The reduced soluble fibrinogen-like protein 2 and regulatory T cells in acute coronary syndrome

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Abstract

Soluble fibrinogen-like protein 2, sfgl2, is the new effector of CD4⁺CD25⁺FOXP3⁺ regulatory T cell (Treg) and exerts immunosuppressive activity. We design this study to investigate the possible role of sfgl2 in atherosclerosis. A total of 58 acute coronary syndrome (ACS) patients, together with 22 stable angina (SA) patients and 31 normal coronary artery (NCA) people were enrolled in our study. Serum level of sfgl2 and plasma level of Treg were respectively measured. In line with the change of Treg, serum level of sfgl2 in ACS (8.70 ng/mL) was significantly decreased ($P = 0.003$), compared with that in SA (11.86 ng/mL) and NCA (17.55 ng/mL). Both sfgl2 and Treg level were obviously decreased in ACS; Sfgl2 may play a protective role in atherosclerosis.

Keywords: Soluble fibrinogen-like protein 2, regulatory T cells, atherosclerosis

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Introduction

With a high prevalence in most regions all over the world, coronary artery disease (CAD) remains the leading cause of death.¹ Increasing evidence shows that immune response plays an essential role in the initiation and progression of CAD.²

Different from Teff, CD4⁺CD25⁺FOXP3⁺ regulatory T cells (Treg) exerts a suppressive role in immunomodulation,³ which has been elucidated in viral hepatitis,⁴ renal transplantation,⁵ and acute coronary syndrome (ACS).⁶ The immunosuppressive activity of Treg maintains through direct cell-to-cell contact and release of cytokines, including interleukin 10 (IL-10) and transforming growth factor- β (TGF- β).⁷ Additionally, another potential effector of Treg, soluble fibrinogen 2 (sfgl2), has been proposed.⁸

Fibroleukin, Fgl2, is encoded by pT49 gene transcript in cytotoxic T cells and presents as two forms, membrane (mfgl2) and soluble protein form (sfgl2).⁹ sfgl2 is mainly secreted by Treg and exerts immunosuppressive activity, which has been corroborated in mouse virus hepatitis and renal transplantation.^{8,10} We design this study to examine serum sfgl2 level in patients with ACS and explore its possible significance of immunomodulation in atherosclerosis.

Materials and methods

Study populations

From September 2013 to June 2014, we prospectively recruited 111 patients with written consent from Union Hospital, Wuhan, Hubei province, China. Approval for the study was got from the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology. All patients were classified into three groups according to European Society of Cardiology (ESC) guidelines for stable CAD and ACS.^{11,12} Patients with either of the following characteristics were excluded: rheumatoid valvular heart disease, malignant arrhythmia cardiogenic shock, known history of malignancies, renal failure, fever of unknown origin, virus hepatitis, immune disorders or receiving immunosuppressive drugs, organ transplantation.

Detection of sfgl2 with enzyme-linked immunosorbent assay (ELISA)

Upon administration into hospital, basic information were recorded. At 6 am in the second morning, peripheral blood was obtained.

An ELISA kit (Bio legend) was used for detection of sfgl2. Briefly, serum samples were added into plates with sfgl2

Table 1 Basic characteristics of study populations

Characteristics	NCA (n = 31)	SA (n = 22)	ACS (n = 58)	P* value
Age (years)	58 ± 2	62 ± 2	59 ± 1	0.386
Sex (male/female)	13/18	16/6	34/24	0.083
Hypertension, n (%)	16 (51.6%)	17 (72.7%)	36 (58.6%)	0.176
Diabetes (%)	6 (19.4%)	9 (40.9%)	13 (22.4%)	0.179
Hyperlipidemia, n (%)	20 (64.5%)	12 (54.6%)	32 (55.2%)	0.667
Smoking, n (%)	10 (32.3%)	10 (45.5%)	25/43.1 (%)	0.532

NCA, normal coronary artery; SA, stable angina; ACS, acute coronary syndrome.
The P* is for comparison among three groups. Data present as mean ± SD or number (%).

Figure 1 Serum level of sfgl2 was significantly reduced in ACS

antibody, followed by 2 h incubation. Then detection antibody was added for 1 h incubation, followed by 30 min incubation of horseradish peroxidase (HRP)-conjugated antibodies (Avidin HRP). Finally, substrate and stop solution were successively added to read absorbance.

Flow cytometry for CD4⁺CD25⁺FOXP3⁺Treg

Fresh peripheral blood mononuclear cells (PBMCs) were isolated after Ficoll density gradient and then were stained with combination of fluorescein isothiocyanate (FITC)-labeled anti-human CD4, phycoerythrin (PE)-labeled anti-human CD25 and APC-labeled anti-human FOXP3 for Treg detection. According to manufacturer instruction, after cell surface antigen staining of CD4 and CD25, fixation and permeabilization were implemented for following intracellular FOXP3 staining. Stained cells were analyzed by flow cytometry using FACS Diva Version 6.1.2 software program.

Statistical analysis

One way-analysis of variance (ANOVA) and chi-square test were, respectively, adopted for comparison of continuous values and dichotomous variables. SPSS17.0 was used in

statistical analysis and statistically significant level was set at $P < 0.05$.

Results

Basic characteristics

Basic characteristics were shown in Table 1 and no statistical difference was present among the three groups ($P > 0.05$).

Serum level of sfgl2 is significantly reduced in ACS patients

AS shown in Figure 1, sfgl2 level was statistically lower in ACS group ($n = 58$, 8.70 ± 3.86 ng/mL) than in normal coronary artery (NCA) ($n = 31$, 17.55 ± 4.77 ng/mL) and stable angina (SA) group ($n = 22$, 11.86 ± 4.10 ng/mL) ($P < 0.001$). The receiver-operating characteristic (ROC) curve assessing the diagnosis efficacy of sfgl2 in ACS was presented in Figure 1. The area under the curve (AUC) was 0.829, enlighting a significant role of sfgl2 in the diagnosis of ACS. The cut-off value for sfgl2 was 14.21 ng/mL, with the sensitivity and specificity being 0.623 and 0.914, respectively.

Figure 2 Frequency of CD4⁺CD25⁺FOXP3⁺Treg was decreased in ACS

Figure 3 Reduced serum level of sfgl2 in ACS was positively correlated with the frequency of Treg

Decreased frequency of CD4⁺CD25⁺FOXP3⁺Treg in ACS

In accordance with others,⁸ Treg frequency (CD4⁺CD25⁺FOXP3⁺/CD4⁺ T cells %) in ACS group ($1.35 \pm 0.59\%$) is markedly lower (see Figure 2). Furthermore, we found a positive correlation between sfgl2 concentration and Treg frequency with the spearman correlation coefficient being 0.671, $P < 0.01$ (see Figure 3). However, the sfgl2 and Treg level among the ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and UA are non-statistically different (data not shown).

Discussion

In this study, we provide evidence for the first time that serum sfgl2 decreases in ACS, in line with the change of Treg frequency in ACS.

Treg has been demonstrated to play an immunosuppressive role in the progression of AS.¹³ In ACS patients, Treg is impaired, as manifested by decreased cell frequency and weaken immunosuppression activity.¹⁴ In line with the finding, decreased Treg frequency in ACS patients was strengthened in our study. The impaired Treg indicates diminished immune tolerance and aggravated immune inflammation, which is a hallmark of ACS.

In atherosclerosis, the protective role of Treg was accomplished through cell-to-cell contact and cytokine release, including IL-10, TGF- β , and IL-35.^{15–17} In addition, sfgl2 deficiency resulted from monoclonal antibody blockade or gene knockout could completely inhibit Treg function and thus aggravate virus hepatitis and lead to autoimmune glomerulonephritis.^{8,18}

In this clinical investigation, it is the first time to present our finding that serum sfgl2 concentration was reduced in vulnerable patients compared with controls. Furthermore, reduction in sfgl2 is strengthened by decreased peripheral Treg frequency and could exactly be explained by it.^{5,14,19} Therefore, we are tempting to speculate that, in addition to IL-10 and TGF- β , sfgl2 may act as another new effector of Treg suppression in atherosclerosis.

sfgl2 has been demonstrated to accomplish its immuno-suppressive activity by binding to Fc γ RIIB and Fc γ RIII receptors expressed on antigen presenting cell (APC) and lymphocytes. The specific engagement can inhibit dendritic cell (DC) maturation, T cell proliferation and induce B cell apoptosis, which play crucial roles in the pathogenesis of atherosclerosis.^{20,21} As a novel downstream effector of Treg, sfgl2 would be able to act as a protector in atherosclerosis through driving negative immune responses by targeting both innate and adaptive immunity.

In summary, serum sfgl2 level is significantly decreased, accompanied by impaired Treg activity in ACS, which highlights an intriguing relationship between plaque stability and serum sfgl2 level. The profile of sfgl2 in atherosclerosis was delineated in our small-scale investigation. Notwithstanding, the defined role will be elucidated in our ongoing efforts.

Author contributions: All authors contributed to the design and conduction of the study and analysis of the result and interpretation of the manuscript; ZHW and KL designed the study, TL conducted the experiments, SYH, RL, YY, and JPL were involved in data analysis, KL and TL wrote the manuscript. ZHW responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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DECLARATION OF CONFLICTING INTERESTS

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