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Highlight on some immune disorders in chronic kidney disease

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Abstract

Introduction

Chronic inflammation and immune inadequacy are present in chronic kidney disease (CKD) patients. T-helper type 17 (Th17) cells give rise to interleukin (IL)-17, IL-22, and IL-23, cause neutrophils to drive to sites of infection, and enhance moderate immune reactions to microbes outside the cells. They are responsible for autoimmune diseases. Regulatory T (Treg) cells secrete anti-inflammatory cytokines IL-10 and transforming growth factor beta and suppress immune reactions to preserve immune homeostasis. The decreased count or disability of Treg leads to immune dysregulation. Endoplasmic reticulum stress (ERS) leads to unfolded protein response that is stimulated by glucose-regulated protein 78 (GRP78). Permanent ERS enhances the transcription of CCAAT-enhancer-binding protein homologous protein (CEBPD or CHOP), which is a proapoptotic gene. The aim of our study was to assess the relationship between immune dysregulation and ERS in CKD for better outcome.

Patients and methods

In all, 68 CKD patients and 20 apparently healthy participants, selected according to estimated glomerular filtration rate, were recruited to our study from the National Institute of Urology and Nephrology. Routine biochemical tests were determined for patients and controls. GRP78 and CEBPD were measured by enzyme-linked immunosorbent assay. Th17 and Treg cells % and median fluorescent intensity (MFI) were estimated by flow cytometric analysis.

Results

We found significant increase in Th17 ($CD4^+$ $IL-17A^+$) cells in both % and MFI and significant decrease in $CD4^+$ cells and Treg ($CD4^+$ $Foxp3^+$) cells in both % and MFI in CKD patients compared with controls ($P<0.05$). Treg ($CD4^+$ $Foxp3^+$) cells % was the lowest in CKD stage 5 patients without dialysis treatment ($P<0.05$). The changes in Th17 and Treg cells % and MFI led to an increase in the ratio of Th17/Treg cells in CKD patients that was positively correlated with disease stage. Also, we found that serum creatinine was positively correlated with Th17 cell frequency and Th17/Treg cell ratio and negatively correlated with Treg cell frequency. Serum creatinine level was strongly correlated with Th17/Treg ratio than with Th17 or Treg cells. Our study showed a significant increase in GRP78 and CEBPD concentrations in CKD patients compared with healthy individuals and positively correlated with CKD stage. We observed no significant change of GRP78 or CEBPD after hemodialysis. We also found positive correlation of serum creatinine with GRP78 and CEBPD in CKD patients. Lastly, we found that the ratio of Th17/Treg cells was positively correlated with serum GRP78 and CEBPD concentrations.

Conclusion

Our study confirmed the link between immune dysregulation and ERS in CKD patients. Further studies are needed for proving this link and the new strategy treatment for Treg cells hoping to protect the patients from disease progression and bad outcomes.

Keywords: Chronic kidney disease, endoplasmic reticulum stress, immune disorders, T-helper type 17, regulatory T cells

INTRODUCTION

Renal disorder is accompanied with an enormous financial problem. The Global Disease 2015 study revealed approximately '5–10 million' deaths every year from chronic kidney disease (CKD) [1]. CKD is identified by the presence

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of either or both renal constitutional and functional anomalies, associated with decreased or normal glomerular filtration rate (GFR), accompanied with illness, existing for a period of more than 3 months [2].

The pathogenesis of CKD has to be understood for effective treatment. Immune imbalance has an essential role in the disease pathogenesis [3]. Chronic inflammation and immune inadequacy are present in uremic and acute kidney injury patients. Uremia is the leading cause of immune imbalance [4], and replacement therapy does not appear to improve immune disorders [5].

CD4 T cell activation leads to evolving of regulatory T (Treg) cells and T-helper type I (Th1), Th2, Th17, T follicular helper (Tfh), and is controlled by the outside environment as cytokines. In the absence of pro-inflammatory cytokines, transforming growth factor beta (TGF-β) causes transformation into Treg cells [6]. These cells secrete anti-inflammatory cytokines interleukin (IL)-10 and TGF-β and suppress immune reactions to preserve immune homeostasis and suppress the stimulation of CD4 and CD8 T-cells and antigen-presenting cells, monocytes/macrophages, and neutrophils [7]. Therefore, the decreased count or disability of Treg leads to immune dysregulation [8].

The expression of the transcriptional factor forkhead box P3 (Foxp3), a member of the forkhead/winged-helix family of transcriptional controllers is the main effector of Treg cells development and function [9].

There are many types of Treg cells, natural or thymic Tregs, formed in the thymus (nTregs or tTreg), induced (adaptive) or peripheral Tregs (iTregs or pTregs) [10], which are produced in the periphery from innocent T cells after stimulation. Other types are Treg type 1 cells, supplying IL-10, and Treg 35 cells forming IL-35, which is linked to the IL-12 family [11].

In the existence of TGF-β with IL-6 or IL-21, innocent CD4$^+$ T cells are transformed to Th17 cells. Th17 cells give rise to IL-17, IL-22, and IL-23, cause neutrophils to drive to sites of infection, and induce moderate immune reactions to microbes outside the cells. Treg and Th17 are opposing each other in function throughout inflammation [12]. Th17 cells are responsible for autoimmune diseases as rheumatoid arthritis and multiple sclerosis. In the presence of IL-6 and TGF-β, T cell receptor/costimulatory signals lead to the activation of STAT3 [13], which enhances the formation of transcription factor (ROR)γt, a member of the retinoic acid-related orphan nuclear hormone receptor family causing Th17 production [14].

Th1 cells provoke primary macrophages and moderate immune reactions to microbes inside the cells while Th2 cells are responsible for the activation of eosinophils, basophils, and mast cells to react against worms. B cell development by Tfh cells occurs at the germinal centers [15].

The function of the endoplasmic reticulum (ER) is protein formation, folding, remodeling, and production. Endoplasmic reticulum stress (ERS) is a state of collection of misfolded proteins in the cavity of the ER due to inflammation, oxidative stress, Ca2+, or energy depletion [16]. ERS leads to unfolded protein response (UPR) that is stimulated by glucose-regulated protein 78 or binding immunoglobulin protein (GRP78), which is an ER lumen protein. GRP78 combines with misfolded proteins to be cleared [17].

Stimulated UPR decreases protein synthesis, increases misfolded protein breakdown, and intensifies the ability of ER protein-folding, trying to decrease ERS and maintain ER homeostasis. In permanent ERS, increased stimulation of the UPR will trigger proapoptotic pathways to get rid of stressed cells [16], and enhances the transcription of CCAAT-enhancer-binding protein homologous protein (CEBPD or CHOP), which is a proapoptotic gene and reduces B-cell lymphoma-2 gene, an anti-apoptotic gene [17]. CHOP is important in cellular differentiation [18] and immune responses [19]. CEBPA, CEBPB, CEBPG, CEBPD, CEBPE, and CEBPZ are six elements of human CEBP gene [20].

In normal state CEBPD is present in the human body in small amounts and increased by the effect of IL-6, IL-1β, and tumor necrosis factor α (TNF-α) [21]. In addition, it increases IL-6, IL-1β, and TNF-α [22]. Also, CEBPD is increased in type 2 diabetes [23] and atherosclerosis [22], which are inflammation-related diseases. So, CEBPD is a crucial controller of inflammation [24].

The aim of our study was to assess the relationship between immune dysregulation and ERS in CKD patients including CKD stages 3, 5, in patients with end-stage renal disease undergoing hemodialysis (HD) for better outcome.

PATIENTS AND METHODS

All study participants were recruited from the National Institute of Urology and Nephrology, Cairo. In all, 68 CKD patients were selected based on the GFR for the classification of kidney disease. Using the abbreviated modification of diet in renal disease equation to calculate the estimated glomerular filtration rate: GFR (ml/min per 1.73 m²)=186×(creatinine/88.4)$^{-1.154}$×(age)$^{-0.203}$×(0.742 if female) [25], 22 patients were in CKD stage 3 [GFR: 30–59 [13 (59%) males and nine (41%) females] and 46 were in CKD stage 5 (GFR <15). They were further subdivided into two groups, 20 CKD patients [eight (40%) males and 12 (60%) females] on conservative treatment and 26 patients [18 (69%) males and eight (31%) females] undergoing HD 4 h thrice weekly for this study. They consisted of 39 (57%) men and 29 (43%) women aged between 20 and 70 years; 20 apparently normal participants [11 (55%) males and nine (45%) females], aged between 18 and 65 years. Primary renal disease was chronic glomerular nephritis (22), diabetic nephropathy (21), hypertensive nephropathy (18), polycystic kidney (four), and unknown (three). Exclusion criteria were a history of acute illness, chronic infectious diseases, tumors, and hematopoietic disease. None of them have received immunosuppressive

drugs. All the patients were informed of the objectives of the study and signed an informed consent form. The study was approved by the institute's ethics committee.

Whole blood was drawn from patients after night fasting. Blood samples from the patients in the HD group were collected before the start of dialysis. Serum creatinine and urea were measured on Vitros 350 autoanalyzer (Ortho Clinical Diagnostics, Mumbai, India). Aliquots of serum were stored at −80°C for the assay of GRP78 and CHOP by enzyme-linked immunosorbent assay using specific detection reagents following the manufacturer's instructions (Huamei Biotech, Wuhan, China and Fine Biotech, Wuhan, China, respectively).

For CD assessment, blood was drawn into tubes containing EDTA. The number of leukocytes in the sample should be less than $5 \times 10^3/\mu l$, diluted if necessary with phosphate buffer saline (PBS). Blood was incubated for 15 min in the dark at room temperature with fluorescein-conjugated monoclonal antibodies: human anti-CD3-APC (allophycocyanin), anti-CD4-FITC (fluorescein isothiocyanate) (Immunotech SAS, Beckman Coulter, Marseille, France). Fixative reagent (formaldehyde, Immunotech SAS, Beckman Coulter) was added and was incubated for 15 min in the dark at room temperature. PBS (0.01 M potassium sulfate and 0.15 M sodium chloride, pH = 7.2 ± 0.2, dissolved in 500 ml distilled water) (Beckman Coulter Life Sciences, Indianapolis, Indiana, USA) was added and centrifuged at 1800 rpm for 5 min IntraPrep Permeabilization Reagent (Saponine, Immunotech SAS, Beckman Coulter) was added after supernatant removal and incubated for 5 min at room temperature. Then anti-IL-17A-Pacific Blue and anti-Foxp3-PE (phycoerythrin) (Immunotech SAS, Beckman Coulter) were added and incubated for 15 min in the dark at room temperature. PBS was added and centrifuged at 1800 rpm for 5 min; PBS was added after supernatant removal. Then, the cells were analyzed on Navios Ex cell analyzer (Beckman Coulter Life Sciences). The data analysis was carried out with Navios Ex software.

Statistical analysis
Categorical data were expressed as numbers (%) and the statistical significance of their independence was tested by χ^2 test or Fisher's exact test. They were all expressed as median (interquartile range) and analyzed by nonparametric tests. Kruskal–Wallis test, Mann–Whitney U test, Bonferroni equation, and Spearman rank correlation test were used. P value less than or equal to 0.05 was considered to be significantly different. The statistical analysis was performed using the statistical package software IBM SPSS, version 24 (IBM Corp., Armonk, New York, USA).

RESULTS
We found significant increase in Th17 (CD4+ IL-17A+) cells in both % and median fluorescent intensity (MFI) and significant decrease in CD4+ cells and Treg (CD4+ FoxP3+)

cells in both % and MFI in CKD stage 5 patients compared with controls ($P<0.05$) (Table 1). Treg (CD4+ FoxP3+) cells % was the lowest in CKD stage 5 patients on conservative treatment ($P<0.05$, Table 2 and Figs. 1-4). The changes in Th17 and Treg cells % and MFI led to an increase in the ratio of Th17/Treg cells in CKD patients that was positively correlated with disease stage (Table 2 and Figs. 5, 6).

We found that serum creatinine was positively correlated with Th17 cell frequency and Th17/Treg cell ratio ($r=0.61$, $P<0.001$ and $r=0.719$, $P<0.001$, respectively), and negatively correlated with Treg cell frequency ($r=−0.35$, $P<0.001$) (Figs. 7 and 8). Serum creatinine level was strongly correlated with serum creatinine level ($r=0.719$) than with Th17 ($r=0.61$) or Treg ($r=−0.345$) cells. So, Th17/Treg cell ratio may be a sign of renal disease progression.

Our study showed a significant increase in GRP78 and CEBPD concentrations in CKD patients compared with healthy participants ($P<0.05$, Fig. 9) and positively correlated with CKD stage ($P<0.05$) (Table 3). We observed no significant change of GRP78 or CEBPD after HD. We also found positive correlation of serum creatinine with GRP78 ($r=0.944$, $P<0.001$) and CEBPD ($r=0.867$, $P<0.001$) (Fig. 10), in CKD patients suggesting a link between the degree of ERS and kidney disease progression.

Lastly, we found that the ratio of Th17/Treg cells was positively correlated with serum GRP78 and CEBPD concentrations ($r=0.653$, $P<0.001$ and $r=0.661$, $P<0.001$, respectively) (Fig. 11), demonstrating that ERS and immune disorder may cooperate to impact CKD progression.

DISCUSSION
T cells are very important in the protecting humans against microorganisms and immune balance. An increase in
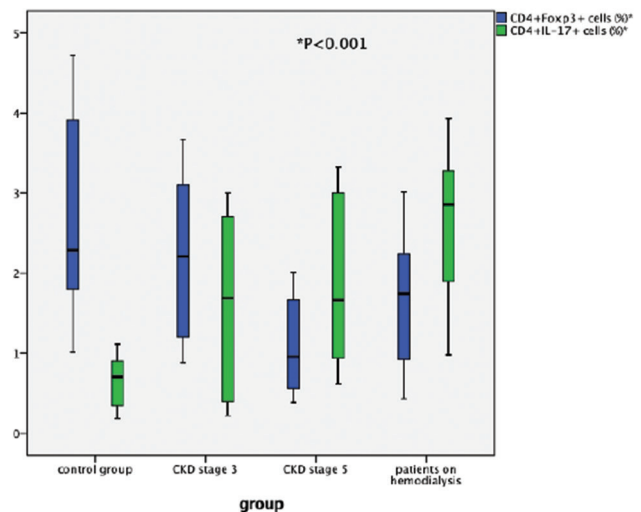

Figure 1: Percentage of Th17, Treg cells in all groups: CKD, chronic kidney disease; CKD stage 5, CKD stage 5 on conservative treatment; Th17, T-helper type 17; Treg, regulatory T, *P value less than 0.001 = significant.

Table 1: Demographics of all groups

Item	Control	CKD stage 3		CKD stage 5 on conservative treatment			CKD stage 5 on HD			
	Value	Value	P with control	Value	P with control	P with stage 3	Value	P with control	P with stage 3	P with stage 5
Sex (male/female)	11/9	13/9	0.26	8/12	0.25	0.35	18/8	0.27	0.36	0.37
Age (years)	37 (18–65)	43 (20–70)	0.061	51 (30–67)	0.07	0.06	49 (22–65)	0.07	0.07	0.059
eGFR (ml/min/1.73 m²)	114.5 (90–127)	36 (31–57)	0.046*	10 (4–14)	<0.001*	0.018*	5.5 (3–9)	<0.001*	<0.001*	0.08
Creatinine (mg/dl)	0.7 (0.5–1)	2 (1.4–2.4)	0.047*	6 (4.9–13.9)	<0.001*	0.015*	9.35 (6.3–16)	<0.001*	<0.001*	0.11
Urea (mg/dl)	25.9 (15.2–30)	56.6 (41–86.4)	0.028*	106.4 (54.8–207)	<0.001*	0.003*	117.45 (75.2–222.9)	<0.001*	<0.001*	0.29
Hb (g/dl)	13 (12–16.1)	10.6 (9–13.5)	0.01*	7.5 (6.6–9.1)	0.001*	0.01*	9.9 (8.1–10.9)	0.001*	0.05	0.02*
WBCs (10³/cmm)	6.4 (4.5–8.7)	6.9 (3.5–10.9)	0.2	5.2 (3.3–7.2)	0.31	0.14*	5.3 (3.1–7.3)	0.3	0.41	0.6
PLT (10³/cmm)	232 (201–359)	148 (119–205)	0.04*	138 (85–198)	0.01*	0.22	146 (112–201)	0.03*	0.33	0.43
CRP (mg/l)	4 (2.1–5)	11.4 (8.1–14)	0.02*	17.5 (10.3–19.2)	0.01*	0.04*	18.6 (9.5–20.9)	0.01*	0.039*	0.35

CKD, chronic kidney disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HD, hemodialysis; PLT, platelets; WBC, white blood cells. *P<0.05=significant.

Table 2: T-helper type 17 and regulatory T cells by flow cytometry

Item	Control	CKD stage 3		CKD stage 5 on conservative treatment			CKD stage 5 on HD			
	Value	Value	P with control	Value	P with control	P with stage 3	Value	P with control	P with stage 3	P with stage 5
CD4 + cells (%)	60.2 (54.6–68.4)	52.1 (41.4–59.6)	0.001*	47.3 (35–51.6)	<0.001*	0.1	44.4 (38.3–55.8)	<0.001*	0.09	0.9
CD4 + IL-17 + cells (%)	0.7 (0.18–1.11)	1.68 (0.22–3)	0.025*	1.66 (0.61–3.32)	0.001*	0.27	2.85 (0.98–3.93)	<0.001*	0.009*	0.29
CD4 + IL-17 + cells (MFI)	1.64 (0.75–2.82)	2.54 (0.73–3.61)	0.13	2.81 (0.99–3.94)	0.054	0.7	3.41 (1.36–4.73)	<0.001*	0.058	0.21
CD4 + Foxp3 + cells (%)	2.28 (1.01–4.72)	2.2 (0.88–3.67)	0.28	0.95 (0.36–2.01)	<0.001*	0.001*	1.74 (0.43–3.01)	0.03*	0.18	0.55
CD4 + Foxp3 + cells (MFI)	3.83 (1.46–6.92)	3.07 (1.08–4.99)	0.45	1.39 (0.51–3.18)	<0.001*	0.001*	2.39 (0.94–5.75)	0.1	0.61	0.097
Th17/Treg cells % ratio	0.24 (0.07–0.45)	0.72 (0.16–1.27)	0.15	1.86 (0.7–2.45)	<0.001*	<0.001*	2.01 (0.89–4.48)	<0.001*	<0.001*	0.99
Th17/Treg cells (MFI) ratio	0.43 (0.25–0.85)	0.68 (0.28–3.34)	0.14	1.68 (0.59–3.94)	<0.001	<0.001	1.28 (0.62–4.97)	<0.001	0.014	0.25

CKD, chronic kidney disease; HD, hemodialysis; MFI, median fluorescent intensity; Th17, T-helper type 17; Treg, regulatory T. *P<0.05=significant.

deaths per year in CKD patients was observed, owing to chronic inflammation and immune disorders sharing in the pathogenesis of CKD [26].

Indoxyl sulfate is a renal toxin in uremic patients, enhances oxidative stress and inflammatory reaction, and disturbs T-cell differentiation causing advancement of kidney disease and CVD [27]. It presents in healthy individuals in very low concentration [28].

Immunological studies demonstrated new T-cell types, such as Th17, Tfh, and Treg [29]. Dysregulations of the Th17/Treg immune hemostasis are linked to diseases such as

systemic lupus erythematosus, atherosclerosis, CKD, and cancer [30]. Our results showed that the Th17 cell percentage was significantly increased while Treg cell percentage was significantly decreased in CKD stage 5 patients on comparing with healthy individuals. This is in accordance with Li *et al*. [31], who found that the frequency of Treg cells from CKD patients was significantly decreased than that in the control group. Their reports stated that Treg cells from CKD patients inhibited Th17 cell response.

Our correlation analysis showed that the Th17 cell percentage was negatively associated with the Treg cell percentage in CKD
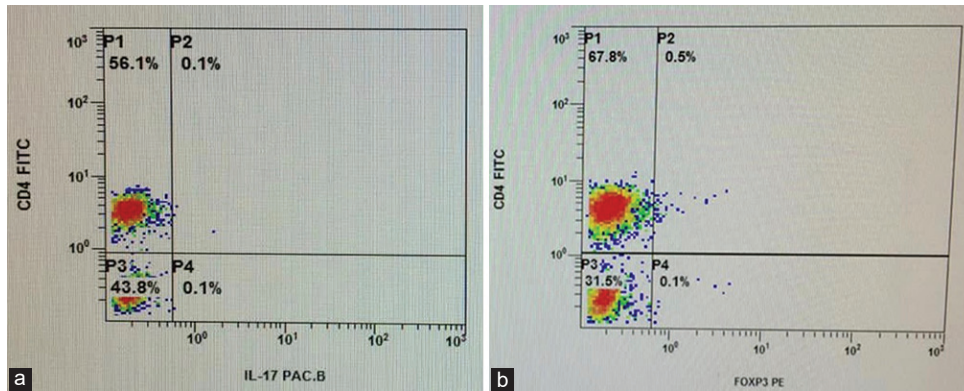

Figure 2.1: Percentage of Th17 (a) and Treg cells (b) in a single normal subject by flow cytometric analysis.
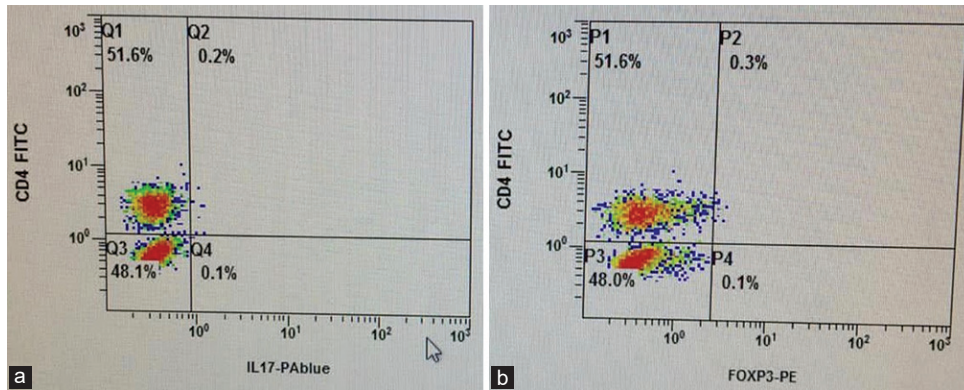

Figure 2.2: Percentage of Th17 (a) and Treg cells (b) in a single patient with chronic kidney disease stage 3 by flow cytometric analysis.
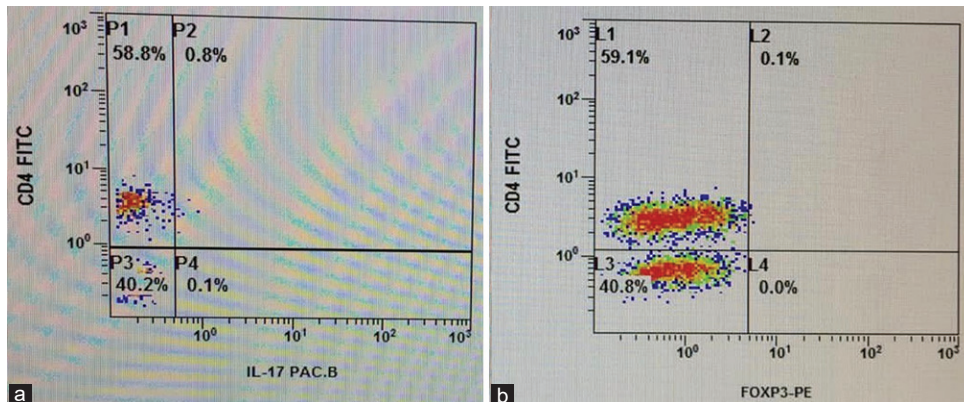

Figure 2.3: Percentage of Th17 (a) and Treg (b) in a single patient with chronic kidney disease stage 5 by flow cytometric analysis.
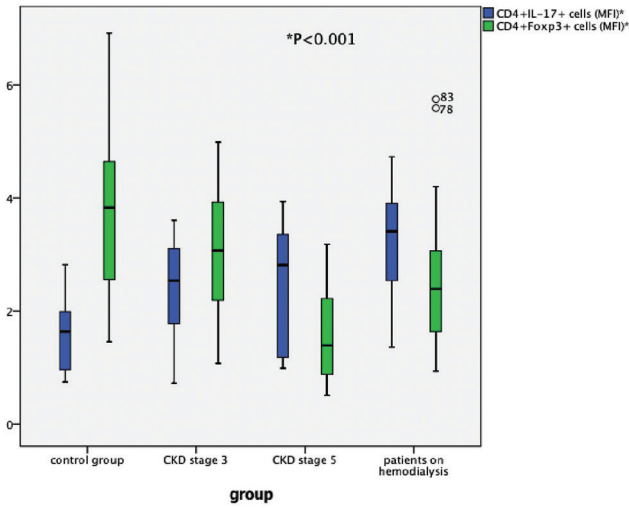
Figure 3: MFI of Th17, Treg cells in all groups: CKD, chronic kidney disease; CKD stage 5, CKD stage 5 on conservative treatment; MFI, median fluorescent intensity; Th17, T-helper type 17; Treg, regulatory T, *P value less than 0.001 = significant.
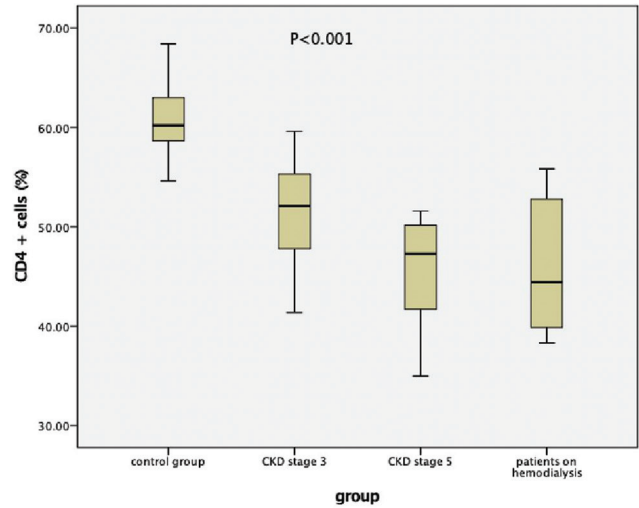

Figure 4: Percentage of CD4+ cells in all groups: CKD, chronic kidney disease; CKD stage 5, CKD stage 5 on conservative treatment, *P value less than 0.001 = significant.
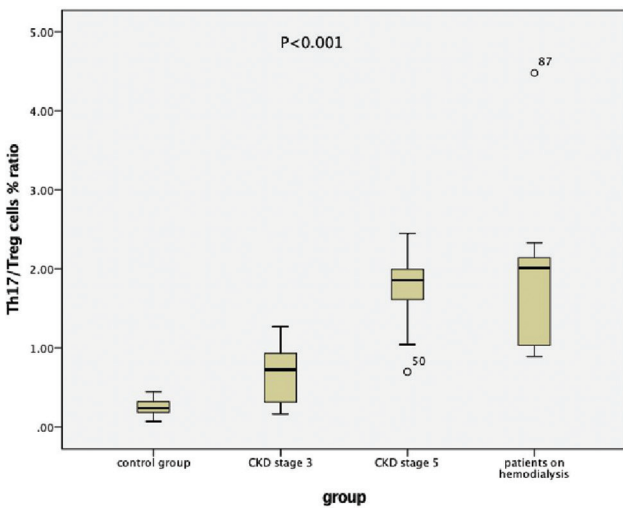

Figure 5: Th17/Treg cells % ratio in all groups: CKD, chronic kidney disease; CKD stage 5, CKD stage 5 on conservative treatment; Th17, T-helper type 17; Treg, regulatory T, *P value less than 0.001 = significant.
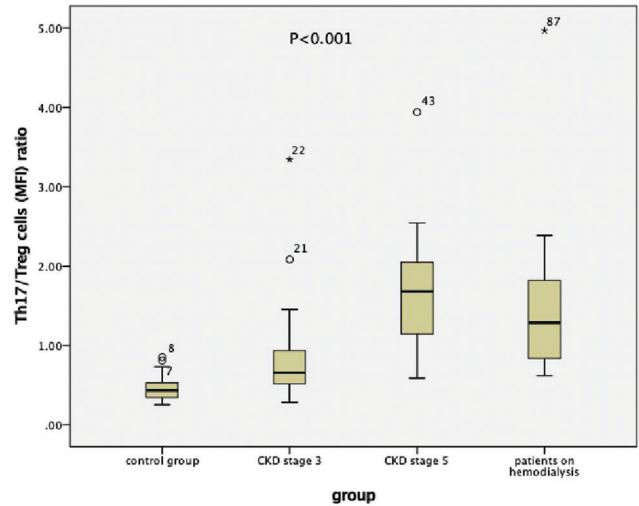

Figure 6: Th17/Treg cells MFI ratio in all groups: CKD, chronic kidney disease; CKD stage 5, CKD stage 5 on conservative treatment; MFI, median fluorescent intensity; Th17, T-helper type 17; Treg, regulatory T, *P value less than 0.001 = significant.

patients. This led to an elevated Th17/Treg cell ratio, which was positively associated with CKD stage, as shown by serum creatinine concentrations. This is in agreement with the results of Zhu *et al*. [32] and Wang *et al*. [30] of researches, which confirm that the imbalance between pro-inflammatory and immunosuppressive cells is responsible for CKD advancement. It is not well known yet whether the imbalance in Th17/Treg ratio leads to or it is a consequence of renal failure [32].

This Treg/Th17 disorder was marked in the HD group with cardiovascular disease on comparing with HD group without cardiovascular disease [33]. A study on lupus nephritis patients reported higher Th17 cells and decreased Treg/Th17 ratio on comparing with the control group, regardless of disease

activity. They revealed that hypofunction of Treg cells may be the cause of evolution of Th17 cells [34].

Our study showed a significant decrease of Treg in the HD group compared with the control group, which is in accordance with the Baron *et al*. [35] report and Caprara *et al*. [36] meta-analysis study. Chen *et al*. [37] results contradict ours, which reported increased Treg cells in HD patients, which may be due to its very small frequency in normal participant group.

In our study, we observed that dialysis did not correct the Th17/Treg imbalance, a data that is in agreement with other researches in renal failure patients [29,36]. On the contrary, another study [38] showed equal percentages of Treg cells

Table 3: Glucose-regulated protein 78 and CCAAT-enhancer-binding protein homologous protein concentration in all groups

Item	Control	CKD stage 3		CKD stage 5 on conservative treatment			CKD stage 5 on HD			
	Value	Value	P with control	Value	P with control	P with stage 3	Value	P with control	P with stage 3	P with stage 5
GRP78 (pg/ml)	47.84 (22.29-87.8)	195.55 (70.2-268.35)	0.03*	359.1 (210.4-856.8)	<0.001*	0.016*	769.15 (300.2-989.4)	<0.001*	<0.001*	0.17
CEBPD (pg/ml)	40.34 (18.23-83.31)	349.39 (210.6-453.33)	0.005*	451.47 (342.2-645.46)	<0.001*	0.1	563.65 (285.9-680.4)	<0.001*	<0.001*	0.55

CEBPD, CCAAT-enhancer-binding protein homologous protein; CKD, chronic kidney disease; GRP78, glucose-regulated protein 78; HD, hemodialysis. *$P<0.05$=significant.
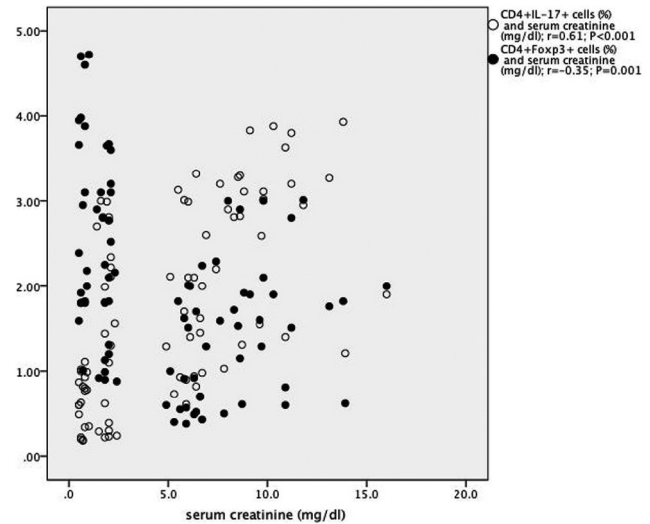

Figure 7: Correlation of Th17 and Treg cells frequency with serum creatinine. Th17, T-helper type 17; Treg, regulatory T, *P value less than 0.001 = significant.

in both HD patients and controls, but revealed hypofunction of Treg cells in HD patients. Immune dysregulation may be exacerbated by dialysis due to severe anemia, hemodynamic instability [39], and dialyzer biocompatibility [40].

Th17 cells are differentiated from the primary Th cells or memory T cells, then exhibit IL-17, IL-22, and RORγt molecules. They promote secretion of IL-6 and TNF-α and chemokines (e.g., MCP-1 and MIP-2) causing accumulation of neutrophils leading to autoimmune and inflammatory reactions [41]. They are included in cancer and immune diseases such as rheumatoid arthritis, systemic lupus [42,43]. While Treg cells produce IL-10 (immune modifier) or TGF-β to inhibit autoimmune reaction [44]. Treg cells can also generate IL-17 during inflammation or autoimmune diseases, leading to disorder progression. Th17 and Treg cells are related to each other in action and opposing each other; the equilibrium between the two cells plays a vital role in human health [45].

Treg cells are responsible for ordinary protection against inflammation, so the immune state can be changed by their variation in CKD [36]. Tregs can inhibit various immune cells directly by the generation of IL-10, TGF-β, IL-35, granzyme, perforin, and enzymes resulting in apoptosis of target cells [46], or indirectly through the expression of CD39/CD73, that produce adenosine and AMP, molecules reducing the surrounding ATP resulting in immunosuppression. On the other hand, Tregs can absorb more IL-2 due to their increased expression of CD25 causing its depletion [47].

Our results revealed that the Th17 cell percentage was markedly elevated in CKD stage 3 patients, while the Treg cell percentage was significantly decreased in CKD stage 5 patients. This is in agreement with the Zhu *et al*. [32] study on their CKD patients. They found that the Th17/Treg cell ratio was significantly increased with the progression of CKD, which is explained by
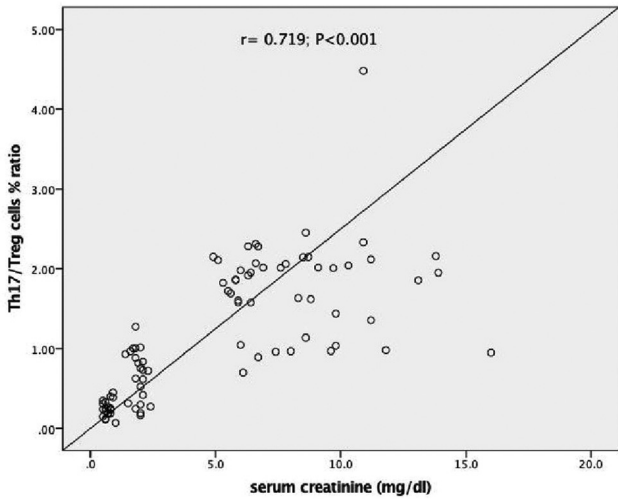
Figure 8: Correlation of Th17/Treg cells % ratio with serum creatinine. Th17, T-helper type 17; Treg, regulatory T, *P value less than 0.001 = significant.
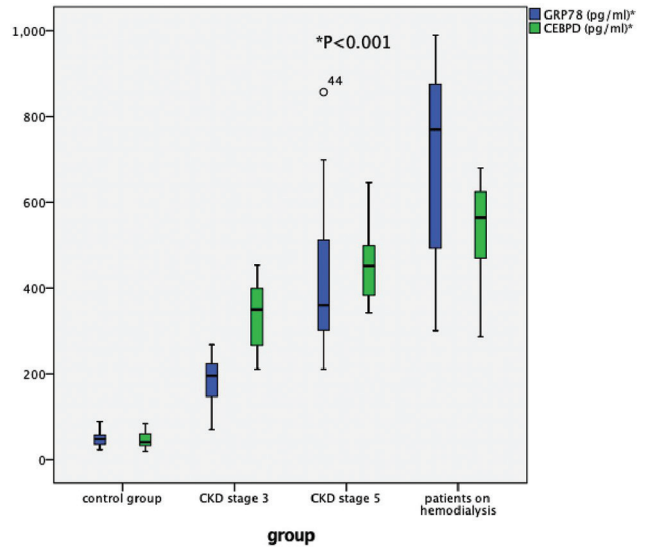

Figure 9: Concentration of GRP78 and CEBPD in all groups: CKD, chronic kidney disease; CKD stage 5, CKD stage 5 on conservative treatment; CEBPD, CCAAT-enhancer-binding protein homologous protein; GRP78, glucose-regulated protein 78, *P value less than 0.001 = significant.
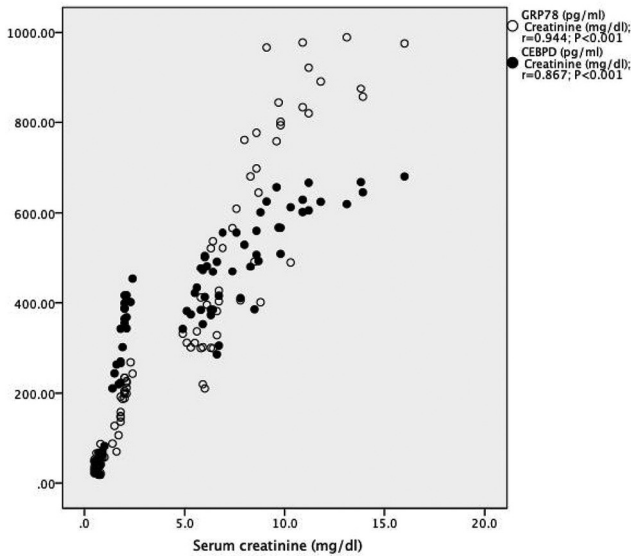

Figure 10: Correlation of GRP78 and CEBPD with serum creatinine. CEBPD, CCAAT-enhancer-binding protein homologous protein; GRP78, glucose-regulated protein 78, *P value less than 0.001 = significant.
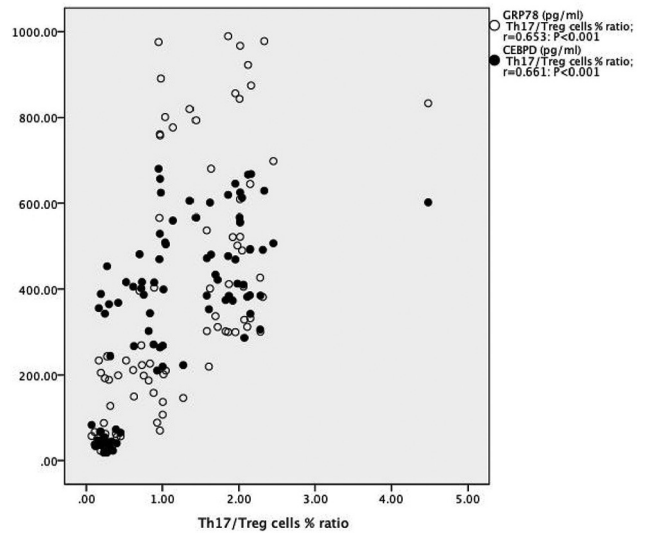

Figure 11: Correlation of Th17/Treg cells % ratio with GRP78 and CEBPD. CEBPD = CCAAT-enhancer-binding protein homologous protein; GRP78, glucose-regulated protein 78; Th17, T-helper type 17; Treg, regulatory T, *P value less than 0.001 = significant.

the reduction of Treg cells occurred after the rise of Th17 cells. So, in the early stage of CKD, a big number of Treg cells oppose the Th17 cell action. The Treg cells are consumed in the late stage, resulting in a rise in Th17 cells and initiating an increase in Th17/Treg cell ratio leading to the bad outcome in CKD progression. Collection of toxins in uremia will result in cell stimulation and enhance apoptosis [8].

Decreased Treg/Th17 cell ratio and uremic toxins are related to cognitive disability in end-stage renal disease patients [30]. Dementia occurs as a result of increased β-amyloid protein generation and deposition in the brain and neurotoxic molecule activation by IL-6 [48].

Ma *et al*. [49] observed that acute and chronic rejection renal transplant groups manifested a decreased frequency of Treg cells and serum IL-10 on comparing with transplant patients without rejection.

Decreasing immunosuppressant is very important in dealing with acute rejection patients. Treg cells can be utilized to obtain tolerance. Treg cells are increased either by rising the count of internal cells or by immediate injection of externally increased Treg cells [50]. Introducing Tregs in the graft may be an indicator of good graft outcome in transplant patients

taking immunosuppressants particularly in those with no evidence of rejection [51].

ERS response is a standard physiological reaction acting as a defence in patients with sufficient kidney function. Researches [16,17] demonstrated that the ERS shares in the occurrence and worsening of CKD involving renal fibrosis that may result in renal failure, regardless of the primary origin of the disease.

UPR is an adaptive response that results as a sequence to enhanced requirement to more protein folding of the ER. The UPR modulates protein synthesis and the evolution of a lot of intended genes which are shared in maintaining ER homeostasis or motivating apoptosis of destructed cells [52]. In other words, the UPR is acting as either a defense throughout mild ERS or a cell-destructive terminator throughout serious or persistent ERS [17].

Renal damage and proteinuria results from misfolded protein buildup and ERS. Proteinuria, uremic toxins, and hyperglycemia promote apoptosis by UPR, impair tubular epithelial cell reconstruction, and worsen CKD [17].

Our results have shown that GRP78 and CHOP concentrations are positively associated with serum creatinine levels in CKD patients. This is in accordance with earlier researches [53,54], demonstrating that the stage of CKD correlates with the extent of ERS. GRP78 function is stimulation of ERS response [55]. GRP78 detaches from ER laminate proteins, combining with misfolded proteins in its cavity throughout UPR. It stimulates more production of GRP78, so it can modify ERS. Persistent ERS induces apoptosis [56].

CEBPD acts as a master key for the stimulation of apoptotic route [57]. CEBPD is a transcription factor initially known as an inflammatory response gene [58]. It was detected in proximal tubular cells with O_2 deficiency inducing hypoxia-inducible factor 1-alpha exhibition. In inflammation, hypoxia-inducible factor 1-alpha amplification by CEBPD, regardless of hypoxia, may take a great part in renal disorders [59].

We observed a positive association between Th17/Treg cells ratio and the serum levels of the ERS markers GRP78 and CEBPD in CKD patients, in agreement with the Zhu *et al*. [32] study. This suggests a strong relation between them. It was noticed that inflammation is a chief intermediary of ERS and immune dysregulation which shares in CKD progression [32].

ERS and immune disorder contribute to CKD pathogenesis but the link between them is unsettled. It is known that oxidative stress affects T-cell stimulation and action [60]; so, ERS may promote immune dysfunction as it is a type of oxidative stress.

The main limitations of this study are that it is a single-center study and only a small number of patients were included. It did not include all stages of CKD or PD patients. Regardless of these limitations, our study showed the relationship between the ratio of Th17/Treg cells and alterations in ERS marker levels and CKD stage.

CONCLUSION

Our study confirmed the link between immune dysregulation and ERS in CKD patients. Further studies are needed for proving this link and for Treg cells' new strategy treatment hoping to protect the patients from disease progression and bad outcomes.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, *et al*. Worldwide access to treatment for end-stage kidney disease: a systematic review. Lancet 2015; 385:1975–1982.
2. Levey AS, Eckardt KU, Tsukamoto Y, Hostetter TH, Lameire N, Eknoyan G, *et al*. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2005; 67:2089–2100.
3. Libetta C, Rampino T, Dal Canton A. Polarization of T-helper lymphocytes toward the Th2 phenotype in uremic patients. Am J Kidney Dis 2001; 38:286–295.
4. Hauser AB, Stinghen AE, Kato S, Bucharles S, Aita C, Yuzawa Y, Pecoits-Filho R. Characteristics and causes of immune dysfunction related to uremia and dialysis. Perit Dial Int 2008; 28(suppl 3):183–187.
5. Zaza G, Granata S, Rascio F, Pontrelli P, Dell'Oglio MP, Cox SN, *et al*. A specific immune transcriptomic profile discriminates chronic kidney disease patients in predialysis from hemodialyzed patients. BMC Med Genomics 2013; 6:17.
6. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006; 24:179–189.
7. Li XC, Turka LA. An update on regulatory T cells in transplant tolerance and rejection. Nat Rev Nephrol 2010; 6:577–583.
8. Hendrikx TK, van Gurp EA, Mol WM, Schoordijk W, Sewgobind VD, Ijzermans JN, *et al*. End-stage renal failure and regulatory activities of CD4+CD25 bright+FoxP3+T-cells. Nephrol Dial Transplant 2009; 24:1969–1978.
9. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+regulatory T cells. Nat Immunol 2003; 4:330–336.
10. Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity 2006; 25:195–201.
11. Feuerer M, Hill JA, Mathis D, Benoist C. Foxp3+regulatory T cells: differentiation, specification, sub phenotypes. Nat Immunol 2009; 10:689–695.
12. Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. Cell 2010; 140:845–858.
13. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, *et al*. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006; 441:235–238.
14. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, *et al*. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+T helper cells. Cell 2006; 126:1121–1133.
15. Zhu J, Paul WE. Peripheral CD4+T-cell differentiation regulated by networks of cytokines and transcription factors. Immunol Rev. 2010; 238:247–262.
16. El Karoui K, Viau A, Dellis O, Bagattin A, Nguyen C, Baron W, *et al*. Endoplasmic reticulum stress drives proteinuria-induced kidney lesions via Lipocalin 2. Nat Commun 2016; 7:10330.
17. Maekawa H, Inagi R. Stress signal network between hypoxia and ER stress in chronic kidney disease. Front Physiol 2017; 8:74.
18. Tanaka T, Yoshida N, Kishimoto T, Akira S. Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene.

Embo J 1997; 16:7432–7443.

19. Zannetti C, Bonnay F, Takeshita F, Parroche P, Menetrier-Caux C, Tommasino M, *et al*. C/EBP {delta} and STAT-1 are required for TLR8 transcriptional activity. J Biol Chem 2010; 285:34773–34780.

20. Ramji DP, Foka P. CCAAT/enhancer-binding proteins: structure, function and regulation. Biochem J 2002; 365:561–575.

21. Lu YC, Kim I, Lye E, Shen F, Suzuki N, Suzuki S, *et al*. Differential role for c-Rel and C/EBPbeta/delta in TLR-mediated induction of proinflammatory cytokines. J Immunol 2009; 182:7212–7221.

22. Takata Y, Kitami Y, Yang ZH, Nakamura M, Okura T, Hiwada K. Vascular inflammation is negatively autoregulated by interaction between CCAAT/enhancer-binding protein-delta and peroxisome proliferator-activated receptor-gamma. Circ Res 2002; 91:427–433.

23. Gao H, Bryzgalova G, Hedman E, Khan A, Efendic S, Gustafsson JA, Dahlman-Wright K. Long-term administration of estradiol decreases expression of hepatic lipogenic genes and improves insulin sensitivity in ob/ob mice: a possible mechanism is through direct regulation of signal transducer and activator of transcription 3. Mol Endocrinol 2006;20:1287–1299.

24. Lai HY, Hsu LW, Tsai HH, Lo YC, Yang SH, Liu PY, Wang JM. CCAAT/enhancer-binding protein delta promotes intracellular lipid accumulation in M1 macrophages of vascular lesions. Cardiovasc Res 2017; 113:1376–1388.

25. Levey AS, Coresh J, Bolton K. Disease outcome quality initiative. K/DOQI clinical practice guidelines for chronic kidney disease evaluation, classification, and stratification. Kidney Am J Kidney Dis 2002; 39:S1–266.

26. Xiang F, Cao X, Shen B, Chen X, Guo M, Ding X, Zou J Transcriptome profiling reveals indoxyl sulfate should be culpable of impaired T cell function in chronic kidney disease. Front Med Nephrol 2020; 7:178.

27. Tan X, Cao X, Zou J, Shen B, Zhang X, Liu Z, *et al*. Indoxyl sulfate, a valuable biomarker in chronic kidney disease and dialysis. Hemodial Int 2017; 21:161–167.

28. Vanholder R, Schepers E, Pletinck A, Nagler EV, Glorieux G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. JASN 2014; 25:1897–1907.

29. Chung BH, Kim KW, Sun IO, Choi SR, Park HS, Jeon EJ, *et al*. Increased interleukin-17 producing effector memory T cells in the end-stage renal disease patients. Immunol Lett 2012; 141:181–189.

30. Wang J, Li X-B, Huang P, Huang M-Y, Gu X-J. Change of peripheral blood Treg/Thl7 in cognitive impairment with chronic renal failure patients. Cell Physiol Biochem 2018; 45:281–290.

31. Li Y, Liu X, Wang W, Wang S, Zhang J, Jiang S, *et al*. Low-dose IL-2 expands CD4+regulatory T cells with a suppressive function *in vitro* via the STAT5-dependent pathway in patients with chronic kidney diseases. Ren Fail 2018; 40:280–288.

32. Zhu X, Li S, Zhang Q, Zhu D, Xu Y, Zhang P, *et al*. Correlation of increased Th17/Treg cell ratio with endoplasmic reticulum stress in chronic kidney disease. Medicine (Baltimore) 2018; 97:e10748.

33. 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 2010; 15:33–41.

34. Jakiela B, Kosałka J, Plutecka H, Bazan-Socha S, Sanak M, Musiał J. Facilitated expansion of Th17 cells in lupus nephritis patients. Clin Exp Immunol 2018; 194: 283–294.

35. Baron M, Belo R, Cathelin D, Moreira-Teixeira L, Cartery C, Rondeau E, *et al*. Innate-like and conventional T cell populations from hemodialyzed and kidney transplanted patients are equally compromised. PLoS One 2014; 9:e105422.

36. Caprara C, Kinsey GR, Corradi V, Xin W, Ma JZ, Scalzotto E, *et al*. The influence of hemodialysis on T regulatory cells: a meta-analysis and systematic review Blood Purif 2016; 42:307–313.

37. Chen D, Huang X, Yang M, Gan H, Gunawan EJ, Tang W. Treg/Th17 functional disequilibrium in Chinese uremia on hemodialysis: a link between calcification and cardiovascular disease. Ren Fail 2012; 34:697–702.

38. Afzali B, Edozie FC, Fazekasova H, Scottà C, Mitchell PJ, Canavan JB, *et al*. Comparison of regulatory T cells in hemodialysis patients and healthy controls: implications for cell therapy in transplantation. Clin J

Am Soc Nephrol 2013; 8:1396–1405.

39. Tzanno-Martins C, Azevedo LS, Orii N, Futata E, Jorgetti V, Marcondes M, Duarte AJ. The role of experimental chronic renal failure and aluminium intoxication in cellular immune response. Nephrol Dial Transplant 1996; 11:474–480.

40. Sanaka T, Koremoto M Selection guidelines for high-performance membrane. Contrib Nephrol 2011; 173:30–35.

41. Shen Y, Wei Y, Wang Z, Jing Y, He H, Yuan J, *et al*. TGF-beta regulates hepatocellular carcinoma progression by inducing Treg cell polarization. Cell Physiol Biochem 2015; 35:1623–1632.

42. Maddur MS, Miossec P, Kaveri SV, Bayry J. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. Am J Pathol 2012; 181:8–18.

43. Su X, Ye J, Hsueh EC, Zhang Y, Hoft DF, Peng G. Tumor microenvironments direct the recruitment and expansion of human Th17 cells. J Immunol 2010; 184:1630–1641.

44. Costantino CM, Baecher-Allan CM, Hafler DA. Human regulatory T cells and autoimmunity. Eur J Immunol 2008; 38:921–924.

45. Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, *et al*. Pathogenic conversion of Foxp3+T cells into TH17 cells in autoimmune arthritis. Nat Med 2014; 20:62–68.

46. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol 2008 8:523–532.

47. Sojka D, Huang Y-H, Fowell D. Mechanisms of regulatory T-cell suppression – a diverse arsenal for a moving target. Immunology 2008 124:13–22.

48. Campbell IL, Abraham CR, Masliah E, Kemper P, Inglis JD, Oldstone MB, Mucke L. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. Proc Natl Acad Sci U S A 1993; 90:10061–10065.

49. Ma L, Zhang H, Hu K, Lv G, Fu Y, Ayana DA. The imbalance between Tregs, Th17 cells and inflammatory cytokines among renal transplant recipients. BMC Immunol 2015; 16:56.

50. Martin-Moreno PL, Tripathi S, Chandraker A Regulatory T Cells and kidney transplantation. Clin J Am Soc Nephrol 2018; 13:1760–1764.

51. Bestard O, Cruzado JM, Rama I, Torras J, Goma M, Seron D, *et al*. Presence of FoxP3+regulatory T Cells predicts outcome of subclinical rejection of renal allografts. J Am Soc Nephrol 2008; 19:2020–2026.

52. Dufey E, Sepúlveda D, Rojas-Rivera D, Hetz C. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 1. An overview. Am J Physiol Cell Physiol 2014; 307:582–594.

53. Chiang CK, Hsu SP, Wu CT, Huang JW, Cheng HT, Chang YW, *et al*. Endoplasmic reticulum stress implicated in the development of renal fibrosis. Mol Med 2011; 17:1295–1305.

54. He F, Chen S, Wang H, Shao N, Tian X, Jiang H, *et al*. Regulation of CD2-associated protein influences podocyte endoplasmic reticulum stress-mediated apoptosis induced by albumin overload. Gene 2011; 484:18–25.

55. Hammadi M, Oulidi A, Gackiere F, Katsogiannou M, Slomianny C, Roudbaraki M, *et al*. Modulation of ER stress and apoptosis by endoplasmic reticulum calcium leak via translocon during unfolded protein response: involvement of GRP78. FASEB J 2013; 27:1600–1609.

56. Tsai YC, Weissman AM. The unfolded protein response, degradation from endoplasmic reticulum and cancer. Genes Cancer 2010; 1, 764–778.

57. Li Y, Guo Y, Tang J, Jiang J, Chenet Z. New insights into the roles of CHOP-induced apoptosis in ER stress. Acta Biochim Biophys Sin (Shanghai) 2014; 46:629–640.

58. Juan TS, Wilson DR, Wilde MD, Darlington GJ. Participation of the transcription factor C/EBP delta in the acute-phase regulation of the human gene for complement component C3. Proc Natl Acad Sci USA 1993; 90:2584–2588.

59. Yamaguchi J, Tanaka T, Eto N, Nangaku M. Inflammation and hypoxia linked to renal injury by CCAAT/enhancer-binding protein δ. Kidney Int 2015; 88:262–275.

60. Larbi A, Kempf J, Pawelec G. Oxidative stress modulation and T cell activation. Exp Gerontol 2007; 42:852–858.