Research Article

Upregulated PD-1 Expression Is Associated with the Development of Systemic Lupus Erythematosus, but Not the PD-1.1 Allele of the PDCD1 Gene

Qingqing Jiao, 1 Cuiping Liu, 2 Ziliang Yang, 1 Qiang Ding, 1 Miaomiao Wang, 1 Min Li, 1 Tingting Zhu, 1 Hua Qian, 3 Wei Li, 3 Na Tu, 1 Fumin Fang, 1 Licai Ye, 1 Zuotao Zhao, 4 and Qihong Qian 1

1 Department of Dermatology, The First Affiliated Hospital of Soochow University, 188 Shizi Road, Suzhou 215006, China
2 Clinical Immunology Laboratory, First Affiliated Hospital, Soochow University, 708 Renmin Road, Suzhou 215007, China
3 Department of Dermatology, Soochow University Affiliated Children’s Hospital, 303 Jingde Road, Suzhou 215003, China
4 Department of Dermatology, First Hospital, Peking University, 8 Xishenku Road, Beijing 100034, China

Correspondence should be addressed to Zuotao Zhao; zhaozuotaotao@163.com and Qihong Qian; qihongqiansz@gmail.com

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Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with complicated genetic inheritance. Programmed death 1 (PD-1), a negative T cell regulator to maintain peripheral tolerance, induces negative signals to T cells during interaction with its ligands and is therefore a candidate gene in the development of SLE. In order to examine whether expression levels of PD-1 contribute to the pathogenesis of SLE, 30 patients with SLE and 30 controls were recruited and their PD-1 expression levels in peripheral blood mononuclear cells (PBMCs) were measured via flow cytometry and quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). Also, whether PD-1 expression levels are associated with the variant of the SNP rs36084323 and the SLE Disease Activity Index (SLEDAI) was studied in this work. The PD-1 expression levels of SLE patients were significantly increased compared with those of the healthy controls. The upregulated PD-1 expression levels in SLE patients were greatly associated with SLEDAI scores. No significant difference was found between PD-1 expression levels and SNP rs36084323. The results suggest that increased expression of PD-1 may correlate with the pathogenesis of SLE, upregulated PD-1 expression may be a biomarker for SLE diagnosis, and PD-1 inhibitor may be useful to SLE treatment.

1. Introduction

Systemic lupus erythematosus (SLE) is a progressive autoimmune disease with a wide range of immunological abnormalities [1]. It is characterized by an immune response against nucleic components, but the etiopathology is not clearly understood yet. Multiple genetic factors relating to SLE have been identified [2–4], which suggest that both immune disorder and genetic factors may play important roles during the SLE process.

The protein programmed death 1 (PD-1), a negative costimulatory molecule, belongs to the CD28 superfamily and is expressed on the surface of activated human CD4+ and CD8+ T cells, B cells, natural killer (NK) cells, activated monocytes, myeloid cells, and CD4−CD8− T cells from the thymus [5, 6]. As an immune inhibitory receptor, PD-1 interacts with its ligands, PD-L1 and PD-L2, which can suppress lymphocyte activation and cytokine production [7]. Current concepts regarding PD-1/PD-L pathway are categorized into immune dysfunction associated with SLE in humans [8]. In addition, it was reported that PD-1 gene polymorphisms were involved in the development of autoimmune diseases, such as SLE, rheumatoid arthritis, and Graves’ disease [9]. However, until now, only few studies have reported a possible link between PD-1 gene polymorphisms and SLE [8, 10–12]. Due to the existence of racial and regional differences in SNPs in PD-1, it is very important to study the relevance of PD-1 to SLE susceptibility in the Chinese Han population, which could
Flow cytometry was performed using 50 μL of EDTA-treated peripheral blood incubated for 30 min at 4°C with fluorochrome-labeled monoclonal antibodies (mAbs): anti-CD4-FITC (Beckman), anti-CD8-FITC (Beckman), anti-CD56-FITC (Beckman), and anti-PD-1-PE (BioLegend). Erythrocyte lysis and cell fixation were carried out using OptiLyse C Lysing Solution (Beckman). Treated blood samples passed through the Coulter Epics XL Flow cytometer (Beckman), and the relevant data were acquired and accordingly examined. Data analysis was accomplished by FlowJo software (Tree Star, Ashland).

2.2. Real-Time RT-PCR Analysis. PBMCs were separated from fresh blood of patient and control groups in order to analyze the mRNA of the PD-1. Total cellular RNA was isolated by Trizol (Invitrogen, USA). After quantification, 1μg of total cellular RNA was used to conduct reverse transcription with Promega RT kit (A3800) and an oligo (dT) primer. PCR was completed in a 50 μL reaction system containing 200 nM PD-1 primers (Table 1), 120 nM TaqMan probe, and premix Ex Taq (Takara, Dalian, China). Samples were amplified in the Applied Biosystems 7900 HT Fast Real-time PCR System (CA, USA) for 40 cycles under the following conditions: denaturation for 10 s at 95°C, anneal...
Figure 1: Increased basal programmed death 1 (PD-1) expression in PBMCs from SLE patients. (a) Representative flow cytometry analysis of PD-1 expression on CD4+, CD8+, and CD56+ T cells in SLE patients and normal healthy controls (NC); (b) upregulated expression of PD-1 on CD4+, CD8+, and CD56+ T cells from SLE patients, as compared with those from NC; (c) mRNA expression of PD-1 in PBMCs from SLE patients. Horizontal bars indicate the mean ± SD.

of SNP rs36084323 was related to the upregulated PD-1 expression or not, the variant of SNP rs36084323 was genotyped. Results indicate that there was no connection between the variant of SNP rs36084323 and upregulated PD-1 expression (Figure 3).

4. Discussion

In this study, we demonstrated that PD-1 expression levels in PBMCs from SLE patients were significantly higher than those in control group. Also, significant relationship was found between SLEDAI scores and upregulated PD-1 expression in PBMCs from PB samples of SLE patients. However, no obvious difference was revealed between PD-1 expression levels and SNP rs36084323. Results show that increased level of PD-1 expression in PBMCs rather than SNP rs36084323 is associated with the development of SLE, and this discovery is presented for the first time. These findings provide more evidence to support the theory that upregulated PD-1 expression may be involved in the pathogenesis of SLE.

SLE is a chronic inflammatory disease of generalized autoimmunity and is characterized by B cell hyperactivity and abnormally activated T cells [1]. PD-1 can be expressed on activated T cells, B cells, and myeloid cells and is considered to play an important role in the regulation of peripheral tolerance [18]. Mice deficient for PD-1 have developed a lupus-like syndrome, with arthritis and glomerulonephritis as phenotypes [19]. In this study, increased expression of PD-1 in PBMCs is found to have significant relationship with SLEDAI scores, and the results suggest that PD-1 is involved in the development of SLE. Although the detailed etiology is still unclear, many genes are considered to have connections with the pathogenesis of SLE. At present, the programmed cell death 1 gene (PDCD1, also called PD-1) was
**Figure 2:** Correlation of upregulated PD-1 expression levels with SLEDAI in PBMCs. The association of SLEDAI with upregulated PD-1 expression on CD4⁺ T cell (a), CD8⁺ T cell (b), CD56⁺ T cell (c), and mRNA expression of PD-1 in PBMCs (d).

**Figure 3:** Analysis of PD-1 expression levels in SLE patients with different genotypes of rs36084323. Increased levels of PD-1 levels in PBMC of SLE ($n=30$) patients, as compared with those from NC ($n=30$). The patients with GG genotype ($n=11$) exhibit higher PD-1 expression levels than those with AG ($n=15$) and AA genotype ($n=4$). Horizontal bars indicate the mean ± SD.
one of the top candidates linking to the disease [20]. Thus, it is therefore necessary to study the interconnection between polymorphisms in PDCD1 and SLE.

135 SNPs (found in the National Center for Biotechnology Information (NCBI) Entrez SNP database) have been identified in the human PDCD1 region. Among them, PD-1.1, PD-1.3, PD-1.5, and others are considered to have connection to autoimmune diseases [21]. PD-1.1 polymorphism is located in the promoter region (position −538 from transcription start site). Previous studies have shown that PD-1.1 G/A (rs36084323) is common in the Chinese Han population (49%), but it is very rare in Europeans (1%) [20], which may indicate that Chinese Han population is more susceptible to SLE. In this study, no connection was found between SNP PD-1.1 G/A (rs36084323) and increased expression of PD-1 in PBMCs from SLE patients (P > 0.05), and the reason might be the limited sample size used in this study or some yet unidentified reason. However, it is observed that frequencies of the GG and AG genotype allele in SNP PD-1.1 were higher in SLE patients when compared with AA in our patients’ population. In addition, PD-1.1 is located within the promoter region of PD-1. This SNP has no function, and further study is required to explore its exact role in the development of SLE.

5. Conclusions

In conclusion, increased expression of PD-1 in PBMCs from SLE patients was significantly related to SLEDAI scores rather than SNP rs36084323. The presented results provide more evidence to support that upregulated expression of PD-1 might be correlated with the pathogenesis of SLE.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Qingqing Jiao, Cuiping Liu, and Ziliang Yang contributed equally to this work.

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