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Increased Plasma Levels of Programmed Death Ligand 1 in Patients with Primary Sjögren's Syndrome

Xin Chang[#], Sisi Ding[#], Lili Sun, Xueguang Zhang, Cuiping Liu[*]

Jiangsu Institute of Clinical Immunology& Jiangsu Key Laboratory of Clinical Immunology, First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[#]**Equally contributed.**

[*]**Corresponding author:** Cuiping Liu, Jiangsu Institute of Clinical Immunology& Jiangsu Key Laboratory of Clinical Immunology, First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China. Tel: +8651265732002; Fax: +8651265125022; Email: liucuiping1980@126.com

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Abstract

To determine the plasma levels of soluble forms of Programmed Death Ligand 1(sPD-L1) in patients with Primary Sjögren's syndrome (pSS), and determine whether altered circulating sPD-L1 levels correlate with cytokines or clinical manifestations. Plasma levels of sPD-L1 in 64 patients with pSS and 61 healthy controls were examined by enzyme-linked immunosorbent assay (ELISA). Cytokines in plasma were detected using human Th1/Th2/Th17 cytometric bead array (CBA) kit. Data were evaluated for correlations between plasma sPD-L1 levels and clinical features. Levels of sPD-L1 differed significantly between patients and healthy controls (0.7025±0.04645 versus 0.3642±0.01713, $P < 0.0001$). Plasma sPD-L1 were positively correlated with IgM ($r = 0.3898$, $P = 0.0129$), ESR ($r = 0.3801$, $P = 0.0084$), RF ($r = 0.4163$, $P = 0.0198$), ALT($r= 0.3028$, $P = 0.0457$), AST ($r=0.4455$,$P=0.0028$), and the ESSDAI score ($r= 0.3866$, $P = 0.0066$). In addition, PD-L1 levels in plasma were correlated significantly with IFN-γ ($r = 0.3685$, $P = 0.0092$) and IL-17 ($r =0.3436$, $P = 0.0127$). Plasma sPD-L1 levels may increase in patients with pSS and correlate with the severity of disease. PD-1/PD-L1 interaction may contribute to the development of pSS.

Keywords: Autoantibodies; Programmed death ligand 1; Primary Sjögren's syndrome

Introduction

Primary Sjögren's syndrome (pSS) is a many-faceted chronic systemic autoimmune disease which primarily affects the salivary and lachrymal glands, but also presenting with a wide range of possible extraglandular manifestations [1]. Accumulating evidence suggests that lymphocytic infiltrate of exocrine glands plays a key role in lesion formation and the subsequent dysfunction of the glands.

Being chronic systemic autoimmune diseases, permanent activation of the adaptive immune system is obvious in pSS. The recognition of the outstanding importance of the costimulatory regulation in many autoimmune diseases and in tumor-immunology has led to highly effective targeted therapies in both fields. Programmed death ligand 1 (PD-L1, also calledCD274 or B7-H1) is a ligand for the programmed death 1 (PDCD-1, PD-1) molecule, which is a member of the CD28/CTLA4 costimulatory family of cell surface receptors. The PDCD-1/PD-L1 interaction acts to attenuate immune responses and thus has broad immunoregulatory roles in peripheral tolerance, as well as immunity to chronic infections, tumors, and autoimmune diseases [2]. Plasma levels of PD-L1 have been shown to be correlated with the disease activity in several autoimmune disorders including allergic rhinitis , systemic sclerosis and type 2 diabetes [3,4,5].

If PD-L1 plays a role during over-activation of selfreactive T cells, soluble PD-L1 levels in the blood may be used as an indicator for pSS. To test this hypothesis, we measured plasma PD-L1 levels and T helper subset-associated cytokines in healthy controls and patients with pSS. Moreover, the relationships between plasma PD-L1, cytokines, and clinical parameters were analyzed.

Material and Methods

Patients and Controls

We recruited 64 patients from the Department of Rheumatology, the first affiliated hospital of Soochow university, China, during January 2015 and December 2016. All were diagnosed with pSS and fulfilled the 2002 American-European Consensus Group Classification Criteria [6]. The exclusion criteria are (1) any other systemic autoimmune diseases; (2) severe infection, malignant tumor, severe organ dysfunction, or any other life-threatening conditions. We also recruited 61 Healthy Controls (HC) from the health examination center of the same hospital, with age and sex matched. All of them were excluded from any autoimmune diseases. The study was approved by the Ethics Committee of first affiliated hospital of Soochow university. All participants of this study had been informed and signed the consent for participation in this study.

Measurement of Plasma PD-L1

Levels of sPD-L1 in the plasma of pSS patients and healthy volunteers were detected using an ELISA system prepared in our laboratory previously [7]. Briefly, blood samples were incubated at 37˚C for 30 min, and then centrifuged for 5 min at 4,000 x g (room temperature) and the plasma were collected. Samples and standards were added to the wells for 2 h at 37˚C in duplicate. The specific binding protein was detected with biotinylated anti-PD-L1 mAb for 1 h at 37˚C, followed by streptavidin-HRP at 1:2000 for 1 h at 37˚C and then revealed with the substrate TMB. The reaction was stopped with 2 M H_2SO_4 and the plates were analyzed at 450 nm using a microplate reader (Bio-Rad, Hercules, CA).

Cytokine Measurement

Cytokines in plasma were detected using human Th1/Th2/Th17 Cytometric Bead Array (CBA) kit, according to the manufacturer's instructions (BD Biosciences). All samples were examined in duplicate.

Statistical Analysis

All statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc, USA). All the quantitative data were presented as the mean±Standard Deviation (SD). Student's t-test was used to analyze the differences between the groups. For correlation analyses, a Spearman's r value derived from Pearson's r was calculated. A p-value of less than 0.05 was considered statistically significant.

Results

Characteristics of pSS Patients and Controls

A total of 64 pSS patients and 61 healthy controls with matched age and gender were included in this study. The mean age of 64 pSS patients at the time of our study was 47.49±11.87 years (range 25-78) and the mean disease duration was 10.5 years ranging from 2 months to 40 years. Demographic, clinical, and laboratory features of pSS patients and healthy controls are shown in Table 1. Anti-SSA antibody and anti-SSB antibody were positive in 40 (62.5%) and 30 (46.8%), respectively. 34 pSS patients (53.13%) showed moderate to high disease activity.

Clinical characteristics	pSS（n=64）	Controls（n=61）	P
Age	47.49±11.87	46.61±11.82	P > 0.05
Sex(F:M)	62:2	59:2	P > 0.05
WBC(×10⁹/L)	6.16±2.746	-	
Hb(g/L)	125.1±14.71	-	
PLT (×10⁹/L)	170.1±67.02	-	
IgA (g/L)	3.491±2.456	-	
IgG (g/L)	18.16±7.19	-	
IgM (g/L)	1.817±1.089	-	
C3 (g/L)*	0.948±0.253	-	
C4 (g/L)*	0.173±0.054	-	
ESR (mm/h)	23.9±22.74	-	
RF (IU/mL)*	169.5±301.9	-	
ANA ≥1:320	80.7%	-	
Anti-SSA≥200RU/ml	62.5%	-	
Anti-SSB≥20RU/ml	46.8%	-	
ALT(IU/L)	25.69±21.15	-	
AST(IU/L)	18.00±11.14	-	
ESSDAI≥5	53.13%	-	

SS= Sjögren's syndrome; WBC= White blood cell; Hb = Hemoglobin; PLT = Platelets; IgA = immunoglobulinA; IgM = immunoglobulin M; IgG = immunoglobulin G; C3 = complement 3; C4 = complement 4; ESR = erythrocyte sedimentation rate; RF = rheumatoid factor; ANA = antinuclear antibody; ALT = alanine aminotransferase; AST = asparate aminotransferase; ESSDAI = EULAR Sjögren's syndrome Disease Activity Index;* indicate that some data are missing. P>0.05 (compared with controls).

Table 1: Characteristics of the studied 64 primary SS patients and 61 healthy controls.

Evaluation of Correlation of Plasma PD-L1 Levels with Clinical Parameters

As shown in Figure 1, the plasma level of sPD-L1 (Figure 1, 0.7025±0.0465 versus 0.3642±0.0171, P < 0.0001) was significantly higher in pSS patients comparing to healthy controls. The relationship between pSS clinical and laboratory features and plasma PD-L1 levels were presented in Table 2, and PD-L1 were positively correlated with IgM (r = 0.3898, P = 0.0129), ESR (r= 0.3801, P = 0.0084), RF (r = 0.4163, P = 0.0198), ALT(r= 0.3028, P = 0.0457) and AST (r=0.4455,P=0.0028). In addition, plasma sPD-L1 levels were correlated positively with the ESSDAI score (r= 0.3866, P = 0.0066).

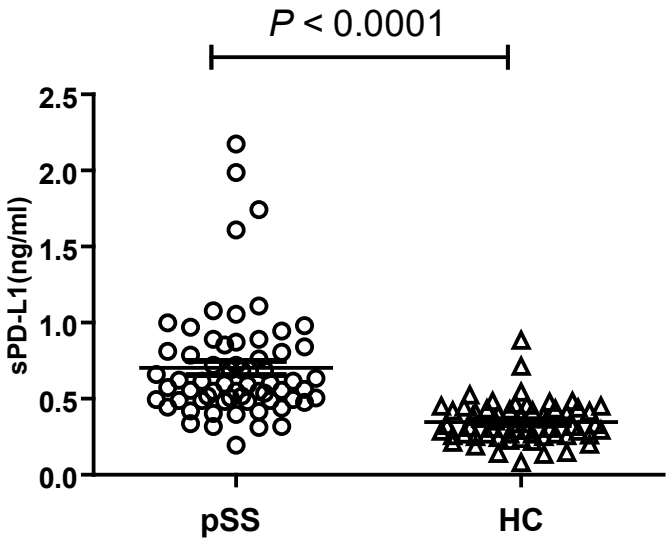

Figure 1: Elevated expression of sPD-L1 in the plasma of patients with primary Sjögren's syndrome (pSS). Plasma levels of sPD-L1 protein were compared between patients with primary SS (n=64) and healthy controls (n=61). Symbols represent individual patients; horizontal lines indicate the median±Standard Deviation (SD).

Relationship Between PD-L1 Levels and Plasma Cytokine Concentrations

We next investigated whether any of the plasma cytokine concentrations determined (IFN-γ, IL-2, IL-4, IL-6, IL-17, IL-10, TNF-a) correlated with PD-L1 levels in the pSS patients. The plasma IFN-γ concentrations were significantly higher in the 49 pSS patients compared with 40 healthy controls (1.703±0.2918 versus0.8897± 0.1840 pg/ml, P = 0.0257), as were also the plasma IL-17 concentrations (26.51±3.939 versus16.39±1.277 pg/ml, P = 0.0126) and IL-6 concentrations (41.31±8.625 versus12.28±2.996 pg/ml, P = 0.0020), respectively. Other plasma cytokine levels did not differ significantly between the pSS patients and the healthy controls (data not shown). PD-L1 levels in plasma were correlated significantly with IFN-γ (r = 0.3685, P = 0.0092, n =49) and IL-17 (r =0.3436, P = 0.0127, n =49) (Table 2).

Clinical characteristics	Spearman r	P
WBC(×10⁹/L)	-0.0769	0.6195
Hb(g/L)	0.2208	0.1498
PLT (×10⁹/L)	-0.2539	0.0963
IgA (g/L)	0.1427	0.3798
IgG (g/L)	0.1162	0.4752
IgM (g/L)	0.3898	0.0129
C3 (g/L)	-0.0581	0.7252
C4 (g/L)	-0.0485	0.7692
ESR (mm/h)	0.3801	0.0084
RF (IU/mL)	0.4163	0.0198
ANA ≥1:320	0.0648	0.6908
Anti-SSA≥200RU/ml	0.0092	0.9523
Anti-SSB≥20RU/ml	0.0881	0.5739
ALT(IU/L)	0.3028	0.0457
AST(IU/L)	0.4455	0.0028
ESSDAI≥5	0.3866	0.0066
IL-2(pg/ml)	0.0145	0.9212
IL-4(pg/ml)	0.1131	0.4390
IL-6(pg/ml)	0.1116	0.4451
IL-10(pg/ml)	0.1719	0.2377
IL-17(pg/ml)	0.3436	0.0157
IFN-γ(pg/ml)	0.3685	0.0092
TNF-α(pg/ml)	0.2271	0.1206

SS= Sjögren's syndrome; WBC = White blood cell; Hb = Hemoglobin;PLT = Platelets; IgA = immunoglobulinA; IgM = immunoglobulin M; IgG = immunoglobulin G; C3 = complement 3; C4 = complement 4; ESR = erythrocyte sedimentation rate; RF = rheumatoid factor; ANA = antinuclear antibody; ALT = alanine aminotransferase; AST = asparate aminotransferase; ESSDAI = EULAR Sjögren's syndrome Disease Activity Index; IFN = Interferon; IL = Interleukin; TNF = Tumor necrosis factor.

Table 2: Correlation of plasma PD-L1 with clinical and laboratory features of pSS patients.

Discussion

Primary Sjögren's syndrome (pSS) is characterized by lymphocytic infiltration of the salivary and lacrimal glands, leading to the destruction of exocrine glands, the inflammatory process often involves other organs too [1]. In this study, we showed that the levels of sPD-L1 were elevated in plasma samples from pSS patients relative to normal healthy controls (Figure 1). Furthermore, the present study is the first to reveal that sPD-L1 levels correlated

positively with several clinical and laboratory features (Table 2). Taken together, these results suggest that the plasma levels of sPD-L1 are useful serologic markers for evaluating the level of disease severity and activity in pSS.

PD-L1 is expressed on the surface of activated antigen presenting cells. In conditions of autoimmunity and inflammation, PD-L1 was strongly expressed by activated T cells and B cells. It is also expressed on the membrane of human tumor cells, which is one of the mechanisms of escaping the host immune system. It has been found that sPD-L1was released from PD-L1-positive tumor cells or immune cells and thought to be a circulating biologically active protein [8]. However, the mechanisms by which sPD-L1 in patients are generated remain poorly understood. An increase in plasma sPD-L1 was reported in patients with non-malignant diseases such as systemic sclerosis and type 2 diabetes mellitus [3,5]. In systemic sclerosis, sPD-L1 levels may positively correlated with the severity of skin sclerosis [3], while in patients with type 2 diabetes, the increased level of sPD-L1 was closely associated with the severity of diabetic atherosclrelotic macorovasucular diseases, especially acute coronary syndrome. Therefore, the authors speculated that sPD-L1 may contribute to continuous T cell activation and development of diabetic macrovascular diseases [5]. By comparing the relative amount of sPD-L1 present in pSS with that in healthy controls, we found plasma levels of sPD-L1 in pSS patients to be significantly higher than those observed in healthy controls, and correlated positively with IgM, ESR and RF, highlighting the significant background activation of this pathway in pSS. Considering the positive correlation between the sPD-L1 and ALT and AST levels, the increase of sPD-L1 may also contribute to T-cell activation in patients with liver damage, which results in an increase in the ALT and AST levels.

Inflammation is a hallmark of autoimmune and can cause disease progression. Dysregulation of the cytokine network contributes to both systemic and exocrine manifestations of Sjögren's Syndrome (SS) [9]. As part of the regulatory homeostatic response, cytokines such as IFN-γ and TNF-α induce expression of PD-L1 in a variety of cancer cells [10,11]. Enhanced expression of programmed death-1 (PD-1)/PD-L1 has been found in salivary glands of patients with Sjögren's syndrome, PD-L1 was expressed on ductal and acinar epithelial cells from 68% of SS patients. In vitro analysis using HSG cells revealed that PD-L1 was induced by interferon-gamma but not by tumor necrosis factor-alpha and IL-1beta [12]. The higher production of IFN-γ in plasma of patients with pSS might account for the release of higher levels of sPD-L1 from epithelial cells of SS patients. Experimental animal models and patients suggest that a shift in Th17/Treg balance toward the proinflammatory Th17 axis exacerbates primary Sjögren's syndrome and other autoimmune disorders [13]. It had been found that IL-17 is consistently expressed in the periductal infiltrates of all minor salivary glands from patients with primary Sjögren's syndrome, with level of expression correlated with severity of glandular inflammation [14,15]. The similar results were found in our study, elevated levels of plasma IL-17 from pSS patients were detected, which supported the pathogenic effects of IL-17 axis in pSS.

The present study has a number of limitations. First, the sample size might have provided inadequate statistical power to detect definitive differences between the patients and healthy controls, and the association with disease activity and severity of pSS, need to be further investigated in future studies. Second, it is essential to evaluate the influence of treatment on plasma levels of sPD-L1. Third, it would be worthwhile to further examine pSS patients for PD-L1 expression in the salivary glands and peripheral blood mononuclear cells by immunohistochemical staining and flow cytometry, respectively; however, this was not included in the approved protocol for this current study. Nonetheless, the results of our study suggested that the PD-1/PD-L1 pathway may be involved in the pathogenesis of pSS.

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Conflict of Interest

All the authors declare that they have no conflict of interest.

Ethical Statement

The study was approved by the Ethics Committee of first affiliated Hospital of Soochow University and the local laws. All participants of this study had been informed and signed the consent for participation in this study.

Authors' Contributions

The research was designed by Cuiping Liu and Xueguang Zhang. Patient samples and data were provided by Xin Chang. ELISA analysis was performed by Sisi Ding. Analysis of cytokine was carried out by Lili Sun. The manuscript was critically reviewed by all authors.

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