Expression of T Helper 17 and Regulatory T Cell Cytokines and Molecules in Glomerulonephritis Class IV Systemic Lupus Erythematosus

Maryam Rastin,[1] Samaneh Soltani,[1] Fatemeh Nazemian,[2] Maryam Sahebari,[3] Seiede Zahra Mirfeizi,[4] Nafiseh Tabasi,[1] Mahmoud Mahmoudi[1]

[1]Immunology Research Center, Bu-Ali Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Department of Internal Medicine, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Rheumatic Diseases Research Center, Ghaem Hospital, Internal Medicine Section, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Rheumatic Diseases Research Center, Imam Reza Hospital, Internal Medicine Section, Mashhad University of Medical Sciences, Mashhad, Iran

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Introduction. Lupus nephritis is a serious organ involvement with unknown etiology, and glomerulonephritis class IV is one of the most severe forms of the disease which correlates with poor prognosis and death. Immunological abnormalities are implicated in the expression of lupus nephritis. In this study, we examined some T helper 17 and regulatory T-related cytokines and molecules in systemic lupus erythematosus patients with glomerulonephritis class IV.

Materials and Methods. The study group comprised of 20 glomerulonephritis class IV SLE patients and 20 sex- and age-matched SLE patients without kidney involvement as control group. Blood samples was collected from each participant, lymphocytes were isolated, and RNA was extracted from lymphocytes. Then cDNA was synthesized using reverse transcription enzyme, and finally using specific primers and probes, the expression levels of forkhead box P3 (Foxp3), transforming growth factor (TGF)-β, interferon (IFN)-γ, interleukin (IL)-6, and IL-17 genes were analyzed by real-time polymerase chain reaction based on the TaqMan method.

Results. The expression levels of IL-6, IL-17, IFN-γ, and Foxp3 genes were significantly higher in SLE patients with glomerulonephritis class IV than those with non-nephritis SLE. However, the expression of TGF-β was not significantly different between the SLE patients with and without glomerulonephritis class IV involvement.

Conclusions. According to our results, it seems that in class IV glomerulonephritis patients, increased Foxp3-producing regulatory T cells has an imperfect capacity to control the pathogenic IL-17- and IFN-g-producing cells.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune multisystem disease characterized by the production of auto-antibodies and deposition of immune complexes in various organs.[1] Kidney involvement is one of the most frequent serious organ manifestations induced by the presence of immune complexes at various sites of the glomeruli.[2] Glomerular pathological findings of lupus nephritis (LN) were originally categorized in 6 classes from class I to VI.[3] Glomerulonephritis class IV is called diffuse proliferative glomerulonephritis and has the highest rate of mortality and prevalence compared to other classes.[4]

The pathogenesis of LN is incompletely understood. Production of nephritogenic anti-double-stranded DNA autoantibodies, in situ formation of immune complexes, and deposition of previously formed immune complexes in the kidney primes inflammatory events leading to the kidney damage.[5] Regulatory T cells ($CD4^+CD25^+Foxp3^+$) are responsible for maintenance of tolerance and play a protective role in SLE.[6] Regulatory T cells play an important role in controlling unwanted immune responses, and distressed balance between regulatory and effector T helper 17 cells are implicated in the pathogenesis of LN.[7] The defective number or function of this population promotes hyperactivated responses in SLE patients.

T helper 17 cells have recently been introduced and are known as an effector subset of T helper cells which are present in the damaged organs in SLE patients. These cells produce interleukin (IL)-17 and have a pathogenic role in the tissue injury in LN.[8] T cells polarization is affected by cytokines milieu and inflammatory conditions.[9] Interleukin-6, in the presence of transforming growth factor (TGF)-β promotes differentiation of naive T helper cells to T helper 17 subset, while presence of TGF-β in the absence of IL-6 converts naive T helper cells to regulatory T cells, with prominent immunomodulatory effects.[10]

In the some classes of LN, predominance of T helper 2 cells was reported, but at the late stages of class IV glomerulonephritis, increased presence of interferon (IFN)-γ and T helper 1 cells was identified,[11] which could worsen nephritis. Overproduction of T helper 1 and T helper 17 cytokines and defective number and function of regulatory T cells could promote nephritogenic conditions in LN.

In this study, we investigated the expression of some T helper 17 and regulatory T cell-related cytokines and molecules in glomerulonephritis class IV and non-nephritis SLE patients to better understand the impression of regulatory T and T helper 17 cells in the pathogenesis of LN.

MATERIALS AND METHODS

Study participants comprised of 40 SLE patients who fulfilled at least 4 of the 1997 revised criteria of American Rheumatism Association for the classification of SLE, and were recruited between 2009 and 2010 by nephrologists or rheumatologists. The SLE patients were divided into 2 groups: 20 patients with glomerulonephritis class IV whose disease was confirmed with renal biopsy by a pathologist based on the World Health Organization classification's system, and 20 sex- and age-matched non-nephritis SLE patients acting as control group. Patients with other classes of LN and those who received cytotoxic drugs were excluded from the study.

The study was approved by the ethics committee of Mashhad University of Medical Sciences and written informed consent was taken from all participants.

RNA extraction and cDNA synthesis

Ten mL peripheral blood in ethylenediaminetetraacetic acid was collected from each participant. Peripheral blood mononuclear cells were isolated using density gradient centrifugation on Ficoll-Hypaque according to the manufacture's instruction (Gibco). Total mRNA was extracted using Tripure Reagent (Roche, Germany). Then, quality and integrity of the total RNA was assessed by electrophoresis on 2% agarose gel (Roche, Germany). Purity of extracted RNA was assessed by relative absorbance at 260:280 nm.

The total amount of mRNA was reverse transcribed using 1 µg of RNA in a volume of 20-µL solution containing 1µL of random hexamers, 4 µL of 5X buffer, 2 µL of dNTP (Roche), 0.5 µL of RNasin (Fermentas), and 1 µL of reverse transcription (RT enzyme, M-MuLV, Fermentas). Reverse transcription was performed as follows: 95°C for 10 minutes, 42°C for 60 minutes, and 70°C for 10 minutes. cDNAs were stored at -20°C for later analysis.

Real-time Polymerase Chain Reaction

The cDNA samples were subjected to real-time quantitative polymerase chain reaction (PCR) analysis. Quantitative real-time PCR was performed using the TaqMan method. The primers and probes were designed using Beacon Designer software to recognize IL-17, IL-6, IFN-γ, TGF-β, forkhead box P3 (Foxp3), and the reference gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*; Table 1). In order to confirm the gene specificity for a single amplification product, a Blast search of the selected primers was performed in the Gene Bank sequence database. TaqMan primers and probes are listed in Table 1. Quantitative real-time PCR amplification

Table 1. Primers and Probes Used in the Study

Genes	Sequences
IL-17	
Primer F	5′- CAgCAAgAgATCCTggTCCTg-3′
Primer R	5′- gACAATCggggTgACACAgg -3′
Probe	5′-FAM AgCCTCCACACTgCCCCAACTCCT TAMRA-3′
IL-6	
Primer F	5′- CTTCggTCCAgTTgCCTTCTC-3′
Primer R	5′- ATTCgTTCTgAAgAggTgAgTgg-3′
Probe	5′-FAM CTgCTCCTggTgTTgCCTgCTgCC TAMRA-3′
IFN-γ	
Primer F	5′- AgCTgACTAATTATTCggTAACTg-3′
Primer R	5′- CTCgAAACAgCATCTgACTC-3′
Probe	5′-FAM TgTCCAACgCAAAgCAA TAMRA-3′
Foxp3	
Primer F	5′- ACATggACTACTTCAAgTTCC-3′
Primer R	5′- AACCAgTggTAgATCTCATTg-3′
Probe	5′-FAM CCgCTgCTTCTCTggA TAMRA-3′
TGF-β	
Primer F	5′- gCAACAATTCCTggCgATACC-3′
Primer R	5′- gCCCTCAATTTCCCCTCCAC-3′
Probe	5′-FAM CTCAACCACTgCCgCACAACTCCg TAMRA-3′
GAPDH	
Primer F	5´-AGCCGGGCATGTTCTTCAAC-3´
Primer R	5´-AGGGAGCTTCACGTTCTTGTATC-3
Probe	5´- FAM CGGAGCTGGACCTGACCTACGGCA TAMRA- 3´

*IL-7 indicates interleukin 7; IL-6, interleukin-6; IFN-γ, interferon- γ; Foxp3, forkhead box P3; TGF-β, transforming growth factor-β; and GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

was performed in a total reaction volume of 20 µL and consisted of initial denaturation (95°C, 10 minutes) and 45 cycles for 95°C, 10 seconds, and 60°C, 45seconds. Gene expression of each target was normalized to *GAPDH* as a reference gene. Each sample was run in duplicate.

Statistically Analysis

Statistical analyses were performed by using the SPSS software (Statistical Package for the Social Sciences, version 21.0, SPSS Inc, Chicago, IL, USA). The Mann-Whitney test was used for nonparametric data and the Student *t* test was used for unpaired data. All data were reported as mean ± standard deviation, and *P* values less than .05 were considered significant.

RESULTS

Clinical Findings

The demographic and clinical data of the present study participants are summarized in Table 2. Clinical baseline data, the hematological and urinary characteristics of each group are shown in Tables 3 and 4. In SLE patients with class IV glomerulonephritis, the total number of leukocytes was significantly higher ($P = .047$), while the number of erythrocytes was significantly lower than the controls ($P = .001$), and erythrocyte sedimentation rate was higher ($P = .001$). In patients with nephritis, blood urea nitrogen level (48.45 ± 35.9 mg/dL) and serum creatinine level (1.52 ± 1.4) were higher.

Gene Expression Levels

The comparison of gene expression of molecules related T helper 1, T helper 17, and regulatory T cells between the glomerulonephritis class IV SLE patients and the control group is shown in the Figure. There was a significant increase in the gene expression of IL-17 ($P < .01$), IL-6 ($P < .01$), IFN-γ, and Foxp3 ($P < .001$) in the SLE patients with LN in comparison to non-nephritis SLE patients. No significant difference was observed in the expression level of TGF-β between the two groups.

Table 2. Characteristics of Systemic Lupus Erythematosus Patients With and Without Lupus Nephritis

Characteristic	Patients With Lupus Nephritis	Patients Without Lupus Nephritis	*P*
Number of patients	20	20	> .05
Sex			
Female	18	19	
Male	2	1	> .05
Mean age, y	30.0 ± 9.3	34.0 ± 11.1	> .05
Hypertension, %	35	5	< .05
Edema, %	50	0	< .05
Arthritis, %	40	25	> .05
Erythrocyte count, × 10^{12}/L	3.7	4.65	< .05
Leukocyte count, , × 10^{3}/L	7.48	5.6	< .05
Platelet count, × 10^{6}/L	2.37	1.86	> .05
Erythrocyte sedimentation rate, mm/h	52.2	27.4	< .05

Table 3. Kidney Function Tests and Serum Potassium Levels in Systemic Lupus Erythematosus Patients With and Without Lupus Nephritis*

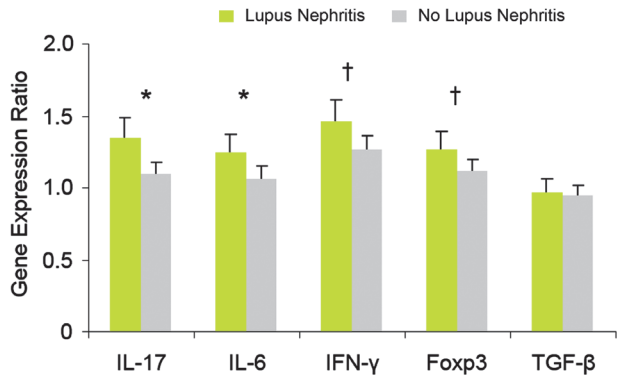
Parameter	Patients With Lupus Nephritis	Patients Without Lupus Nephritis	*P*
Blood urea nitrogen, mg/dL			
8 to 20	15	75	< .05
20 to 25	55	25	< .05
25 to 50	30	0	< .05
Serum creatinine, mg/dL			
< 1	30	85	< .05
1 to 1.5	60	15	< .05
> 1.5	10	0	< .05
Serum potassium			
3.5 to 4.0	50	60	> .05
4.0 to 5.5	35	40	> .05
5.5 to 6.0	15	0	> .05

*Reference ranges were 8 mg/dL to 25 mg/dL for blood urea nitrogen, up to 1.5 mg/dL for serum creatinine, and 3.5 mEq/dL to 5.5 mEq/dL for serum potassium.

Table 4. Urine Parameters in Systemic Lupus Erythematosus Patients With and Without Lupus Nephritis*

Parameters	Patients With Lupus Nephritis	Patients Without Lupus Nephritis
Hematuria, /HPF*		
0 to 5	20	95
6 to 10	25	5
11 to 15	10	0
16 to 20	10	0
21 to 25	35	0
Proteinuria		
+	25	0
++	45	0
++	30	0
Leukocyturia, HPF*		
0 to 5	35	85
6 to 10	30	15
11 to 15	10	0
16 to 20	5	0
21 to 25	20	0

*Reference range was zero to 1 cells per high-power field.



Gene expression in patients with class IV lupus glomerulonephritis compared to lupus patients with no nephritis.
*$P < .01$
†$P < .001$

DISCUSSION

Lupus nephritis is a serious organ involvement in SLE characterized by inflammation within the glomerulus. Glomerulonephritis class IV is one of the most severe forms of LN which correlates with poor prognosis and develops to end-stage disease and death.[4] Loss of tolerance and deposition and in situ production of immune complexes are one of the hallmarks of LN,[12] which promotes a chronic inflammatory process in the kidney.[13] Pathogenesis of the LN is unknown and complex. Regulatory T cells are known to have a key role in limiting unwanted inflammatory responses in SLE,[14-15] and recent studies have shown that there is a distressed balance between regulatory T cells ($CD4^+CD25^+Foxp3^+$) and pathogenic T helper 17 cells in SLE.[16] Altered rate and function of regulatory T cells and pathogenic T helper 17 cells appear to be involved in the immunopathogenesis of LN.[17]

This study showed that the expression level of IL-17 increased in class IV glomerulonephritis SLE compared to non-nephritis SLE patient. These results are in line with some previous studies.[18] Interleukin-17 has powerful pro-inflammatory activity and is an important cytokine which promotes kidney damage in SLE, [19] and in some studies, an increased number of IL-17-positive T cells were reported as the main pathogenic cells in the kidney of patients with LN.[20] These studies suggested a crucial role for IL-17-producing cells in triggering glomerulonephritis in lupus patients, and our results showed increased expression of this cytokine in the peripheral blood mononuclear cells of LN patients. Cytokine situation affects the generation of T helper 17 cells,[21] and in accordance with others, increased expression of IL-6 in the presence of TGF-β in our class IV glomerulonephritis SLE patients was evident, which could provoke the differentiation of naive T helper cells into the pathogenic T helper 17 lymphocytes.

Interleukin-6 is a critical mediator of kidney damage and in LN; increased presence of IL-6 was associated with increased auto-antibody production

and disease activity.[22] In some SLE patients, T helper 1 responses predominated and promoted the progression of SLE to active nephritis.[11, 23] Our results showed increased expression of IFN-γ in glomerulonephritis class IV SLE patients, which is the significant cytokine of T helper 1 cells. Some previous studies demonstrated that IFN-g could induce B cells for production of nephritogenic anti-nuclear antibodies that could worsen nephritis in glomerulonephritis class IV.[24,25] A correlation between the overexpression of IFN-γ with the histological activity index of LN was reported in some previous studies.[23,26] Several studies demonstrated that double-negative T cells are the main source of both IL-17 and IFN-g in lupus patients with nephritis.[27] Our results showed concurrent increase in the expression of IL-17 and IFN-γ in LN. It is predictable that increased presence of T helper 17 and T helper 1 cells is implicated in worsening the glomerular inflammatory conditions in SLE nephritis. Concomitant presence of IL17-positive and IFN-g-producing cells in LN is resistant to the modulatory effects of regulatory T cells.[28] In the present study, we showed that in SLE patients with nephritis, in spite of the increased expression of Foxp3 (the transcription factor of regulatory T cells), the expression rate of IL-17 and IFN-γ also increased. Considering our results, it seems that regulatory T cells were not able to control the increased population of IL-17-producing cells in glomerulonephritis class IV, which is compatible with some previous studies.[28]

It is well accepted that regulatory T cells play an important role in the control of autoimmune responses, and a decreased number and function of regulatory T cells was implicated in lupus patients. However, the results in different other studies are inconclusive.[29,30] Some studies confirmed the reduced number and function of regulatory T cells in patients suffering from LN compared to normal subjects[31]; however, Wang and colleagues indicated an increased number of regulatory T cells in LN.[32] Some studies showed increased Foxp3 expression in active LN,[33-35] while the suppressive function of regulatory T cells decreased. Analysis of Foxp3 mRNA in our study demonstrated increased levels of this molecule in the peripheral blood mononuclear cells of patients with active nephritis. This absorbing paradox between different studies is probably due to the fact that although the overall expression of Foxp3 increases, the suppressive function of regulatory T cells may be imperfect,[32] which is compatible with our results. We showed that in SLE patients with active nephritis, in spite of the increased expression of Foxp3, the expression rate of IL-17 and IFN-g also increased, which demonstrates the defective suppressive function of regulatory T cells. A high level of IL-6 was also supposed to interfere with the regulatory function of regulatory T cells.[36]

CONCLUSIONS

According to our results, it seems that in active LN, increased Foxp3-producing regulatory T cells have an imperfect capacity to control the pathogenic IL-17- and IFN-g-producing cells. Enhanced understanding of the pathogenic mechanisms of LN could foster effective approaches for monitoring disease progression and targeted depletion of effector cells to reduce tissue damage in the kidney.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Rahman A, Isenberg DA. Mechanisms of disease systemic lupus erythematosus. N Engl J Med. 2008;358:929-39.
2. Mortensen ES, Fenton KA, Rekvig OP. Lupus nephritis: the central role of nucleosomes revealed. Am J Pathol. 2008;172:275-83.
3. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol. 2004;15:241-50.
4. Schwartz MM, Korbet SM, Lewis EJ. The prognosis and pathogenesis of severe lupus glomerulonephritis. Nephrol Dial Transplant. 2008;23:1298-306.
5. Bijl M, Dijstelbloem HM, Oost WW, et al. IgG subclass distribution of autoantibodies differs between renal and extra-renal relapses in patients with systemic lupus erythematosus. Rheumatology (Oxford). 2002;41:62-7.
6. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self tolerance and negative control of immune responses. Annu Rev Immunol. 2004;22:531-62.
7. Yang J, Chu Y, Yang X, et al. Th17 and natural Treg cell population dynamics in systemic lupus erythematosus. Arthritis Rheum. 2009;60:1472-83.

8. Chen DY, Chen YM, Wen MC, Hsieh TY, Hung WT, Lan JL. The potential role of Th17 cells and Th17-related cytokines in the pathogenesis of lupus nephritis. Lupus. 2012;21:1385-96.
9. Reiner SL. Development in motion: helper T cells at work. Cell. 2007;129:33-6.
10. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. Eur J Immunol. 2010;40:1830-5.
11. Summers SA, Steinmetz OM, Li M, et al. Th1 and Th17 cells induce proliferative glomerulonephritis. J Am Soc Nephrol. 2009;20:2518-24.
12. Mannik M, Merrill CE, Stamps LD, Wener MH. Multiple autoantibodies form the glomerular immune deposits in patients with systemic lupus erythematosus. J Rheumatol. 2003;30:1495-504.
13. Urbonaviciute V, Furnrohr BG, Meister S, et al. Induction of inflammatory and immune responses byHMGB1-nucleosome complexes: implication for the pathogenesis of SLE. J Exp Med. 2008;205:3007-18.
14. Horwitz DA. Regulatory T cells in systemic lupus erythematosus: past, present and future. Arthritis. Res Ther. 2008;10:227-35.
15. Mellanby RJ, Thomas DC, Lamb J. Role of regulatory T-cells in autoimmunity. Clin Sci (London). 2009;116:639-49.
16. Yang J, Yang X, Chu Y, Li M. Recovery of the immune balance between Th17 and regulatory T cells as a treatment for systemic lupus erythematosus. Rheumatology (Oxford). 2011;50:1366-72.
17. Mucida D, Park Y, Kim G, et al. Reciprocal Th17 and regulatory T cell differentiation mediated by retinoic acid. Science. 2007;317:256-60.
18. Zickert A, Amoudruz P, Sundström Y, Rönnelid J, Malmström V, Gunnarsson I. IL-17 and IL-23 in lupus nephritis - association to histopathology and response to treatment. BMC Immunol. 2015;16:7.
19. Zhang Z, Kyttaris VC, Tsokos GC. The role of IL-23/IL-17 axis in lupus nephritis. J Immunol. 2009;183:3160-9.
20. Perl A. Systems biology of lupus: Mapping the impact of genomic and environmental factors on gene expression signatures, cellular signaling, metabolic pathways, hormonal and cytokine imbalance, and selecting targets for treatment. Autoimmunity. 2010;43:32-47.
21. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441:235-8.
22. Richards HB, Satoh M, Shaw M, Libert C, Poli V, Reeves WH. Interleukin 6 dependence of anti-DNA antibody production: evidence for two pathways of autoantibody formation in pristane-induced lupus. J Exp Med. 1998;188:985-90.
23. Uhm WS, Na K, Song GW, et al. Cytokine balance in kidney tissue from lupus nephritis patients. Rheumatology (Oxford). 2003;42:935-8.
24. Teramoto K, Negoro N, Kitamoto K, et al. Microarray analysis of glomerular gene expression in murine lupus nephritis. J Pharmacol Sci. 2008;106:56-67.
25. Chan RW, Lai FM, Li EK, et al. Intrarenal cytokine gene expression in lupus nephritis. Ann Rheum Dis. 2007;66:886-92.
26. Masutani K, Akahoshi M, Tsuruya K, et al. Predominance of Th1 immune response in diffuse proliferative lupus nephritis. Arthritis Rheum. 2001;44:2097-106.
27. Crispin JC, Oukka M, Bayliss G, et al. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. J Immunol. 2008;181:8761-6.
28. Zhao XF, Pan HF, Yuan H, et al. Increased serum interleukin 17 in patients with systemic lupus erythematosus. Mol Biol Rep. 2010;37:81-5.
29. Oukka M. Interplay between pathogenic Th17 and regulatory T cells. Ann Rheum Dis. 2007;66 suppl 3.iii 87-90.
30. Xing Q, Wang B, Su H, Cui J, Li J. Elevated Th17 cells are accompanied by FoxP3+ Treg cells decrease in patients with lupus nephritis. Rheumatol Int. 2012;32:949-58.
31. Xing Q, Su H, Cui J, Wang B. Role of Treg cells and TGF-β1 in patients with systemic lupus erythematosus: A possible relation with lupus nephritis. Immunol Invest. 2012;41:15-27.
32. Wang G, Lai FM, Tam LS, et al. Urinary FOXP3 mRNA in patients with lupus nephritis--relation with disease activity and treatment response. Rheumatology (Oxford). 2009;48:755-60.
33. Yazici MU, Orhan D, Kale G, Besbas N, Ozen S. Studying IFN-gamma, IL 17 and FOXP3 in pediatric lupus nephritis. Pediatr Nephrol. 2014;29:853-62.
34. Bonelli M, Von Dalwigk K, Savitskaya A, Smolen JS, Scheinecker C. FOXp3 expression in CD4+ T cells of patients with systemic lupus erythematosus (SLE): a comparative phenotypic analysis. Ann Rheum Dis. 2008;67:664-71.
35. Muthukumar T, Dadhania D, Ding R, et al. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. N Engl J Med. 2005;353:2342-51.
36. Zheng SG, Wang J, Horwitz DA. Cutting edge: Foxp3+ CD4+CD25+ regulatory T cells induced by IL-2 and TGF-beta are resistant to Th17 conversion by IL-6. J Immunol. 2008;180:7112-6.

Correspondence to:
Mahmoud Mahmoudi
BuAli Sq, Ferdowsi Sq, BuAli Research Institute, Mashhad, Iran
Tel: +98 915 115 6304
E-mail: mahmoudim@mums.ac.ir

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