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Expansion and characterization of regulatory T cell populations from Korean kidney transplant recipients

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Abstract

The development of immunosuppressants has enabled remarkable progress in kidney transplantation (KT). However, current immunosuppressants cannot induce immune tolerance, and their nonspecific immunosuppressive effects result in many adverse effects. Regulatory T cells (Tregs) play crucial roles in controlling all specific immune responses. This study evaluated the distribution of Tregs and their effects on kidney allograft function in Korean KT recipients. We enrolled 113 KT recipients with stable graft function. The differentiation and expansion of Tregs were examined by flow cytometry to compare the Tregs subpopulations. Among the 113 patients, 73 (64.6%) were males, and the mean follow-up period from KT to Tregs collection was 147.5 ± 111.3 months. Patients receiving lower doses of cyclosporine had higher proportions of Tregs than those with higher doses of cyclosporine (36.3 ± 21.6 vs 17.0 ± 12.7, P = .010, respectively). Patients taking cyclosporine tended to have higher Tregs numbers than those taking tacrolimus (94.7 ± 158.1 vs 49.3 ± 69.4, P = .095, respectively). However, no significant association was observed between Tregs and allograft dysfunction in the cox proportional hazard model. Tregs counts may be associated with the type and dose of immunosuppressants. However, no significant relationship was found between Tregs and kidney allograft function in stable KT recipients.

Abbreviations: BMI = body mass index, CNI = calcineurin inhibitor, CsA = cyclosporine, HBV = hepatitis B virus, KT = kidney transplantation, MMF = mycophenolate mofetil, PBMCs = peripheral blood mononuclear cells, PBS = phosphate-buffered saline, Tregs = regulatory T cells.

Keywords: graft survival, immunosuppressive agents, kidney transplantation, regulatory, T-lymphocytes

1. Introduction

Kidney transplantation (KT) is the best treatment for patients with end-stage renal disease. During the past decades, advances in immunosuppressants reduced the acute rejection episodes and improved short-term kidney allograft survival. However, long-term allograft survival may be suboptimal mainly because of chronic rejection.[1] Current immunosuppressive drugs cannot induce immune tolerance and their nonspecific immunosuppressive effects result in complications such as infection, nephrotoxicity, and malignancies.[2] Thus, there has been great interest in achieving of immune tolerance to minimize or eliminate immunosuppression.

Regulatory T cells (Tregs), derived from the thymus or peripheral tissues, constitute approximately 5% to 10% of peripheral circulating $CD4^+$ T cells.[3] Tregs play crucial roles in maintaining immune homeostasis.[4,5] They have been shown to downregulate activated allospecific T cells.[6] Studies have revealed that Tregs can maintain hyporesponsiveness to alloantigens in KT recipients.[7,8] Previous animal model studies revealed that tolerance of an allograft was a process largely affected by the presence of Tregs.[9] Interestingly, a study including murine models demonstrated that inducible Treg treatment decreased donor specific antibody levels within allografts, suggesting a potential role in the treatment of antibody mediated rejection.[10] Recently, regulatory cell therapy is being evaluated in living donor KT in the 1 study, which is a multicenter phase 1/2A trial.[11] Furthermore, Tregs are believed to be a key modulator of tolerance in the aspect of autoimmune, infection, and tumor entities.[12] Although, many pieces of evidence show the role of Tregs in the immune mechanisms, their exact roles remain uncertain in KT recipients.

In the present study, we investigated the association between the distribution of Tregs and clinical parameters that may affect the level of Tregs. Furthermore, we evaluated the clinical

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significance of Tregs in kidney allograft function in Korean KT recipients.

2. Materials and Methods

2.1. Study design and participants

This study included adult patients who underwent KT at Kosin University Gospel Hospital from 1989 to 2018. In this study, 113 patients who survived at least 6 months after KT and provided informed consent were enrolled. All patients received basiliximab as an induction therapy. Maintenance immunosuppressant regimens consisted of calcineurin inhibitor (CNI), mycophenolate mofetil (MMF), and prednisolone. Heparinized blood samples were collected at the initiation of the study.

The Institutional Review Board of Kosin University Gospel Hospital approved this study. (Approval number: KUGH 2019-08-033). Informed consent was obtained from all participants. All procedures were performed in accordance with the Declaration of Helsinki and Istanbul.

2.2. Patients

This study enrolled primary KT recipients who were more than 18 years of age with stable renal function (estimated glomerular filtration rate changes of < 30 mL/minute/1.73 m^2 for the recent 3 months) and no history of rejection. Maintenance immunosuppressive regimens were standard triple therapy, which consisted of CNI (cyclosporine [CsA] or tacrolimus) combined with MMF and prednisolone. Patients were treated with following immunosuppressive protocol in early stage of KT. Initial dose of tacrolimus was 0.1 mg/kg and CsA was 5 mg/kg daily. Trough levels for tacrolimus were 5 to 10 ng/mL for the first month and 4 to 8 ng/mL thereafter. Trough levels for CsA were 200 to 300 ng/mL for 3 months and 50 to 150 ng/mL for subsequent months. We gave MMF at 2g daily for the first month, followed by MMF at 1g per day. Then, we modified the immunosuppressants according to the patient condition.

Patients who had evidence of active infection; autoimmune disease; a history of cancer, except for adequately cured basal cell carcinoma; and insufficient data were excluded from this study.

2.3. Data collection and definitions

We collected the demographic characteristics and laboratory data from the electronic medical records of the study population. Clinical data included age, sex, body mass index (BMI), and dose and trough level of immunosuppressants. Creatinine (Cr), sodium, potassium, albumin, total CO_2, and lipid profile were included in the laboratory findings. BMI was calculated using the following formula: BMI = body weight [kg]/height $[m]^2$. Patients who had a serum Cr level below 1.5 mg/dL were considered to have a stable allograft kidney function.

2.4. Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll. Whole blood from KT recipients was diluted with phosphate-buffered saline (PBS). An equal volume of Ficoll was layered over and centrifuged. The middle phase containing PBMCs was obtained and washed with PBS. The isolated PBMCs were counted and resuspended in freezing medium containing 10% dimethyl sulfoxide. The samples were placed in a deep freezer at – 80°C for 24 to 72 hours. Then, the PBMCs were stored in a liquid nitrogen tank at – 150°C for long-term storage.

2.5. Cell thawing

The frozen PBMCs were thawed in a water tank and transferred to a conical tube. Five mL of fetal bovine serum was added and centrifuged at 1000 rpm for 5 minutes at 25°C. After the supernatant was removed, the wash step was repeated. The cells were resuspended in 1 mL of fetal bovine serum. The samples were divided into 3 tubes: tube 1: surface and intracellular Ab staining test; tube 2: unstaining test; and tube 3: surface Ab test.

2.6. Flow cytometry

The PerFix-nc Kit (Beckman Coulter, France) was used to prepare the samples for analysis by flow cytometry. The PerFix protocol was used for tube 1 only. Five hundred µL of sample was moved to the E-tube and centrifuged at 2000 rpm for 5 minutes at 25°C. Four hundred µL of the upper layer was removed and 100 µL of the remaining sample was moved to the test tube. Five µL of the fixative reagent was added to the tube and vortexed immediately. The samples were incubated in the dark for 15 minutes. Thirty µL of permeabilizing agent was added to the tube and vortexed immediately. Then, 5 µL of CD4, 10 µL of CD25, and 10 µL of CD127 were added to each tube and vortexed immediately. The samples were kept in the dark for 20 minutes at room temperature and vortexed. Five µL of FoxP3 was added and vortexed immediately. The samples were incubated in the dark for 20 minutes at room temperature. Three µL of 1 X PerFix-nc buffer was added and vortexed immediately. The sample was analyzed using flow cytometry. In tube 2, 300 µL of PBS was added. In tube 3, 5 µL of CD4, 10 µL of CD3, and 10 µL of CD28 were added to each tube and vortexed immediately. The samples were incubated in the dark for 20 minutes at room temperature. Then, 300 µL of PBS was added. Tregs were identified as $CD4^{+}CD25^{high}CD127^{low/-}FoxP3^{+}$. CD3-PC7, CD4-PC5.5, CD25-PC7, CD28-FITC, CD127-FITC, and FoxP3 were used from Beckman Coulter, France.

2.7. Statistical analysis

Continuous variables were expressed as means ± standard deviations. Categorical variables were presented as numbers and percentages. Student *t* test or the Mann–Whitney test was used to analyze the intergroup comparisons of continuous variables. The 1-way analysis of variance test was used to analyze more than 2 groups of continuous variables. Cox proportional hazard regression analysis was performed to identify the risk factors for kidney allograft dysfunction. The p for trend analysis was performed using R (version 3.0.3; The R Foundation). P values of < .05 were used to denote statistical significance. With the exception of the p for trend analysis, we conducted all statistical analyses using Statistical Package for the Social Sciences (version 24; IBM Corp., Armonk, NY).

3. Results

In this study, 113 patients were enrolled. The demographic features of the patients are shown in Table 1. The mean age of the patients was 54.5 ± 9.7 years, and 73 (64.6%) patients were males. The mean follow-up duration was 147.5 ± 111.3 months; the mean creatinine level was 1.2 ± 0.7 mg/dL. All patients received basiliximab as an induction therapy. Furthermore, 109 (96.5%) patients received CNI, 73 (64.6%) patients received MMF, and 79 (69.9%) patients received prednisolone as maintenance immunosuppression regimens. The mean tacrolimus trough level was 5.8 ± 2.2 ng/mL, and the mean cyclosporine trough level was 98.1 ± 45.4 ng/mL.

Table 2 shows the Tregs subpopulation according to the patients characteristics. Patients receiving CsA doses of below 100 mg had higher proportions of Tregs population than

Table 1

Demographics of patients.

Variables	All patients (n = 113)
Age (yr)	54.5 ± 9.7
Sex (Male)	73 (64.6)
Height (cm)	163.7 ± 13.5
Body weight (kg)	63.9 ± 11.7
Follow-up duration from KT to Tregs collection (months)	147.5 ± 111.3
DDKT (%)	23 (21.3)
Desensitization	2 (0.2)
HLA mismatch	2.0 ± 1.5
Induction therapy	
Basiliximab (%)	113 (100)
Maintenance therapy	
CNI (%)	109 (96.5)
Tacrolimus (%)	70 (61.9)
Cyclosporine (%)	39 (34.5)
MMF (%)	73 (64.6)
PDN (%)	79 (69.9)
CNI + MMF + PDN (%)	58 (51.3)
Drug level	
Tacrolimus trough level (ng/mL)	5.8 ± 2.2
Cyclosporine trough level (ng/mL)	98.1 ± 45.4
Drug dose	
Tacrolimus (mg)	2.6 ± 1.2
Cyclosporine (mg)	106.7 ± 45.0
HBV (%)	7 (6.2)
Laboratory findings	
Creatinine (mg/dL)	1.2 ± 0.7
Creatinine > 1.5 mg/dL	17 (15.0)
Sodium (mg/dL)	138.2 ± 2.7
Potassium (mg/dL)	4.2 ± 0.5
Albumin (mg/dL)	4.2 ± 0.4
Total CO_2 (mg/dL)	26.7 ± 2.7
Total Cholesterol (mg/dL)	177.2 ± 42.1
Low-density lipoprotein (mg/dL)	100.9 ± 30.4
Triglyceride (mg/dL)	127.7 ± 84.3
High-density lipoprotein (mg/dL)	53.0 ± 13.4
Triglyceride/High-density lipoprotein	1.3 ± 0.9

Values are expressed as means ± SDs, n (%).

CNI = calcineurin inhibitor, DDKT = deceased donor kidney transplantation, HBV = hepatitis B virus, KT = kidney transplantation, MMF = mycophenolate mofetil, PDN = prednisone, Tregs = regulatory T cells.

those receiving CsA doses of more than 100 mg (36.3 ± 21.6 vs 17.0 ± 12.7, P = .010, respectively). Patients with total cholesterol of below 172.8 mg/dL had higher Tregs numbers than those with total cholesterol of more than 172.8 mg/dL (90.6 ± 140.6 vs 45.8 ± 74.8, P = .044, respectively). Patients without hepatitis B virus (HBV) infection had higher Tregs numbers than those infected with HBV (72.8 ± 117.0 vs 12.4 ± 13.0, P < .001, respectively). Patients followed up for more than 147.5 months tended to have more gating Tregs than those with shorter follow-up durations. Among the patients who received CNI as a maintenance therapy, those who received tacrolimus tended to have lower Tregs numbers than those who received CsA.

Figure 1 shows the Tregs subsets according to the patients' follow-up duration. Tregs numbers appeared to be increased in the long-term follow-up group, although the difference was not statistically significant.

Cox proportional hazards models were developed to identify the risk factors for allograft dysfunction (Table 3). In the crude analysis, younger age, deceased donor KT, lower albumin levels, and lower total CO_2 levels were associated with an increased risk of allograft dysfunction (Cr > 1.5 mg/dL). However, no significant association was observed between Tregs and allograft dysfunction. Since Tregs were not a risk factor in the univariate analysis, we did not perform multivariate analysis.

Table 4 shows the T-cell subpopulation according to allograft function. No significant differences in the T-cell subpopulation were observed between patients with Cr ≤ 1.5 mg/dL and those with Cr < 1.5 mg/dL.

4. Discussion

In this study, we explored the distribution and characterization of Tregs in stable Korean KT recipients. Patients receiving lower doses of CsA had higher proportions of Tregs than those receiving higher doses of CsA. Patients taking cyclosporine tended to have higher Tregs numbers than those taking tacrolimus. However, no significant relationship was found between Tregs and kidney allograft function.

Among the diverse types of Tregs, $CD4^+$ Tregs were the most examined. Tregs account for approximately 5% to 10% of peripheral circulating $CD4^+$ T cells.[3] Because $CD4^+CD25^+$ T cells contribute to maintaining self-tolerance, many studies have focused on the role of $CD4^+CD25^+$ T cells in transplantation and autoimmune diseases.[13] FoxP3 was identified as a transcription factor considered the master gene of Tregs.[14] Furthermore, the different mutations of FoxP3 cause fatal autoimmune disorder such as immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome.[15] Recently, low CD127 expression, the alpha chain of the interleukin-7 receptor, was recognized as a standard Treg marker along with CD25 and Foxp3 in humans. Low levels of CD127 indicate a highly suppressive Tregs population.[16] In this study, we defined Tregs using $CD4^+CD25^{high}CD127^{low/-}FoxP3^+$ as a phenotype.

Tregs are crucial mediators of immune homeostasis and could reduce rejection episodes by downregulating activated all specific T cells.[4–6] However, several immunosuppressive drugs have been developed to target effector T cells, which also negatively affect Tregs by inhibiting crucial intracellular signaling pathways in T cells. Among the currently used maintenance immunosuppressive drugs, CNIs were the most commonly prescribed. CNIs have negative effects on immune homeostasis in humans by inhibiting intracellular signaling pathway. Previous report revealed that CNIs inhibited Tregs population dose dependently in an in vitro model.[17] Although the quantitative analysis of 6 KT recipients showed that no significant association between the dose of CNIs and the frequency of Tregs,[18] our study revealed that patients receiving higher doses of CsA had lower proportions of Tregs than those receiving lower doses of the drug. We included more patients than previous studies, our findings may suggest that reducing the dose of CNIs could not only minimize CNI nephrotoxicity but spare Tregs.

There are only a few data concerning Tregs population according to the type of CNI. A study involving 41 patients who underwent deceased donor KT found that those receiving tacrolimus-based regimens had a higher percentage of Tregs than those receiving CsA-based regimens.[19] However, in this study, patients taking CsA tended to have higher Tregs numbers compared to those taking tacrolimus. In former report, similar to most studies investigating Tregs, CD127 was not addressed as a phenotype of Treg. Furthermore, since CsA was developed before tacrolimus and tacrolimus is used more often than CsA recently, patients who received CsA may have been followed up longer and more stable than those taking tacrolimus. Furthermore, this study showed only tendencies, not statistical significance. These differences may affect the results. Further clinical trials are warranted to clarify the impact of the types of CNI on the Tregs population.

Nowadays, atherosclerosis is accepted as a chronic autoimmune disease by self- and nonself-antigens contributing to the excessive activation of T and B cell immune responses.[20,21] As Tregs are important modulators of immune homeostasis, numerous studies have indicated that reduced numbers of Tregs and their dysfunction might contribute to the development of atherosclerosis in both mouse models and humans.[22] To prevent and treat atherosclerosis, recently, strategies for

Table 2

Regulatory T cell subpopulation according to the patients characteristics.

	Gating cell number	*P* value	Gated (%)	*P* value
Male (n = 73) vs Female (n = 40)	76.7 ± 129.5 vs 56.6 ± 73.5	.295	29.1 ± 22.4 vs 37.6 ± 27.1	.078
LDKT (n = 85) vs DDKT (n = 23)	75.6 ± 121.5 vs 44.5 ± 72.1	.113	32.0 ± 24.1 vs 34.7 ± 26.7	.640
Follow-up duration ≤ 147.5 mo (n = 57) vs Follow-up duration > 147.5 mo (n = 56)	50.6 ± 76.9 vs 89.0 ± 138.5	.073	36.4 ± 27.9 vs 27.8 ± 19.5	.060
Tacrolimus (n = 70) vs Cyclosporine (n = 39)	49.3 ± 69.4 vs 94.7 ± 158.1	.095	34.3 ± 26.2 vs 29.9 ± 21.4	.378
MMF (n = 73) vs No MMF (n = 40)	65.0 ± 121.7 vs 78.1 ± 95.8	.558	33.2 ± 25.3 vs 30.1 ± 22.8	.515
PDN (n = 79) vs No PDN (n = 34)	58.1 ± 89.6 vs 96.3 ± 152.4	.181	34.1 ± 25.8 vs 27.6 ± 20.3	.194
Tacrolimus/MMF/PDN (n = 49) vs Cyclosporine/MMF/PDN (n = 9)	44.0 ± 66.4 vs 88.9 ± 158.8	.427	36.8 ± 26.8 vs 29.7 ± 20.7	.459
Mean tacrolimus level ≤ 5.8 ng/mL (n = 39) vs Mean Tacrolimus level > 5.8 ng/mL (n = 31)	57.6 ± 73.3 vs 38.9 ± 63.8	.266	30.9 ± 20.6 vs 38.5 ± 31.9	.053
Mean tacrolimus dose ≤ 2.6 mg (n = 36) vs Mean tacrolimus dose > 2.6 mg (n = 22)	46.6 ± 62.5 vs 45.7 ± 80.2	.963	37.6 ± 25.7 vs 33.9 ± 30.1	.614
Mean cyclosporine level ≤ 98.1 ng/mL (n = 23) vs Mean cyclosporine level > 98.1 ng/mL (n = 16)	52.9 ± 105.5 vs 154.8 ± 201.1	.078	30.7 ± 21.9 vs 28.7 ± 21.4	.776
Mean cyclosporine dose ≤ 106.7 mg (n = 18) vs Mean cyclosporine dose > 106.7 mg (n = 12)	119.8 ± 197.7 vs 66.2 ± 110.5	.401	36.3 ± 21.6 vs 17.0 ± 12.7	.010
Creatinine ≤ 1.5 mg/dL (n = 96) vs Creatinine > 1.5 mg/dL (n = 17)	67.6 ± 111.9 vs 81.2 ± 121.1	.647	33.0 ± 24.6 vs 27.3 ± 23.3	.382
Mean total cholesterol ≤ 172.8 mg/dL (n = 53) vs Mean total cholesterol > 172.8 mg/dL (n = 52)	90.6 ± 140.6 vs 45.8 ± 74.8	.044	32.3 ± 25.1 vs 33.0 ± 24.9	.886
nonHBV (n = 95) vs HBV (n = 7)	72.8 ± 117.0 vs 12.4 ± 13.0	< .001	30.8 ± 23.1 vs 55.2 ± 37.0	.134
BMI ≥ 25 (n = 35) vs BMI < 25 (n = 78)	73.9 ± 111.4 67.7 ± 114.3	.787	28.2 ± 23.1 33.9 ± 24.9	.255

Values are expressed as means ± SDs.

BMI = body mass index, DDKT = deceased donor kidney transplantation, HBV = hepatitis B virus, LDKT = living donor kidney transplantation, MMF = mycophenolate mofetil, PDN = prednisone.

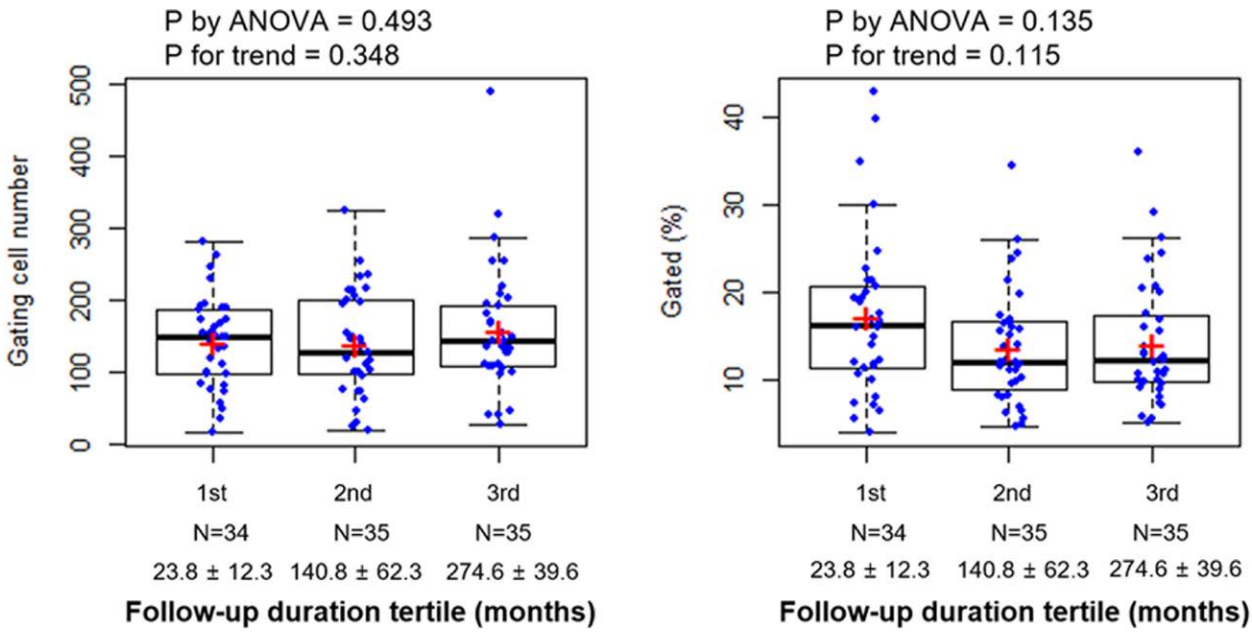

Figure 1. Regulatory T cell subset according to the patients follow-up duration. ANOVA = analysis of variance.

targeting the regulation of Tregs have gained specific attention because they could induce the downregulation of the immune system. In accordance with previous reports, our study revealed that patients with higher total cholesterol levels had lower Tregs numbers than those with lower total cholesterol levels.

Table 3

Cox proportional hazard model to identify risk factors for allograft dysfunction.

	Univariable		
Variables	**HR**	**95% CI**	***P* value**
Age	0.928	0.871–0.988	.020
Sex, male	3.161	0.902–11.081	.072
DDKT	4.362	1.051–18.101	.043
Hepatitis B virus	7.947	0.790–79.953	.078
Albumin	0.222	0.083–0.595	.003
Total CO_2	0.819	0.704–0.952	.010
Total cholesterol	0.999	0.987–1.012	.931
Low-density lipoprotein	1.000	0.983–1.017	.998
Triglyceride	1.000	0.993–1.008	.910
High-density lipoprotein	0.963	0.923–1.004	.077
Triglyceride/High-density lipoprotein	0.982	0.454–2.125	.964
$CD127^{low/-}FoxP3^{+}$			
Gating cell number	1.000	0.996–1.004	.966
Gated (%)	1.002	0.978–1.027	.871

CI = confidence interval, Cr = creatinine, DDKT = deceased donor kidney transplantation, HR = hazard ratio.

Table 4

T cell subpopulation according to the allograft function.

	All patients	Cr ≤ 1.5 mg/dL	Cr > 1.5 mg/dL	
Variables	**(n = 113)**	**(n = 96)**	**(n = 17)**	***P* value**
Lymphocyte number	5702.4 ± 1468.6	5754.2 ± 1454.8	5409.8 ± 1557.4	.375
CD4 T cell				
Gating cell number	3490.4 ± 1389.3	3509.3 ± 1393.9	3383.9 ± 1400.7	.733
Gated (%)	60.4 ± 17.0	60.1 ± 17.1	61.9 ± 17.0	.687
$CD3^{+}CD28^{+}$ T cell				
Gating cell number	2870.9 ± 1283.1	2851.2 ± 1280.5	2982.2 ± 1332.2	.700
Gated (%)	82.6 ± 14.6	81.6 ± 15.1	88.0 ± 10.2	.098
$CD127^{low/-}FoxP3^{+}$ T cell				
Gating cell number	69.6 ± 112.9	67.6 ± 111.9	81.2 ± 121.1	.647
Gated (%)	32.1 ± 24.4	33.0 ± 24.6	27.3 ± 23.3	.382

Values are expressed as means ± SDs, n (%).
Cr = creatinine.

Whether the Tregs population is increased in HBV infection is a controversial concern.[23] HBV is a hepatotropic virus that causes liver inflammation.[24] Virus-specific T cell responses are crucial for manipulating HBV infection. However, Tregs suppressed protective T cell responses and helped establish viral persistence. Several reports have indicated that Treg frequency was higher in patients with chronic HBV infection than in controls.[25,26] In contrast, Tregs might be beneficial by limiting immunopathological T cell mediated liver damage. A study reported similar Tregs frequencies between patients with chronic HBV infection and controls. In this study, patients without HBV infection had higher Tregs numbers than those infected with HBV. This finding is consistent with those of a latter study. A recent study revealed that the phase of chronic HBV infection is important in Tregs population.[27] However, we could not verify the phase of HBV infection in this study. Further studies are needed to identify the Tregs population in individuals with HBV infection.

There are many pieces of evidence that Tregs could be associated with clinical outcomes of KT. Several reports showed that patients with stable allograft function have higher Tregs than those with acute or chronic rejection.[28–30] Furthermore, Tregs in kidney allograft biopsy were associated with better allograft function.[31] However, no significant association was found between Tregs and allograft dysfunction in our study. We enrolled stable KT recipients, and this might have affected the results. Recent study also reported that the frequency of Tregs did not correlate with allograft function in stable KT recipients.[32]

This study has some limitations. First, we could not perform flow cytometry analysis immediately after PBMC isolation. After we isolated the PBMCs, we stored them in a liquid nitrogen tank for long-term storage. These may have affected the results. In the process of cell thawing, cell death may have occurred. Second, since this study was a cross-sectional study, the time from KT to blood collection was not consistent between patients and we only took 1 measurement. This may weaken our results. Third, this was a single center study that included a relatively small sample size.

In conclusion, the present study demonstrated a significant association between the distribution of Tregs and the type and dose of immunosuppressants in KT recipients. However, there was no significant relationship between Tregs and kidney allograft function in stable KT recipients. Further prospective randomized large-scale studies are warranted.

Author contributions

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