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Aberrant expression of PD-1 on B cells and its association with the clinical parameters of systemic lupus erythematosus

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Abstract

Background: Programmed death 1 (PD-1) is an immunoregulatory receptor that inhibits T cell activation and proliferation upon binding to its cognate ligand (PD-L1). However, the role of the PD-1/PD-L1 axis in B cell function, especially in inflammatory and autoimmune disorders, is less clear. The aim of this study was to analyze the PD-1 expression patterns on multiple B cell subpopulations isolated from systemic lupus erythematosus (SLE) patients, and determine their clinical relevance. Results: The frequency of B cells increased significantly in patients with active SLE compared with healthy controls and patients with inactive SLE. In particular, the frequencies of the IgD CD27 and IgD CD27high (plasmablast cells) subpopulations were significantly higher in the patients compared to healthy individuals. Interestingly, the patients with active SLE harbored an increased proportion of the PD-1+ B cells, which correlated significantly with the disease severity (SLEDAI scores), incidence of lupus nephritis, and the circulating levels of autoantibodies and complement factors. Furthermore, the primary PD-1+ B cells isolated from the peripheral blood of SLE patients proliferated faster and secreted more anti-dsDNA antibodies and immunoglobulins in vitro compared to the PD-1+/- B cells from healthy controls. Conclusions: PD-1 is overexpressed on all B cell subpopulations of SLE patients and associated with disease progression.

Background

Systemic lupus erythematosus (SLE) is a chronic inflammatory condition that affects the connective tissues of multiple organs, and is the result of excessive autoimmune response. It manifests as fatigue, fever, joint and muscular pain, and the characteristic “butterfly rash” across the cheeks and nose[1]. Although the exact etiological and pathological mechanisms underlying SLE are unknown, auto-reactive T and B cells have been frequently implicated[2-5].

The activation of T cells is primarily regulated by the programmed death 1 (PD-1) receptor and its ligands PD-L1 and PD-L2, which form an immune checkpoint that is essential for maintaining tolerance to self-antigens[6-8] and preventing autoimmune disorders[9-11]. Furthermore, the PD-1/PD-L axis is often disrupted in animal models simulating human autoimmune diseases[12-14], and directly affects immune activation and homeostasis[15-17]. Interestingly, blocking either PD-1 or PD-L1 in a murine model of lupus-like nephritis significantly alleviated tissue inflammation and other symptoms by inhibiting the autoreactive T cells and concomitantly increasing the proportion of the immunosuppressive CD8+ subset[17-19]. In addition, anti-PD-L1 immunoglobulin improved the survival of these mice by delaying proteinuria onset[20]. However, the exact pathological relevance of the PD-1/PD-L1 axis in human SLE remains to be elucidated.

Studies show that antigen-primed SLE patients that are recalcitrant to immunosuppressive therapy harbor an expanded IgD^- $CD27^+$ class-switched memory B cell population[2, 21], which can be attributed to aberrant B-cell receptor (BCR) editing and somatic hypermutation in the peripheral memory B cells[22]. These abnormal memory B cells significantly increase the risk of autoimmune responses on account of their lower antigen-dependent activation thresholds, as well as antigen-independent activation through the B-cell-activating factor, Toll-like receptor agonists or cytokines[23]. The IgD^- $CD27^-$ memory B-cell subset is also enriched in the SLE patients[24], and correlates with increased disease severity and renal involvement[25]. Interestingly, IgD^- $CD27^-$ B cells harboring mutated BCRs have been detected in the peripheral blood and lymphoid tissues of healthy donors as well[26, 27]. However, the functional relationship between the PD-1/PD-L1 axis and memory B cell activity in SLE is not clear. To this end, we analyzed the expression patterns of PD-1 on different B cell populations in SLE patients, and determined their correlation with clinical indices.

Methods

Patients

Seventy-four Asian-origin SLE patients diagnosed as per the 1997 American College of Rheumatology revised criteria[28], and 54 matched healthy controls were enrolled at the Department of Rheumatology of the First Affiliated Hospital of Bengbu Medical College, China. The medical records of all participants were screened for age, gender, blood cell counts, 24-h urinary protein secretion, circulating levels of anti-dsDNA, anti-nucleosome, anti-Smith (anti-Sm), anti-Sjogren syndrome A (anti-SSA) and anti-Sjogren syndrome B (anti-SSB) antibodies, complement component 3 (C3) and C4, IgG, IgM and IgA, and the erythrocyte sedimentation rates (ESR). The disease activity was scored according to the SLE Disease Activity Index (SLEDAI) and the patients were classified into the inactive (SLEDAI <10) and active (SLEDAI ≥10) groups.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood (3 mL) of SLE patients and controls using Ficoll-Hypaque density gradient centrifugation, and stained with FITC-anti-CD3, PE-anti-CD3, APC-anti-CD3, APC-Cy7-anti-CD19, FITC-anti-IgD, APC-anti-CD27, and PE-anti-PD-1 antibodies (all from Biolegend, 9727 Pacific Heights Blvd, San Diego, CA 92121, USA, 1:1000) as appropriate. The cells were acquired in a FACS Verse flow cytometer (BD Bioscience, San Jose, CA 95131, USA) and gated as previously described[29,30], and analyzed using the Flowjo software (Version X; Tree Star, Ashland, OR, USA). Furthermore, PBMCs isolated from 20 ml fasting blood samples were stained with the anti-CD19 and anti-PD-1 antibodies, and the $CD19^+PD\text{-}1^+$ and $CD19^+PD\text{-}1^-$ populations were sorted using FACSAria II (**BD Biosciences,** 2350 Qume Drive, San Jose, CA 95131, 877.232.8995, USA). After establishing >95% purity, the $PD\text{-}1^+$ and $PD\text{-}1^-$ B cells were stained with 5M CFSE (Molecular Probes, Waltham, MA, USA) in phosphate-buffered saline/0.1% bovine serum albumin at 37°C for 10 min to determine their viability. The cells were seeded in RPMI 1640 medium supplemented with 10% fetal calf serum and 10 ng/ml IL-2 and IL-10 (PeproTech Rocky Hill, NJ, USA) at the density of 2×10^4/well, and cultured for 7 days in the presence of 2.5 μg/ml CpG2006 oligonucleotide (Invivogen, San Diego, CA, USA) and/or 2.5 μg/ml goat F(ab)2 anti-human IgM. The medium was changed every 2 days, and the ensuing clones were then stained with anti-PD-1 antibody for flow cytometry analysis.

Enzyme linked immunosorbent assay (ELISA)

The levels of anti-dsDNA antibody and IgG in the supernatants (see above) were analyzed on days 1, 3, 5 and 7 of culture by ELISA (Biorbyt, San Francisco, CA, USA).

Statistical analysis

All data were presented as mean ± standard deviation, and compared by one-way analysis of variance or two-tailed Student *t* test as appropriate. The correlation between two variables was analyzed by Spearman or Pearson correlation coefficient. *P* values < 0.05 were considered statistically significant. All data were analyzed using SPSS 16.0 (IBM, Armonk, NY, USA).

Results

The B cell subpopulations are skewed in SLE

As shown in Table 1, the SLE patients and controls did not differ significantly in terms of age and gender, and the patients displayed the clinicopathological features of SLE. The relative proportion of $CD19^+$ B cells was significantly higher in the SLE patients compared to controls (P<0.05), and slightly higher among those with active as opposed to inactive disease (Fig 1A-B). Furthermore, the SLE patients also harbored significantly expanded $CD19^+$ IgD^- $CD27^-$ (double negative or DN) and $CD19^+$ IgD^- $CD27^{high}$ plasmablast cell (PC) populations compared to the healthy controls (Fig 1C-D). Interestingly, while the overall high B cell frequency did not affect the clinical symptoms or circulating autoantibody levels in the patients (data not shown), it correlated positively with the SLEDAI score and 24-h urinary protein levels, and negatively with C3 levels (Fig 1E). In contrast, a positive correlation was seen between the frequency of PCs and the IgM and C3 levels. Furthermore, the $CD27^+$ class-switched memory (SM) and $CD27^-$ non-switched memory (NSM) B cells respectively correlated with higher SLEDAI cores and 24h urinary protein levels. Both populations showed a significant positive correlation with IgG levels and a negative correlation with that of IgM. The naïve B cells on the other hand were negatively associated with both SLEDAI and IgG levels (Table 2). Patients exhibiting the malar rash and positive for anti-histones and anti-SSA52 antibodies showed an increased proportion of both SM and naïve B cells whereas the presence of only anti-SSB and anti-SSA52 antibodies correlated with an increase in the NSM population (Table 3). The other B cell subsets however did not show any significant association with the clinical and biochemical indices of SLE (Table 3).

B cells of SLE patients express PD-1 and correlate with the clinical progression

The frequency of the $PD\text{-}1^+$ B cells was significantly higher in the SLE patients compared to the healthy controls, as well as in the patients with active as opposed to inactive disease (Fig. 2A-B). Furthermore, PD-1 was overexpressed on all B cell subpopulations in SLE patients (Fig. 2C-D). The expanded $PD\text{-}1^+$ B cell population in the patients was associated with increased SLEDAI scores, as well as higher 24-h urinary protein levels. In addition, the serum levels of IgG and IgM respectively correlated positively and negatively with these cells (Fig. 2E). The frequency of $PD\text{-}1^+$ B cells was significantly higher in patients positive for the anti-dsDNA (P = 0.040), anti-histone (P = 0.025) and anti-SSA52 (P = 0.048) antibodies, and those presenting lupus nephritis (P <0.0001) and oral ulcers (P = 0.05). In contrast, no significant association was observed between $PD\text{-}1^+$ B cells and the hematological manifestations of SLE, arthritis or serositis (Table 4). We also analyzed the clinical significance of the distinct B-cell subsets expressing PD-1 (Table 5), and found that the $PD\text{-}1^+$ PCs correlated positively associated with SLEDAI scores, 24-h urinary protein secretion and IgG levels, $PD\text{-}1^+$ SM B cells with SLEDAI scores and 24-h urinary protein levels, and the $PD\text{-}1^+$ NSM and naive B cells with only IgG levels (Fig. 2F). Based on these findings, we surmised that the PD-1-expressing B cells are the effectors of SLE progression.

$PD\text{-}1^+$ B cells from SLE patients secrete large amounts of autoantibodies

To validate the above hypothesis, we isolated primary $PD\text{-}1^+$ and $PD\text{-}1^-$ B cells from the SLE patients and controls, and cultured them *in vitro* in the presence of CpG DNA. As shown in Fig. 3A-B, the $PD\text{-}1^+$ B cells from SLE patients were highly responsive to CpG DNA stimulation and showed markedly higher proliferation rates compared to the $PD\text{-}1^+$ B cells from healthy controls, as well as the $PD\text{-}1^-$ B cells isolated from SLE patients or controls. In addition, the SLE $PD\text{-}1^+$ B cells secreted significantly higher levels of anti-dsDNA antibodies (P <0.01, P <0.001; Fig. 3C) and IgG (P = 0.0261; Fig. 3D) compared to the control $PD\text{-}1^+$ and SLE/control $PD\text{-}1^-$ cells. Thus, the auto-reactive $PD\text{-}1^+$ B cells likely mediate the pathological changes in SLE by secreting large amounts of autoantibodies.

Discussion

Activated B cells are the key effectors of SLE development and progression, and induce the pathological changes by producing autoantibodies and inflammatory cytokines. In addition, they also activate specific T cells by functioning as antigen presenting cells[31]. Previous studies have reported significant changes in the proportion of different B-cell subsets in SLE patients[32, 33]. Consistent with this, we detected a significant increase in the frequency of different B cell phenotypes in SLE patients, and particularly of the SM and DN cells among those with active disease. A previous study reported increased frequency of DN B cells in SLE patients, which correlated to higher SLEDAI scores and elevated anti-dsDNA and anti-Sm antibodies in circulation[27]. Other studies have identified an aberrant $CD19^+$ IgD^- $CD27^-$ $CXCR5^-$ B cell subset in SLE patients, which is closely associated with the inflammatory changes characteristic of the disease[34, 35]. In agreement with our findings, Kubo et al[36] also detected increased proportion of both the DN and PC subsets in SLE patients compared to the healthy controls. In our study however, only the expanded $PD\text{-}1^+$ $CD19^+$ sub-population was associated with increased disease severity, overproduction of autoantibodies and the clinical manifestations, indicating that it plays a key role in SLE progression as opposed to the other B cell subsets.

PD-1/PD-L1 binding suppresses T-cell activation and expansion by inhibiting TCR-dependent signaling[7, 37]. Not surprisingly therefore, the immunosuppressive PD-1-expressing CD8$^+$ T cells are exhausted during chronic viral infection[38, 39]. In stark contrast, clonal expansion of antigen-primed PD-1$^+$CD8$^+$ T effector cells has been observed during chronic inflammation[40], and the PD-1$^+$ CD4$^+$ T cell population in mice with lupus-like symptoms secrete excessive amounts of interferon (IFN)-γ[17]. Consistent with this, PD-1$^+$CD4$^+$ T cells isolated from the blood of SLE patients activated B cells *in vitro* in the presence of interleukin (IL)-10[41]. These findings point to an immunogenic role of PD-1 in chronic inflammatory and autoimmune disorders, which is contradictory to its inhibitory effect on the phagocytic activity of tumor-associated macrophages[42]. This strongly indicates the existence of multiple functionally distinct immune cell subsets with differential PD-1 expression. Indeed, Thibult et al identified several B-cell subpopulations with divergent PD-1 levels[43], and found that inhibiting PD-1 signaling activated B cells, promoted their clonal expansion and increased the production of effector cytokines[43]. Similarly, PD-1 blockade in *Streptococcus pneumoniae* capsule-primed B cells significantly enhanced proliferation and immunoglobulin production[44].

Although the above findings clearly implicate PD-1 signaling in B cell survival and function, its potential role in SLE is largely unknown. We detected a substantial CD19$^+$ PD-1$^+$ B cell population in the SLE patients, which correlated significantly with disease severity, inflammation and high levels of circulating autoantibodies. *In vitro* expansion of these cells was also associated with increased proliferation and secretion of IgG and anti-dsDNA antibodies. Contradictory to a previous study that reported an inhibitory effect of PD-1 on B cell activation[45], our findings indicate that an aberrant PD-1-expressing B cell subset is the likely autoimmune effector in SLE. It is possible that the abnormal activation of these auto-reactive B cells is due to certain SLE-related pathological factors rather than PD-1, wherein the latter is merely a marker of this population and not functionally relevant. Furthermore, PD-1 might be upregulated on the B cells following their activation and in fact exert an inhibitory effect via negative feedback. A previous study showed elevated PD-1 on the IgM$^+$ IgD$^+$ CD27$^+$ memory B cells as opposed to the naïve and SM populations[43]. Although all B-cell subpopulations in the SLE patients of our cohort overexpressed PD-1, only some of these subsets were associated with autoantibody production and clinical parameters. The mechanism underlying PD-1 overexpression in the autoreactive B cells, the functional importance of specific PD-1$^+$ B cell subsets in SLE, and the potential interactions between the PD-1$^+$ B cells and T cells remain to be elucidated.

Conclusions

To summarize, the B cell phenotypes and PD-1 expression pattern are skewed in SLE patients, and the expanded CD19$^+$PD-1$^+$ population is primarily associated with the pathological changes in SLE.

Abbreviations

C3 :	complement 3
C4 :	complement 4
dsDNA:	double-stranded DNA
IgA:	immunoglobulin A
IgG:	immunoglobulin G
LN:	lupus nephritis
N	naïve
NSM	non-switched memory
PC	plasmablast cells
PD-1	programmed death 1
PD-L1	programmed death ligand 1
Sm:	smith
SSA:	Sjögren syndrome antigen A
SLE:	systemic lupus erythematosus
SLEDAI:	systemic lupus erythematosus disease activity index
SM	switched memory.
SSB:	Sjögren syndrome antigen B
U1snRNP:	U1 small nuclear ribonucleoprotein

Declarations

Ethics approval and consent to participate

All participants provided informed written consent. This study was approved by the institutional review board of the First Affiliated Hospital of Bengbu Medical College.

Consent for publication

The consent to publish has been acquired from each patient at the beginning of study.

Availability of data and material

The data are owned by Changhao Xie. All data are available from the corresponding author on reasonable request.

Competing interests

The authors declare no financial interests.

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Authors' contributions

CX, YuanWang conceived and designed the work. QW, CJ contribute to collect peripheral blood samples of subjects. YLu, QZ and YLi performed the experiments. QZ, YanWang, WZ analyzed data and statistical analysis. YLu and CX drafted the manuscript. ZL and HW critically revised the manuscript for important intellectual content.

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Tables

Table 1. Characteristics of SLE patients and healthy controls (*mean* ± SD, %).

Characteristic	SLE	Control	*P* value
Cases	*N* = 74	*N* = 54	
Number of males/females	3/71	3/51	NS
Age (year)	31.42 ± 12.02	27.1 ± 8.1	NS
Disease duration (month)	49.13 ± 9.331	-	
Clinical features			
SLEDAI≥10	43	-	
Lupus nephritis	48	-	
Raynaud’s phenomenon	27	-	
Malar rash	32	-	
Fever	47	-	
Oral ulcer	16	-	
Arthritis	35	-	
Serositis	32	-	
Neurological disorder	18	-	
Interstitial lung	17	-	
Laboratory findings			
Anti-dsDNA(+)	54	-	
Anti-SmD1(+)	60	-	
Anti-U1snRNP(+)	47	-	
Anti-SSA60(+)	55	-	
Anti-SSA52(+)	27	-	
Anti-SSB(+)	25	-	
C3↓	78	-	
C4↓	68	-	
IgA↑	60	-	
IgG↑	59	-	

Values are number (%) of patients unless indicate otherwise.

NS, No significance; SLE, systemic lupus erythematosus. SLEDAI, SLE Disease Activity Index; Anti-SmD1, Anti-Smith D1; Anti-dsDNA, Anti-double-stranded DNA; Anti-U1snRNP, Anti-U1 small nuclear ribonucleoprotein; Anti-SSA, Anti- Sjögren syndrome antigen A; Anyi-SSB, Anti-Sjögren syndrome antigen B; C3/C4, complement component 3/4; IgG/IgM/IgA, immunoglobulin G/M/A.

Table 2. Correlation of the frequencies of PC, SM, NSM, DN and N B cells in SLE patients with SLEDAI or biochemical indices.

Laboratory test parameters	Cases	PC B cells	SM B cells	NSM B cells	DN B cells	N B cells
		r	*r*	*r*	*r*	*r*
SLEDAI	74	0.03501	0.08304*	0.01213	0.00436	-0.0845*
Amounts of proteinuria (g/24 h)	69	-0.01255	0.01846	0.09926*	-0.02297	-0.03198
Immunoglobulin G (g/L)	74	0.02445	0.07461*	0.08158*	0.05188	-0.2087*
Immunoglobulin A (g/L)	74	0.06296	0.03587	0.1280	-0.1827	-0.04001
Immunoglobulin M (g/L)	74	0.09914*	-0.02337*	-0.07387*	0.005707	-0.02223
Complement 3 (g/L)	74	0.1162*	0.006449	0.03364	0.001436	-0.06764
Complement 4 (g/L)	74	-0.1857	0.1340	0.09826	-0.1698	0.00045

DN, double negative; naïve; NSM, non-switched memory; PC, plasmablast cells; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index; SM, switched memory.

*$P < 0.05$.

Table 3. Association between the percentages of PC, SM, NSM, DN and N B cells and the clinic-pathological parameters in SLE patients (*mean* ± SD, %).

Parameters		Cases	PC Bcells (%)	*P* value	SM B cells (%)	*P* value	NSM B cells (%)	*P* value	DN B cells (%)	*P* value	NB cells (%)	*P* value
Anti-dsDNA	+	44	7.799 ±0.9774	0.300	19.54 ±1.208	0.896	16.43 ±2.100	0.641	12.04 ±1.612	0.084	50.35 ±3.049	0.198
	-	30	6.449 ±0.626		19.88 ±2.527		14.97 ±2.196		43.99 ±3.049		50.35± 3.880	
Anti-histones	+	26	8.536 ±1.167	0.138	24.15 ±2.613	0.007	17.52 ±3.652	0.420	13.90 ±2.231	0.017	35.85 ±4.009	0.001
	-	48	6.556 ±0.739		17.26 ±1.169		14.92 ±1.297		8.729 ±1.002		52.37 ±2.692	
Anti-smD1	+	45	7.919 ±0.929	0.193	20.32±1.549	0.522	16.31 ±2.045	0.702	11.07 ±1.399	0.537	44.20 ±3.164	0.225
	-	29	6.216 ±0.718		18.68 ±2.082		15.10 ±2.293		9.735 ±1.587		50.23 ±3.669	
Anti-U1snRNP	+	35	7.856 ±1.176	0.371	20.30 ±1.854	0.641	14.95 ±1.662	0.586	11.82 ±1.717	0.253	44.87 ±3.594	0.508
	-	39	6.709 ±0.5865		19.13 ±1.684		16.63 ±2.497		9.406 ±1.255		48.09 ±3.269	
Anti-nucleo	+	35	7.625 ±0.954	0.571	18.42 ±1.839	0.326	14.12 ±2.425	0.277	12.28 ±1.807	0.108	47.52 ±3.960	0.704
	-	39	6.898 ±0.853		20.87 ±1.675		17.46 ±1.879		8.904 ±1.075		45.67 ±2.870	
Anti-SSA60	+	41	7.151 ±0.679	0.832	21.38 ±1.710	0.062	16.74 ±2.194	0.424	11.60 ±1.399	0.173	53.01 ±3.811	0.049
	-	33	7.437 ±1.323		16.54 ±1.428		14.17 ±1.570		8.596 ±1.458		43.08 ±2.999	
Anti-SSA52	+	20	7.444±1.179	0.856	24.30 ±3.274	0.022	23.26 ±4.333	0.02	8.155 ±1.205	0.167	50.18 ±2.671	0.013
	-	54	7.180 ±0.759		17.9 ±1.131		13.09 ±1.172		11.43 ±1.356		36.82 ±4.695	
Anti-SSB	+	15	6.865 ±1.446	0.761	21.24 ±3.548	0.530	22.85 ±5.868	0.019	9.406 ±1.879	0.587	39.57 ±6.199	0.145
	-	59	7.350 ±0.713		19.28 ±1.283		14.05 ±1.137		10.83 ±1.231		48.34 ±2.559	
Anti-P0	+	30	7.653 ±1.066	0.605	17.26 ±1.560	0.108	12.82 ±1.146	0.103	11.78 ±1.774	0.334	50.24 ±3.656	0.210
	-	44	6.978 ±0.791		21.33 ±1.767		17.90 ±2.408		9.704 ±1.287		44.06 ±3.176	
LN	Yes	36	6.569 ±0.657	0.299	20.07 ±2.042	0.761	17.65 ±2.414	0.250	10.70 ±1.567	0.889	44.98 ±3.355	0.525
	No	38	7.898 ±1.067		19.31 ±1.474		14.12 ±1.886		10.40 ±1.425		48.07 ±3.481	
Malar rash	Yes	24	9.554 ±1.404	0.011	25.54 ±2.717	0.001	15.96 ±2.656	0.957	10.92 ±1.755	0.807	38.00 ±4.333	0.013
	No	50	6.146 ±0.607		16.87 ±1.111		15.78±1.883		10.36 ±1.316		50.68 ±2.743	
Arthritis	Yes	26	6.391 ±0.694	0.322	18.61 ±2.247	0.529	13.95 ±1.137	0.367	8.935 ±1.429	0.261	52.08 ±3.650	0.093
	No	48	7.718 ±0.902		20.26 ±1.488		16.86 ±2.267		11.42 ±1.415		43.58 ±3.089	
Serositis	Yes	18	6.234 ±1.018	0.368	18.57 ±2.465	0.615	11.14 ±1.003	0.081	9.713 ±1.915	0.655	54.32 ±4.281	0.068
	No	56	7.579 ±0.772		20.04 ±1.445		17.35± 1.953		10.81 ±1.250		44.07 ±2.812	
Interstitial lung	Yes	13	6.985 ±1.018	0.848	20.25 ±2.737	0.834	11.78 ±1.163	0.222	9.840 ±2.703	0.758	51.09 ±4.689	0.390
	No	61	7.308 ±0.742		19.56 ±1.397		16.70 ±1.819		10.70 ±1.145		45.60 ±2.748	

Anti-SmD1, anti-Smith D1; Anti-dsDNA, Anti-double-stranded DNA; Anti-U1snRNP, Anti- U1 small nuclear ribonucleoprotein; Anti- nucleo, Anti-nucleosomes, Anti-SSA, Anti- Sjögren syndrome antigen A; Anyi-SSB, Anti-Sjögren syndrome antigen B ,Anti-P0, anti-ribosomal P0 antibody ;DN, double negative; LN, lupus nephritis; N, naïve; NSM, non-switched memory; PC, plasmablast cells; SLE, systemic lupus erythematosus; SM, switched memory.

Table 4. Association between the percentage of PD-1^{+}B cells and clinic-pathological parameters in SLE patients (*mean* ± SD, %).

Parameters		Cases	PD-1⁺B cells (%)	P
Anti-dsDNA	+	44	12.96±2.023	0.040
	-	30	8.109±1.060	
Anti-histone	+	26	15.03±2.454	0.025
	-	48	10.22±0.798	
Anti-smD1	+	45	13.06±1.834	0.373
	-	29	11.17±1.220	
Anti-U1snRNP	+	35	12.53±1.617	0.571
	-	39	11.35±1.326	
Anti-nucleo	+	35	12.21±1.397	0.766
	-	39	11.59±1.540	
Anti-SSA60	+	41	12.82±1.459	0.234
	-	33	10.23±1.128	
Anti-SSA52	+	20	15.25±2.829	0.048
	-	54	10.67±0.9151	
Anti-SSB	+	15	13.47±2.930	0.451
	-	59	11.52±1.066	
Anti-P0	+	30	11.03±1.294	0.486
	-	44	12.51±1.498	
C3↓	Yes	58	12.21±1.475	0.881
	No	16	11.83±1.256	
IgG↑	Yes	44	12.52±1.686	0.621
	No	30	11.47±1.305	
Lupus nephritis	Yes	36	15.55±1.912	<0.0001
	No	38	8.468±0.386	
Malar rash	Yes	24	14.08±2.459	0.146
	No	50	10.87±0.956	
Fever	Yes	35	12.14±1.584	0.838
	No	39	11.71±1.363	
Oral ulcer	Yes	12	16.49±3.818	0.049
	No	62	11.02±0.964	
Arthritis	Yes	26	12.10±1.937	0.895
	No	48	11.81±1.210	
Serositis	Yes	18	9.87±1.491	0.265
	No	56	12.57±9.501	
Interstitial lung	Yes	13	9.503±1.869	0.283
	No	61	12.42±1.181	

Anti-SmD1,anti-Smith D1,Anti-dsDNA, Anti-double-stranded DNA; Anti-U1snRNP,Anti- U1 small nuclear ribonucleoprotein; Anti-nucleo, Anti-nucleosomes, Anti-SSA, Anti- Sjögren syndrome antigen A; Anti-SSB, Anti-Sjögren syndrome antigen B ,Anti-P0, anti-ribosomal P0 antibody, C3complement component 3; IgG, immunoglobulin G; SLE, systemic lupus erythematosus.

Table 5. Association between PD-1 expression on PC, SM, NSM, DN and N B cell subsets and the clinic-pathological parameters in SLE patients (*mean* ± SD, %).

Parameters		Cases	PC PD-1+B cells (%)	*P* value	SM PD-1+B cells (%)	*P* value	NSM PD-1+B cells (%)	*P* value	DN PD-1+B cells (%)	*P* value	N PD-1+B cells (%)
Anti-dsDNA	+	44	7.922±1.685	0.040	10.38±2.195	0.010	6.976±0.492	0.454	17.67±1.212	0.928	7.894±0.600
	-	30	4.873±0.373		5.425±0.372		6.415±0.540		17.50±1.495		6.613±0.647
Anti-histone	+	26	7.800±1.928	0.09	9.113±2.459	0.196	7.000±0.743	0.615	18.98±1.711	0.280	7.521±0.726
	-	48	5.193±0.404		6.526±0.6131		6.612±0.397		16.85±1.103		7.296±0.569
Anti-smD1	+	45	6.369±0.867	0.661	7.029±1.130	0.599	7.155±0.502	0.166	17.03±1.041	0.450	7.376±0.514
	-	29	5.705±1.314		8.065±1.699		6.117±0.495		18.49±1.768		7.372±0.824
Anti-U1snRNP	+	35	6.227±1.105	0.880	7.640±1.509	0.840	7.047±0.595	0.441	16.74±1.427	0.389	7.548±0.672
	-	39	6.003±0.988		7.251±1.212		6.480±0.442		18.37±1.234		7.219±0.600
Anti-nucleo	+	35	6.397±1.044	0.705	7.435±1.397	1.000	6.978±0.598	0.544	17.75±1.352	0.875	6.600±0.567
	-	39	5.836±1.039		7.435±1.315		6.531±0.432		17.45±1.311		8.108±0.668
Anti-SSA60	+	41	6.807±1.100	0.198	7.970±1.444	0.449	6.646±0.4523	0.707	18.08±1.197	0.489	7.022±0.542
	-	33	4.820±0.397		6.448±0.4802		6.936±0.6262		16.71±1.496		8.025±0.779
Anti-SSA52	+	20	8.752±2.518	0.027	10.19±3.281	0.077	6.683±0.7926	0.914	17.75±1.722	0.923	7.690±0.908
	-	54	5.130±0.323		6.413±0.4535		6.773±0.4091		17.54±1.121		7.258±0.514
Anti-SSB	+	15	8.457±2.422	0.107	9.016±3.275	0.406	7.133±0.9511	0.598	19.14±1.641	0.411	6.294±0.615
	-	59	5.512±0.676		7.033±0.8705		6.650±0.3917		17.21±1.096		7.649±0.533
Anti-P0	+	30	5.945±0.458	0.855	7.599±0.8584	0.888	7.460±0.6516	0.107	17.19±1.591	0.720	7.299±0.604
	-	44	6.220±1.198		7.323±1.498		6.263±0.4132		17.88±1.152		7.426±0.632
C3↓	Yes	58	1.256±0.910	0.417	7.486±1.172	0.920	6.684±0.4223	0.739	17.46±1.061	0.787	7.234±0.485
	No	16	4.967±0.747		7.251±1.197		6.981±0.7278		18.09±2.037		7.886±1.098
IgG↑	Yes	44	6.414±0.890	0.627	7.700±1.219	0.745	6.666±0.497	0.793	16.81±1.316	0.323	6.909±0.483
	No	30	5.686±1.252		7.067±1.538		6.862±0.539		18.70±1.282		8.021±0.824
LN	Yes	36	7.557±1.424	0.054	9.6884±1.861	0.020	6.394±0.474	0.347	18.82±1.413	0.207	8.421±0.795
	No	38	4.737±0.373		5.301±0.3515		7.084±0.550		16.45±1.223		6.383±0.378
Malar rash	Yes	24	7.344±2.100	0.246	8.811±2.699	0.320	7.443±0.775	0.189	15.76±1.686	0.174	7.200±0.725
	No	50	5.516±0.409		6.775±0.5732		6.415±0.388		18.48±1.112		7.459±0.565
Oral ulcer	Yes	12	7.816±3.071	0.309	10.04±3.767	0.231	5.722±0.674	0.218	20.49±2.405	0.176	8.323±1.094
	No	62	5.779±0.653		6.931±0.879		6.947±0.412		17.04±1.007		7.191±0.489
Arthritis	Yes	26	6.233±1.449	0.902	8.494±1.873	0.417	6.505±0.578	0.627	16.09±1.732	0.238	7.188±0.796
	No	48	6.042±0.824		6.861±1.066		6.880±0.470		18.42±1.090		7.476±0.541
Serositis	Yes	18	4.698±0.675	0.278	6.602±1.173	0.623	6.905±0.7578	0.810	19.11±1.780	0.363	6.546±0.729
	No	56	6.562±0.938		7.703±1.202		6.698±0.419		17.11±1.096		7.641±0.539
Interstitial lung	Yes	13	4.205±0.726	0.233	5.163±0.720	0.273	5.705±0.744	0.188	19.58±1.891	0.331	6.752±0.973
	No	61	6.515±0.868		7.919±1.136		6.971±0.409		17.18±1.059		7.507±0.501

Anti-SmD1,Anti-Smith D1,Anti-dsDNA, Anti-double-stranded DNA; Anti-U1snRNP, Anti-U1 small nuclear ribonucleoprotein; Anti- nucleo, Anti-nucleosomes, Anti-SSA, Anti- Sjögren syndrome antigen A; Anti-SSB, Anti-Sjögren syndrome antigen B, Anti-P0, anti-ribosomal P0 antibody, C3, complement component 3; DN, double negative; IgG, immunoglobulin G; LN, lupus nephritis; N, naïve; NSM, non-switched memory; PC, plasmablast cells; SLE, systemic lupus erythematosus; SM, switched memory.

Figures

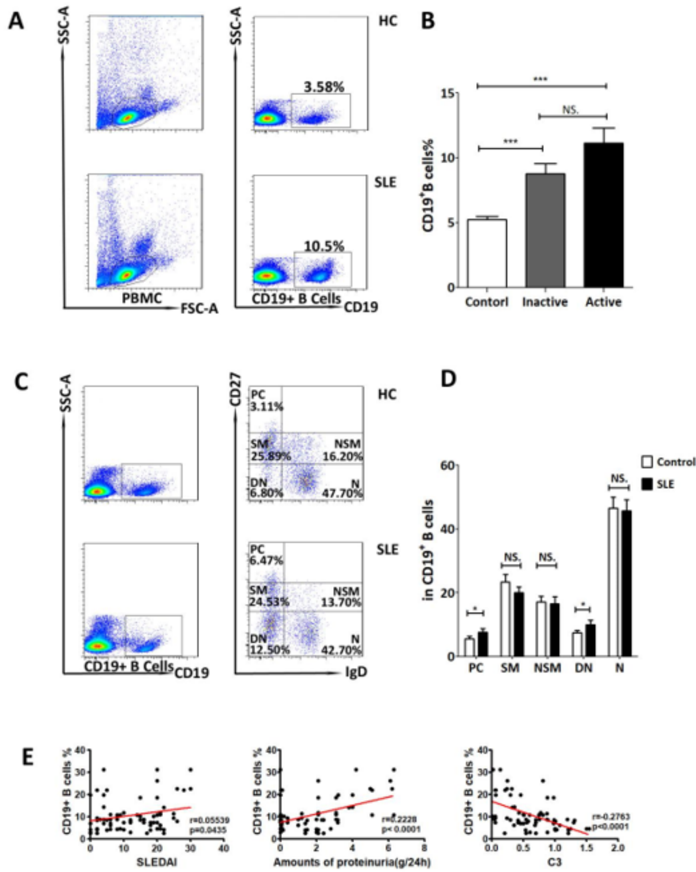

Figure 1

The proportion of B cell subsets is aberrant in SLE patients. (A) Representative dot plots from one patient and one control sample indicating the gating strategy for CD19+ B cells. (B) Percentage of CD19+ B cells in the controls and patients with inactive (SLEDIA <10) and active (SLEDIA ≥10) SLE. ***P < 0.00001 (one-way analysis of variance). (C) Representative dot plots from one patient and one control sample indicating the distribution of B-cell subsets in the peripheral blood. PC - plasmablast cells (CD27high IgD−); SM - switched memory (CD27+ IgD−); NSM - non-switched memory (CD27+ IgD+); DN - double negative (CD19+ IgD− CD27−); N - Naïve (CD27− IgD+). (D) Proportion of B-cell subsets in controls and patients (x ± s, %); *P<0.05 (Student t test). (E) Correlation of CD19+ B cell frequency with SLEDAI (SLE Disease Activity Index) scores, proteinuria and C3 levels. All data were expressed as mean ± standard deviation.

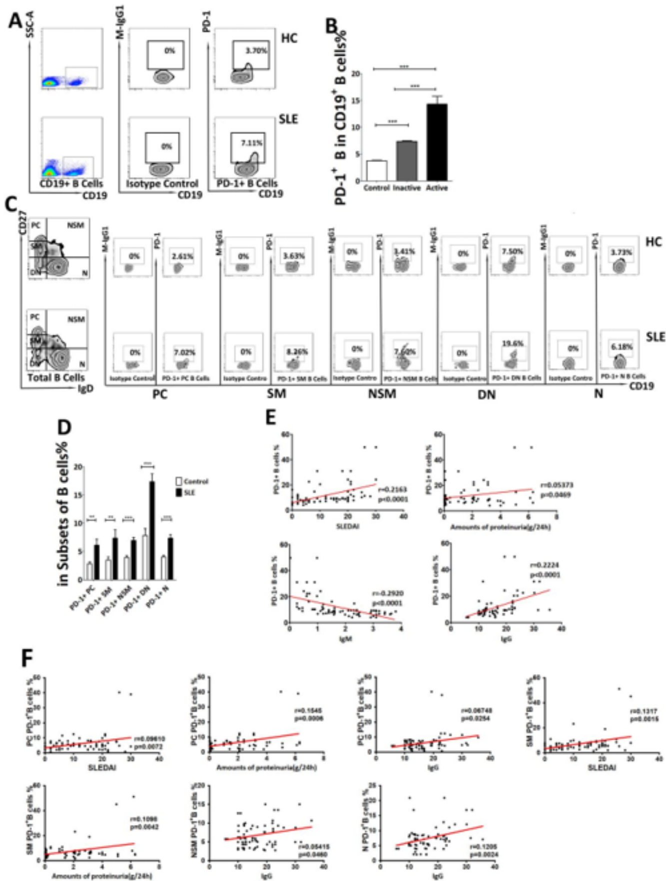

Figure 2

PD-1+ is differentially expressed on the B cells from SLE patients and controls. (A) Representative dot plots from one patient and one control sample indicating the gating strategy for CD19+ PD-1+ B cells. (B) Percentage of PD-1+ B cells in the controls and patients with inactive (SLEDIA <10) and active (SLEDIA ≥10) SLE. ***P < 0.0001 (one-way analysis of variance). (C) Representative dot plots from one patient and one control sample indicating the gating strategy for PD-1+ PC, SM, NSM, DN and N subsets. (D) Percentage of PD-1+ B-cell subsets in controls and patients. **P <0.001, ***P <0.0001 (mean ± SD, %; Student t test). (E) Correlation of CD19+ PD-1+ B cell frequency with SLEDAI (SLE Disease Activity Index) scores, proteinuria and C3 levels. All data were expressed as mean ± standard deviation. (F) Correlation of CD19+ PD-1+ B cell subsets with SLEDAI (SLE Disease Activity Index) scores, proteinuria and C3 levels. All data were expressed as mean ± standard deviation.

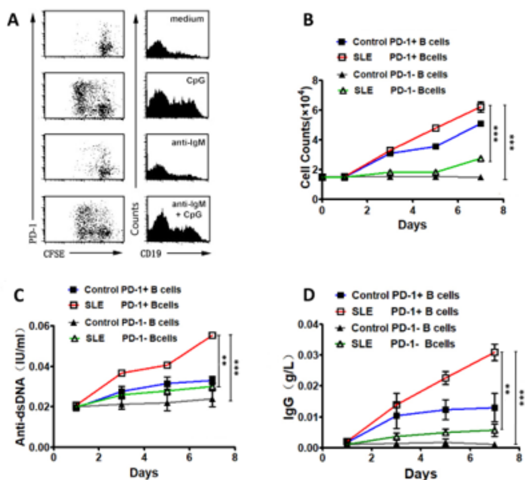

Figure 3

Proliferation of PD-1+ or PD-1− B cells in response to CpG DNA stimulation. (A) Proliferation of B cells in the presence of CpG DNA and/or anti-IgM antibody. (B) Proliferation rates of SLE/control PD-1+ and PD-1− B cells at days 1, 2, 3 and 7 of CpG DNA stimulation. (C-D) The levels of (C) anti-dsDNA antibodies and

(D) IgG secreted by the control PD-1+, SLE PD-1+, control PD-1− and SLE PD-1− B cells at days 1, 2, 3 and 7 of CpG DNA stimulation. All data were expressed as mean ± standard deviation. *$P < .05$, **$P < .01$, ***$P < .001$. One-way analysis of variance followed by a Newman–Keuls post hoc test.