Dysfunction of CD27+IgD+B cells correlates with aggravated systemic lupus erythematosus

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Abstract

The apoptotic signaling pathway is obviously disordered in systemic lupus erythematosus (SLE). Concurrent occurrence of induced apoptotic cell death and altered phagocytosis promotes autoantigen production, which leads to the biosynthesis of autoantibodies and autoimmune disorders. Natural IgM (nIgM) is important in clearing apoptotic cells and preventing them from triggering deleterious autoimmunity. B-1- and innate-like B- (ILBs) cells are the main nIgM producers. Human CD27+IgD+B cells (un-switched memory B cells) are considered ILBs. However, their functional properties in SLE remain undefined. Here, individuals with SLE showed markedly reduced CD27+IgD+B cell amounts. Moreover, these cells had altered function in terms of natural antibody-like IgM production. CD27+IgD+B cells also showed negative correlations with clinical and immunological properties in SLE patients. Following effective treatment achieving SLE remission, CD27+IgD+B cell amounts were restored. Jointly, these findings suggest that CD27+IgD+B cell dysfunction potentially contributes to the exacerbation of SLE, and modulating their features may represent a powerful tool for treating this persistent disease.

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Summary

The apoptotic signaling pathway is obviously disordered in systemic lupus erythematosus (SLE). Concurrent occurrence of induced apoptotic cell death and altered phagocytosis pro-

motes autoantigen production, which leads to the biosynthesis of autoantibodies and autoimmune disorders. Natural IgM (nIgM) is important in clearing apoptotic cells and preventing them from triggering deleterious autoimmunity. B-1- and innate-like B- (ILBs) cells are the main nIgM producers. Human CD27$^+$IgD$^+$B cells (un-switched memory B cells) are considered ILBs. However, their functional properties in SLE remain undefined. Here, individuals with SLE showed markedly reduced CD27$^+$IgD$^+$B cell amounts. Moreover, these cells had altered function in terms of natural antibody-like IgM production. CD27$^+$IgD$^+$B cells also showed negative correlations with clinical and immunological properties in SLE patients. Following effective treatment achieving SLE remission, CD27$^+$IgD$^+$B cell amounts were restored. Jointly, these findings suggest that CD27$^+$IgD$^+$B cell dysfunction potentially contributes to the exacerbation of SLE, and modulating their features may represent a powerful tool for treating this persistent disease.

Keywords: systemic lupus erythematosus, CD27$^+$IgD$^+$ B cells, innate-like B cells , natural IgM

Introduction

Systemic lupus erythematosus (SLE), a major systemic autoimmune disorder, affects humans with genetic susceptibility in certain environmental conditions(1). Its pathogenetic mechanisms remain unclear. It is admitted that autoantibodies represent the main determinants of SLE's pathological signs. Antibodies could harm self-tissues via complement-mediated inflammatory reactions, programmed cell death and immune-complexes associated injury. However, the exact role of antibodies in SLE remains unraveled(2).

Apoptosis is strictly controlled by cell surface proteins (e.g., Fas) and intracellular proto-oncogenes such as Bcl-2 and Bax family members(3). It has been demonstrated that the apoptosis signaling pathway is obviously disordered in SLE. Abnormal production of a large number of apoptotic signaling molecules, including TNF-related apoptosis inducing ligand (TRAIL), TNF-like weak inducer of apoptosis (TWEAK) and death ligand FasL (Fas ligand), leads to abnormal increase of apoptosis(4, 5). In addition, individuals with SLE have trouble clearing apoptotic cells. Cell death (programmed cell death, necrosis and NETosis [affecting neutrophils via NETs]), provides most of the self-dsDNA that induces immune reactions and causes autoimmune disorders(6).

Natural immunity enables the identification and elimination of injured and apoptotic cells. Meanwhile, it also performs the intricate regulation of inflammatory response and enhances immune tolerance. Natural IgM (nIgM) represents a critical part of innate immunity in humans, reacting with multiple epitopes found in self-and non-self antigens(7). Therefore, nIgM deficiency increases the tendency toward the development of autoimmune disorders; nIgM is indeed very important in clearing apoptotic cells and preventing them from triggering autoimmunity.

B-1- and innate-like B- (ILBs) cells constitute the main natural IgM producers. It is admitted that 80% of serum natural IgM is produced by B1 cells(8). On the other hand, ILBs represent a heterogeneous group of atypical B cells possessing innate sensing and responding features. Mouse ILBs comprise B1-, marginal zone (MZ) B- and other related B cells. CD19$^+$CD27$^+$IgD$^+$B cells (or unswitched memory B cells) have been considered ILBs in humans(9). ILBs keep natural IgM amounts at the steady state, and quickly gain immune modulatory features via secretion of natural IgM and IL-10 after innate activation(10). Our previous study indicated CD27$^+$IgD$^+$B cell impairment in rheumatoid arthritis (RA), likely contributing to disease perpetuation(11). However, CD27$^+$IgD$^+$B cell properties and function in SLE remain undefined.

Here, we determined the amounts and natural antibody-like IgM-producing capacity of CD27$^+$IgD$^+$B cells in SLE patients. In addition, we analyzed their clinical associations and revealed their tendency after therapy.

Materials and Methods

Patients and samples

Totally 50 SLE and 50 healthy control (HC) patients were enrolled. Individuals with SLE were treated in

the First Affiliated Hospital of Baotou Medical College that applied the American College of Rheumatology criteria for the classification of SLE(12). The main features of the SLE cases are listed in Table 1. Disease activity was measured by the modified Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2K)(13), and classified as low disease activity (LDA) (SLEDAI of 0-4, n=34) and active disease (SLEDAI above 4, n=16). This trial had approval from the Institutional Medical Ethics Review Board of Baotou Medical College, and signed informed consent was obtained from each participant.

Twelve SLE patients were assessed in a follow-up study. All SLE cases were administered immunosuppressants, with disease remission. Blood specimens were collected prior to treatment and at 4 weeks post-treatment. Patient features pre- and post-treatment are summarized in Table 2

Flow cytometry analysis

For $CD27^+IgD^+$ B cell detection, 2-ml blood specimens were collected from SLE and HC patients, and 100μl fresh whole blood cells was extracted and incubated with the following antibodies:APC-CY7-labeled anti-CD19 (BioLegend, USA), APC-linked anti-CD27 (eBioscience, USA) and FITC-labeled anti-IgD (eBioscience). Then, erythrocyte lysis was carried out with the RBC lysis buffer (MultiSciences, China) and the remaining cells were assessed on a FACS Aria II. Dead cells were excluded based on scatter properties and 7-AAD staining.

Cell sorting and culture

Fluorescence-activated cell sorting (FACS) was utilized to purify $CD27^+IgD^+$ B cells. Briefly, 10-ml blood specimens were obtained from SLE and HC patients, followed by peripheral blood mononuclear cell (PBMC) isolation by Ficoll density-gradient centrifugation and staining as described above. Next, the target cells were collected in RPMI 1640 containing 10% FBS and 2% antibiotics on a FACS Aria II flow cytometer as directed by the manufacturer (95–99% purity).

Quantitative real-time RT-PCR (qRT-PCR) analysis

Total RNA extraction from $CD27^+IgD^+$B cells utilized RNeasy Mini Kit (Qiagen, Germany). Reverse transcription was carried out with the RevertAid First Strand cDNA synthesis kit (Fermentas, USA) as instructed by the manufacturer, followed by qRT-PCR assessment. GAPDH and β-actin were employed for normalization, and the $2^{-\Delta\Delta^{\tilde{}}T}$ method was applied for data analysis(11).

ELISPOT

ELISPOT was carried out with ELISpotPLUS Human IgM Kit (MABTECH AB, Sweden). In brief, 10^4 purified $CD27^+IgD^+$ B cells and other B cell types from SLE and HC patients, respectively, were assessed for IgM after incubation with anti-CD40 (3μg/ml, eBioscience) and CpG (10μg/ml, Invivogen, USA) for 24h. An ImmunoSpot Analyzer (Cellular Technology Ltd., USA) was utilized for data analysis.

Statistics analysis

SPSS 17.0 (SPSS, USA) was employed for data analysis. Student's t-test, paired t-test, one-way ANOVA and Spearman rank correlation, respectively, were performed for statistical analysis as appropriate. $P<0.05$ indicated statistical significance.**Results$CD27^+IgD^+$B cell amounts are significantly decreased in SLE patients**

B cells were grouped into 4 subtypes according to CD27 and IgD levels, including naïve ($CD27^-IgD^+$), innate-like ($CD27^+IgD^+$), switched memory ($CD27^+IgD^-$) and double negative ($CD27^-IgD^-$) B cells (Figure 1A). To examine the function of $CD27^+IgD^+$B cells in SLE pathogenesis, their amounts were firstly compared between SLE and HC patients. The 50 HCs showed remarkably higher circulatory $CD27^+IgD^+$B cell amounts compared with the 50 individuals with SLE (Figure 1B and C).

$CD27^+IgD^+$ B cell amounts correlate with clinical and immunological features in SLE patients

The associations of $CD27^+IgD^+$ B cell amounts with demographic and clinical characteristics were analyzed. As shown in Figures 2A–L and Table 3, WBC, platelet count and serum C3 levels were positively correlated with $CD27^+IgD^+$B cell amounts. Besides, $CD27^+IgD^+$B cell amounts showed negative correlations with serum creatinine levels, SLEDAI and anti-dsDNA. However, no associations of $CD27^+IgD^+$B cells with disease duration, ESR, serum C4 levels and serum IgA, IgG and IgM levels were found. The above findings indicated a numerical deficiency of $CD27^+IgD^+$B cells in SLE.

$CD27^+IgD^+$B cells are less competent in producing IgM in SLE

To examine $CD27^+IgD^+$B cells' functional changes, their ability to produce IgM was assessed in SLE patients. $CD27^+IgD^+$B cells isolated from active SLE cases (SLEDAI>5) and HCs underwent ELISPOT and qRT-PCR. As depicted in Figure 3A, ELISPOT revealed significantly reduced IgM-producing ability for $CD27^+IgD^+$B cells in SLE, which was confirmed by decreased IgM mRNA levels as examined by qRT-PCR (Figure 3B). Jointly, the above data indicated that $CD27^+IgD^+$B cells had impaired function regarding IgM production in SLE.

$CD27^+IgD^+$B cells are recovered in SLE cases with treatment-related disease remission

To ascertain $CD27^+IgD^+$ B cells' usefulness as a biomarker of disease activity, whether they are restored following effective treatment was assessed. Totally 12 patients previously diagnosed with SLE were assessed during relapse and 4 weeks post-treatment initiation. As expected, treatment markedly decreased disease activity based on the SLEDAI (Table 2). In addition, anti-dsDNA and 24h urinary protein amounts were reduced, with serum C3 and C4 level normalization to a certain degree. Total leukocyte and platelet levels were heightened post-treatment.

Next, $CD27^+IgD^+$B cell amounts were assessed pre-treatment and at 4 weeks post-treatment initiation (Figure 4A). As measured by the SLEDAI, all cases had a significant reduction in disease activity after treatment (Figure 4B); meanwhile, $CD27^+IgD^+$B cell amounts were remarkably elevated (Figure 4C). Jointly, the above findings suggested that $CD27^+IgD^+$ B cell amount impairment in SLE patients could be alleviated by effective treatment.

Discussion

The current study revealed that $CD27^+IgD^+$B cells displayed reduced amounts and functional impairment regarding the production of natural antibody-like IgM in patients with SLE compared with healthy controls. In addition, $CD27^+IgD^+$B cell amounts were associated with clinical characteristics in SLE patients, and were restored by effective treatment.

In SLE, the number of apoptotic cells increases while phagocytosis is impaired(14, 15). Concurrent occurrence of increased apoptotic cell death and deficient phagocytosis represents a major factor in SLE pathogenesis, promoting autoantigen accumulation and subsequent autoantibody production and autoimmune disorders(16). Apoptotic cells undergo phagocytosis by professional phagocytic cells (e.g., macrophages) under normal conditions. However, apoptotic cell clearance depends not only on functional phagocytes, but also on soluble proteins acting as opsonins and/or bridging materials. Phagocytosis of apoptotic cells can be increased by C-reactive protein (CRP), serum amyloid P component (SAP), C1q, IgM, MBL and other proteins, forming a redundant backup mechanism. Previous studies have found that macrophages are three- to four-fold less phagocytic in the absence of IgM(17). Decreased amounts of natural IgM in SLE patients might reduce apoptotic cell clearance, with dead cells accumulating in blood(18). Individuals with SLE show anti-PC natural IgM level reduction, which correlates with disease duration. In addition, decreased anti-PC natural IgM amounts are associated with a higher frequency of cardiovascular events in human SLE (19). However, the causes of natural IgM defects in SLE patients remain largely unknown.

In a previous study, we demonstrated that $CD27^+IgD^+$B cells can readily secrete

IgM with poly-reactivity and low affinity. These $CD27^+IgD^+$B cell-associated IgM were coined natural antibody-like IgM. In this study, $CD27^+IgD^+$B cell amounts in SLE patients were remarkably reduced

and negatively associated with SLEDAI and anti-dsDNA autoantibodies. The above findings corroborate previously reported data(20, 21), jointly indicating that B cell-subsets are disordered in SLE patients. Since distinct B cell subsets have different functional characteristics, the imbalance of their proportions would lead to altered immune homeostasis and promote pathological events to some extent. Cytokines including IFN-γ, BAFF, TNF-α, IL-6 and IL-21 in the serum of SLE patients affect the B cell signaling pathway, thereby increasing B cell activation and differentiation(22-24). Therefore, we speculated that the inflammatory environment in SLE patients is one of the factors explaining the reduced $CD27^+IgD^+$B cell amounts, which deserves further investigation.

In addition, qRT-PCR and ELISPOT analyses showed that the ability of $CD27^+IgD^+$B cells to secrete IgM in human SLE was markedly reduced, indicating that in SLE patients, $CD27^+IgD^+$B cells have defects not only in quantity, but also in function. Therefore, this may also account for apoptotic cell accumulation and autoimmunity development in human SLE. Reduced TCR and/or BCR diversities have been reported in cancer and autoimmune disorders, as potential etiologic factors(25). Our previous research revealed that BCR profile in $CD27^+IgD^+$B cells is abnormal in RA. Therefore, we speculated that BCR profile in $CD27^+IgD^+$B cells might also be changed in SLE, and a follow-up study is underway to score $CD27^+IgD^+$B cells' BCR repertoire in SLE patients.

Overall, $CD27^+IgD^+$B cell amounts and function are altered in SLE. Therefore, $CD27^+IgD^+$B cells could help reliably detect active SLE, although their precise role in SLE development deserves further investigation.

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Disclosures

The authors have no conflict of interest.

Authors' contributions

Study conception and design, W. Z., Y. W. and K.L.; experiments, W. Z. and T.W.; data analysis, W. Z., Y. W. and F. H.; contribution with reagents, materials and analytical tools, L.F., F. H. and Y. W.; manuscript writing, W. Z.; manuscript revision , F. H. and K.L.

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Table 1 SLE patient features

Characteristic	SLE (N=50)
Age, mean(range), years	45.6(17-67)
Female/male	46/4
Duration of diagnosis, mean(range), years	6.8(1-30)
SLEDAI score, mean(range)	3.8(0-12)
Anti-dsDNA, IU/mL, mean(range)	244.5(100-659)
IgG, g/L, mean(range)	14.35(7.4-29.2)
Serum C3, g/L, mean(range)	0.94(0.26-1.51)
Serum C4, g/L, mean(range)	0.19(0.03-0.40)
Serum Creatinine, μmol/L, mean(range)	87.99(56-189)
WBC, 109/L (mean ± SD)	3.75±1.04
Platelet, 1012/L (mean ± SD)	171.76±70.76
Urinary proteins, g/24h, mean(range)	0.4(0-3.4)

Note: SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index; WBC, White blood cell

Table 2 Pre- and post-treatment clinicodemographic features of

SLE patients experiencing remission

Characteristic	Before treatment (N=12)	After treatment (N=12)
Age, years	50±12	50±12
Female/male	10/2	10/2
SLEDAI score	8±2	2±2***
Anti-dsDNA, IU/mL	177.8±51.32	98.92±28.55**
Serum C3, g/L	0.72±0.34	1.25±0.46*
Serum C4, g/L	0.09±0.07	0.21±0.10*
WBC, 10^9/L	2.98±1.57	3.53±0.63
Platelet, 10^{12}/L	124.0±65.9	157.08±69.94
Serum Creatinine, μmol/L	102.58±31.16	87.83±20.39
Urinary proteins, g/24h	1.73±1.58	0.20±0.19**

Note: Data are mean±SD. *p<0.05, **p < 0.01, ***p <0.001 vs. pre-treatment values

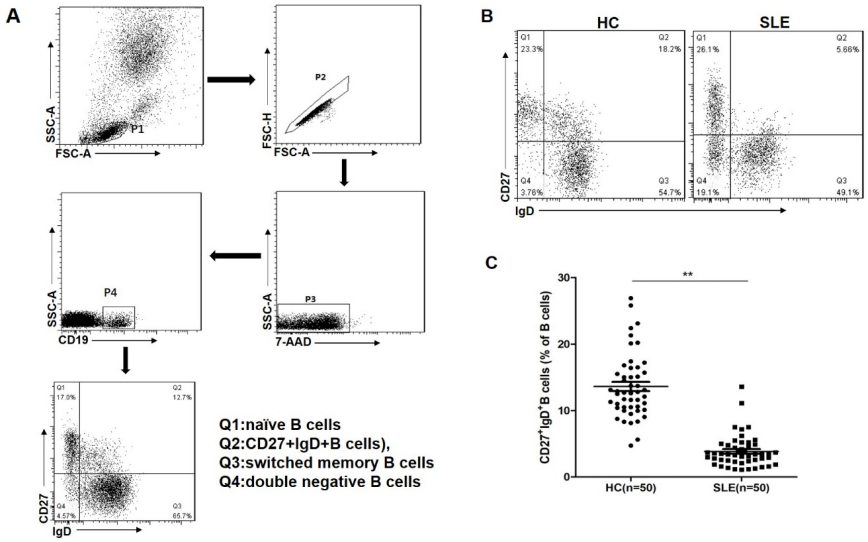

Fig. 1. Circulatory $CD27^{+}IgD^{+}$B cell amounts are reduced in SLE cases in comparison with healthy control (HC) patients. (A) Gating strategy for identifying naïve ($CD27^{-}IgD^{+}$), innate-like ($CD27^{+}IgD^{+}$), switched memory ($CD27^{+}IgD^{-}$) and double negative ($CD27^{-}IgD^{-}$) B cells in human blood. (B) $CD27^{+}IgD^{+}$B cells obtained from representative HC (left panel) and SLE (right panel) patients. (C) Percentages of $CD27^{+}IgD^{+}$ B cells in SLE (n=50) and HC (n=50) patients.

Data are mean±SD or median and interquartile range. Data points represent individual participants. NS, not significant. **p<0.01, ***p<0.001

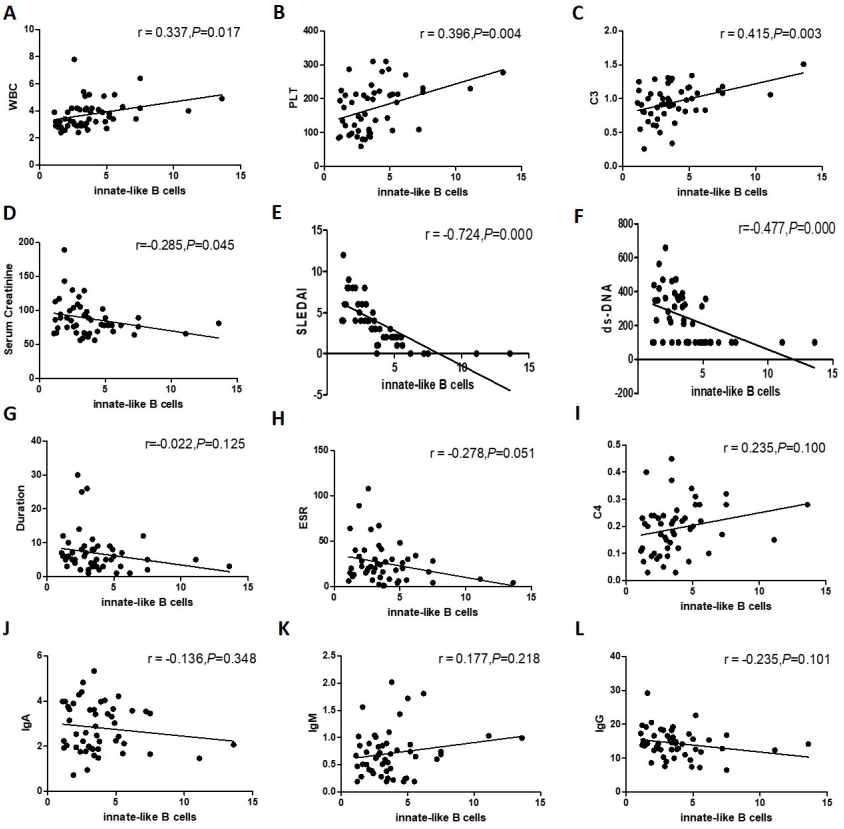

Fig.2 Associations of $CD27^{+}IgD^{+}$ B cell amounts with SLE patient disease manifestations, including WBC count (A), Platelet count (B), serum C3 (C), Serum Creatinine (D), SLEDAI (E), Anti-dsDNA (F), disease duration (G), ESR (H), serum C4 (I), IgA (J), IgG (K) and IgM (L). The Spearman test was applied (*P<0.05, **P<0.01).

Table 3 Associations of CD27+IgD+ B cell amounts with patient features in SLE

clinical manifestation	*r*	*P*
WBC	**0.337**	**0.017***
PLT	**0.396**	**0.004***
C3	**0.415**	**0.003***
Serum Creatinine	**0.285**	**0.045***
SLEDAI	**-0.724**	**0.000****
ds-DNA	**-0.477**	**0.000****
Duration	-0.022	0.125
ESR	-0.278	0.051
C4	0.235	0.100
IgA	-0.136	0.348
IgM	0.177	0.218
IgG	-0.235	0.101

Bold font indicates having statistical significance. *P<0.05, **P<0.01.

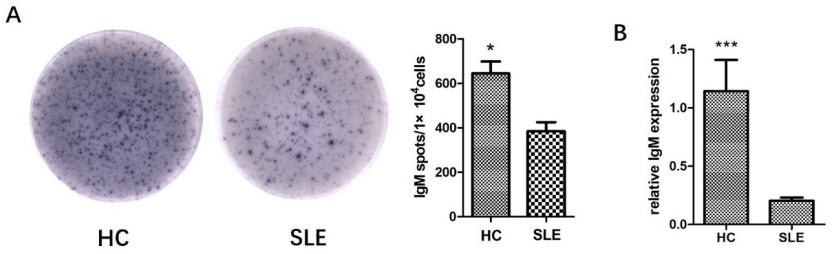

Figure 3 IgM-producing abilities of $CD27^+IgD^+$B cells in SLE and healthy control patients. Flow cytometry-purified $CD27^+IgD^+$B cells from four healthy donors and four active SLE cases (SLEDAI>5) were assessed by ELISPOT (A) and real-time PCR (B) for IgM detection. Representative charts and quantitation are shown (t-test, *P<0.05, ***P<0.001).

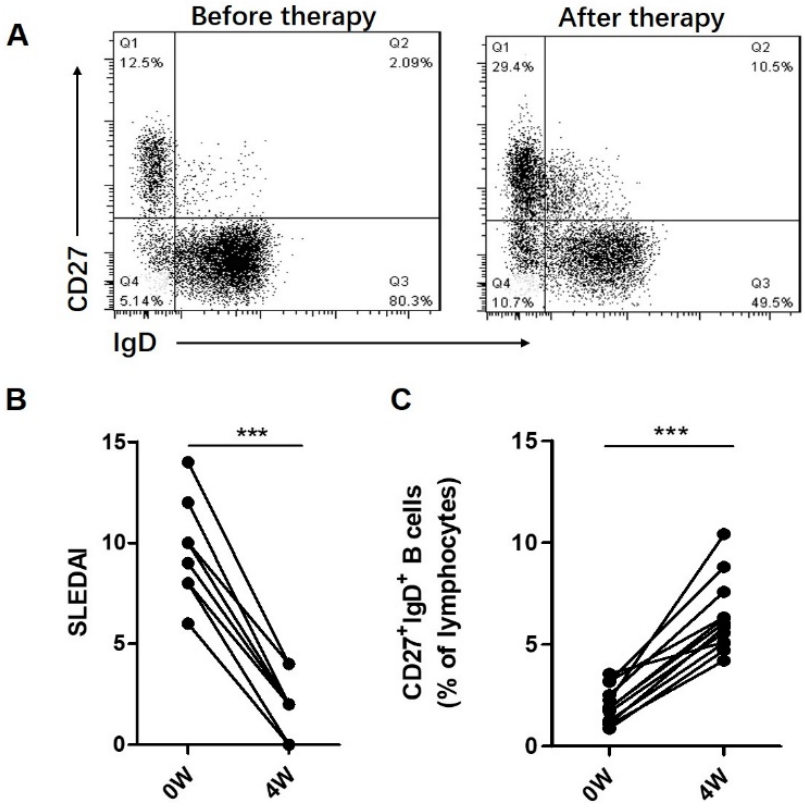

Fig. 4. $CD27^+IgD^+$ B cell amounts are increased after treatment of human SLE. (A) Flow cytometry assessment of $CD27^+IgD^+$ B cell levels pre-treatment and at 4 weeks post-treatment initiation. (B) SLEDAI scores before and after treatment in SLE patients (n=12). (C) $CD27^+IgD^+$ B cell percentages in SLE patients before and after treatment (n=12). (*P<0.05, **P<0.01).