NEPHROLOGY

Openventio PUBLISHERS

ISSN 2380-0445 Open Journal http://dx.doi.org/10.17140/NPOJ-3-117

Research

*Corresponding author
JianBin Zhang, MD
Department of Nephrological Diseases
Affiliated Yong Chuan Hospital of Chong Qing Medical University
Chong Qing, China
E-mail: zjb7526@163.com

Volume 3 : Issue 1
Article Ref. #: 1000NPOJ3117

Article History
Received: August 9th, 2017
Accepted: September 6th, 2017
Published: September 6th, 2017
Retracted: November 6th, 2017

Citation
Tang D, Guo J, Zhang J. Variation of peripheral Th17/Treg imbalance in patients with idiopathic membranous nephropathy after cyclosporin A treatment: A prognostic marker of idiopathic membranous nephropathy. *Nephrol Open J.* 2017; 3(1): 9-15. doi: 10.17140/NPOJ-3-117

Variation of Peripheral Th17/Treg Imbalance in Patients with Idiopathic Membranous Nephropathy after Cyclosporin A Treatment: A Prognostic Marker of Idiopathic Membranous Nephropathy

Dan Tang, MD; JiGuang Guo, MD; JianBin Zhang, MD*

Department of Nephrological Diseases, Affiliated Yong Chuan Hospital of Chong Qing Medical University, Chong Qing, China

ABSTRACT

Objective: To investigate the variation and effect of peripheral T-helper 17 (Th17) and regulatory T (Treg) cells upon the clinical prognosis of idiopathic membranous nephropathy (IMN) patients before and after cyclosporin A (CsA) treatment.
Methods: Twenty-four patients diagnosed with IMN and 12 healthy controls at the Yong Chuan Hospital of Chong Qing Medical University between September 2015 and October 2016, were recruited for this study. All enrolled IMN patients received prednisolone acetate in combination with CsA on the basis of supportive treatment. After CsA therapy for 6 months, patients were assigned into the responsive and unresponsive groups according to the serum levels of albumin (ALB) and 24-hour urinary protein. The serum levels of ALB and 24-hour urinary protein were measured by full automatic biochemical analyzer. The peripheral Th17% and Treg% were detected and calculated by flow cytometry. The expression levels of Interleukin-17 (IL-17), tumor necrosis factor-alpha (TNF-α) and transforming growth factor beta (TGF-β) in the peripheral blood were quantitatively measured by ELISA.
Results: Compared with the healthy controls, in the peripheral blood of IMN patients, Th17 percentage and the expression levels of IL-17 and TNF-α were upregulated, whereas Treg percentage and TGF-β level were downregulated. All patients were assigned into the high-, middle- and low-risk groups according to quantitative analysis of the 24-hour urinary protein. In the high-risk group, the expression levels of IL-17, TNF-α and TGF-β were significantly upregulated, whereas the Treg% and TGF-β level were dramatically downregulated compared with those in the middle and low-risk groups. The 24-hour urinary protein level was positively correlated with Th17% and Th17/Treg ratio, whereas negatively correlated with Treg%. After a 6 month combined therapy of CsA and prednisone, 18/24 IMN patients fell into the effective group. In these patients, the 24-hour urinary protein level, Th17%, IL-17 and TNF-α levels were significantly downregulated, whereas the peripheral Treg% and TGF-β level were dramatically upregulated in the effective group (all $p<0.05$). 6/24 IMN patients fell into the in effective group, no significant changes were noted in these parameters in the ineffective group.
Conclusion: In IMN patients, present peripheral Th17/Treg imbalance is correlated with the severity of IMN. CsA treatment is an effective approach to improve peripheral blood Th17/Treg imbalance in a sub-population of IMN patients, which is associated with the clinical efficacy of CsA treatment. Monitoring the variations in peripheral concentration of Treg and Th17 is of significance for evaluation of the severity of IMN and clinical efficacy.

KEY WORDS: Th17; Treg; Idiopathic membranous nephropathy; Cyclosporin A; Efficacy evaluation.

Openventio PUBLISHERS

ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-3-117

ABBREVIATIONS: IMN: Idiopathic Membranous Nephropathy; CsA: Cyclosporin A; ALB: Albumin; Treg: regulatory T; Scr: Serum creatinine; CYP: Cyclophosphamide; IL-17: Interleukin-17; TNF-α: Tumor Necrosis Factor-alpha; TGF-β: Transforming Growth Factor-beta; ELISA: Enzyme-Linked Immunosorbent Assay.

INTRODUCTION

Idiopathic membranous nephropathy (IMN) is a common cause of nephrotic syndrome in adults. Previous investigations reveal that IMN is the most common single cause of nephritic syndrome, accounting for approximately 1/3 of cases globally.[1] A major fraction of these patients do not achieve remission with immune therapy, and eventually progress to end-stage renal disease. The exact pathogenesis of IMN remains to be fully elucidated. Recent investigations have demonstrated that cellular immunity disturbance probably plays a vital role in the pathogenesis of IMN.[2]

In recent years, the T-helper 17 (Th17)/regulatory T (Treg) balance, which is different from the $CD4^+$ T-helper lymphocyte subset of Th1 and Th2, has been identified to play an influential role in the regulation of host immune tolerance, resistance of rejection reaction, infection, malignant tumor, inflammation and alternative diseases.[3] Multiple studies[4,5] have suggested that Th17 and its primary secretion interleukin-17 (IL-17) are involved with the pathogenesis of lupus nephritis, crescentic glomerulonephritis and proliferative glomerulonephritis, etc. Previous investigations have demonstrated that the peripheral Treg% in IMN patients is significantly lower compared to that in the healthy controls. After rituximab monoclonal antibody therapy, peripheral Treg% is elevated, which is intimately correlated with clinical efficacy.[6] Nevertheless, existence of Th17/Treg imbalance in IMN patients, its correlation with the progression and clinical prognosis of IMN patients has not yet been thoroughly investigated.

Cyclosporin A (CsA) is a highly selective potent immunosuppressive agent, which can effectively inhibit the proliferation and activation of T-lymphocytes. Recent studies[7,8] have reported that use of CsA exerts significant effect upon the Th17/Treg ratio in patients with autoimmune diseases and after organ transplantation. In addition, the variation in Th17/Treg ratio is probably correlated with clinical efficacy. However, the effect of CsA therapy upon the changes in Th17/Treg ratio in IMN patients has been seldom studied. Consequently, this study was designed to investigate the variation in Th17/Treg ratio before and after CsA therapy and its correlation with the clinical prognosis of IMN patients.

MATERIALS AND METHODS

Baseline Data

Twenty-four IMN patients (48.83±4.92 years, range 20-77 years, 15 males, and 9 females) admitted to the Yong Chuan Hospital between September 2015 and October 2016 were recruited for the clinical trial, 12 healthy people (48.55±5.82 years, range 21-73 years, 7 males, and 5 females) were enrolled as controls (Table 1). All subjects were informed and signed informed consent. Membranous nephropathy was diagnosed *via* renal biopsy. The possibility of alternative secondary membranous nephropathy was excluded.

Inclusion criteria were as follows: (1) patients of both genders aged ≥18 years; (2) those pathologically diagnosed with membranous nephropathy *via* renal biopsy; (3) those diagnosed with IMN for the first time and had no medical history of cyclophosphamide (CYP), CsA or other immunosuppressive agent use. Exclusion criteria were as below: (1) patients with a complicated infection, malignant tumor, hypertension and diabetes mellitus, etc.; (2) pregnant women. Twelve, age and gender-matched healthy volunteers, of which 7 were male and 5 females were recruited as the control group. None of the enrolled individuals had any history of immune or infectious diseases. All patients had signed the informed consents and completely cooperated with the study procedures.

All enrolled patients were administered with prednisone (0.5 mg/kg*d) in combination with CsA (3-5 mg/kg*d). The blood drug concentration of CsA was monitored and maintained to the standard range of 100-180 ng/ml. According to the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline, the patients were divided into three groups, viz., low- middle- and high-risk. In the low-risk group, the kid-

Table 1: Baseline Data of the Enrolled Study Subjects.

	IMN Patients (n=24)	Health Control (n=12)
Sex (male:female)	15/9	7/5
Age (year)	48.86±7.31	48.55±5.82
Process (month)	11.04±17.31	-
IL-17 (pg/ml)	56.02±9.54*	43.6±8.81
TNF-α (pg/ml)	149.8±13.09*	36.51±7.79
TGF-β (pg/ml)	935.16±417.02*	264.30±66.23
Th17%	1.15±0.62*	0.77±0.22
Treg%	0.96±0.59*	1.67±0.52
Th17/Treg	1.15±0.33*	0.41±0.18

*$p<0.05$ *vs.* heathy control

NEPHROLOGY
Open Journal
Openventio PUBLISHERS
ISSN 2380-0445
http://dx.doi.org/10.17140/NPOJ-3-117

ney function was normal and the albuminuria level <4 g/24 h within 6 months. In the middle-risk group, the renal function was normal and the albuminuria level ranged from 4 g/24 h to 8 g/24 h. In the high-risk group, renal insufficiency or kidney atrophy was noted, and the albuminuria level exceeded 8 g/24 h. Quantitative analysis of the 24-hour urinary protein was utilized as a parameter to evaluate the clinical efficacy.

After 6 months treatment, according to the KDIGO guidelines, the patients were divided into two groups: the effective group and the ineffective group, the effective group classification criteria were as follows: (1) Complete remission: 24UPro<0.3g/L (UPro/Scr<300 mg/g or <30 mg/mmol), the two determinations are at least one week apart, with normal ALB and serum creatinine (Scr), (2) Partial remission: 24 UPro<3.5 g/d (UPro/Scr <3500 mg/g or <350 mg/mmol), and urinary protein reduction to reach or exceed the peak of 50%, the two determinations are at least one week apart, accompanied by ALB and Scr improved or return to normal.

METHODS

8-20 ml of fasting venous blood samples were collected from both the IMN patients and healthy controls before and 6 months after corresponding treatment. The plasma was separated from the cells within 2 hours after blood sampling collection. The serum levels of ALB and 24-hour urinary protein were detected by full automatic biochemical analyzer. The peripheral blood levels of IL-17, tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β) were measured by enzyme-linked immunosorbent assay (ELISA) and the serum Th17% and Treg% were calculated by flow cytometry.

Statistical Analysis

SPSS 20.0 software package was utilized for the statistical analysis. Measurement data were expressed as mean±standard deviation and analyzed using *t*-test. Enumeration data were expressed in percentage and statistically processed by *chi*-square test. A *p* value of less than 0.05 was considered as statistical significance.

RESULTS

Compared to Healthy Controls, IMN Patients Exhibited Significantly Higher Th17% and Significantly Lower Treg% in their Peripheral Blood

Baseline data of the IMN patients and healthy controls were illustrated in Table 1. No statistical significance was identified in gender and age between two groups (both $p>0.05$).

Compared to the healthy group, IMN group exhibited higher Th17% (1.15±0.62% *vs*. 0.77±0.22%), and lower Treg% (0.96±0.59% *vs*. 1.67±0.52%) (Table 2). The related cytokine concentrations IL-17 and TNF-a in IMN group were higher (56.02±9.54 *vs*. 43.6±8.81 and 149.8±13.09 *vs*. 36.51±7.79) while TGF-β was lower than the healthy group (935.16±417.02 *vs*. 264.30±66.23)

In IMN Patients, Th17/Treg Ratio Shows Positive Correlation with the 24-Hour Urinary Protein Levels

Correlation analysis between 24-hour urinary protein level analysis and Treg and Th17 levels of all 24 IMN patients revealed that there was a strong positive correlation between the 24-hour urinary protein level with peripheral Th17% as well as Th17/Treg ratio. However, these analyses also showed a negative correlation between the 24-hour urinary protein level and peripheral Treg% in IMN patients (Figure 1).

Th17/Treg Ratio Imbalance is more Pronounced in Middle-Risk and High-Risk Patients Compared to Low-Risk Patients

According to the 24-hour urinary protein level, all IMN patients were divided into the low-, middle- and high-risk groups. As illustrated in Table 3, the peripheral Th17% and Th17/Treg ratio in the middle and high-risk groups were significantly upregulated compared with those in the low-risk group (all $p<0.05$). In the middle and high-risk groups, the peripheral Treg% was considerably downregulated than that in the low-risk group (all $p<0.05$), Furthermore, compared with the middle-risk group, significant variations were observed in terms of the parameters above in the high-risk group (all $p<0.05$).

Cyclosporin A Treatment Effectively Corrected the Th17-Treg Imbalance and Significantly Decreased the 24-Hour Urinary Protein Levels in a Majority of Patients

As outlined in the methods section, flow cytometry was used to measure peripheral Th7 and Treg cell levels. Typical flow cytometry diagrams of peripheral Th7 and Treg cell levels in IMN patients before and after CsA treatment are depicted in Figure 2.

As illustrated in Table 3, the 24-hour urinary protein level, Th17%, IL-17 and TNF-α levels were significantly upregulated, whereas the peripheral Treg% and TGF-β level were

Table 2: Baseline Peripheral Th17 and Treg Cell Levels in IMN Patients Stratified by Risk Level.

	Th17%	Treg%	Th17/Treg
Low-risk (9)	1.06±0.54	1.73±0.49	0.54±0.22
Middle-risk (11)	1.33±0.65*	1.12±0.41*	1.02±0.35*
High-risk (4)	1.75±0.70*#	0.73±0.64*#	1.48±0.49*#

*$p<0.05$ *vs*. low-risk; #$p<0.05$ *vs*. middle-risk.

NEPHROLOGY
Open Journal

Openventio PUBLISHERS

ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-3-117

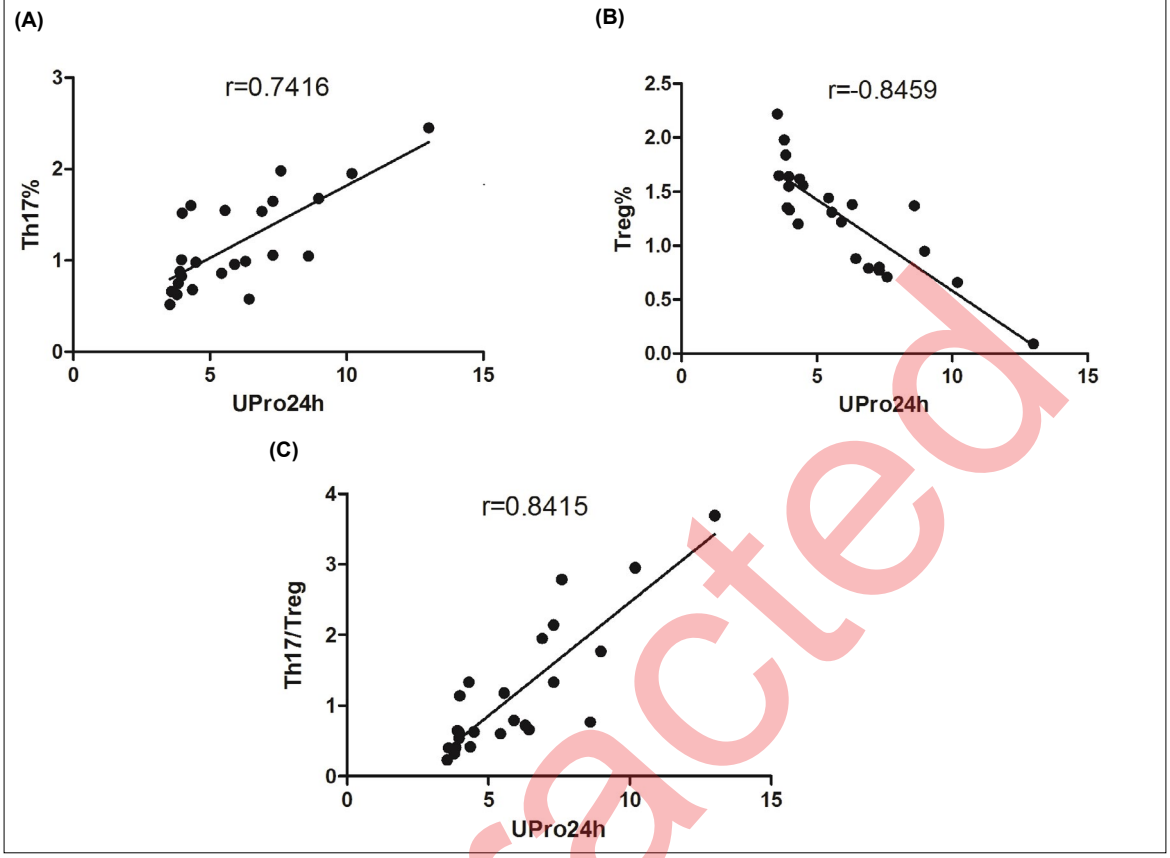

Figure 1: Correlation Analysis between 24-Hour Urinary Protein Level and Th17% (A), Treg% (B) and Th17/Treg Ratio (C) at Baseline in IMN Patients.

Table 3: Changes in Clinical Parameters and Peripheral Th17, Treg and Relevant Cytokines in IMN Patients before and after CsA Treatment.

	Effective group (n=18)		Ineffective group (n=6)	
	Before treatment	After treatment	Before treatment	After treatment
24-hour urinary protein (g/24 h)	6.82±3.89*	4.64±2.63	9.06±2.35	8.75±3.35
Alb (g/L)	29.44±4.91*	32.21±6.50	23.44±3.24	24.48±4.09
IL-17 (pg/mL)	52.90±10.4*	43.15±7.34	63.43±8.65	61.63±12.08
TNF-α (pg/mL)	123.8±7.91*	81±6.5	179.5±12.6	175±5.14
TGF-β (pg/mL)	869.15±369.21*	654.21±158.23	1032.98±542.60	986.21±593.77
Th17%	1 .05±0.54*	0.81±0.43	1 .38±0.68	1.36±0.51
Treg%	1.11±0.60*	1.64±0.39	0.88±0.39	0.91±0.41
Th17/Treg	0.99±0.48*	0.45±0.22	1.57±0.44	1.47±0.37

*p<0.05 *vs*.after treatment.

remarkably downregulated in IMN patients compared with those in healthy controls (all p<0.05). After CsA therapy for 6 months, patients were assigned into the effective and ineffective groups according to the serum levels of Alb and 24-hour urinary protein. In the effective group, the 24-hour urinary protein level, peripheral Th17%, IL-17 and TNF-α levels were significantly downregulated, whereas the serum level of ALB, peripheral Treg% and TGF-β were considerably upregulated after CsA treatment (all p<0.05). In the unresponsive group, the 24-hour urinary protein level, serum level of ALB, peripheral Th17%, Treg%, IL-17, TNF-α and TGF-β levels did not significantly differ before and after the CsA treatment (all p>0.05).

Cyclosporin A Treatment has a Significantly Increased impact on Low-Risk Patients Compared to the Middle-Risk and High-Risk Patients

Stratification of data presented in Table 4 among various risk-levels indicates that CsA treatment improved the clinical parameters of 8 out 9 patients in the low-risk group and 8 out of 11 patients in the middle-risk group but only 2/4 patients in the high-risk group.

NEPHROLOGY
Open Journal

Openventio PUBLISHERS

ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-3-117

Figure 2: Typical Flow Cytometry Diagrams of Peripheral Th7 and Treg Cell Levels in IMN Patients before and after CsA Treatment.

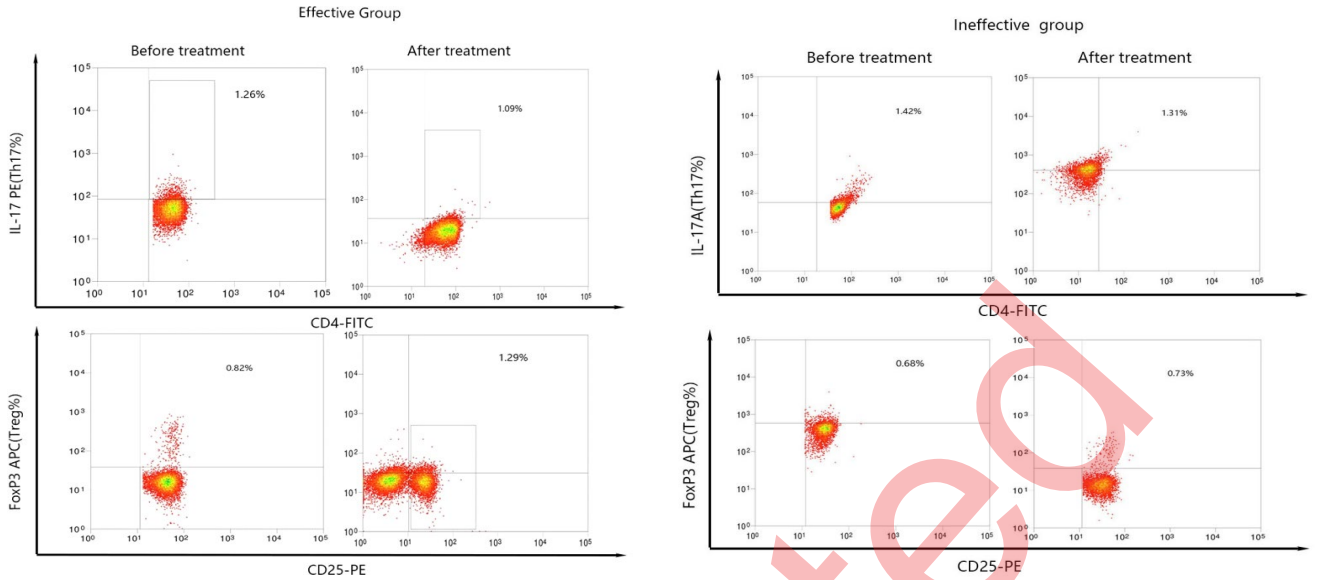

Table 4: Effect of CsA Treatment among Various Risk-Levels Patients.

	Effective Group (n=18)	Ineffective Group (n=6)
Low risk	8/9	1/9
Middle risk	8/11*	3/11*
High risk	2/4*#	2/4*#

*$p<0.05$ *vs.* low risk; #$p<0.05$ *vs.* middle risk.

DISCUSSION

At present, the pathogenesis of IMN is that the circulating autoantibody recognizes the target antigen of glomerular podocytes. After the antigen-antibody binding, an immune complex forms subepithelially, which activates the complement to form membrane-attack complex, leading to basement membrane and glomerular filtration barrier injury and generates albuminuria.[9] Until now, no biomarker has been utilized to monitor the immune activity in IMN patients in clinical practice. Recent investigations have demonstrated that the pathogenesis of IMN is correlated with the immune disorder of T/B lymphocytes. Zhihong et al reported that the quantities of multiple immunologically competent cells were substantially altered in IMN patients, including a decrease in Treg, increase in B-cells, and an elevated CD4/CD8 ratio.[10] Masutani et al[11] employed flow cytometry to quantitatively analyze the ratio of each cell subset and found that the quantity of IL-4$^+$ T-cells is significantly upregulated, whereas the Th1/Th2 ratio was considerably downregulated in the IMN patients compared to healthy controls. In addition, the serum level of IL-4 is intimately correlated with the quantity of urinary protein. The immune responses of T lymphocytes dominated by Th1 downregulation and Th2 polarization disrupt the host immune balance, which probably promotes the incidence of IMN. As the CD4$^+$ T-cell subset, Treg and Th17 cells have been proven to differ from Th1 and Th2. Th17/Treg balance plays a pivotal role in maintaining immune homeostasis and preventing the occurrence of autoimmune diseases.[12] Consequently, we investigated the association of Th17/Treg imbalance with IMN severity and treatment response.

As a common drug for membranous nephropathy, CsA can be utilized as the initial treatment of IMN or alternative therapy if other medications are ineffective. The primary mechanism underlying the decrease of albuminuria is to inhibit immune response, selectively suppress the activation of T-cells, repress the production of IL-2, inhibit the secretion of calcineurin, block the dephosphorylation of synaptopodin induced by calcineurin and stabilize the cytoskeleton of kidney podocyte, thereby reducing the generation of protein.[13] Previous studies have reported that administration of CsA exerts a significant effect upon immune diseases and after organ transplantation, which is possibly associated with clinical efficacy.[14,15] Our observation that CsA treatment has a significant impact on Th17/Treg imbalance offers a novel mechanism.

CONCLUSION

The prime objective of this study was to assess Treg/Th17 imbalance in patients with IMN. The study revealed Th17/Treg ratio was different in IMN *vs.* control, Th17% and related cytokines level was higher while Treg% and serum TGF-β levels

ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-3-117

were lower in IMN compared to healthy controls, suggesting that Th17/Treg immune imbalance (reduced Treg cells and increased Th17 cells) exists in IMN patients. Downregulation of Treg cells may activate the immune system and promote pathological reactions that contribute to the pathogenesis of IMN. In addition, it was also found that the Th17 cells and the levels of IL-17, TNF-α and IL-6 were upregulated in IMN patients. We suggest that IMN initiates the Th17-type immune response through the release of IL-17, TNF-α and other pro-inflammatory cytokines, thereby provoking regional kidney tissue inflammation and upregulating the expression of pro-inflammatory cytokines and chemokines.

The secondary objective of this study was to investigate the relationship between Treg/Th17 imbalance with IMN progression and severity. Treg/Th17 cell imbalance was found to correlate with 24 hours total urine protein, and the patients with a greater Treg/Th17 imbalance had more 24 hours total urine protein. Further subgroup analysis revealed that the Th17% and Th17/Treg ratio was higher while Treg% was lower in the middle-risk and high-risk group when compared to low-risk and healthy control group. The We suggest that Treg/Th17 imbalance is associated with severity and progression of IMN through immune-mediated injury. Therefore, immunotherapy with the goal of decreasing the inflammations caused by Treg/Th17 imbalances may have a protective effect in patients with IMN.

The third question addressed by this study was to observe the variations of peripheral Th17/Treg imbalance after CsA therapy and its correlation with the clinical prognosis of IMN patients. In this study, we observed that after 6 months of CsA, the 24-hour urinary protein level, peripheral Th17%, IL-17 and TNF-α levels were significantly downregulated, whereas the serum level of ALB, peripheral Treg% and TGF-β were considerably upregulated in 18/24 responsive patients, hinting that CsA probably affects clinical efficacy and prognosis of IMN patients by regulating the Th17/Treg immune imbalance. Moreover, Th17/Treg ratio did not significantly alter in 6/24 non-responsive group. However, we did not have further research on why CSA treatment of IMN was unresponsive. CsA does not prevent continuing autoantibody formation, production and deposition of IgG4 in the glomeruli may promote the development of MN,[16] but still need larger samples, a long-term follow-up to confirm.

In conclusion, IMN patients present with peripheral Th17/Treg imbalance are correlated with the severity of IMN. CsA therapy is an efficacious approach to improve the peripheral Th17/Treg imbalance, which is linked to the clinical efficacy of CsA treatment. Dynamic monitoring of the variation in the peripheral levels of Treg and Th17 contributes to evaluate the severity of IMN and assess the clinical efficacy.

ACKNOWLEDGMENTS

The authors thank Mrs. Liu Juan for experimental support.

CONFLICTS OF INTEREST

The authors have no potential conflicts of interest.

REFERENCES

1. Kwatra IS, Prasher PK. Pathogenesis of membranous nephropathy: Update. *J Assoc Physicians India*. 2013; 61(11): 807-810.

2. Shi X, Qu Z, Zhang L, et al. Increased ratio of ICOS(+) /PD-1(+) follicular helper T cells positively correlates with the development of human idiopathic membranous nephropathy. *Clin Exp Pharmacol Physiol*. 2016; 43(4): 410-416. doi: 10.1111/1440-1681.12555

3. Schmitt V, Rink L, Uciechowski P. et al. The Th17/Treg balance is disturbed during aging. *Exp Gerontol*. 2013; 48(12): 1379-1386. doi: 10.1016/j.exger.2013.09.003

4. Jia XY, Hu SY, Chen JL, et al. The clinical and immunological features of patients with combined anti-glomerular basement membrane disease and membranous nephropathy. *Kidney Int*. 2014; 85(4): 945-952. doi: 10.1038/ki.2013.364

5. Iannitti RG, Carvalho A, Cunha C, et al. Th17/Treg imbalance in murine cystic fibrosis is linked to indoleamine 2,3-dioxygenase deficiency but corrected by kynurenines. *Am J Respir Crit Care Med*. 2013; 187(6): 609-620. doi: 10.1164/rccm.201207-1346OC

6. Rosenzwajg M, Languille E, Debiec H, et al. B- and T-cell subpopulations in patients with severe idiopathic membranous nephropathy may predict an early response to rituximab. *Kidney Int*. 2017; 92(1): 227-237. doi: 10.1016/j.kint.2017.01.012

7. Tang B, Ren H, Liu H, et al. CCR5 blockade combined with cyclosporine A attenuates liver GVHD by impairing T cells function. *Inflamm Res*. 2016; 65(11): 917-924. doi: 10.1007/s00011-016-0974-6

8. Sakai R, Taguri M, Oshima K, et al. A comparison of tacrolimus and cyclosporine combined with methotrexate for graft-versus-host disease prophylaxis, stratified by stem cell source: A retrospective nationwide survey. *Int J Hematol*. 2016; 103(3): 322-333. doi: 10.1007/s12185-016-1939-9

9. Mercadal L. Membranous nephropathy. *Nephrol Ther*. 2013; 9: 507-517. doi: 10.1016/j.nephro.2013.10.002

10. Bo W, Zhi-hong L, Yan W, et al. Regulatory T cells and B cells in patients with idiopathic membranous nephropathy. *Chinese Journal of Nephrology, Dialysis & Transplantation*. 2009; 18: 322-328.

11. Masutani K, Taniguchi M, Nakashima H, et al. Up-regulated interleukin-4 production by peripheral T-helper cells in idiopath-

ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-3-117

ic membranous nephropathy. *Nephrol Dial Transplant*. 2004; 19: 580-586. doi: 10.1093/ndt/gfg572

12. Zhang J, Hua G, Zhang X, Tong R, Du X, Li Z. Regulatory T cells/T-helper cell 17 functional imbalance in uraemic patients on maintenance haemodialysis: A pivotal link between microinflammation and adverse cardiovascular events. *Nephrology (Carlton)*. 2010; 15: 33-41. doi: 10.1111/j.1440-1797.2009.01172.x

13. Foxwell BM, Ruffel B. The mechanisms of action of cyclosporine. *Cardiol Clin*. 1990; 8(1): 107-117.

14. Jaiswal A, Prasad N, Agarwal V, et al. Regulatory and effector T cells changes in remission and resistant state of childhood nephrotic syndrome. *Indian J Nephrol*. 2014; 24(6): 349-355. doi: 10.4103/0971-4065.132992

15. Hunemorder S, Treder J, Ahrens S, et al. TH1 and TH17 cells promote crescent formation in experimental autoimmune glomerulonephritis. *J Pathol*. 2015; 237(1): 62-71. doi: 10.1002/path.4559

16. Rosenzwajg M, Languille E, Debiec H, et al. B- and T-cell subpopulations in patients with severe idiopathic membranous nephropathy may predict an early response to rituximab. *Kidney Int*. 2017; 92(1): 227-237. doi: 10.1016/j.kint.2017.01.012