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The effect of PD-1/PD-L1 signaling axis on the interaction between CD19^{+}B cells and CD4^{+}T cells in peripheral blood of patients with systemic lupus erythematosus

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Abstract

Background The defect of B cell self-tolerance and the continuous antigen presentation by T cells (TCs) mediated by autoreactive B cells (BCs) play a key role in the occurrence and development of systemic lupus erythematosus (SLE). PD-1/PD-L1 signaling axis negatively regulates the immune response of TCs after activation and maintains immune tolerance. However, the effect of PD-1/PD-L1 signaling axis on the interaction between CD19^{+}B/CD4^{+}TCs in the peripheral blood of patients with SLE has not been studied in detail.

Methods PD-1/PD-L1 and Ki-67 levels in peripheral blood (PB) of 50 SLE patients and 41 healthy controls (HCs) were detected through flow cytometry, and then the expression of PD-1$^{+/-}$cells and PD-L1$^{+/-}$cells Ki-67 was further analyzed. CD19^{+}B/CD4^{+}TCs were separated for cell culture and the supernatant was collected to determine proliferation and differentiation of TCs. IL-10 and IFN-γ secretion in the supernatant was also determined using ELISA.

Results The PD-1, PD-L1, and Ki-67 levels on CD19^{+}B/CD4^{+}TCs in patients with SLE were higher than HCs. In CD19^{+}B/CD4^{+}TCs of SLE patients, the proliferative activity of PD-L1^{+} cells was higher than that of PD-L1^{-} cells, and the proliferative activity of PD-1^{+} cells was higher than that of PD-1^{-} cells. In the system co-culturing CD19^{+}B/CD4^{+}TCs from HCs/SLE patients, activated BCs promoted TCs proliferation and PD-L1 expression among TCs. Addition of anti-PD-L1 to co-culture system restored the proliferation of TCs, and inhibited IL-10/IFN-γ level. The addition of anti-PD-L1 to co-culture system also restored Tfh and downregulated Treg in HCs.

Conclusions Axis of PD-1/PD-L1 on CD19^{+}B/CD4^{+}TCs in PB of SLE patients is abnormal, and cell proliferation is abnormal. In CD19^{+}B/CD4^{+}TCs of SLE patients, the proliferative activity of PD-L1^{+} and PD-1^{+} cells compared with PD-L1^{-} and PD-1^{-} cells in SLE patients, respectively. CD19^{+}B/CD4^{+}TCs in SLE patients can interact through PD-1/PD-L1.

Keywords SLE, CD19^{+}B cells, CD4^{+}T cells, PD-1/PD-L1 signal axis, Ki-67

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Introduction

The autoimmune disease SLE has complex and diverse clinical manifestations. It is characterized by the activation of innate/adaptive immunity, which causes the activation of autoreactive B cells (BCs) through T cells (TCs), resulting in the deposition of immune complexes and causing an autoimmune cascade possibly limited to an organ, as well as extensive systemic involvement [1–3]. Its pathogenesis remains obscure but insufficient tolerance of BCs and continuous antigen presentation by TCs exert a pivotal effect on the disease development [4]. $CD4^{+}$TCs are helper cells that play an integral role in B cell development/activation [5]. Lupus TCs are related to the immunopathogenesis of lupus through several pathways: excessive production of pro-inflammatory cytokines or increased adhesion between cells attack target cells or tissues leading to apoptosis of target cells; TCs initiate precursors of autoimmune B cell through cytokines along with costimulatory molecules to produce anti-apoptotic signals of autoimmune cells [6, 7]; increased $CD4^{+}$TCs in lupus blood has the potential to help BCs through IL-10/succinate [8].

PD-1 (CD279) is a transmembrane molecule of the immunoglobulin CD28 family [9] **and its ligand PD-L1 (CD274 or B7-H1) is expressed on BCs, TCs, monocytes, as well as dendritic cells. In addition, it is also extensively expressed in non-blood cells, including the lung, vascular endothelial cells, reticular fibroblasts, etc.** [10]. **Toll-like receptors together with type I interferon can modulate the** expression level **of PD-1/PD-L1 via activating NF-κB and/or STAT1** [11]. **As antigen-presenting cells (APC), PD-L1 on BCs is able to combine with PD-1 on TCs to negatively modulate the immune response as well as sustain immune tolerance** [12]. **Ki-67 protein is widely considered to be an indicator for cell proliferation in human tumors** [13] **and it is a necessary nuclear protein for cell proliferation, and its expression is confined to the phases of G1/S/G2/M during the cell cycle** [14]. **However, the role as well as clinical significance of Ki-67 expression in PD-1$^{+/-}$and PD-L1$^{+/-}$cells, especially for SLE, are unclear. Therefore, this study examined the expression of PD-1 and PD-L1 as well as Ki-67 on $CD4^{+}$TCs and $CD19^{+}$BCs in healthy controls (HCs) together with SLE patients.**

As the main component of cellular immune response, $CD4^{+}$TCs exert a crucial impact on infection control [5]. **Regulatory TCs (Treg) belong to a subset of $CD4^{+}$TCs, potentially inhibiting T cell-based immunity to avoid damage caused by excessively activated TCs** [9]. **TFH cells are the major TCs in humans and mice and are responsible for supporting the proliferation, survival, and differentiation of BCs. In mammalian germinal centers (GCs), Tfh exerts a crucial effect on the selection/differentiation of BCs into activated antibody-secreting cells** [15, 16]. **Studies have demonstrated a lower PD-L1 expression on $CD4^{+}CD25^{+}Foxp3^{+}$Treg cells after induction culture in patients with in contrast to HCs and a negative association with SLE Disease Activity Index (SLEDAI)** [17]. **Our previous studies demonstrated higher expression of $CD4^{+}$TCs among patients with active SLE in contrast to those with stable SLE and HCs, with a higher expression among patients with stable SLE when further compared to HCs. Besides, the increased frequency of $CD19^{+}$PD-L1^{+}BCs is strongly related to the disease activity index as well as the frequency of Tfh cells** [18].

The PD-1/PD-L1 signaling axis transmits a negative signal to TCs to prevent SLE progression but the abnormal activation of TCs/BCs results in the continuous progression of SLE. Therefore, in the present study, immunomagnetic cell sorting, flow cytometry (FCM), ELISA, and cell culture were used to test the influence of signaling axis of **PD-1/PD-L1 on the system** co-culturing **$CD19^{+}$B and $CD4^{+}$TCs in the peripheral blood (PB) of patients with** SLE. **$CD19^{+}$BCs together with $CD4^{+}$TCs were cocultured in vitro to assess the proliferation of TCs and the differentiation of Tfh/Treg cells. The expression of IFN-γ and IL-10 was detected by ELISA.**

Materials and methods

Patients and healthy controls

In total, 50 patients complying with the American College of Rheumatology criteria for lupus were enrolled from the Rheumatism Department of the First Affiliated Hospital of Bengbu Medical College in Bengbu, China. Any patient would be clinically diagnosed if≥4 of the following 11 criteria were observed complying with the 1997 American College of Rheumatology (ACR) criteria for SLE [19].The disease activity was evaluated according to SLEDAI-2000 based on the conditions of central nervous system, blood vessels, kidneys, joints, skin, serous membrane, blood, and systemic symptoms within the first 10 days of treatment. The higher the score, the more severe the disease activity and the more critical the condition.

We recruited healthy individuals who were age and sex-matched as the controls. The study was ratified by the Human Ethics Committee of the First Affiliated Hospital of Bengbu Medical College. Informed consent was obtained from all participants and Supplementary Table S1 displays their baseline characteristics.

Cell staining and FCM analysis

The measurement of PD-1, PD-L1, as well as Ki-67 on $CD4^{+}$TCs/$CD19^{+}$BCs in blood samples from HCs / SLE patients using the following antibodies: AF488

anti-human Ki-67 (Ki-67, Biolegend), APC anti-human PD-L1 (MIH2, Biolegend), BV421 anti-human PD-1 (MIH4, BD), PE/Cy7 anti-human CD4 (OKT4, Biolegend), as well as APC/Fire^{TM}-750 anti-human CD19 (HIB19, Biolegend). For Ki-67, the cells were permeabilized (eBiosciences) before staining with the indicated antibodies.

TC subsets were detected following coculture with BCs using the antibodies as folllows: APC/Cy7 anti-human CD19 (HIB19, Biolegend), AF647 anti-human FOXP3 (259D, Biolegend), FITC anti-human CD185 (CXCR5) (J252D4, Biolegend), PE anti-human CD25(M-A251, BD), and BV421 anti-human CD4 (RPA-T4, BD). Fixable Viability Dye eFlour 506 (eBiosciences) was used for the exclusion of dead cells. Treg cells were permeabilized before staining using the indicated antibodies. The data obtained from D×P Athena were analyzed using FlowJo.

Co-culture of T and B cells

$CD4^+$T/$CD19^+$BCs were isolated using CD4/CD19 magnetic beads (Miltenyi) yielding > 95% $CD4^+$TCs with >95% $CD19^+$BCs. Subsequently, BCs were stimulated by 40 ng/ml of IL-2 (PeproTech) as well as 1 μg/ml of cytosine-phosphate-guanine oligodeoxynucleotide (CPG-ODN) 2006 (Invivogen) for 24 h and then co-cultured with autologous $CD4^+$TCs in complete medium in 96-well plates that were pre-coated with anti-CD3 (1 μg/ml, OKT3, Biolegend) as well as anti-CD28 (1 μg/ml, CD28.2, Biolegend) for 3 days at 37℃ and 5% CO_2. After 24 h, anti-PD-L1 (10 μg/ml, 29E.2A3, Biolegend) and isotype control were added to the co-culture. On day 3, cells were examined by FCM. Furthermore, we measured the production of IL-10 and IFN-γ, which were secreted into the supernatant of the cell culture, using ELISA.

Cell proliferation

$CD4^+$TCs were incubated with carboxyfluorescein succinimidyl ester (CFSE; BD) in phosphate-buffered saline (PBS) for a period of 10 min at 37 °C. Before ending the labeling process, an equivalent amount of bovine serum albumin was added to the cells. Subsequently, after triplicately washing the cells, they were kept gentle shaking in the dark. Besides, CFSE signal was quantified by FCM.

ELISA

IFN-γ and IL-10 (DAKEWE) secretion in the supernatants of cell culture was analyzed by ELISA complying with the instructions provided by the manufacturer.

Data analysis

FCM data were analyzed using Flowjo10.4 software and the experimental data were analyzed using SPSS26.0. The data were described with mean ± standard deviation ($x \pm s$); t-tests were carried out to compare the differences between groups. Pearson correlation analysis was conducted. A P value less than 0.05 was indicative of statistical significance.

Results

Differential expression of PD-L1, PD-1 and Ki-67 on $CD19^+$B/$CD4^+$TCs

Compared to HCs, the expression of PD-L1, PD-1, and Ki-67 was significantly higher on $CD4^+$TCs from patients with SLE; and the frequency of the expression of PD-1 / PD-L1 on $CD19^+$BCs among SLE patients was also higher with statistical significance (Fig. 1). In addition ,we analyze the expression of PD-1, PD-L1, and Ki-67 on $CD4^+$ T cells and $CD19^+$ B cells in relation to drug therapy. The result showed no correlation between the expression of PD-1, PD-L1 and Ki67 on $CD4^+$T cells and $CD19^+$B cells and drug treatment status (Supplementary Table S2 and S3).

Correlation of $CD19^+$BCs with $CD4^+$TCs

The linear regression analysis revealed a positive correlation of $CD4^+$Ki-67^+TCs with $CD4^+$PD-1^+TCs ($r=0.332$, $P<0.05$), and the percentage of $CD19^+$Ki-67^+BCs ($r=0.384$, $P<0.05$) in HCs. The average fluorescence intensity of $CD4^+$PD-1^+TCs was in a positive association with $CD4^+$PD-$L1^+$TCs ($r=0.576$, $P<0.001$), and the percentage of $CD4^+$PD-$L1^+$TCs was positively associated with $CD19^+$PD-$L1^+$BCs ($r=0.512$, $P<0.001$) and $CD19^+$PD-1^+BCs ($r=0.403$, $P<0.001$). For SLE patients, there was a positive correlation of $CD4^+$Ki-67^+TCs with $CD19^+$Ki-67^+BCs ($r=0.323$, $P<0.05$); besides, the average fluorescence intensity of $CD19^+$PD-$L1^+$BCs was in a positive association with $CD19^+$PD-1^+BCs ($r=0.572$, $P<0.01$), as shown in Table 1.

Ki-67 expression on PD-$1^{+/-}$ and PD-$L1^{+/-}$ cells

Further analysis revealed a higher ratio of $CD4^+$PD-$L1^-$Ki-67^+TCs as well as $CD4^+$PD-$1^{+/-}$Ki-67 TCs among SLE patients compared to HCs (Fig. 2A and B). In addition, there was a higher ratio of $CD19^+$PD-$L1^-$Ki-67^+ BCs & $CD19^+$PD-1^-Ki-67^+BCs in SLE patients compared to HCs. The proportions of $CD19^+$PD-$L1^+$Ki-67^+, $CD19^+$PD-$L1^-$Ki-67^+, as well as $CD19^+$PD-1^+Ki-67^+ BCs were not significantly different between groups but the ratio of $CD19^+$PD-1^-Ki-67^+ (SLE: 2.63%±2.04% vs. HC: 1.92%±0.74%, $P=0.038$) BCs between groups was significantly different (Fig. 2C and D).

There were also more $CD4^+$PD-$L1^+$Ki-67^+TCs than $CD4^+$PD-$L1^-$Ki-67^+TCs among SLE patients with SLE ($P<0.001$), with more $CD4^+$PD-$L1^+$Ki-67^+TCs than $CD4^+$PD-$L1^-$Ki-67^+TCs in HCs and SLE patients. Meanwhile, there was a

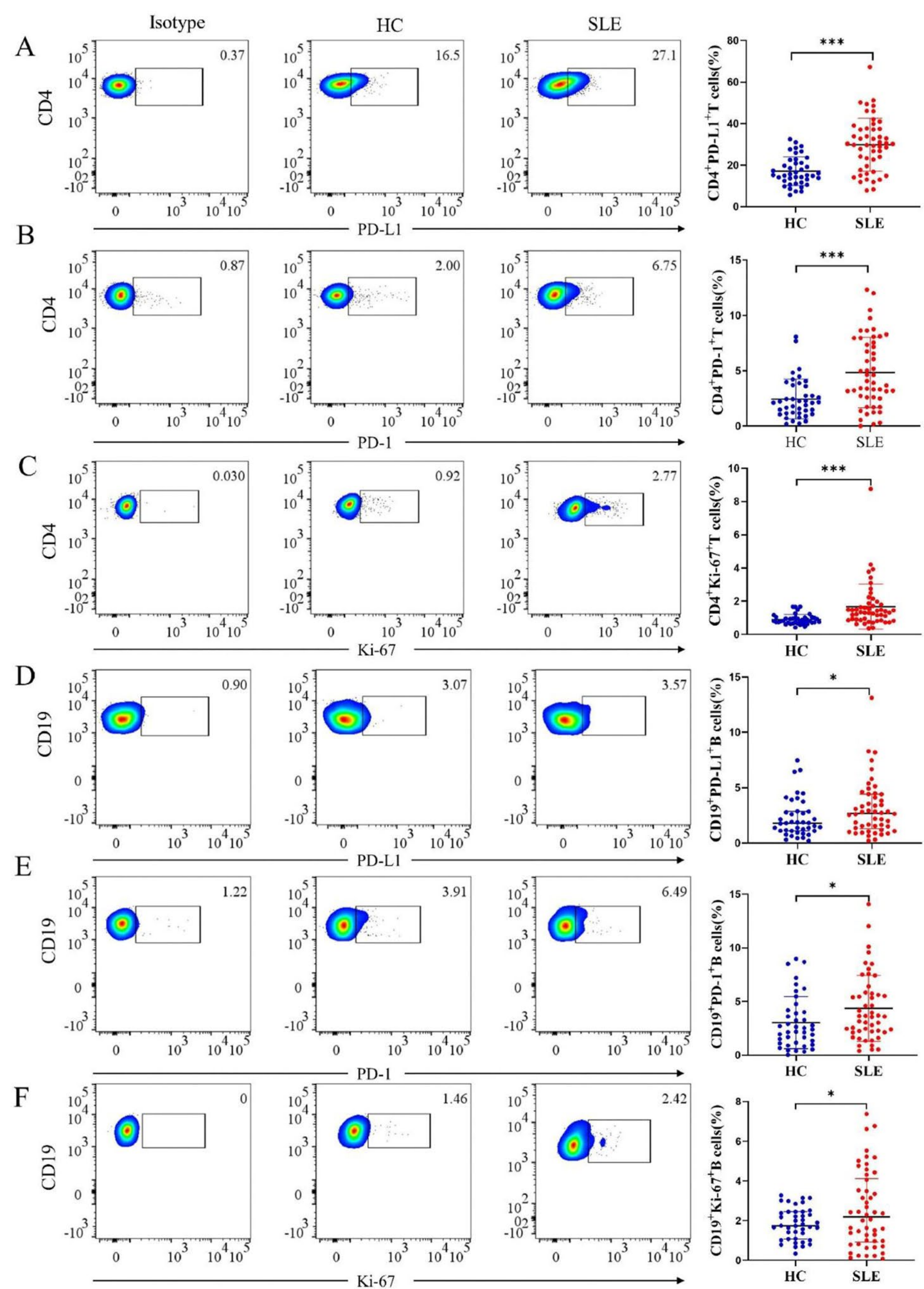

Fig. 1 Expression of PD-L1, PD-1, and Ki-67 on CD4$^+$TCs and CD19$^+$BCs. (**A-C**) Flow and statistical plots of PD-L1 **(A)**, PD-1 **(B)**, and Ki-67 **(C)** expression frequency on peripheral blood CD4$^+$TCs from SLE patients and HC. (**D-F**) Flow and statistical plots of PD-L1 **(D)**, PD-1 **(E)**, and Ki-67 **(F)** expression frequency on peripheral blood CD19$^+$BCs from SLE patients and HC. ***$P < 0.001$, *$P < 0.05$

higher proportion of CD19$^+$PD-L1$^+$Ki-67$^+$BCs to CD19$^+$PD-L1$^-$Ki-67$^+$BCs among HCs and SLE patients with more CD19$^+$PD-1$^+$Ki-67$^+$BCs compared to CD19$^+$PD-1$^-$Ki-67$^+$BCs.

Activated BCs are more conducive to T cell proliferation and PD-L1 expression

It has been shown that CD19$^+$BCs collected from untreated patients with RA and HCs similarly upregulated PD-L1 expression once stimulated by CpG/IL-2 [20]. To explore PD-L1 expression on CD19$^+$BCs from SLE patients, CD19$^+$BCs were isolated and purified from the PB collected from four SLE patients as well as four

Table 1 Correlation analysis of PD-L1, PD-1 and Ki-67 on $CD19^{+}$B and $CD4^{+}$T cells

		$CD4^{+}Ki\text{-}67^{+}$(%)	$CD4^{+}PD\text{-}L1^{+}$(%)	$CD4^{+}PD\text{-}1^{+}$(%)	$CD19^{+}Ki\text{-}67^{+}$(%)	$CD19^{+}PD\text{-}L1^{+}$(%)
HC	$CD4^{+}PD\text{-}L1^{+}$(%)	0.094				
	$CD4^{+}PD\text{-}1^{+}$(%)	**0.332***	**0.576****			
	$CD19^{+}Ki\text{-}67^{+}$(%)	**0.384***	0.149	0.184		
	$CD19^{+}PD\text{-}L1^{+}$(%)	-0.155	**0.512****	**0.452****	0.192	
	$CD19^{+}PD\text{-}1^{+}$(%)	-0.144	**0.403****	**0.638****	-0.058	**0.597****
SLE	$CD4^{+}PD\text{-}L1^{+}$(%)	-0.064				
	$CD4^{+}PD\text{-}1^{+}$(%)	0.201	0.106			
	$CD19^{+}Ki\text{-}67^{+}$(%)	**0.323***	-0.063	-0.111		
	$CD19^{+}PD\text{-}L1^{+}$(%)	-0.021	0.098	-0.015	-0.011	
	$CD19^{+}PD\text{-}1^{+}$(%)	-0.110	-0.003	0.257	-0.006	**0.572****

*$p<0.05$; **$p<0.01$

HCs, and cultured with either CPG or IL-2. There was a significantly higher frequency and mean fluorescence intensity (MFI) of PD-L1 expression on $CD19^{+}$BCs collected from HCs and SLE patients compared to untreated cells after one day of culture (Fig. 3A and B).

After 24 h of cell stimulation, BCs were washed two times using PBS and then divided into activated and non-activated groups for co-culture with $CD4^{+}$TCs (1:1 for 48 h). The results revealed that for HCs and patients with SLE, expression of PD-1/PD-L1 was upregulated on the stimulated TCs, while that of PD-1 was very low on unstimulated TCs, consistent with studies by Shi et al. [12] and Said et al. [21]. In addition, activated $CD19^{+}$BCs in HCs promoted the proliferation of $CD4^{+}$TCs ($P<0.001$) together with PD-1 expression on $CD4^{+}$TCs ($P<0.001$). Activated $CD19^{+}$BCs of SLE patients promoted $CD4^{+}$T cell proliferation along with PD-L1 expression ($P<0.05$) but there was no significance in PD-1 expression (Fig. 3C-F).

Effects of activated TCs on PD-1 / PD-L1 on $CD19^{+}$BCs

After stimulated for 24 h, TCs were co-cultured with $CD19^{+}$BCs in the proportion of 1:1 for 48 h, showing that activated $CD4^{+}$TCs promoted the PD-L1/PD-1 expression ($P<0.05$) in $CD19^{+}$BCs of HCs. Activated $CD4^{+}$TCs also promoted PD-L1 expression ($P<0.01$), accompanied with a trend toward upregulating PD-1 expression in $CD19^{+}$BCs of SLE patients but without statistical significance (Fig. 4).

Impact of the PD-1/PD-L1 signaling pathway on the proliferation and differentiation of $CD4^{+}$ TCs in the co-culture system

The proliferation and differentiation of $CD4^{+}$TCs were detected by adding anti-PD-L1 and the isotype to the co-culture system, confirming that the $CD4^{+}$TC proliferation was restored via the pathway of PD-1/PD-L1 among HCs / SLE patients. In HC, anti-PD-L1 restored Tfh expression; meanwhile, it reduced the frequency of Treg ($P<0.05$). Also, no obvious change in the $CD4^{+}$T cell proliferation rate, Tfh, and Treg change ratio was observed in SLE patients and HC (Fig. 5).

Detection of IL-10 / IFN-γ using ELISA

Next, we examined whether anti-PD-L1 could modulate IFN-γ / IL-10 secretion in the T and B co-culture system, showing that IFN-γ / IL-10 secretion decreased when anti-PD-L1 was added ($P<0.05$, Fig. 6).

Discussion

The etiology of SLE has not been fully elucidated but is related to many factors including genetic, environmental, infection, and hormones [22]. The production of BCs and autoantibodies is the key driving factor in SLE pathogenesis but without the proper support of TCs, BCs are ineffective and TCs provide BCs with the necessary costimulatory signals [23]. In the RA patient study, the ratio of $CD19^{+}PD\text{-}L1^{+}$BCs among untreated patients was significantly lower than that in HC patients, whereas after treatment, there was an increased frequency of $PD\text{-}L1^{+}$BCs among patients with a positive response to treatment [20]. However, in patients with melanoma, the expression of B cell PD-L1 increased significantly and was positively correlated with the tumor stage [24]. SLE patients have been reported to have increased PD-1, Ki-67, or PD-L1 expression on $CD11c^{+}$BCs [25]. Additionally, the ratio of $CD4^{+}PD\text{-}1^{+}$cells in psoriatic patients was much lower than compared to the control group [26]. In summary, PD-1 / PD-L1 expression is disease-specific on immune cells. In our study, there was a higher expression frequency of PD-L1 and PD-1 on $CD4^{+}$T/ $CD19^{+}$BCs from the PB of SLE patients compared to HCs, indicating the aberrant expressed pathway of PD-1/PD-L1 during the interaction of $CD4^{+}$TCs with $CD19^{+}$BCs.

Ki-67 protein, a common marker of proliferating cells, encodes a 359 KD non-histone nuclear protein [14] and was originally identified as an antigen in the nucleus of Hodgkin's lymphoma, which is highly expressed in circulating cells but strongly downregulated in quiescent

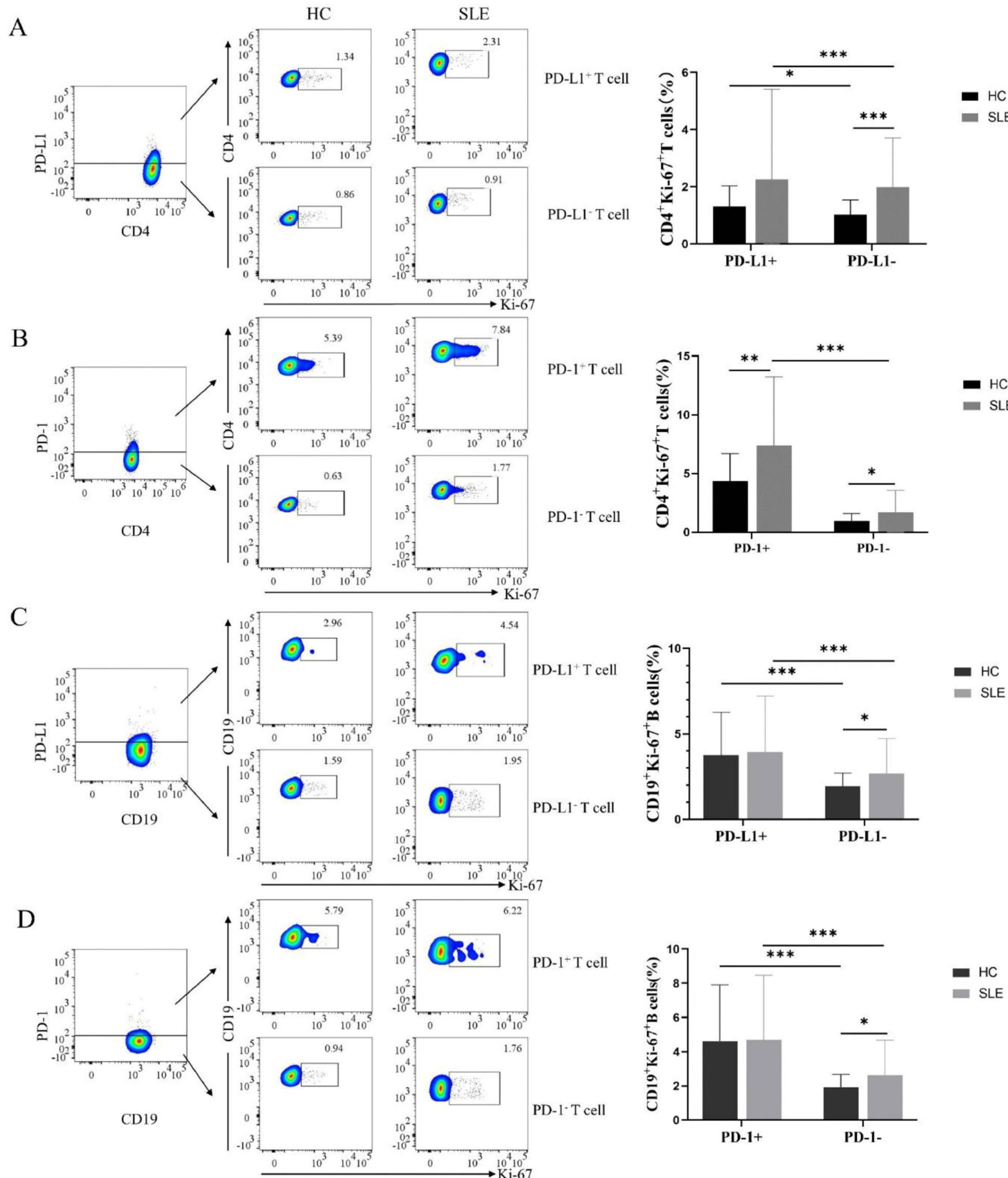

Fig. 2 Expression of Ki-67 in PD-1$^{+/-}$ cells and PD-L1$^{+/-}$ cells. (**A-B**) Flow plots and statistical results of Ki-67 in CD4$^+$PD-L1$^{+/-}$TCs (**A**) versus CD4$^+$PD-1$^{+/-}$TCs (**B**) from the peripheral blood of SLE patients and HCs. (**C-D**) Flow plots and statistical results of Ki-67 in CD19$^+$PD-L1$^{+/-}$BCs (**A**) and CD19$^+$PD-1$^{+/-}$BCs (**B**) from the peripheral blood of SLE patients and HCs. *P < 0.05, **P < 0.01, ***P < 0.001

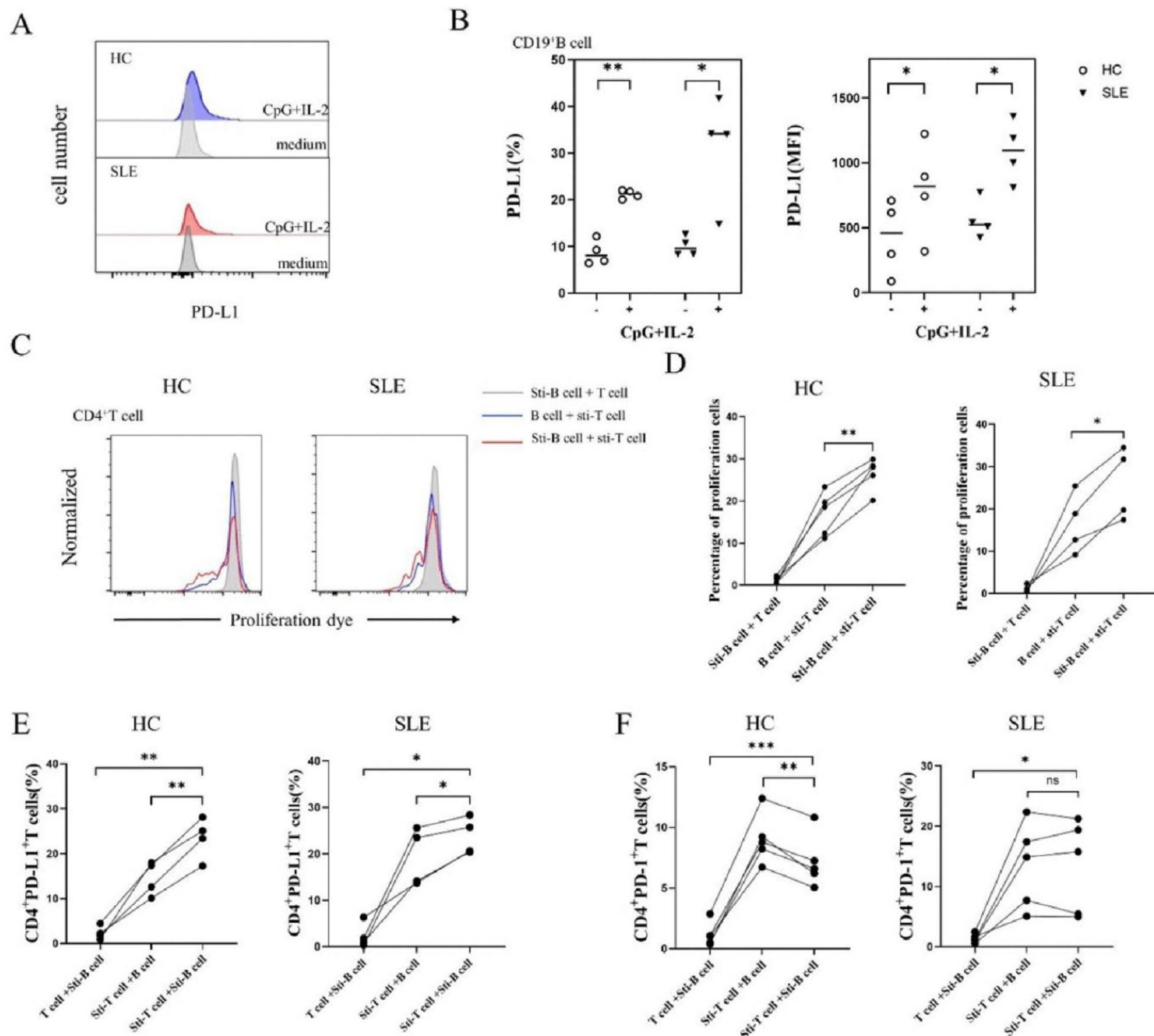

Fig. 3 Effect of activated CD19^{+}BCs on the expression and proliferation of PD-L1 and PD-1 in CD4^{+}TCs. **(A)** Representative histograms of PD-L1 expression on total CD19^{+}BCs. **(B)** Expression (frequency, left panel, and MFI, right panel) of CD19^{+}PD-L1^{+}BCs. **(C)** Flow cytometry of the effects of activated CD19^{+}BCs on the proliferation of CD4^{+}TCs, and statistical results of the effects of activated CD19^{+}BCs on the proliferation of CD4^{+}TCs **(D)** and the expression of PD-L1 **(E)** and PD-1 **(F)**. *P < 0.05, **P < 0.01, ***P < 0.001

G0 cells [27]. Some studies have found that in early SLE, several expanded lymphocyte populations commonly express Ki-67 [28]. Ki-67^{+} cell populations, peripheral helper T (Tph) cells as well as age-related B cells (ABCs) remained elevated during the first year, and similar increases were observed in diagnosed SLE, suggesting these pathways to be activated early in SLE and characteristic of SLE pathologic immune responses [28]. Ki-67^{+}NK cells are related to the increased production of autoantibodies, decreased levels of complement, and nephritis in SLE patients [29]. In vitro experiments have proved the influence of IL-15 on upregulating Ki-67 expression in NK cells [29]. Compared to HCs, Ki-67 expression in CD4^{+}TCs and CD19^{+}BCs of PB of patients with SLE was relatively higher. Also, the proportion of CD4^{+}PD-L1^{-}Ki-67^{+}TCs, CD4^{+}PD-1$^{+/-}$Ki-67^{+}TCs, CD19^{+}PD-L1^{-}Ki-67^{+}, as well as CD19^{+}PD-1^{-}Ki-67^{+}BCs in SLE patients, was higher compared to HCs. This suggests that the proliferative activity of CD4^{+}TCs, CD19^{+}BCs, CD4^{+}PD-L1^{-}TCs, CD4^{+}PD-1$^{+/-}$TCs, CD19^{+}PD-L1^{-} and CD19^{+}PD-1-BCs was abnormal. In the research of tumors, it is generally believed that Ki67 is considered to be related to the prognosis of patients [30–34], while there are few relevant researches in SLE patients.

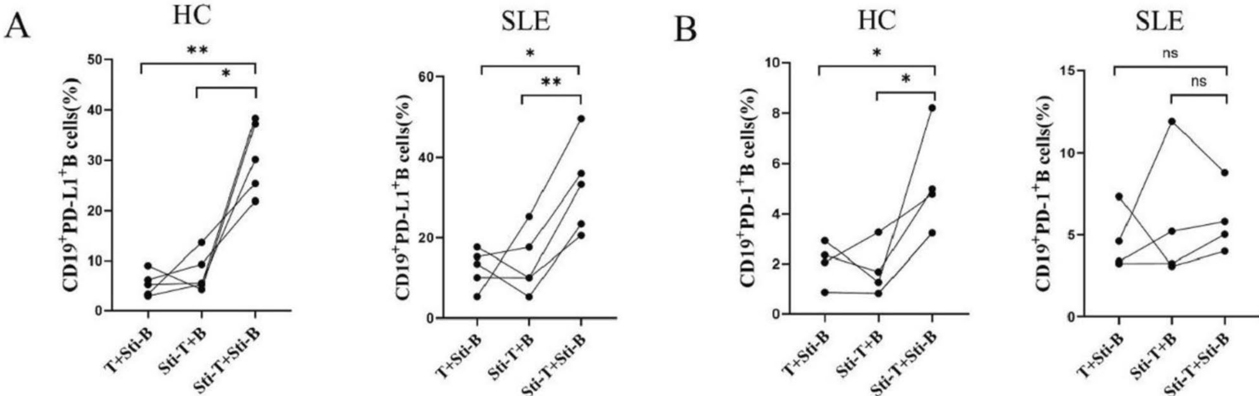

Fig. 4 Effect of activated T cells on the expression of PD-L1 and PD-1 on CD19$^+$BCs. (**A-B**) The plot of the effect of activated TCs on the expression of PD-L1 (**A**) and PD-1 (**B**) on the surface of CD19$^+$BCs. ns: no statistical significance ($P > 0.05$), $^*P < 0.05$, $^{**}P < 0.01$

Some studies demonstrated among new patients with SLE, an upregulated serum level of PD-1 antibody, which is linked to the disease activity, was observed; besides, PD-1 autoantibody promoted the proliferation of CD4$^+$TCs [12]. As reported by studies on patients with RA, CD19$^+$BCs has a potentical in inhibiting CD8$^+$TCs proliferation as well as cytokine production in a manner depending on PD-L1 [20]. In addition, PD-L1 immature BCs inhibit TCs depending on the level of PD-L1 among patients with melanoma (stage III and IV) [24]. In our study, activated CD19$^+$B in HCs/SLE patients promoted the proliferation and PD-L1 expression in CD4$^+$TCs. Thus, it was speculated that after activation, the negative signal increased and the proliferation decreased because of the increased expression of PD-L1. Therefore, PD-L1 blockers were added to investigate the impact of the axis of PD-1/PD-L1 on the interaction of CD4$^+$TCs with CD19$^+$BCs, showing that CD4$^+$TC proliferation was restored by PD-1/PD-L1 pathway among HCs and SLE patients. In summary, we hypothesized that PD-1/PD-L1 signaling axis exerts a crucial impact on activating pathogenic cell responses of TCs and BCs in SLE patients; Besides, BCs have the potential to regulate the T cell immune response by expressing regulatory molecules including PD-L1.

PD-L1^{hi}B cells can regulate the expansion of TFH cells [35]. PD-1 limits the upregulation of CXCR3 on Tfh cells, thus concentrating cells in the germinal center, where the PD-L1/PD-1 interaction of a single Tfh cell with BCs optimizes the competition as well as affinity maturation of BCs [36]. Also, PD-L1 solely has a potential in inducing the immature CD4$^+$TCs to differentiate into Foxp3-induced regulatory T (iTreg) cells [37]. The upregulation of PD-1 expression among SLE patients with the PTPN22 Trp^{620} missense allele is related to the decrease of Treg inhibition ability and the increase of T cell proliferation ability [38]. In addition, CTLA-4 expressed by Treg depleted CD80/CD86 through macrophages and released free PD-L1 on antigen-presenting cells [39]. In our study, anti-PD-L1 restored the expression of Tfh but decreased that of Treg among HCs. Therefore, we believe that the inhibition of Tfh and the recovery of Treg expression by PD-1/PD-L1 signaling axis when TCs interacts with BCs is key for TC inhibition along with the maintenance of peripheral tolerance. However, we speculate that based on individual differences or the impairment of PD-1/PD-L1 signaling pathway among SLE patients, the role of the PD-1/PD-L1 signaling pathway in the expression frequency of Tfh/Treg is not statistically significant.

As reported by experimental studies, PD-1$^{+/-}$TCs are beneficial for the increase of IFN-γ/IL-17 production [39]. In studies of melanoma patients, B cell subsets could suppress IFN-γ expression by TCs in a manner depending on PD-L1 [24]. Several researchers have also suggested that the IFN-γ gene may occur early in patients with SLE [40], possibly playing a critical role in LN [41]. IL-10 can specifically block B cell responses in SLE mice [42]. It is commonly believed that IL-10 is paradoxical pathogenic in SLE. Our results proved that PD-L1 inhibition inhibited IL-10 / IFN-γ secretion, therefore, roles of IFN-γ and IL-10 for SLE cannot be ignored.

The roles of PD-L1 are various in immune regulation. Besides, PD-1/PD-L1 signaling axis has complex pathogenicity in SLE. In addition to ITIM (inhibitory motif), the intracellular segment of PD-1 also has a structure called immune receptor complex tyrosine conversion motif (ITFM) which can bind tyrosine kinase or tyrosine phosphatase (such as SHP-2) according to the existence of a binding protein called SAP, to initiate negative regulation. The classical PD-1 pathway is related to the trans-interaction of PD-1 (on TCs) with PD-L1 (on APC or tumor cells), negatively regulating the immune response after the activation of TCs, thereby maintaining immune tolerance [12]. Recently, the existence of cis-interactions

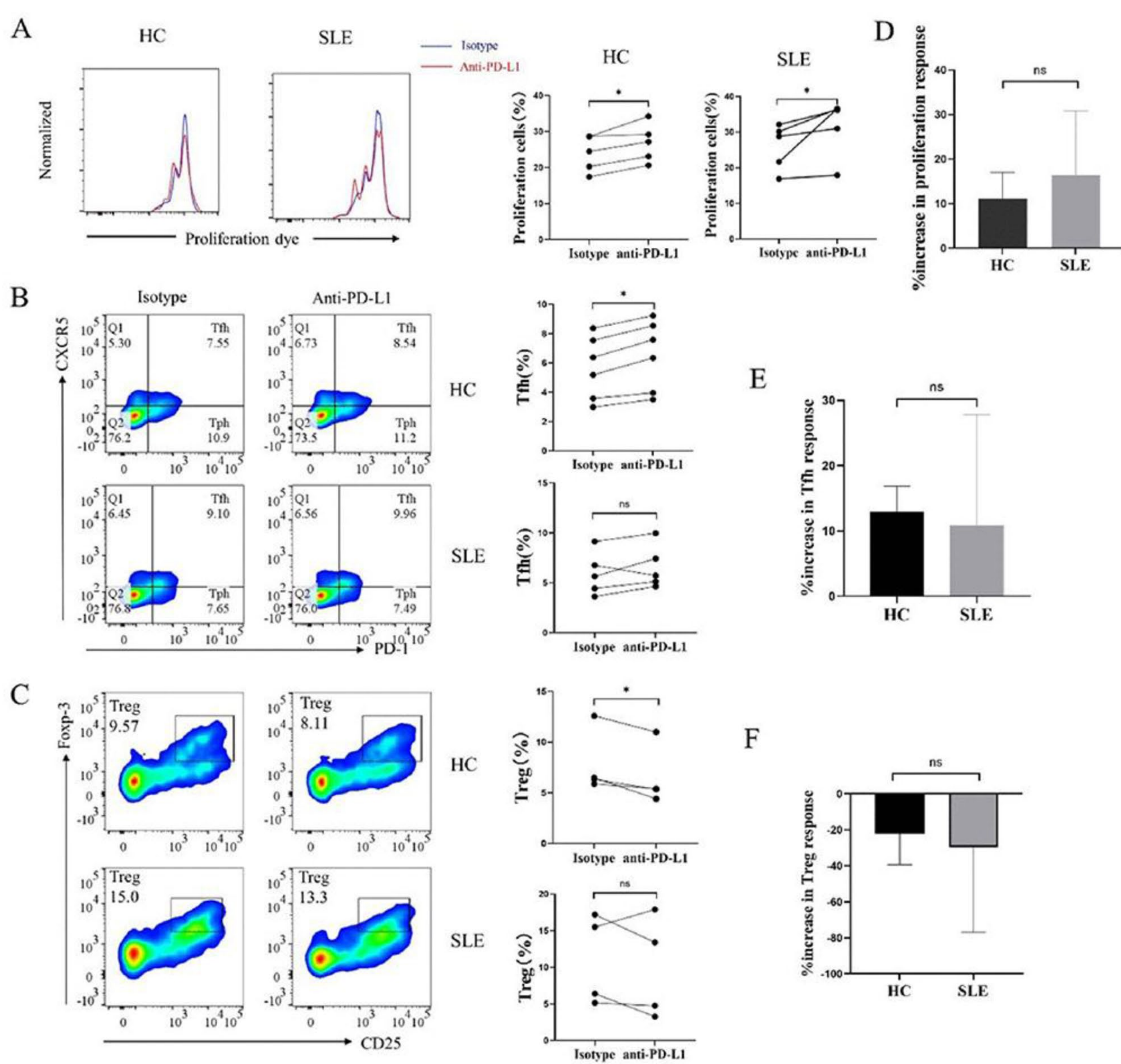

Fig. 5 Effect of the PD-1/PD-L1 signaling axis on the proliferation and differentiation of CD4^{+}TCs in the CD4^{+}T/CD19^{+}BCs co-culture system. CD19^{+}B/CD4^{+}TCs isolated from PBMC of HCs and SLE patients were co-cultured with anti-PD-L1 or isotype antibodies. The proliferation, Tfh, and Treg expression of CD4^{+}TCs were detected 72 h later, and the corresponding statistical results are shown. (**A-C**) shows the statistical results of CD4^{+}TC proliferation (**A**), Tfh (**B**), and Treg(**C**). (**D-F**) The increase rate of proliferating cells (**D**), Tfh (**E**), and Treg (**F**) cells was calculated as follows: $\frac{\text{Proliferation, Tfh, Treg frequencies anti-PD-L1 group} - \text{Proliferation, Tfh, Treg frequencies isotype group}}{\text{Proliferation, Tfh, Treg frequencies anti-PD-L1 group}} \times 100\%$ ns had no statistical significance ($P > 0.05$), $^{*}P < 0.05$

of PD-1 and APCs has been revealed on the same tumor cells or PD-L1 [43]. In addition, blocking [44] or activating [45] PD-1 can improve the survival of lupus mice. In addition, the combination of PD-1 agonist and low-dose IL-2 may have better therapeutic efficacy in SLE [46]. The susceptibility to SLE is also related to PD1.3/PD1.5 polymorphism, while the PD1.6 polymorphism is possibly protective for SLE [47].

In addition, this study has some limitations. (1) TCs and BCs were isolated for co-culture in vitro, while there are countless other cell types potentially expressing PD-L1, including dendritic cells, macrophages as well as bone marrow suppressor cells in the in vivo microenvironment. (2) In future studies, each cell type should be blocked by PD-L1 to distinguish effects of PD-L1 blockers on BCs or TCs. (3) However, its mechanism of action and its relationship with PD-1/PD-L1 signaling axis

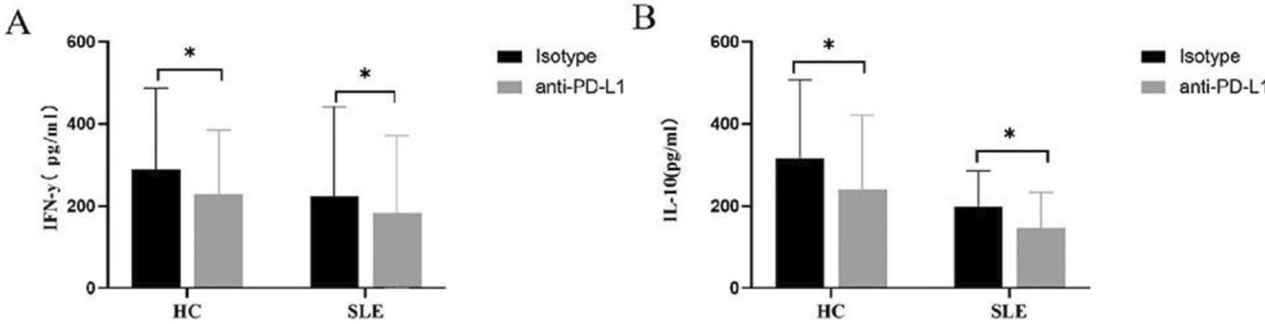

Fig. 6 Effect of the PD-1/PD-L1 signaling axis on the secretion of cytokines IFN-γ and IL-10 in the $CD4^{+}T/CD19^{+}BCs$ co-culture system. (A-B) $CD19^{+}BCs$ and $CD4^{+}TCs$ isolated from PBMC of HC and SLE patients were co-cultured with anti-PD-L1 or isotype antibodies and IFN-γ (**A**) and IL-10 (**B**) were quantified 72 h later by ELISA. $^{*}P < 0.05$

need to be further studied. Reseach has shown that PD-L1-Ig can inhibit the formation of Th17 cells in several organs such as spleen and the kidney, clear the abnormal production of cytokines (IFN-γ, IL-17, and IL-10) and anti-dsDNA autoantibodies in the serum, inhibit the deposition of IgG in the glomeruli with decreased proteinuria, and activate TCs in the urine [45]. In addition, PD-L1 binding to PD-1 on the surface of $CD8^{+}TCs$, weakens the function of cytotoxic $CD8^{+}TCs$ and promotes immune escape in nasopharyngeal carcinoma [48]. In conclusion, both dysfunction of the cytokine IL-17 and regulatory $CD8^{+}TCs$ are involved in the pathogenesis of SLE. However, its mechanism of action and its relationship with PD-1/PD-L1 signaling axis need to be further investigation.

Conclusion

Our study found that the proliferative activity of $PD-L1^{+}$ cells and $PD-1^{+}$ cells in $CD4^{+}T$ cells and $CD19^{+}B$ cells of SLE patients was higher than that of the corresponding negative cells. We considered that $PD-L1^{+}$ cells and $PD-1^{+}$ cells in SLE patients may play a role in disease activity. In addition, we further added anti-PD-L1 to T and B cell co-culture system, and found that anti-PD-L1 could restore the proliferation of $CD4^{+}T$ cells in SLE patients. The secretion of IFN-γ and IL-10 was inhibited. In summary, we suggest that $CD19^{+}B$ and $CD4^{+}T$ cells can interact through PD-1/PD-L1 in SLE patients, and the PD-1/PD-L1 signaling axis has potential value in the treatment of SLE.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

ZX, CX and YW presented the idea and design. WX, XC and XD helped to collect samples. ZX, LD, HH and HT participated in the experiments and writing the manuscript. CX, HW and BL supervised the whole study and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Data Availability

The authors confirm that the data supporting the findings of this study are available within the corresponding author when requested.

Declarations

Supplementary Information

Refer to Web version on PubMed Central for supplementary material.

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by Human Ethics Committee of Bengbu Medical College. Written informed consent to participate in this study was provided.

Conflicts of interest

The authors declare no financially oriented conflicts of interest.

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