$Treg cells in the peripheral blood of patients with IgAN before and after tonsillectomy and evaluated its clinical significance.

Materials and methods

Subjects

IgAN patient group

Thirty-five IgAN patients were admitted into our hospital for renal diseases from September 2006 to August 2011. IgAN patients (18 men and 17 women; age range 15–47 years; mean age 24.5 ± 4.3 years) were identified by immuno histopathological examination of renal biopsy specimens. IgAN was diagnosed on the basis of mesangial cell proliferation and mesangial matrix expansion under light microscopy and detection of predominantly granular mesangial IgA deposits by immunofluorescence. Systemic lupus erythematosus, Henoch–Schonlein purpura and hepatic diseases were excluded by clinical history, physical examination and negative laboratory test results. All IgAN patients underwent again kidney biopsy 2 years after tonsillectomy. Clinical and laboratory data such as

age, gender, serum creatinine, estimated glomerular filtration rate (eGFR) (eGFR was calculated with an abbreviated modification of dietin renal disease (MDRD) equation modified for Chinese subjects [6]), urinary protein and blood pressure were recorded at the time of renal biopsy. All patients did not undergo treatment with steroid, immunosuppressive agents, angiotensin-converting enzyme (ACE) inhibitor or AT1 receptor blockers before tonsillectomy, but six patients were treated with steroid therapy after tonsillectomy. Other 17 IgAN patients who did not receive second biopsy 2 years after tonsillectomy from September 2006 to August 2011 were excluded.

Control group

Thirty-five patients with chronic tonsillitis but without renal disease (15 men and 20 women; age range 11–45 years; mean age 21.8 ± 5.4 years) were selected as the control group. All patients with chronic tonsillitis had not hematuria and proteinuria who did not undergo treatment with steroid, immunosuppressive agents, ACE inhibitor or AT1 receptor blockers.

The indications of tonsillectomy in patients with IgAN include mainly the deterioration of urinary findings after tonsillar infection, mild or moderate renal damage. Otolaryngologically, the two main indications for tonsillectomy are upper airway obstruction due to tonsillar hypertrophy and recurrent chronic tonsillitis. The study was approved by the Ethical Committee of second Xiangya Hospital and the Hunan Government Medical Research Council.

Clinical samples collected and tested

After acquiring informed consent, peripheral blood and urine samples were collected before and after tonsillectomy, respectively. Twenty-four-hour urine samples were collected to determine the protein. Urine protein concentrations were measured by the Coomassie Blue binding reagent. Fresh urine samples were collected in the morning to determine urine erythrocytes. At least three urinalyses are required for the diagnosis of urinary abnormalities, and each test should include microscopic examination of urinary sediment in addition to routine urinalysis (the number of replicates of each sample was three).

Flow cytometry analysis of $CD_4^+CD_{25}^+$Treg cells

Peripheral blood was mixed and incubated for 30 min at room temperature with 10 μl monoclonal Cy5-labeled antihuman CD3 (Jingmei Biotech), FITC-anti-CD4 and PE-anti-CD25. After a short incubation period, the samples were fixed with 1 % paraformaldehyde and analyzed by flow cytometry (Coulter EpicsXL, System2 software;

Beckman-Coulter). The analysis and gates were restricted to lymphocytes (the number of replicates of each sample was three).

Isolation of peripheral blood mononuclear cells and culture

PBMC were isolated from heparinized peripheral blood by density gradient centrifugation, using Lymphocyte Separation Medium (Flow Labs, McLean, VA). Cells recovered at the interface were resuspended in RPMI1640 supplemented with penicillin (100 U/ml), streptomycin (100 pg/ml), glutamine (2 mM) and 10 % heat-inactivated fetal calf serum (FCS), at a concentration of 3×10^6 cells/ml. Duplicate cultures, with phytohaemagglutinin (PHA; Sigma, St Louis, MO) 20 ug/ml, were maintained for 24 h at 37 °C in a 5 % CO_2 atmosphere. At the end of this period, cell-free supernatant was obtained by centrifugation at 800g for 10 min and frozen at −70 °C until assayed.

Enzyme-linked immunosorbent assay

The serum concentrations of IL-2, IL-6 and the cell-free supernatant concentration of IL-4 were measured, respectively, by an enzyme-linked immunosorbent assay (ELISA) with ELISA kits (R&D Systems, USA) according to the manufacturer's instruction (the number of replicates of each sample was three).

Histopathological evaluation

Kidney Biopsy for histological evaluation was repeated shortly before tonsillectomy and again 2 years after tonsillectomy in 35 IgAN patients. Renal specimens obtained from the first and the second of these biopsies were evaluated by the same pathologist, who was unaware of patients' clinical profiles. All kidney biopsy samples were subjected to fluorescence, and light microscopic examination. For light microscopy, kidney biopsy samples were fixed in 10 % buffered formalin, dehydrated, and embedded in paraffin by conventional techniques. Sections were stained with hematoxylin and eosin, and periodic acid–schiff. Proteinuria, IgA, complement component C3, activity (AI) and chronicity (CI) indices, as well as semiquantitative renal histological evaluation of renal biopsy specimens obtained at time of diagnosis, were compared. AI includes glomerular hypercellularity, leukocyte exudation, fibrinoid necrosis, cellular crescents, hyaline deposits and interstitial inflammation, while CI includes glomerular sclerosis, fibrous crescents, renal tubular atrophy and interstitial fibrosis. These alterations were graded semiquantitatively on a 1+–3+ scale (mild, moderate or marked) [7]. The renal pathological lesions were graded single-blindedly according to the Haas classification [8].

Statistical analysis

Data were expressed as mean standard deviation. Comparison between the two groups was done by independent-sample t test or nonparametric Mann–Whitney U test as appropriate. Differences between multiple groups were compared by the ANOVA test. Relationships between different values were examined using Spearman's correlation tests. A p value <0.05 was considered statistically significant. All statistical analyses were performed using Statistical Package for Social Sciences version 16.0 (SPSS Inc., USA).

Results

Flow cytometry analysis of $CD_4^+CD_{25}^+$Treg cells

We analyzed the population of $CD_4^+CD_{25}^+$Treg cells in peripheral blood by flow cytometry (Fig. 1). $CD_4^+CD_{25}^+$Treg cells significantly decreased in IgAN patients compared to those of the control groups before tonsillectomy ([a]$p < 0.05$). After tonsillectomy, the number of $CD_4^+CD_{25}^+$cells gradually increased (however, 3 months after tonsillectomy, increase of $CD_4^+CD_{25}^+$Treg cells tended to be stable) (Fig. 2), but was still lower than those of the control groups (*$p < 0.05$). There was statistically significant difference before and after tonsillectomy between IgAN patients and the control groups (all $p < 0.05$) (Table 1). Although $CD_4^+CD_{25}^+$Treg cells in 6 IgAN patients treated with steroid after tonsillectomy tended to decrease compared to those of the IgAN patients without steroid therapy (data not shown), they were still higher than those of themselves before tonsillectomy.

Clinical and histopathological findings in patients on pre-/post-tonsillectomy

Clinical and histopathological findings in patients on pre-/post-tonsillectomy were listed in Table 2. Serum IgA, IL-2, IL-6 and supernatant IL-4 significantly increased in IgAN patients compared to those of the control groups before tonsillectomy (all *$p < 0.05$). Twenty-four months after tonsillectomy, serum IgA and supernatant IL-4 significantly decreased (all [Δ]$p < 0.05$), but were still higher than those of the control groups. There was statistically significant difference before and after tonsillectomy between IgAN patients and the control groups (all *$p < 0.05$). Serum IL-2 and IL-6 tended to decrease, although the difference was not significant in the IgAN patients after tonsillectomy. In contrast, serum levels of C3 did not differ between IgAN patients and control groups ($p > 0.05$), and there was no significant change in serum C3 levels in IgAN patients before

Fig. 1 $CD_4^+CD_{25}^+$Treg cells in peripheral blood by flow cytometry. $CD_4^+CD_{25}^+$Treg cells were counted using the indicated gates and are enumerated in Table 1. α: $CD_4^+CD_{25}^+$Treg cells, I: IgAN patients, II: control patients. **a** $CD_4^+CD_{25}^+$Treg cells in peripheral blood, before tonsillectomy, **b** $CD_4^+CD_{25}^+$Treg cells in peripheral blood, 24 months after tonsillectomy. $CD_4^+CD_{25}^+$Treg cells significantly decreased in IgAN patients compared to the control group before tonsillectomy ($p < 0.05$). Twenty-four months after tonsillectomy, the number of $CD_4^+CD_{25}^+$cells significantly increased, but were still lower than those of the control group ($p < 0.05$)
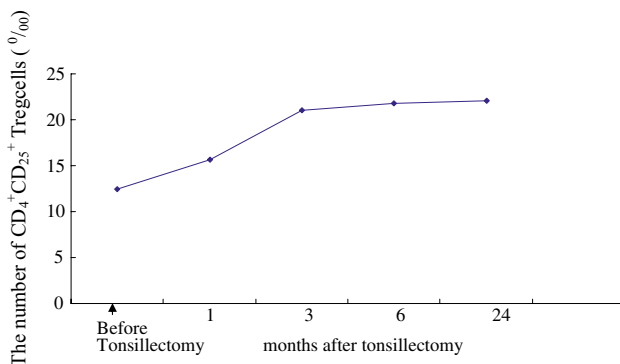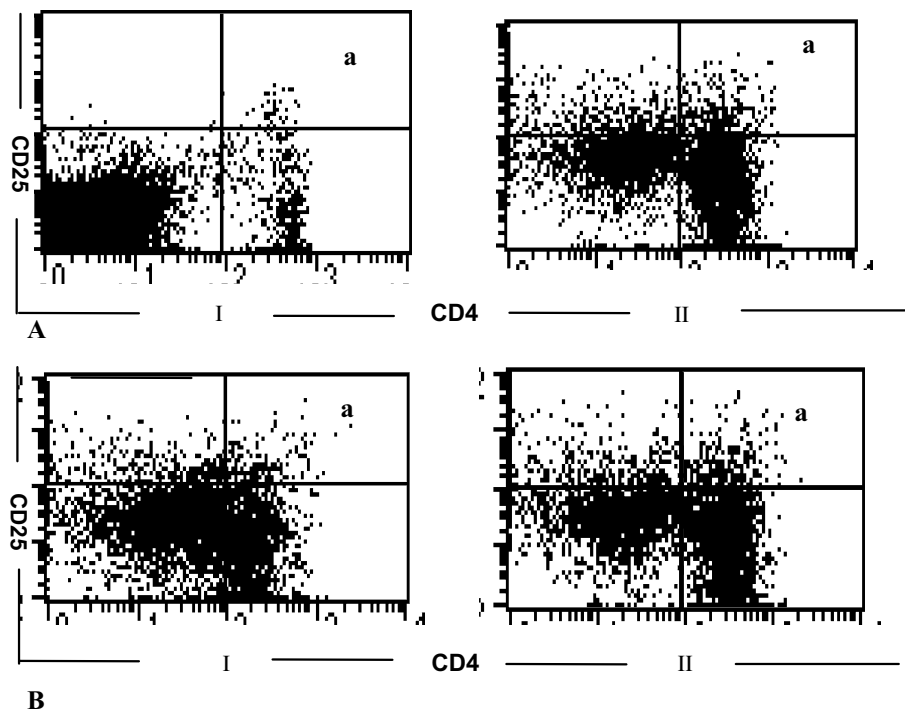

Fig. 2 The percentage of $CD_4^+CD_{25}^+$Treg cells in patients with IgAN before and after tonsillectomy. The percentage of $CD_4^+CD_{25}^+$Treg cells in IgAN patients who had undergone tonsillectomy was gradually increased compared to those before tonsillectomy. However, 3 months after tonsillectomy, increase of $CD_4^+CD_{25}^+$Treg cells tends to be stable

Table 1 The number of $CD_4^+CD_{25}^+$cells in peripheral blood (mean ± SD) %

	Preoperative	After 24 months
IgAN group	1.25 ± 0.118^a	2.19 ± 0.369*
Controls	3.04 ± 0.452	2.97 ± 0.562

$CD_4^+CD_{25}^+$Treg cells significantly decreased in IgAN patients compared to the control group before tonsillectomy ($^ap < 0.05$). Twenty-four months after tonsillectomy, the number of $CD_4^+CD_{25}^+$cells significantly increased, but were still significantly lower than those of the control group (*$p < 0.05$)

and after tonsillectomy ($p > 0.05$). Urine protein and urine erythrocytes were significantly decreased when decreased $CD_4^+CD_{25}^+$Treg cells in the peripheral blood in IgAN were significantly increased after tonsillectomy. After tonsillectomy, the AI scores were significantly decreased, while CI showed worsened slightly compared to preoperative findings in IgAN groups, although the change was not significant ($p > 0.05$). The intensity of IgA, C3 deposition in mesangial region was significantly decreased in IgAN groups after tonsillectomy (all $^\Delta p < 0.05$) (Table 2; Fig. 3).

$CD_4^+CD_{25}^+$Treg cells and clinical data

The correlations between $CD_4^+CD_{25}^+$Treg cells and values for numerous clinical parameters were examined in IgAN patients (Table 3). $CD_4^+CD_{25}^+$Treg cells were negatively correlated with serum blood urea nitrogen (BUN) in IgAN patients, and $CD_4^+CD_{25}^+$Treg cells were positively correlated with eGFR before and after tonsillectomy (all $p < 0.05$) in IgAN patients. These results indicate that $CD_4^+CD_{25}^+$Treg cells were associated with renal function in IgAN patients. $CD_4^+CD_{25}^+$Treg cells were negatively correlated with urine protein and supernatant IL-4 in IgAN patients before and after tonsillectomy (all $p < 0.05$).

Correlation between $CD_4^+CD_{25}^+$Treg cells and histological classification of IgA nephropathy

$CD_4^+CD_{25}^+$Treg cells in IgAN patients tended to decrease in parallel with the severity of the histological classification

Table 2 Clinical and histopathological findings in patients on pre-/post-tonsillectomy

	IgAN group (pre-/post-tonsillectomy)	Control group (pre-/post-tonsillectomy)
Proteinuria (g/day)	$1.62 \pm 0.54/0.62 \pm 0.23^{\Delta}$	
Hematuria ($\times 10_4$ cells/ml)	$14.87 \pm 5.12/7.29 \pm 0.72^{\Delta}$	
BUN (mg/dl)	$15.8 \pm 0.59*/14.9 \pm 0.90*$	$14.2 \pm 0.89/13.5 \pm 0.71$
Serum Cr (mg/dl)	$0.92 \pm 0.15*/0.89 \pm 0.14*$	$0.77 \pm 0.16/0.75 \pm 0.16$
Serum uric acid (μmol/l)	$347.84 \pm 73.96*/324.17 \pm 56.27*$	$238.59 \pm 40.11/244.10 \pm 54.74$
eGFR (ml/min/1.73 m^2)	$94.14 \pm 16.42*/101.16 \pm 14.73*$	$108.77 \pm 10.84/110.56 \pm 10.11$
Serum IgA (mg/dl)	$341.2 \pm 83.7*/276.7 \pm 50.7^{*,\Delta}$	$206.7 \pm 59.3/202.9 \pm 47.3$
Serum C3 (mg/dl)	$122.1 \pm 22.4/116.8 \pm 24.9$	$124.8 \pm 21.7/127.5 \pm 26.7$
Serum IL-2(pg/ml)	$102.4 \pm 12.8*/101.5 \pm 11.2*$	$24.6 \pm 12.2/22.9 \pm 8.3$
Supernatant IL-4 (pg/ml)	$513 \pm 33.03 */337 \pm 72.40^{*,\Delta}$	$211.48 \pm 34.81/219.42 \pm 89.72$
Serum IL-6 (pg/ml)	$49.21 \pm 10.30*/45.97 \pm 7.04*$	$31.41 \pm 10.49/29.14 \pm 6.56$
AI (median)	$8.3 \pm 1.1/5.1 \pm 0.8^{\Delta}$	
CI (median)	$4.6 \pm 0.4/4.7 \pm 0.3$	
Intensity of IgA deposition	$2.12 \pm 0.63/1.67 \pm 0.39^{\Delta}$	
Intensity of C3 deposition	$2.04 \pm 0.54/1.54 \pm 0.42^{\Delta}$	

AI activity index, *CI* chronicity index

* $p < 0.05$ compared with control

$^{\Delta}$ $p < 0.05$ compared with before tonsillectomy

of nephropathy as determined by the Haas classification [8] (Fig. 4), although the difference was not significant (all $p > 0.05$).

Discussion

Tonsil tissues are located at the gateway of the respiratory and alimentary tract and belong to the mucosa-associated lymphoid tissue. The generation of B cells in the germinal centers of the tonsil is one of the most essential tonsillar functions. Pathogenic bacteria easily remain the tonsillar fossae. Tonsillitis may induce nephritis or make nephritis. This indicates that tonsils were closely related to IgAN. Depletion of the minor $CD_4^+CD_{25}^+$Treg cells results in the development of organ-specific autoimmunity [9]. Autoimmune diseases can be prevented by reconstitution of the animals with $CD_4^+CD_{25}^+$Treg cells [9]. Powrie et al. [10] demonstrated that transfer of $CD_4^+CD_{25}^+$Treg cells protected mice from the development of inflammatory bowel disease and even reversed established gastrointestinal inflammation. $CD_4^+CD_{25}^+$Treg cells are regulators in almost all of the animal models of human organ-specific diseases, transplant rejection and allergic diseases [4]. The most notable immunomodulatory property of $CD_4^+CD_{25}^+$Treg cells is their ability to limit the development of a proinflammatory CD_4^+ Th2 phenotype [11]; this inhibition is characterized by reduced cytokine production. Abnormality of peripheral *T* cell and increased IgA can result from an inappropriate balance between allergen activation of $CD_4^+CD_{25}^+$Treg cells and effector Th2 cells [12, 13]. This imbalance could result from a deficiency in suppression by $CD_4^+CD_{25}^+$Treg cells or strong activation signals that overcome such regulation [14]. Recent work has shown that following antigen inhalation, $CD_4^+CD_{25}^+$Treg cells play a key immunomodulatory role [15]. It has been reported that Th1 responses are more prone to regulation by $CD_4^+CD_{25}^+$Treg cells than Th2 responses [16]. Some patients with recurrent chronic tonsillitis do not suffer from renal disease, implying that it is possible to find a balance between immunity and tolerance. However, other patients do suffer from IgAN along with recurrent chronic tonsillitis. We therefore hypothesize that a numerical and/or functional deficit of $CD_4^+CD_{25}^+$Treg cells IgAN patients might trigger the development of disease. The purpose of the present study was to observe the number of $CD_4^+CD_{25}^+$Treg cells in the peripheral blood of patients with IgAN before and after tonsillectomy. $CD_4^+CD_{25}^+$Treg cells significantly decreased in IgAN patients compared with controls before tonsillectomy. The number of $CD_4^+CD_{25}^+$Treg cells gradually increased after tonsillectomy, but was still lower than those of the control groups. Although $CD_4^+CD_{25}^+$Treg cells in 6 IgAN patients treated with steroid after tonsillectomy tend to decrease compared to those of the IgAN patients without steroid therapy (data not shown), they were still higher than those of themselves before tonsillectomy. In addition, two patients with lower levels of $CD_4^+CD_{25}^+$Treg cells exhibited a decrease after tonsillectomy in this study. These changes might be a result of other transient disease, such as acute inflammatory disease,

Fig. 3 Histopathological findings in IgAN patients on pre-/post-tonsillectomy (×40) **a** pre-tonsillectomy, **b** post-tonsillectomy. The intensity of IgA and C3 deposition in mesangial region and the AI (activity index) score were significantly decreased after tonsillectomy. However, CI (chronic index) score showed worsened slightly compared to preoperative findings, although the change was not significant after tonsillectomy
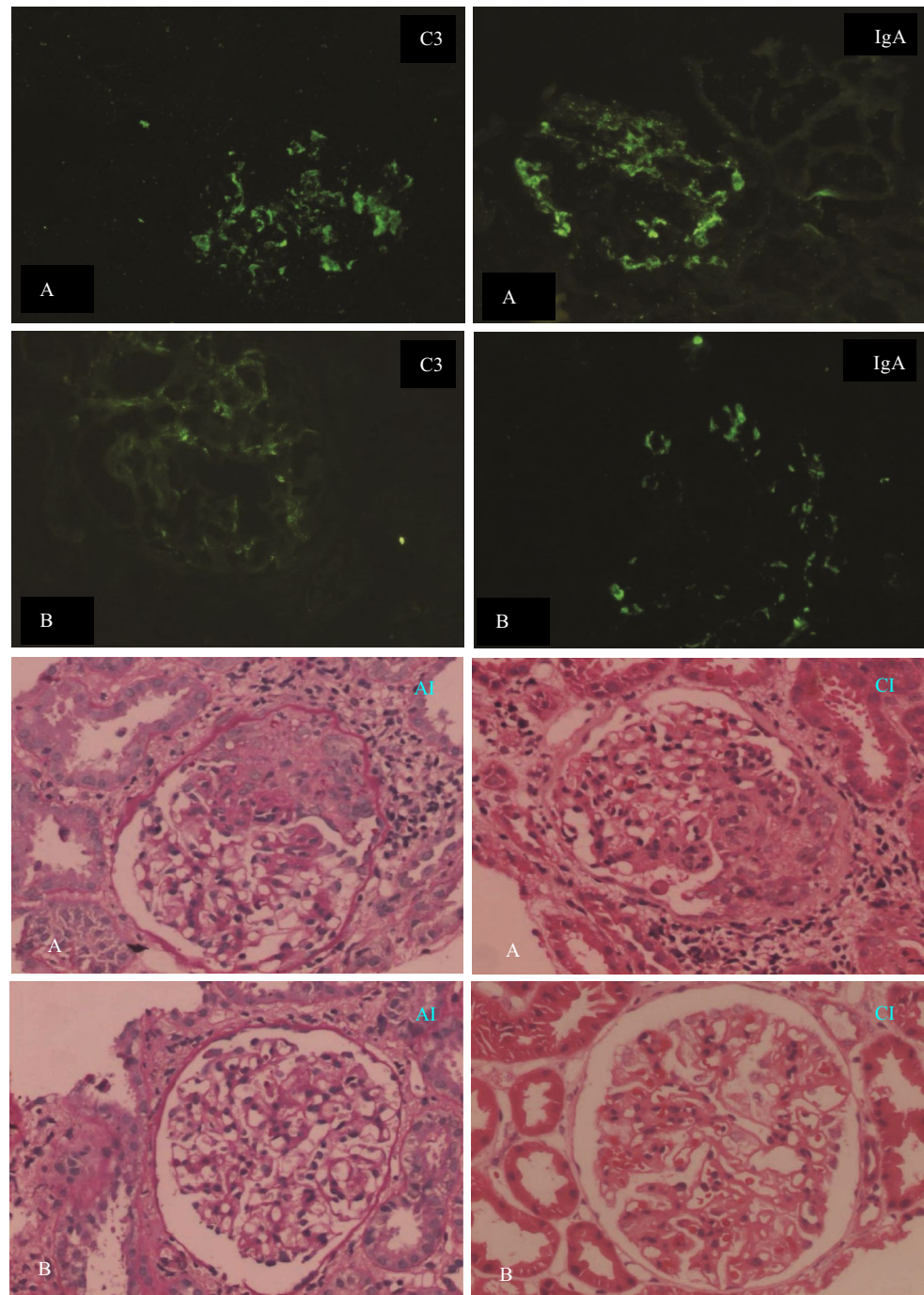
although the exact cause was not elucidated. Evaluation of $CD_4^+CD_{25}^+$Treg cells in patients with IgAN should therefore be carefully performed.

$CD_4^+CD_{25}^+$Treg cells were negatively correlated with serum BUN, supernatant IL-4 and urinary protein and were positively correlated with eGFR. The $CD_4^+CD_{25}^+$Treg cells tended to gradually decrease with increasing severity of histologically assessed nephropathy. The intensity of IgA and C3 deposition in mesangial region was significantly decreased after tonsillectomy. Serum IL-2 and

IL-6 tended to decrease, although the difference was not significant in the IgAN patients after tonsillectomy. Supernatant IL-4 and serum IgA were significantly decreased in the IgAN patients after tonsillectomy. Twenty-four months after tonsillectomy, urine protein and urine erythrocytes were significantly decreased that are consistent with previous reports [17–19]. Decreased $CD_4^+CD_{25}^+$Treg cells of peripheral blood were increased when urine protein, urine erythrocytes, serum IgA, IL-2, IL-6 and supernatant IL-4 were decreased in IgAN patients after tonsillectomy.

Table 3 Correlation between the frequency of $CD_4^+CD_{25}^+$Treg cells and clinical parameters in IgA nephropathy patients

	$CD_4^+CD_{25}^+$ cells (%) (preoperative)		$CD_4^+CD_{25}^+$ cells (%) (postoperative)	
	r	p	r	p
SBP	−0.259	n.s.	−0.276	n.s.
DBP	−0.310	n.s.	−0.202	n.s.
BUN	−0.403	<0.05	−0.370	<0.05
SUA	−0.171	n.s.	−0.193	n.s.
eGFR	0.858	<0.01	0.428	<0.05
24-h UP	−0.562	<0.01	−0.367	<0.05
Hematuria	−0.206	n.s.	−0.117	n.s.
Serum IgA	−0.221	n.s.	−0.188	n.s.
Serum C3	−0.250	n.s.	−0.116	n.s.
Serum IL-2	−0.102	n.s.	−0.012	n.s.
Supernatant IL-4	−0.727	<0.01	−0.876	<0.01
Serum IL-6	−0.238	n.s.	−0.248	n.s.

n.s. not significant, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *Scr* serum creatinine, *SUA* serum uric acid, *eGFR* estimated glomerular filtration rate, *24-h UP* 24-hour urinary protein
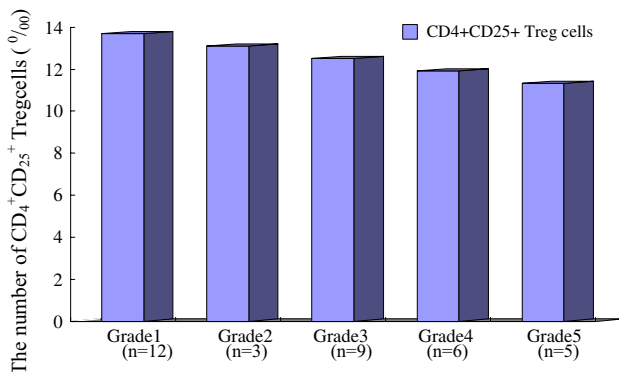

Fig. 4 The number of $CD_4^+CD_{25}^+$Treg cells in five grades of IgAN patients classified by Haas classification. The number of $CD_4^+CD_{25}^+$Treg cells in IgAN patients tended to decrease in parallel with the severity of the histological classification of nephropathy, although the difference was not significant (all $p > 0.05$)

When IgAN patient experiences a decrease in $CD_4^+CD_{25}^+$Treg cells, his/her lymphocytes react significantly more strongly to antigens, leading to higher levels of cytokine production (IL-2, IL-4, IL-6). Excessive cytokine production may enhance the switching of antibody production from one isotype to another (e.g., from IgM to IgA [20]). IL-4 regulates IgE and IgA synthesis, and increased production of IL-2 augments the IgA immune response [21, 22].

Therefore, large amounts of IgA by the blood circulation are present in the mesangial area. IgA is deposited on the glomerular mesangium where it can activate complement or disturb the balance between blood coagulation and plasminogen and stimulate renal mesangial cells to produce cytokine and inflammatory substances, altogether resulting in injured renal tissue [23]. $CD_4^+CD_{25}^+$Treg cells may play an important role in the pathogenesis of IgAN.

Tonsillectomy is thought to be effective in improving renal function in IgAN patients [24]. Akagi et al. [25] reported that tonsillectomy leads to favorable long-term outcomes in IgAN patients. Although the mechanism by which tonsillectomy is an effective treatment for IgAN is not fully understood, there duction of IgA mesangial deposition after tonsillectomy may in part explain the mechanism of this treatment. The present study indicates that increase of $CD_4^+CD_{25}^+$Treg cells is another mechanism by which tonsillectomy may function. In addition, $CD_4^+CD_{25}^+$Treg cells significantly increased after tonsillectomy, and $CD_4^+CD_{25}^+$Treg cells were not increased to the levels seen in healthy subjects in our study. Wang et al., based on their meta-analysis, concluded that whereas neither tonsillectomy nor steroid treatment alone increased remission rates in IgAN patients, tonsillectomy combined with either normal steroid or steroid pulse treatment resulted in higher remission rates with favorable long-term efficacy [26–30]. We speculate that tonsillectomy is effective in increasing $CD_4^+CD_{25}^+$Treg cells in IgAN patients, but that this effect is limited and combination therapy with tonsillectomy plus steroid-based treatment may be necessary to obtain significant increased of $CD_4^+CD_{25}^+$Treg cells and favorable long-term outcome. Kusano et al. [31] reported that the prevalence of tonsillar *H. pylori* in IgAN patients was 14 (100 %) of 14 patients and was significantly higher than in patients with recurrent pharyngotonsillitis. Anti-*H. pylori* IgA antibody in the serum of IgAN patients was more frequent and levels were higher compared to controls without renal diseases [32], and this antibody showed positive staining in the glomerular capillary walls [33]. Basal levels of reactive oxygen species (ROS) were greater in epithelial cells isolated from gastric mucosal biopsy specimens from *H. pylori*-infected subjects than in cells from un-infected individuals [34]. It is possible that immune complexes formed by *H. pylori* and this antibody in the palatine tonsils are a potential cause of IgAN pathogenesis [31]. In a recent study, Camilla et al. reported that the circulating oxidative stress predicted best the progressive kidney disease in a cohort of 292 patients with IgA nephropathy [35].

Our experiments have shown [5] that $CD_4^+CD_{25}^+$Treg cells were significantly decreased and dimeric IgA-producing cells were dramatically increased in tonsils in IgA nephropathy patients compared with the controls. Tonsillectomy may reduce these complexes and dimeric IgA-producing cells, improve immune function, and increase $CD_4^+CD_{25}^+$Treg cells, leading to attenuation of the severity of IgAN.

In conclusion, the present study indicated that $CD_4^+CD_{25}^+$Treg cells were not only lower in patients with IgAN, but also were correlated with disease severity in IgAN patients. In addition, the effects of tonsillectomy in IgAN patients may be associated with an increase of $CD_4^+CD_{25}^+$Treg cells. Additional studies are necessary to demonstrate a functional deficit of tonsillar $CD_4^+CD_{25}^+$Treg cells in IgAN patients and other biological properties of $CD_4^+CD_{25}^+$Treg cells.

Conflict of interest None.

References

1. Donadio JV, Grande JP (2002) IgA nephropathy. N Engl J Med 347:738–748
2. Xie Y, Chen X, Nishi S, Narita I, Gejyo F (2004) Relationship between tonsils and IgA nephropathy as well as indications of tonsillectomy. Kidney Int 65(4):1135–1144
3. Sakaguchi S (2004) Naturally arising CD4 + regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 22:531–562
4. Shi H-Z, Qin X-J (2005) CD4+CD25+ regulatory T lymphocytes in allergy and asthma. Allergy 60:986
5. Huang H, Peng Y, Liu H, Yang X, Liu F (2010) Decreased CD4+CD25+ cells and increased dimeric IgA-producing cells in tonsils in IgA nephropathy. J Nephrol 23(02):202–209
6. Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, Xu JS, Huang SM, Wang LN, Huang W, Wang M, Xu GB, Wang HY (2006) Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. J Am Soc Nephrol 17:2937–2944
7. Jiang L, Liu G, Lv J, Huang C, Chen B, Wang S, Zou W, Zhang H, Wang H (2009) Concise semiquantitative histological scoring system for immunoglobulin A nephropathy. Nephrology (Carlton) 14:597–605
8. Haas M (1997) Histologic subclassification of IgA nephropathy: a clinicopathologic study of 244 cases. Am J Kidney Dis 29:829–842
9. Shevach EM, McHugh RS, Piccirillo CA, Thornton AM (2001) Control of T-cell activation by CD4+CD25+ suppressor T cells. Immunol Rev 182:58–67
10. Powrie F, Read S, Mottet C, Uhlig H, Maloy K (2003) Control of immune pathology by regulatory T cells. Novartis Found Symp 252:92–98; discussion 98–105, 106–114
11. Mottet C, Uhlig HH, Powrie F (2003) Cutting edge: cure of colitis by CD4 +CD25+ regulatory T cells. J Immunol 170:3939–3943
12. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 190:995–1004
13. Thornton AM, Shevach EM (2000) Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol 164:183–190
14. Huang H, Peng Y, Liu F, Lei H (2007) Is IgA nephropathy induced by abnormalities of $CD_4^+CD_{25}^+$Treg cells in the tonsils? Med Hypotheses 69:410–413
15. Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT (2002) Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nat Med 8:1024–1032
16. McGuirk P, McCann C, Mills KH (2002) Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. J Exp Med 195:221–231
17. Béné MC, Hurault de Ligny B, Kessler M, Foliguet B, Faure GC (1993) Tonsils in IgA nephropathy. Contrib Nephrol 104:153–161
18. Barta J, Kovács T, Fazekas A, Jeges S, Nagy G, Nagy J (1996) Does tonsillectomy cause any change in long-term course of IgA nephropathy? Orv Hetil 137:2903–2906
19. Tamura S, Masuda Y, Inokuchi I, Terasawa K, Sugiyama N (1993) Effect of and indication for tonsillectomy in IgA nephropathy. Acta Otolaryngol Suppl 508:23–28
20. Suzuki S, Fujieda S, Sunaga H, Yamamoto C, Kimura H, Gejyo F (2000) Synthesis of immunoglobulins against Haemophilus parainfluenzae by tonsillar lymphocytes from patients with IgA nephropathy. Nephrol Dial Transplant 15:619–624
21. Yano N, Endoh M, Miyazaki M, Yamauchi F, Nomoto Y, Sakai H (1992) Altered production of IgE and IgA induced by IL-4 in peripheral blood mononuclear cells from patients with IgA nephropathy. Clin Exp Immunol 88:295–300
22. van den Wall Bake AW, Crowley-Nowick PA, Kulhavy R, Hermans J, Jackson S, Julian BA, Mestecky J (1992) Cytokine-induced immunoglobul in production in primary IgA nephropathy. Am J Kidney Dis 20:611–617
23. Falk R, Jennette C, Nachman PH (2000) IgA Nephropathy. In: Brenner BM (ed) The kidney, 6th edn. Saunders, Philadelphia, pp 1302–1310
24. Hotta O, Miyazaki M, Furuta T, Tomioka S, Chiba S, Horigome I, Abe K, Taguma Y (2001) Tonsillectomy and steroid pulse therapy significantly impact on clinical remission in patients with IgA nephropathy. Am J Kidney Dis 38:736–743
25. Akagi H, Kosaka M, Hattori K, Doi A, Fukushima K, Okano M, Kariya S, Nishizaki K, Sugiyama N, Shikata K, Makino H, Masuda Y (2004) Long-term results of tonsillectomy as a treatment for IgA nephropathy. Acta Otolaryngol 555:38–42
26. Wang Y, Chen J, Chen Y, Chen Y, Wang L, Lv Y (2011) A meta-analysis of the clinical remission rate and long-term efficacy of tonsillectomy in patients with IgAnephropathy. Nephrol Dial Transplant 26:1923–1931
27. Liu Y, Liu H, Tu X, Peng Y, Liu F, Zhang F, Guo C, Liu Y, Yang X, Chen G, Liu Y, Yuan F (2014) Study of tonsillectomy for IgA nephropathy patients: short- and longer-term observation. Int Urol Nephrol 46(6):1153–1159. doi:10.1007/s11255-013-0606-9
28. Fang J, Li W, Tan Z, Li D (2014) Comparison of prednisolone and lamivudine combined therapy with prednisolone monotherapy on carriers of hepatitis B virus with IgA nephropathy: a prospective cohort study. Int Urol Nephrol 46(1):49–56. doi:10.1007/s11255-013-0480-5
29. Ochi A, Moriyama T, Takei T, Uchida K, Nitta K (2013) Comparison between steroid pulse therapy alone and in combination with tonsillectomy for IgA nephropathy. Int Urol Nephrol 45(2):469–476. doi:10.1007/s11255-012-0251-8
30. Moriyama T, Nakayama K, Iwasaki C, Ochi A, Tsuruta Y, Itabashi M, Tsukada M, Takei T, Uchida K, Nitta K (2012) Severity of nephrotic IgA nephropathy according to the Oxford classification. Int Urol Nephrol 44(4):1177–1184. doi:10.1007/s11255-011-0109-5
31. Kusano K, Inokuchi A, Fujimoto K, Miyamoto H, Tokunaga O, Kuratomi Y, Shimazu R, Mori D, Yamasaki F, Kidera K, Tsunetomi K, Miyazaki J (2010) Coccoid *Helicobacter pylori* exists

in the palatine tonsils of patients with IgA nephropathy. J Gastroenterol 45:406–412

32. Barratt J, Bailey EM, Buck KS, Mailley J, Moayyedi P, Feehally J, Turney JH, Crabtree JE, Allen AC (1999) Exaggerated systemic anti-body response to mucosal *Helicobacter pylori* infection in IgA nephropathy. Am J Kidney Dis 33:1049–1057

33. Nagashima R, Maeda K, Yuda F, Kudo K, Saitoh M, Takahashi T (1997) *Helicobacter pylori* antigen in the glomeruli of patients with membranous nephropathy. Virchows Arch 431:235–239

34. Ding SZ, Minohara Y, Fan XJ, Wang J, Reyes VE, Patel J, Dirden-Kramer B, Boldogh I, Ernst PB, Crowe SE (2007) *Helicobacter pylori* infection induces oxidative stress and programmed cell death in human gastric epithelial cells. Infect Immun 75:4030–4039

35. Zhu C, Mertens PR (2012) IgA nephropathy and oxidative stress: news on clinically evaluated biomarkers hits the stage. Int Urol Nephrol 44(4):1277–1280. doi:10.1007/s11255-012-0201-5