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Soluble Tim-3 and Gal-9 are associated with renal allograft dysfunction in kidney transplant recipients: A cross-sectional study

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ABSTRACT

Background: T cell immunoglobulin mucin-3 (Tim-3) has been reported to participate in the regulation of immune response and the induction of allograft tolerance. However, the association between Tim-3 and renal allograft dysfunction is unclear. We studied the expression of cellular and soluble Tim-3 (sTim-3), soluble galectin-9 (sGal-9) and carcinoembryonic antigen-related cell adhesion molecule-1 (sCEACAM-1) in kidney transplantation recipients (KTRs) to explore their roles in allograft dysfunction.

Methods: 96 KTRs (53 with stable graft and 43 with graft dysfunction) and 30 healthy controls (HC) were enrolled. Among the KTRs, 55 used Tacrolimus (TAC) and 41 used Sirolimus (SRL). In the dysfunction group, 29 recipients have undergone graft biopsy and 14 were classified as biopsy-proven rejection (BPR). Cellular Tim-3 was determined by flow cytometry. sTim-3 was determined by ELISA. sGal-9 and sCEACAM-1 were determined by Bio-Plex® suspension array system.

Results: KTRs with renal dysfunction showed significantly higher levels of sTim-3 and sGal-9 but similar levels of cellular Tim-3 and sCEACAM-1 compared with stable recipients. Correlation analysis revealed that estimated glomerular filtration rate (eGFR) was negatively associated with sTim-3 and sGal-9. Both BPR and non-BPR groups showed comparable levels of Tim-3, Gal-9 and CEACAM-1. Moreover, SRL group showed significantly higher levels of sCEACAM-1 than TAC and HC groups.

Conclusions: sTim-3 and sGal-9 were promising biomarkers for allograft dysfunction, but unable to differentiate allograft rejection from other causes of renal dysfunction in KTRs. Moreover, long-term administration of sirolimus would up-regulate sCEACAM-1 level, while exert similar regulatory effects on Tim-3 and Gal-9 compared to tacrolimus.

1. Introduction

Kidney transplantation (KT) is currently the optimal renal replacement therapy for most patients with end-stage renal disease (ESRD). Although the development of immunosuppressive drugs has remarkably decreased the incidence of acute rejection (AR) and achieved excellent short-term outcomes, long-term graft survival remains to be dissatisfied [1]. Allograft loss is primarily due to progressive chronic renal allograft dysfunction, which involves in both immune and non-immune mechanisms [2]. Noteworthily, some causes of allograft dysfunction are recognizable, mainly immune mediated and possible to be intervened early [3]. Therefore, exploring the key immunoregulatory markers involved in chronic allograft dysfunction is crucial to improve long-term renal allograft outcomes.

In recent years, the T-cell immunoglobulin and mucin domain (Tim) family members were emerging as important immune modulators in kidney transplant recipients (KTRs) [4–6]. As the first discovered Tim molecule, Tim-3 was initially found to express on Th1 cells but now has been described on Th17, regulatory T cells (Treg) and CD8 + T cells [7]. It was identified as a novel co-inhibitory receptor, which existed in human with two forms, namely full length Tim-3 that anchored on immune cells and soluble Tim-3 (sTim-3) that existed in body fluid [8]. Previous studies revealed that cellular Tim-3 played an important role in inducing T cell exhaustion and immunological tolerance by binding to its ligands [9–11], while sTim-3 played an opposite role by interfering the interaction between cellular Tim-3 and Tim-3 ligands [10,11]. Recently, accumulating evidence demonstrated that Tim-3

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mRNA in urine, blood and graft was a promising biomarker for acute rejection in KTRs [5,12–16]. However, there has been no study assessing the expression of Tim-3 protein on the immune cells or in serum of KTRs so far.

Galectin-9 (Gal-9), the best-known ligand for Tim-3, is a mammalian β-galactoside binding lectin, which was originally identified as an eosinophil chemoattractant, and subsequently clarified as a multifunctional molecule involved in many biological activities such as cell apoptosis, cell proliferation and differentiation of Tregs [17]. In addition, a newly identified heterophilic ligand for Tim-3 was carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1) [9], the only member of CEA-family to contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) [18]. It was expressed on epithelial cells and a variety of hemopoietic cells, such as T cells, natural killer (NK) cells, B cells, monocytes and granulocytes, and has been reported to exert multiple immune regulatory functions that involved in inflammation, cell apoptosis and inhibitory activities [18,19]. Moreover, clinical studies found that the higher levels of Gal-9 and CEACAM-1 inside renal allografts were significantly associated with the acute rejection, which indicated that they might be promising biomarkers of allograft rejection in KTRs [16,20]. However, no research so far has evaluated the expression of Gal-9 and CEACAM-1 in the peripheral blood of KTRs. In addition, whether these promising molecules are associated with renal allograft dysfunction remains to be explored.

Several previous studies have investigated the immunoregulatory effects of two major representative immunosuppressants, tacrolimus (TAC) and sirolimus (SRL), in KTRs [21–23]. Previously, our group found that TAC and SRL affected the proportions of Th1, Th17, Treg and T follicular helper cells (Tfh) in different ways [21,24]. Moreover, it was reported that the regulation of these T cell subsets was partly mediated by Tim-3 pathway [25,26]. However, whether immunosuppressants would affect the expression of Tim-3, Gal-9 and CEACAM-1 in KTRs was unclear till now.

Therefore, in this study, we investigated the expression of Tim-3 on CD4 + and CD8 + T cells, along with sTim-3, sGal-9 and sCEACAM-1 in peripheral blood of KTRs with different renal allograft functions, aiming to explore the associations between these molecules and allograft dysfunction. Meanwhile, whether these molecules could distinguish chronic allograft rejection from non-allograft rejection in KTRs with allograft dysfunction was further investigated. Finally, we analyzed the regulatory effects of different immunosuppressive drugs on those molecules in KTRs.

2. Materials and methods

2.1. Patients

This is a cross-sectional study enrolled 96 KTRs (77 males and 19 females) and 30 healthy controls (HC) (21 males and 9 females) between May 2016 and April 2017 from West China Hospital of Sichuan University. 55 KTRs received consecutive Tacrolimus (TAC)-based immunosuppressive regimen (TAC + mycophenolate mofetil (MMF) + steroid) and 41 KTRs received consecutive Sirolimus (SRL)-based immunosuppressive treatment (SRL + MMF + steroid) for more than 6 months. Among all the KTRs, 53 recipients with stable estimated glomerular filtration rate (eGFR) levels (eGFR ≥ 60 ml/min/1.73 m^2) and no history of acute rejection were included in stable renal function group. The other 43 recipients with obviously impaired allograft function (eGFR < 60 ml/min/1.73 m^2) were enrolled in renal dysfunction group. Among those renal dysfunction recipients, 29 KTRs treated with TAC-based regimen have undergone core needle biopsy. According to 2013 Banff classification [27], 14 KTRs were classified as having biopsy-proven rejection (BPR, 12 antibody mediated rejection and 2 T cell-mediated rejection) and the other 15 KTRs were enrolled in non-BPR group (4 BK virus nephropathy, 4 interstitial fibrosis tubular atrophy, 2 recurrent glomerulonephropathy and 5 transplant glomerulonephropathy). This study was approved by the Ethics Committee of West China Hospital and written informed consent was obtained from each participant before the enrollment.

2.2. Sample collection

We collected the heparin-anticoagulated whole blood samples from HC and KTRs before the morning dose of TAC or SRL. For 29 KTRs with biopsy results, blood samples were collected before graft biopsy. Detection of cellular Tim-3 was carried out immediately after sample collection. Serum samples were collected after centrifugation and then stored in aliquots at − 80 °C until the detection of sTim-3, sGal-9 and sCEACAM-1.

2.3. Flow cytometry analysis

To detect cellular Tim-3, 50 μl whole blood was stained with the following antibodies: CD3-PerCP (SP34-2), CD4-FITC (RPA-T4), CD8-APC (SK1) and Tim-3-PE (7D3). Mixtures were incubated at 4 °C for 30 min in the dark. Then, the uncombined antibodies and erythrocyte fragments were washed off after erythrocytes were lysed by the hemolytic agent (NH4CL, 0.15 mol/l). IgG1, k-PE (MOPC-21) was used as isotype control for gating purpose and eliminating the nonspecific combination background. All antibodies were purchased from BD Pharmingen (San Diego, USA). Flow cytometry detections were performed on FACS canto II instrument (BD Bioscience, San Diego, USA) and the data analysis was done with FlowJo software (Tree Star, Ashland, Oregon, USA).

2.4. Detection of soluble molecules

The serum concentration of sTim-3 was quantified by enzyme-linked immunosorbent assay (ELISA), sGal-9 and sCEACAM-1 were determined by Bio-Plex® suspension array system (Bio-Rad Laboratories Inc., Hercules, CA, USA) strictly according to the manufacturer's instruction. Both Human sTim-3 Quantikine ELISA Kit and Human Premixed Multi-Analyte Kit were purchased from R&D Systems (Minneapolis, MN, USA). All serum samples were measured in duplicate.

2.5. Statistical analysis

Data were presented as absolute number, mean ± standard deviation or median (interquartile range) according to the data type. Chi-square or Fisher exact tests were utilized to compare categorical variables between groups. Student's *t*-test or Mann-Whitney *U* test were utilized to compare continuous variables with normal distribution and skewed distribution, respectively. Spearman's rank correlation coefficient test was performed to analyze correlations between variables. Receiver-operating characteristic (ROC) curve was conducted to assess the diagnostic efficiency and the optimal cut-off value was determined by maximum value of the Youden-J indexes (sensitivity + specificity − 1). All statistical analyses were done with SPSS software (version 23.0, SPSS Inc., Chicago, IL, USA) and two tailed p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinical data

Main characteristic data of HC and KTRs were summarized in Table 1. In general, KTRs showed decreased renal function than healthy controls, but no significant difference was found in age and gender distributions between two groups. As for subgroups, lower rate of living donor was found in renal dysfunction group compared to that in stable group (60.5% vs. 83.0%, $p = 0.036$). As the values were within normal range, the difference of ALT levels among groups showed no clinical