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Original Article

Loss of regulatory T cell function on anti-inflammation is correlated with increased risk of acute kidney injury development in patients with primary glomerulonephritis

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Abstract: Inflammation is believed to play a major role in the pathophysiology of acute kidney injury (AKI). The injury induces the generation of inflammatory mediators like cytokines and chemokines by tubular and endothelial cells which contribute to the recruiting of leukocytes into the kidneys. Early AKI risk evaluation is limited to demographic characteristics and past clinical histories, and no specific treatment is available. To better identify patients at risk of developing AKI, and devise more targeted treatment and prevention regimen, we tracked 158 primary glomerulonephritis patients for their occurrence of AKI, and analyzed the characteristics of their adaptive immune system. We found that in patients that later developed AKI, peripheral blood T cell composition is shifted toward IFN-g-producing Th1-like cells. While the composition of $CD4^+CD25^+$ T cells were similar between patients that later developed AKI and patients without AKI development, in patients that later developed AKI, their $CD4^+CD25^+$ T cells secreted less regulatory cytokine IL-10, and was unable to suppress proinflammatory cytokine production by $CD4^+$ T cells, while in patients without AKI development, $CD4^+CD25^+$ T cells were able to suppress $CD4^+$ T cell-mediated IFN-g and IL-17 expression under stimulation, partially through IL-10 secretion. Collectively, we identified a defect in $CD4^+CD25^+$ T cell regulatory function in patients at risk of developing AKI.

Keywords: Regulatory T cell, AKI, glomerulonephritis

Introduction

Acute kidney injury (AKI) is the sudden loss of normal kidney function and can occur as a complication of various health issues, including but not limited to ischemic, toxic or septic insults, as well as following kidney transplant [1, 2]. It is a severe condition that worsens the clinical outcome of affected patients, substantially increases the risk for developing chronic kidney diseases and ends-stage renal diseases, with no specific therapies available [1, 3, 4]. Therefore, the prevention of AKI, including devising reliable markers to prospectively identify patients at risk of developing AKI, is of crucial importance.

Adaptive immunity is primarily mediated by T cells and B cells. Pathogenic T cell and B cell responses are implicated in the etiology of AKI in experimental rodent models and human renal diseases. At first, infiltration of $CD3^+$ T cells were found in human AKI biopsies [5, 6]. Since then, the role of T cells in the induction and development of AKI were examined in various experimental mouse models, including septic AKI, which reflects the renal injury following complex secondary inflammatory responses in sepsis, and aseptic ischemic AKI or nephrotoxic AKI, which reflects the renal injury following ischemia-reperfusion or induced by nephrotoxic drugs, respectively [1, 7]. Although the specific findings were variable, depending on the specific models used and treatment conditions, proinflammatory $CD4^+$ T cell-mediated response of the T helper 1 (Th1) type were found to contribute to the induction of renal injury [8], which was attenuated in $CD4^+$ T cell-deficient mice in

both ischemic and nephrotoxic models [9, 10]. Higher infiltration of $CD8^+$ T cells and stronger production of IFN-g by $CD8^+$ T cells were found the post-ischemic kidneys [9, 11]. Interestingly, a subtype of T cells, termed regulatory T cells (Treg cells), was shown to have a protective role in renal injury through IL-10 mediated suppression of the innate immune system. Partial depletion of $CD25^+$ Treg cells lead to increases in the infiltration of IFN-g-producing activated neutrophils and macrophages [12]. In nephrotoxic AKI, Treg cells were found to attenuate renal injury through decreasing the infiltration of macrophages [13]. The role of B cells in AKI was not entirely clear, but studies have shown that mature B cell-deficient mice were partially protected from early renal injury [14], and B cells trafficking into kidneys were differentiating into antibody-producing plasma cells and were interfering with post-ischemic repair [15], suggesting an adverse role of B cells in AKI. Follicular helper T cells (Tfh cells) is specialized in assisting B cell differentiation and antibody production [16]. The role of Tfh cells in AKI is unknown, but increased number of circulating Tfh-like $CD4^+CXCR5^+$ T cells is associated with systemic lupus erythromatosus, one of the preceding causes of AKI, and more severe kidney damage [17, 18]. These data together demonstrated the participation of T cells and B cells in the initiation, development, and repair stages of AKI.

Due to the wide variety in the preceding events and causes of human AKI, the interindividual genetic variabilities and disease histories, as well as the different treatment schemes, no reliable marker currently exists to prospectively identify patients at risk of developing AKI, posing a significant difficulty in AKI prevention. Here, we designed this group-matched study to investigate the role of T cells in AKI development in patients suffering glomerulonephritis. We tracked 158 primary glomerulonephritis patients for their occurrence of AKI, and analyzed the characteristics of their adaptive immune system. We found that in patients that later developed AKI, peripheral blood T cell composition is shifted toward IFN-g-producing Th1-like cells. While the composition of $CD4^+CD25^+$ T cells were similar between patients that later developed AKI and patients without AKI development, in patients that later developed AKI, their $CD4^+CD25^+$ T cells secreted less regulatory cytokine IL-10, and was unable to suppress proinflammatory cytokine production by $CD4^+$ T cells, while in patients without AKI development, $CD4^+CD25^+$ T cells were able to suppress $CD4^+$ T cell-mediated IFN-g and IL-17 expression under stimulation, partially through IL-10 secretion. Together, our study demonstrated a role of T cell responses in AKI development and revealed potential markers in primary glomerulonephritis patients at risk of developing AKI.

Methods

Subjects and study design

Written informed consent was obtained from all participants. The experimental protocol was established according to the guidelines of the Declaration of Helsinki and was approved by Ethics Committee of Shanghai Corps Hospital. A total of 158 patients with newly diagnosed glomerulonephritis were recruited in the hospital from September 2011 to December 2013 and followed for disease status and progression. Blood samples were collected at the time of diagnosis, prior to the start of treatment. A subset of patients were diagnosed with IgA nephropathy by histological examination of the biopsied kidney tissue, while in most cases the precise cause of glomerulonephritis were unknown. In several cases, preceding infections prior to the diagnosis of glomerulonephritis were suspected to be the cause. Exclusion criteria included secondary glomerulonephritis and presence of concurrent lupus, diabetes, or other diseases that involves complications in the immune system. All 158 were followed for at least one year after diagnosis. Patients that developed AKI within one year were termed AKI+ patients in this study. 14 other patients were then randomly selected for comparison from the pool of all patients without AKI-development after 1 year, and were termed AKI-null patients. The demographic characteristics of the AKI+ and AKI-null patients at the time of diagnosis and sample collection were then assembled. None of the AKI-null patients had developed AKI by the end of the study.

Sample collection and preparation

Peripheral blood samples were collected by venous puncture. PBMCs were purified by standard Ficoll-Paque gradient centrifugation, and was frozen in 10% DMSO plus 90% fetal bovine serum (FBS) immediately at -150°C until use. Fresh PBMCs were used in some experiments.

Table 1. Demographic, serological, and clinical characteristics in AKI+ and AKI-null patients

	AKI+ (n = 7)	AKI-null (n = 14)	*P*-value
Age (year)	57 (45-67)	54 (42-66)	>0.05
F/M	4/3	9/5	>0.05
Serum IgA (g/L)	2.78 (1.08-5.01)	2.55 (0.98-4.88)	>0.05
Urinary proteins (g/24 h)	2.45 (1.17-5.98)	2.75 (0.88-7.70)	>0.05
Serum uric acid (μmol/L)	378 (255-564)	334 (212-525)	>0.05
Cholesterol (mmol/L)	5.02 (2.98-7.90)	4.85 (2.77-8.01)	>0.05
eGFR (mL/min/1.73 m^2)	75 (36-90)	77 (32-95)	>0.05
Serum albumin (g/L)	30.5 (17.0-41.4)	35.2 (21.5-43.1)	>0.05
Microscopic hematuria (rbc/hpf)	10.3 (1.5-52.1)	12.6 (2.7-49.6)	>0.05
Serum creatinine	3.5 (1.7-6.7)	4.1 (1.3-6.8)	>0.05
WBC (10^9/L)	8.85 (4.8-9.1)	7.98 (4.6-9.8)	>0.05
Lymphocytes (10^9/L)	1.5 (1.1-3.2)	1.7 (1.2-2.85)	>0.05

During incubation, cells were cultured in culture medium (RPMI 1640 supplemented with Penicillin/Streptomycin and 10% FBS) at 37°C 5% CO_2.

Flow cytometry

The following monoclonal antibodies were used in PBMC staining: CD3, CD4, CD8, CD25, CD28, IFN-g, IL-4, IL-10, and IL-17 (BioLegend). Recombinant sIL-10R (R&D systems) were added at 10 μg/mL for some experiments. For phenotypical and cytokine expression analysis, BD LSRII was used. For live cell sorting, BD FACSAria was used. Positively selected $CD4^+CD25^+$ T cells were used in IL-10 Luminex experiments and discarded to obtain $CD4^+CD25^+$ T cell-depleted fraction. To account for potential alterations in cellular function during the sorting process, the whole fraction went through a mock sorting. Flow cytometry data were analyzed using FlowJo (TreeStar).

Luminex assay

50,000 per well $CD4^+CD25^+$-sorted T cells were cultured in 200 μL culture medium without or with 10 ng/mL PMA plus 2.5 μM ionomycin in the presence of IL-10 capture beads (Millipore). After 12 h, the IL-10 capture beads were harvested and the concentration of IL-10 in medium was measured by Luminex assay according to manufacturer's protocol.

Statistical analysis

The significance of the difference between two groups was evaluated by Mann Whitney non-parametric test. The significance of the difference between two conditions subjected to samples from the same individual was evaluated by Wilcoxon matched-pairs signed rank test. Friedman test followed by Dunn's multiple comparison test was used for comparisons between multiple matched conditions. All statistical analyses were done using Prism software (GraphPad).

Results

No significant serologic or clinical differences in glomerulonephritis disease symptoms were found between AKI+ patients and AKI-null patients

Previous studies on human AKI development have used diagnosed AKI patient samples, when the injury is already present [1, 19, 20]. Therefore, the clinical and cellular markers for reliable identification of patient at risk of AKI prior to its development are still lacking, representing a huge burden in disease prophylaxis. To resolve this issue, we followed the disease status of 158 hospitalized primary glomerulonephritis patients over the course of this study. Peripheral blood samples were collected at the time of glomerulonephritis diagnosis. 7 of the patients later developed AKI within one year. For comparison between patients that developed AKI (AKI+ subjects) and patients that did not develop AKI after one year (AKI-null subjects), we randomly selected 14 patients' samples from the pool of AKI-null subjects. The resulting group of AKI-null subjects were matched with the groups of AKI subjects in age, male-to-female ratio, smoking habits, absence of diabetes, lupus, and other complications that may affect their immune profile (data not shown). The clinical correlates in blood and urine tests at the time of diagnosis and peripheral sample collection were compared between the AKI+ group and the AKI-null group. No significant differences in serologic and clinical characteristics between AKI+ and AKI-null groups were observed at this time (**Table 1**). These data demonstrated that at the time of diagnosis and sample collection, patients that later developed AKI within 1 year of glomerulo-

nephritis and patients that did not develop AKI had comparable disease severity.

AKI+ subjects had elevated levels of IFN-g-producing, Th1-like cells compared to AKI-null subjects

Next, we examined potential immunological markers for patients at risk of developing AKI. Since $CD4^+$ T cell infiltration and cytokine production, as well as the presence of IFN-g-producing $CD8^+$ T cells, were implicated in renal injury in mouse models, we first examined the peripheral T cell composition in AKI+ group and AKI-null group. Blood samples were obtained at the time of diagnosis and peripheral blood mononuclear cells (PBMCs) were stained with T cell-specific markers and examined by flow cytometry (**Figure 1A**). We found that the frequencies of total $CD4^+$ T cells and total $CD8^+$ T cells were comparable in AKI-null and AKI+ patients (**Figure 1B**).

Previously, Th1 type $CD4^+$ T cells were found to exacerbate renal injury while Th2 type $CD4^+$ T cells were found to ameliorate injury. Therefore, we decided to examine the $CD4^+$ T cell composition in AKI-null and AKI+ groups. $CD4^+$ T cell subsets can be distinguished by their differential expression of cytokines. Thus, IFN-g, IL-4, and IL-17 productions were used as markers for Th1-, Th2-, and Th17-like circulating T cells, respectively, and was measured both under unstimulated condition (Medium) for assessment of ex vivo composition and under PMA/ionomycin-stimulated condition (PMA/ionomycin) for total cytokine production potency (**Figure 1C**). When measured ex vivo, patients from the AKI+ group contained significantly higher frequencies of IFN-g-producing $CD4^+$ T cells than those in the AKI-null group, while no significant differences were found in the frequencies of IL-4-producing or IL-17-producing $CD4^+$ T cells (**Figure 1D**). After stimulation with PMA/ionomycin, all subjects upregulated their cytokine production, which is expected because PMA/ionomycin directly elevates intracellular calcium levels in T cells and enables assessment of all cells with cytokine production potency. The increase of IFN-g-producing $CD4^+$ T cells in AKI+ patients is retained under this stimulated condition (**Figure 1E**). Collectively, these data showed the circulating $CD4^+$ T cells in glomerulonephritis patients at risk of developing AKI contained elevated levels of IFN-g producing cells ex vivo or under stimulated conditions.

AKI+ subjects had comparable levels of $CD4^+CD25^+$ circulating Treg cells with AKI-null subjects

Treg cells can refer to multiple subsets of T cells with the capacity to maintain self-antigen tolerance, restrain excessive inflammation, and abrogate autoimmune diseases. $CD4^+CD25^+$ Treg cell is the best-characterized subset, and partial depletion of Treg cells with anti-CD25 monoclonal antibody were shown to exacerbate kidney damage in experimental ischemic AKI [12, 21]. Therefore, we also examined the composition of $CD4^+CD25^+$ T cells in our study groups (**Figure 2A**). No significance difference in the percentage of circulating $CD4^+CD25^+$ T cells between the AKI-null group and the AKI+ group were found (**Figure 2B**).

CD25 is the IL-2 receptor alpha chain and can be expressed on non-Treg cells during immune activation [22]. Using $CD4^+CD25^+$-expression to identify Treg cells in glomerulonephritis patients with ongoing inflammation may be problematic. Since Treg cells were found to modulate injury through IL-10-mediated suppressions in experimental AKI [12], we decided to utilize IL-10 to functionally analyze Treg cells in AKI-null patients and AKI+ patients. As shown in **Figure 2C** and **2D**, IL-10 production in $CD4^+$ T cells were concentrated in the $CD25^+$ compartment. When examining IL-10 production by $CD4^+CD25^+$ T cells, we found that $CD4^+CD25^+$ T cells from AKI+ patients contained significantly less IL-10-expressing cells than those from AKI-null patients (**Figure 2E**). In addition, we also analyzed the amount of IL-10 secretion from $CD4^+CD25^+$ T cells. Live-sorted $CD4^+CD25^+$ T cells were cultured in medium with IL-10 capture beads for 12 h, after which the IL-10 production in culture was measured by Luminex assay. We found that $CD4^+CD25^+$ T cells from AKI+ subjects secreted significantly less IL-10 than those from AKI-null patients under unstimulated (medium) condition (**Figure 2F**). After PMA/ionomycin-stimulation, $CD4^+CD25^+$ T cells from AKI+ subjects appeared to secrete less IL-10 but the result is not significant.

Collectively, these data suggested that the $CD4^+CD25^+$ T cells from AKI+ subjects secreted

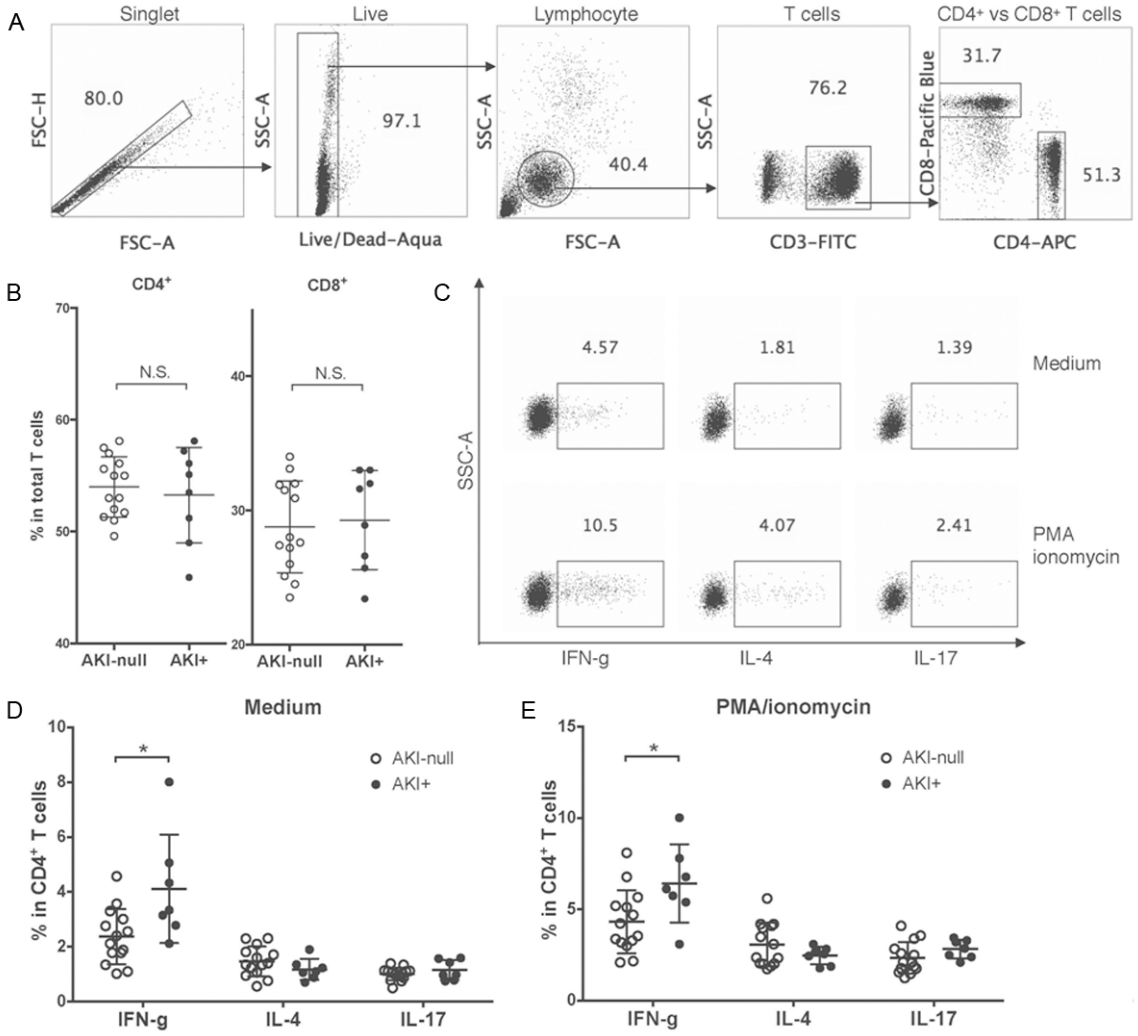

Figure 1. T cell compositions of AKI+ and AKI-null patients. A. Gating strategy for total T cells as well as $CD4^+$ vs. $CD8^+$ T cells. Shown is a representative from AKI-null patients. B. Frequencies of circulating $CD4^+$ T cells and $CD8^+$ T cells in all AKI-null and AKI+ patients. C. Gating strategy for IFN-g-, IL-4-, and IL-17-producing T cells. PBMCs were first cultured in medium without or with 10 ng/mL PMA plus 2.5 μM ionomycin (PMA/ionomycin) for 6 h in the presence of GolgiStop and GolgiPlug, and then stained with surface markers, after which cells were permeabilized with CytoFix/CytoPerm and stained for intracellular expression of IFN-g, IL-4, and IL-17. Shown is gated on $CD4^+$ T cells from a representative in the AKI-null group. D. Frequencies of IFN-g-, IL-4-, and IL-17-producing $CD4^+$ T cells in AKI-null and AKI+ subjects directly ex vivo. E. Frequencies of IFN-g-, IL-4-, and IL-17-producing $CD4^+$ T cells in AKI-null and AKI+ subjects after PMA/ionomycin-stimulation. *$P < 0.05$. N.S.: not significant. Mann-Whitney test. $P < 0.05$ is considered significant.

less IL-10 and may have lowered capacity to suppress inflammation.

$CD4^+CD25^+$ T cells from AKI+ patients had reduced capacity to suppress IFN-g secretion than those from AKI-null patients

Treg cells were known to suppress Th1- and Th17-mediated inflammations in chronic infections and autoimmune diseases [22]. Moreover, in mouse post-ischemic kidneys, depletion of $CD25^+$ Treg cells resulted in increased infiltration of IFN-g-producing proinflammatory neutrophils and macrophages at the injury site [12]. Based on the elevation in the frequencies of IFN-g-producing T cells and the reduction in IL-10 production from $CD4^+CD25^+$ T cells in AKI+ patients, we investigated whether these two phenomena were connected. $CD4^+CD25^+$ T cells were depleted from PBMCs by live sorting, and the frequency of cytokine producing T cells

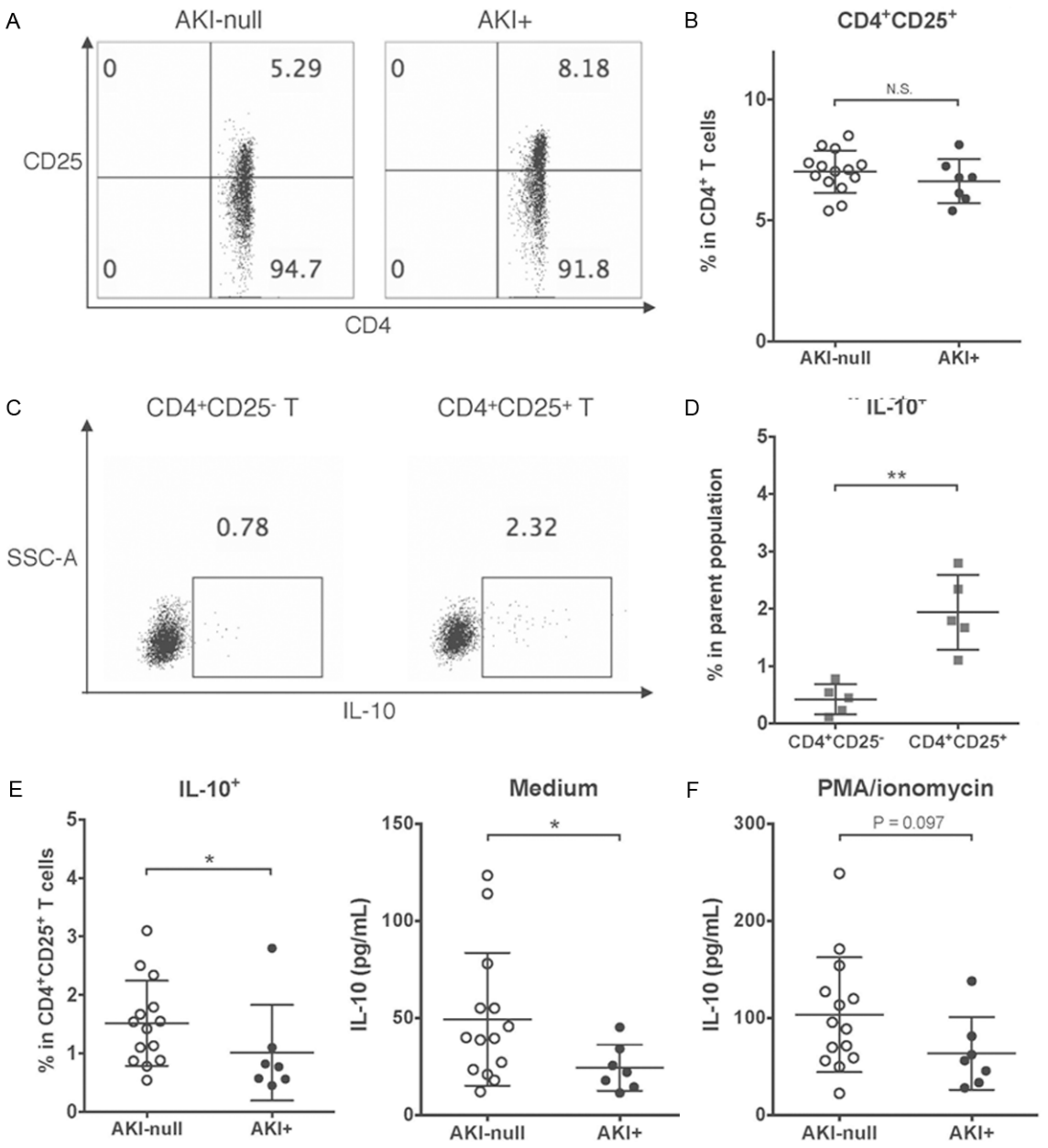

Figure 2. Frequencies of $CD4^+CD25^+$ T cells and IL-10 production in AKI-null and AKI+ patients. A. Gating strategy of $CD4^+CD25^+$ T cells. Shown are gated on $CD4^+$ T cells from one representative from each of AKI-null and AKI+ groups. PBMCs were stained for surface marker expression directly ex vivo. B. Frequencies of $CD4^+CD25^+$ T cells in total $CD4^+$ T cells in AKI-null and AKI+ patients. C. Gating strategy of IL-10 expression in $CD4^+CD25^-$ T cells and $CD4^+CD25^+$ T cells. Shown is from a representative AKI-null patient. PBMCs were first cultured in medium for 6h in the presence of GolgiStop and GolgiPlug, and then stained with surface markers, after which cells were permeabilized with CytoFix/CytoPerm and stained for intracellular expression of IL-10. D. The percentage of IL-10-expressing T cells in $CD4^+CD25^-$ T cells and $CD4^+CD25^+$ T cells, obtained from three AKI-null patients and two AKI+ patients. E. The intracellular expression of IL-10 in $CD4^+CD25^+$ T cells from AKI-null patients and AKI+ patients. F. The concentration of IL-10 secreted by live-sorted unstimulated (medium) or stimulated (PMA/ionomycin) $CD4^+CD25^+$ T cells from AKI-null patients and AKI+ patients. Live-sorted $CD4^+CD25^+$ T cells were cultured in medium without or with 10 ng/mL PMA plus 2.5 μM ionomycin in the presence of IL-10 capture beads. After 12 h, the IL-10 capture beads were harvested and the concentration of IL-10 in medium was measured by Luminex assay. *$P < 0.05$. **$P < 0.01$. N.S.: not significant. Mann-Whitney test. $P < 0.05$ is considered significant.

when cultured ex vivo in medium (**Figure 3**) or in the presence of PMA/ionomycin stimulation (**Figure 4**) was examined. To account for the individual differences in cytokine secretion in

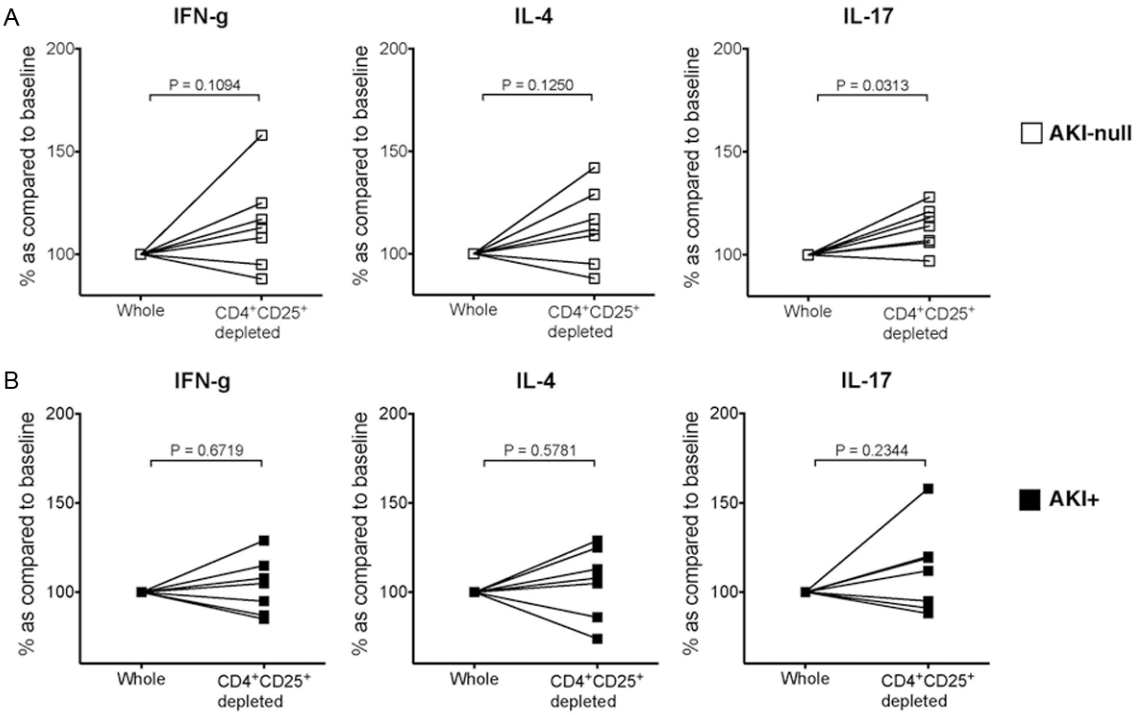

Figure 3. Expression of IFN-g, IL-4, and IL-17 from unstimulated $CD4^+$ T cells before (whole) or after $CD4^+CD25^+$ T cell depletion ($CD4^+CD25^+$ depleted). PBMCs were stained with anti-CD4/anti-CD25 antibodies and sorted as whole (mock sorted) or $CD4^+CD25^+$ depleted ($CD4^+CD25^+$ removed). The sorted cells were then plated in a 96-well plate pre-coated with anti-CD3/CD28 at 200,000 cells per well in culture medium for 6 h, in the presence of GolgiStop and GolgiPlug. The cells were then harvested and stained for surface marker expression and intracellular cytokine expression. IFN-g-, IL-4- and IL-17-expressing $CD4^+$ T cells were gated in a similar fashion as in 1A and 1C. Data were normalized using the cytokine expression in the unstimulated whole cell fraction of each individual as the baseline expression (100%). A. The IFN-g-, IL-4- and IL-17-expressing cells in AKI-null patients. B. The IFN-g-, IL-4- and IL-17-expressing cells in AKI+ patients. Wilcoxon matched-pairs signed rank test. $P < 0.05$ is considered significant.

AKI-null patients and AKI+ patients (**Figure 1D**), we normalized the data by setting the baseline cytokine production (whole T cells in medium) of each individual at 100%. As shown in **Figure 3A**, in AKI-null patients under ex vivo condition, frequencies of IL-17-producing cells were significantly elevated when $CD4^+CD25^+$ T cells were depleted. 5 out of 7 AKI-null patients also upregulated their frequencies of IFN-g- and IL-4-producing cells, but the trend was not statistically significant. In AKI+ patients (**Figure 3B**), no significant differences were found in the frequencies of IFN-g-, IL-4-, and IL-17-producing cells when $CD4^+CD25^+$ T cells were depleted. Interestingly, when examining cytokine secretion after PMA/ionomycin stimulation, in AKI-null patients, the frequencies of IFN-g-producing cells and of IL-17-producing cells were significantly upregulated when $CD4^+CD25^+$ T cells were depleted (**Figure 4A**). In AKI+ patients (**Figure 4B**), no significant differences were found in the frequencies of IFN-g-, IL-4-, and IL-17-producing cells when $CD4^+CD25^+$ T cells were depleted. Together, these data suggest that $CD4^+CD25^+$ T cells from AKI-null subjects were able to suppress IL-17-production in T cells ex vivo, and suppress IFN-g- and IL-17-production after stimulation. This suppressive function was lost in the AKI+ patients.

The suppressive capacity of $CD4^+CD25^+$ T cells in AKI-null patients were partially attributed to IL-10 secretion

IL-10 is known to repress proinflammatory responses and limit unnecessary tissue damage during inflammation [23]. Here, we wondered whether the suppressive effects of $CD4^+CD25^+$ T cells from AKI-null subjects were mediated by IL-10 production. We repeated the above experiments in three more AKI-null sub-

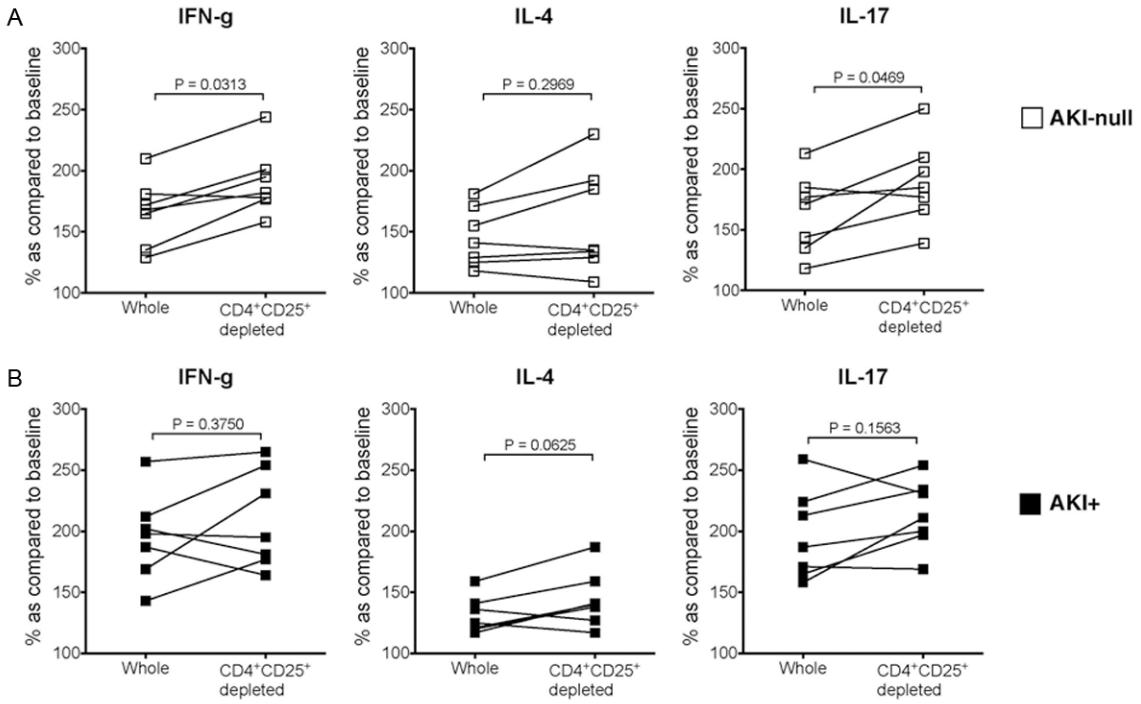

Figure 4. Expression of IFN-g, IL-4, and IL-17 from PMA/ionomycin-stimulated $CD4^+$ T cells before (whole) or after $CD4^+CD25^+$ T cell depletion ($CD4^+CD25^+$ depleted). PBMCs were sorted with the aforementioned method, plated in anti-CD3/CD28 pre-coated plate at 200,000 cells per well with 10 ng/mL PMA and 2.5 μM ionomycin for 6 h, in the presence of GolgiStop and GolgiPlug, and then harvested and stained for surface marker expression and intracellular cytokine expression. Data were normalized using the cytokine expression in the unstimulated whole cell fraction of each individual as the baseline expression (100%). A. The IFN-g-, IL-4- and IL-17-expressing cells in AKI-null patients after PMA/ionomycin stimulation. B. The IFN-g-, IL-4- and IL-17-expressing cells in AKI+ patients after PMA/ionomycin stimulation. Wilcoxon matched-pairs signed rank test. $P < 0.05$ is considered significant.

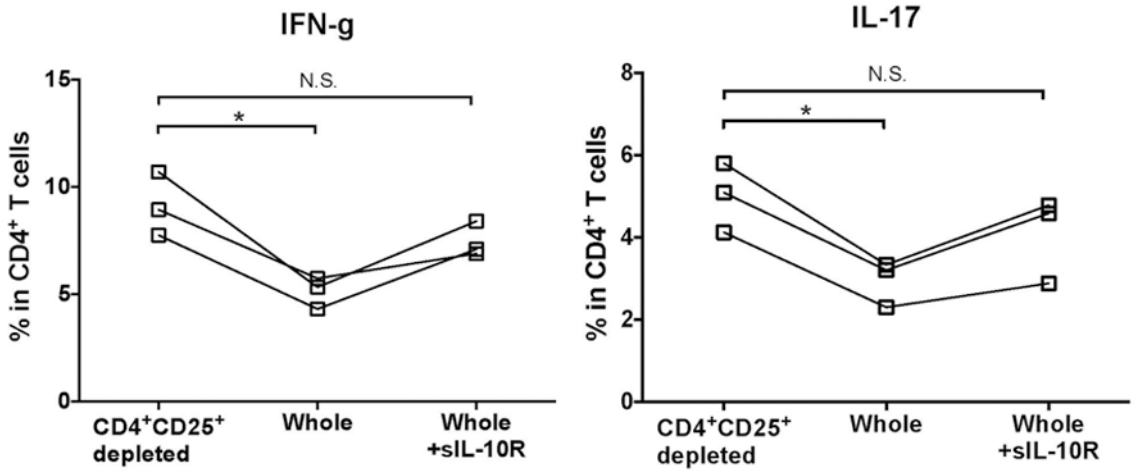

Figure 5. The frequency of IFN-g- and IL-17-expressing cells in AKI-null patients, with the addition of sIL-10R. PBMCs were sorted, cultured and stained with the aforementioned method, with addition of 10 μg/mL sIL-10R in a subset of whole cell cultures during the 6 h incubation time. IFN-g- and IL-17-expressing $CD4^+$ T cells were gated in a similar fashion as in **Figure 1A** and **1C**. *$P < 0.05$. N.S.: not significant. Friedman test followed by Dunn's multiple comparison test. $P < 0.05$ is considered significant.

jects with soluble IL-10 receptor (sIL-10R) added in some of the cultures. As shown in **Figure 5**, frequency of IFN-g- and IL-17-producing cells were downregulated in whole T cells compared to $CD4^+CD25^+$-depleted T cells. The addition of sIL-10R during incubation into the whole T cell culture partially abrogated the suppression of IFN-g and IL-17 production.

Discussion

A major challenge in prevention and early treatment of AKI is the lack of reliable predictive markers for identifying patients at risk of AKI development. Current risk assessment schemes rely heavily on demographic characteristics and past clinical histories targeted at a broad group of patients [24]. In addition, early identifiers of potential AKI is still unknown. Most diagnoses were done when the renal injury is already established, limiting treatment options to conservative methods such as fluid therapy and dialysis. Furthermore, the direct induction of AKI appears to be multifactorial; many diseases with different clinical presentations, symptoms, and treatment schemes may lead to AKI as a complication [7]. Controlled renal injury induction in experimental mouse models has been helpful in elucidating the underlying mechanisms but the relevance in applying animal model findings in clinical research requires more investigation.

In this study, we attempted to identify early markers for primary glomerulonephritis patients at risk of AKI development prior to the induction of AKI. At first, we examined serological parameters and clinical symptoms between AKI-null patients and AKI+ patients, but no significant differences between the two groups were found, suggesting that the induction of AKI is not a direct result of disease severity. Based on the role of the adaptive immune system in experimental AKI [2, 7, 25], we focus the study on the compositions and functions of T cells. We found that in AKI+ patients, peripheral blood T cell composition is shifted toward IFN-g-producing Th1-like cells, a cell type shown to increase susceptibility of ischemic kidney injury [9]. Although the compositions of $CD4^+CD25^+$ T cells were similar between AKI+ patients and AKI-null patients, AKI+ patients' $CD4^+CD25^+$ T cells secreted less regulatory cytokine IL-10. When $CD4^+CD25^+$ T cells were depleted, IFN-g and IL-17 production in $CD4^+$ T cells were elevated in AKI-null subjects, while no such effects were observed in in AKI+ patients. The suppression by the presence of $CD4^+CD25^+$ T cells was mediated partially through IL-10, as when sIL-10R was added to sequester supernatant IL-10, the downregulation in IFN-g and IL-17 expression was partially reversed. Together, our study demonstrated a change in T cell profile and function prior to AKI development, and revealed potential functional markers in primary glomerulonephritis patients at risk of developing AKI.

Treg-mediated regulation in animal models were found to attenuate early injury by IL-10-mediated suppression of the infiltrating innate cells, reduce macrophage infiltration, enhance tubular repair and fasten the healing process after injury. In this study, we illustrated a role of $CD4^+CD25^+$ T cells in suppressing IFN-g and IL-17 responses and maintaining immune balance under stimulation in primary glomerulonephritis. Since AKI is thought to occur through multiple mechanisms, suppression of excessive inflammation upon acute kidney insult is expected to improve the prognosis of disease. Indeed, the suppressive function of $CD4^+CD25^+$ T cells was impaired in AKI+ patients. In the future, preventive and treatment schemes targeted toward the immune system and inflammation may be considered. Other functions involving the participation of immune cells, including modulation of injury repair through cytokine secretion, and infiltration of immune cells into the site of injury, need to be studied further.

Disclosure of conflict of interest

None.

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