The expression of Siglec-10 on naive B cells is involved in the pathology of systemic lupus erythematosus

B. Ju[1], J. Luo[1], N. Hu[1], J. Zhang[1], L. Zhu[1], Q. Li[1], Y. Wang[1], J. Huang[1], Q. An[1], Q. Xu[1], Z. Hao[1], D. Pu[1], X. Lv[1], X. Li[1], Y. Huo[2], B. Zhang[3,4], L. He[1]

[1]Department of Rheumatology and Immunology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi; [2]Department of Human Anatomy and Histology and Embryology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi; [3]Department of Pathogenic Microbiology and Immunology, School of Basic Medical Sciences, Xi'an Jiaotong University, Xi'an, Shaanxi; [4]Institute of Infection and Immunity, Translational Medicine Institute, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, China.

Abstract

Objective

Previous studies have reported the expansion of CD19$^+$Siglec-10$^+$ B cells in systemic lupus erythematosus (SLE) patients. However, the composition of this cell population, phenotype and characteristics are still unknown.

Methods

We examined this memory B-cell subset's composition and phenotype and determined the SYK and AKT phosphorylation levels by flow cytometry. Additionally, we explored the relationship between Siglec-10 expression on B-cell subsets and clinical manifestations.

Results

Our results indicated elevated levels of Siglec-10 on naive B cells in active SLE patients. Compared with healthy controls (HCs) and inactive SLE patients, the Siglec-10$^+$ B cells in active SLE patients exhibited elevated CD40 and CD21^{low} levels. The levels of Siglec-10 on naive B cells were positively correlated with the proportion of CD21^{low} double negative (DN) B cells and the SLEDAI-2K score.

Conclusion

The results indicate that the upregulation of Siglec-10$^+$/naive B cells may function as a feedback mechanism to regulate B cell hyperreactivity. Monitoring the proportion of Siglec-10$^+$/naive B cells may contribute to the evaluation of disease progression in SLE.

Key words

systemic lupus erythematosus, sialic acid binding Ig like lectin 10, B-cell subsets, biomarker, autoreactive B cells

*Bomiao Ju, PhD**
*Jing Luo, MD**
Nan Hu, MD, PhD
Jing Zhang, MD, PhD
Li Zhu, MD, PhD
Qian Li, MD, PhD
Yanhua Wang, MD
Jing Huang, MD, PhD
Qi An, MD
Qianyun Xu, MD
Zhiming Hao, MD, PhD
Dan Pu, MD
Xiaohong Lv, MD
Xin Li, MD
Yongwei Huo, MD, PhD
Baojun Zhang, MD, PhD
Lan He, MD, PhD

**Contributed equally.*

Please address correspondence to:
Lan He
Department of Rheumatology and Immunology,
The First Affiliated Hospital of Xi'an Jiaotong University,
277 Yanta West Road
Xi'an, Shaanxi 710061, China.
E-mail: xajdhl87@mail.xjtu.edu.cn

and to:
B. Zhang: bj.zhang@mail.xjtu.edu.cn
Y. Huo: hyw301@xjtu.edu.cn

Received on June 24, 2024; accepted in revised form on November 20, 2024

Funding: this work was supported by the Shaanxi Province Natural Science Basic Research Program (grant no. 2022JQ-958), the Key Research and Development Program of Shaanxi Province (grant no. 2023-ZDLSF-11).

Competing interests: none declared.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterised by diverse and heterogeneous clinical and serological manifestations (1). Significant progress has been made in the management and treatment of SLE through efforts in clinical and translational medicine research (2). However, the pathogenesis of SLE remains challenging. Multiple abnormalities in the B-cell lineage have been identified in SLE, which is characterised by the production of autoantibodies and the formation of pathogenic immune complexes (3). These abnormalities encompass changes in the composition and function of the B-cell compartment, including autoreactivity (4). In SLE patients, the composition of the B-cell compartment is altered, with a decrease in $CD27^+IgD^+$ non-switched and $CD27^+IgD^-$ switched B cells, and an increase in $CD27^-IgD^+$ naive and $CD27^-IgD^-$ DN B cells (5, 6) Recent studies have also shown expansions of certain subsets, including extrafollicular effector double negative type 2 ($IgD^-CD27^-CD21^{low}CXCR5^-CD11c^+$) (DN2) and activated naive ($IgD^+CD27^-CD21^{low}CXCR5^-CD11c^+$) (aNAV) B cells (7, 8), age-/autoimmune-associated B cells ($CD19^+CD21^-$ $CD11c^+$T-bet$^+$) (9, 10) Syk^{++} B cells (11) and $FCRL4^+$ B cells (12).These subsets share several characteristics and are associated with disease activity and autoantibody formation (13). Recently, Liu *et al*. demonstrated that the type 2 cytokine IL-4 can reverse B cell anergy by increasing surface immunoglobulin M (sIgM) and promote the reactivation of anergic autoreactive B cells (BND cells) (14). Thus, investigating the mechanisms underlying pathogenic autoreactive B cells is crucial for monitoring disease progression in SLE. Siglec-10 is a transmembrane protein that binds to sialic acid and belongs to the immunoglobulin-like lectin family. It consists of one V-set Ig-like domain and three C2-set domains in its extracellular region, as well as an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. When tyrosine phosphorylation occurs, the ITIM recruits SH2 family phosphatases, including SHP-1, SHP-2, and PTPN6, thereby inhibiting signal transduction (15, 16). Ligands for Siglec-10 include CD24, CD52, and vascular adhesion protein-1 (VAP-1) (17-19). Recently, Robinson *et al* reported that the CD52 protein, which is the ligand for Siglec-10, was found to be significantly up-regulated in B cells of SLE patients based on single-cell sequencing and *in vivo* flow cytometry. Additionally, the level of CD52 in the plasma of SLE patients was also found to be significantly elevated. In healthy B cells, BCR activation promotes the cleavage of CD52 from the cell membrane through phospholipase C, resulting in a reduction in the surface expression level of CD52 on B cells. Furthermore, soluble CD52 can bind to the Siglec-10 receptor to inhibit BCR signalling. CD52-Fc is capable of reducing the surface expression of IgM immunoglobulin molecules and CXCR5 on B cells, suggesting a potential increase in anergy B cells and a decrease in the proportion of DN2 cells. The up-regulation of CD52 in SLE may act as a feedback regulatory mechanism to suppress the sensitivity of B cells to antigen stimulation and maintain the immune homeostasis of B cells (18). However, the expression of the Siglec-10 receptor in B cell subpopulations of SLE patients remains unclear.

Our previous study reported an expansion of $CD19^+$Siglec-10^+ B cells in SLE patients (20). The current study further delineated the composition, phenotype, and characteristics of Siglec-10^+ B cells. Therefore, the aim of this study was to compare memory B-cell subsets and phenotypic features (CD40, CD80, CD95, and $CD21^{low}$), as well as the levels of phosphorylation of SYK and AKT following BCR engagement, in Siglec-10^+ B cells and Siglec-10^- B cells. Additionally, the correlation between Siglec-10 expression on B-cell subsets, defined autoreactive specificities of B cells, and clinical manifestations was further studied in patients with SLE.

Materials and methods

Study subjects

The study included 36 patients diagnosed with SLE (11 with active SLE and 25 with inactive SLE) and 22 HCs. To be included in the SLE patient

group, individuals had to meet the 2019 criteria established by the European League Against Rheumatism/American College of Rheumatology (21) and be at least 18 years old. Patients with confirmed infections, cancer, or end-stage disease were excluded from the study. Disease activity was evaluated using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (22). The active subgroup comprises patients who have never received glucocorticoids, conventional disease-modifying anti-rheumatic drugs (csDMARDs), targeted synthetic disease-modifying anti-rheumatic drugs, or biological disease-modifying anti-rheumatic drugs, and who have a SLEDAI-2K score greater than 4. In contrast, the inactive SLE group had a SLEDAI-2K score of 4 or lower after receiving steroids or conventional antirheumatic drugs. The inclusion criteria for HCs were based on sex and age and were matched with those of the SLE patient group. This research received approval from the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (no. XJTU1AF2022LSK-144). The detailed characteristics of the patients are listed in Table I.

Table I. Demographics and characteristics of SLE patients and HC.

Characteristic	HC (n=22)	Total SLE patients (n=36)
Age (years)	36.09 ± 7.82	38.86 ± 11.27
Sex, no. female/male	21/1	34/2
disease duration, months		29.5 (5.25–93)
SLEDAI-2K		4.88 ± 3.35
SLEDAI >4 (n=11)		8 (7-8)
SLEDAI ≤4 (n=25)		2 (0-4)
Untreated, n (%)		11 (30.6)
Treatment SLE, n (%)		25 (69.4)
Prednisolone		18 (72)
Prednisolone (mg/day)		7.42 ± 2.36
Hydroxychloroquine		19 (76)
Mycophenolate		12 (48)
Tacrolimus		3 (12)
Cyclosporine,		2 (8)
Leflunomide		3 (12)
Serological features		
anti-dsDNA (IU/ml)		17 (11.5-100)
IgG (g/L)		16.4 (13.2-21.6)
IgM (g/L)		1.08 (1.03-1.38)
C3 (g/L)		0.79 ± 0.15
C4 (g/L)		0.13 (0.1-0.22)
ESR (mm/h)		30 (16-48)
CRP (mg/L)		10.83 ± 2.9

SLE: systemic lupus erythematosus; HC: healthy controls; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; anti-dsDNA: anti-double stranded DNA antibody, IgG: immunoglobulin G; IgM: immunoglobulin M; C3: complement 3; C4: complement 4; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein. All the data were demonstrated as mean ± SD or median [IQR, 25th-75th percentile].

Data collection

The data collection process encompasses a range of demographic and clinical variables, including individuals' ages and sex measurements. The quantitative laboratory indicators collected included the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complement C3, complement C4, immunoglobulin G (IgG), immunoglobulin M (IgM) and anti-double-stranded DNA (anti-dsDNA) antibodies.

Antibodies used in this study

The following antibodies were used: Siglec-10-APC (BioLegend, 347606), Siglec-10-FITC (Bio-Rad, MCA-5783F), CD19-APC (BD Biosciences, 555415), CD19-PE-Cy7 BV421 (BD Biosciences, 302215), IgD-FITC (BD Biosciences, 555778), CD27-PE (BD Biosciences, 555441), CD95-PerCP-C5.5 (BioLegend, 305629), CD40-APC- Cy7 (BioLegend, 334323), CD21-BV421 (BD Biosciences, 566-260), CD80-BV510 (BD Biosciences, 563084), pSYK-PerCP-Cy5.5 (BioLegend, 683710), pAKT-BV421 (BD Biosciences, 562599), and anti-human IgM (H+L chain) (BioLegend, 397302).

Flow cytometry

- Isolation of PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using Ficoll-Paque™ PLUS (Cytiva, Sweden AB) according to standard protocols (23).

- Siglec-10+ B-cell subsets and phenotyping

To examine the composition of Siglec-10+ B-cell subsets, isolated PBMCs were incubated with fluorochrome-conjugated human monoclonal antibodies (mAbs) against CD19, Siglec-10, IgD, and CD27. Siglec-10+ B cells and Siglec-10- B cells were gated into $CD27^+IgD^-$ switched B cells, $CD27^+IgD^+$ non-switched B cells, $CD27^-IgD^-$ DN B cells, and $CD27^-IgD^+$ naive B cells.

To analyse the phenotypic features of the Siglec-10+ B cells, the cells were incubated with fluorochrome-conjugated human mAbs against CD19, Siglec-10, CD40, CD80, CD95, and CD21. The assessment of cells was conducted using CytoFlex (Beckman Coulter, USA), and the data analysis was performed using CytExpert.

Measurement of BCR-induced signalling

In this experiment, PBMCs were first incubated in RPMI medium at 37 °C for 30 minutes. After that, the sections were stained with APC-labeled human monoclonal antibodies against CD19 and Siglec-10 for another 30 minutes at 37 °C. Next, the PBMCs were stimulated with F(ab')2-conjugated anti-IgM (at a concentration of 2 μg/ml) for 5 minutes. Following stimulation, the cells were fixed using BD Cytofix™ Fixation Buffer at a volume equal to the initial staining volume, for 10 minutes at 4 °C. After fixation, the PBMCs were centrifuged at 400 × g for 3 minutes and washed twice with BD Phosflow™ Perm/Wash buffer. Then, the cells were stained with antibodies specific for phosphorylated-spleen tyrosine kinase (p-SYK) and p-AKT for 1.5 hours at

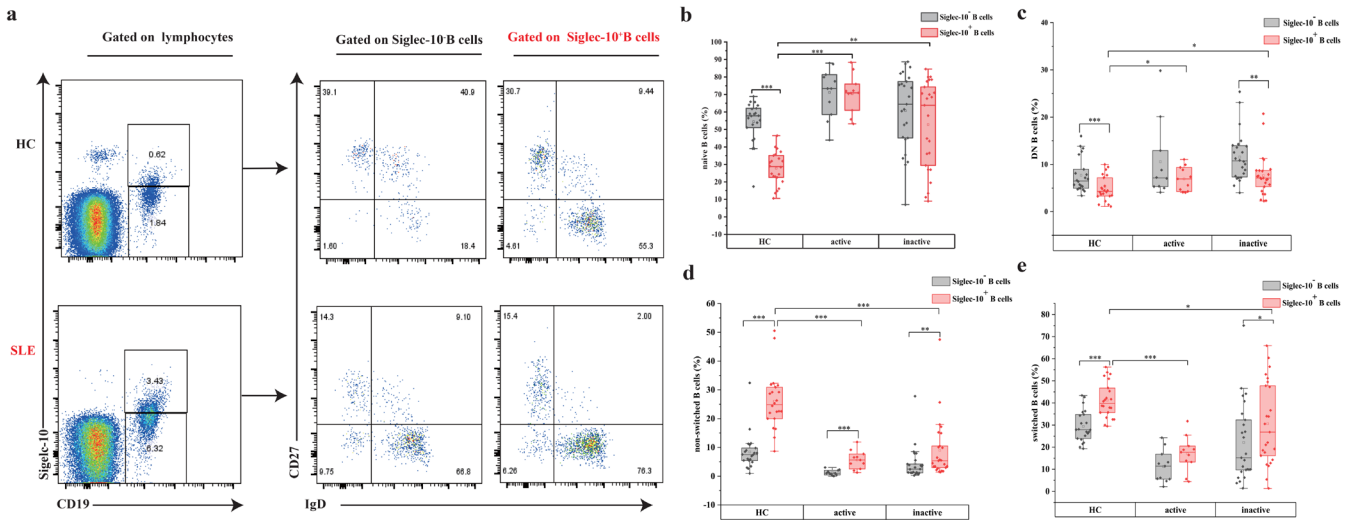

Fig. 1. Diverse composition of the Siglec-10⁺ B-cell subpopulation.
a. Gating strategy for identifying Siglec-10⁺ and Siglec-10⁻ B cells in one representative HC and one representative patient with SLE. The populations were further differentiated based on the expression of IgD and CD27, which included switched ($CD27^+IgD^-$), non-switched ($CD27^+IgD^+$), DN ($CD27^-IgD^-$), and naive ($CD27^-IgD^+$) B cells.
b. Comparative analysis of naive, DN, non-switched and switched B cells was conducted to evaluate the composition of Siglec-10⁺ and Siglec-10⁻ B cells in the peripheral blood of HCs, active SLE patients, and inactive SLE patients. Siglec-10⁺ and Siglec-10⁻ B cells are represented by red and grey rectangles, respectively.
The bars represent the medians with interquartile ranges. *p*-values were determined by the Mann-Whitney U-test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.
HCs: healthy controls; SLE: systemic lupus erythematosus; DN: double negative.

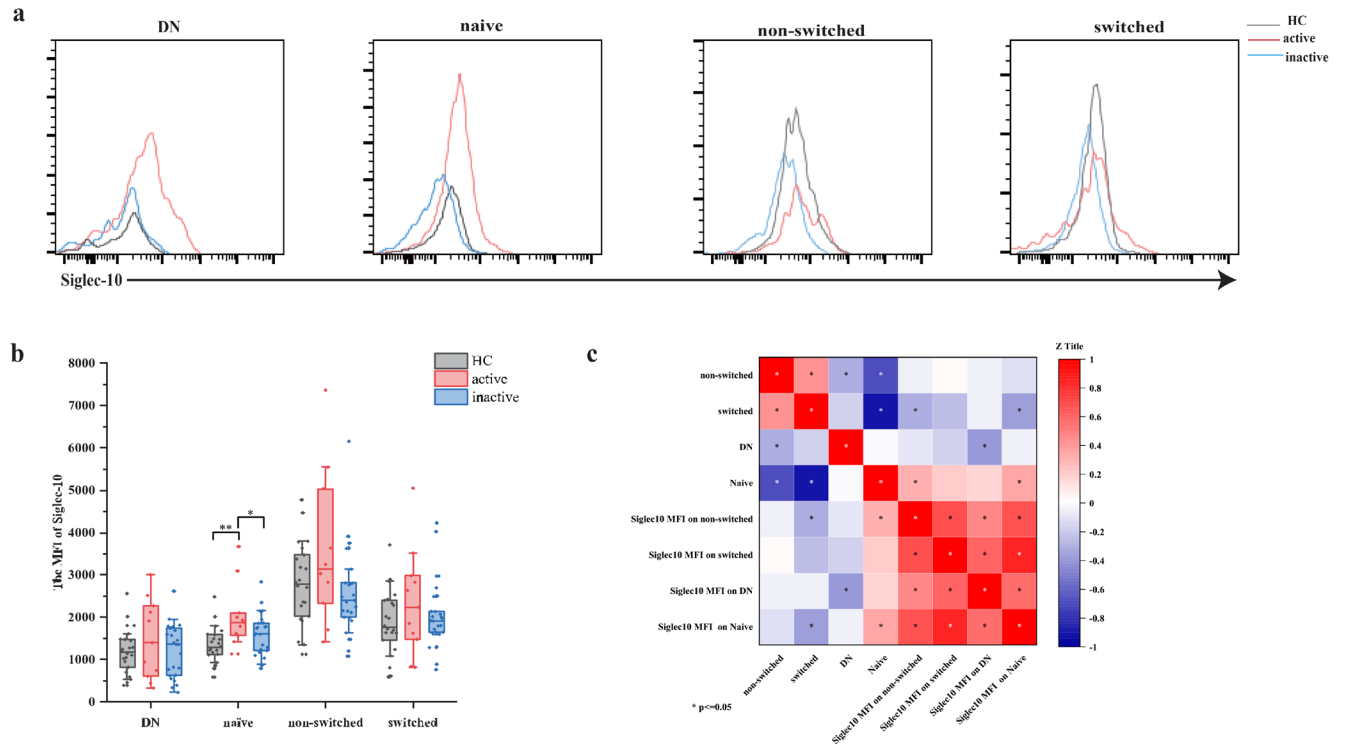

Fig. 2. The expression of Siglec-10 is upregulated on naive B cells in active SLE patients.
a. Representative flow histogram displaying surface Siglec-10 expression on switched, non-switched, DN, and naive B cells from one HC (grey), one active SLE patient (red) and one inactive patient (blue).
b. A comparative analysis was conducted to examine the expression levels of Siglec-10 on different B-cell subsets.
c. A correlation analysis was performed to evaluate the relationships between switched, non-switched, DN, and naive B cells in SLE patients and the MFI of Siglec-10 on B-cell subsets. The bars represent the medians with interquartile ranges. *p*-values were determined by the Mann-Whitney U-test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.
Spearman's correlation coefficient (r) was utilised as a metric to calculate the correlations between the numerical data.
HCs: healthy controls; SLE: systemic lupus erythematosus; DN: double negative; MFI: median fluorescent intensity.

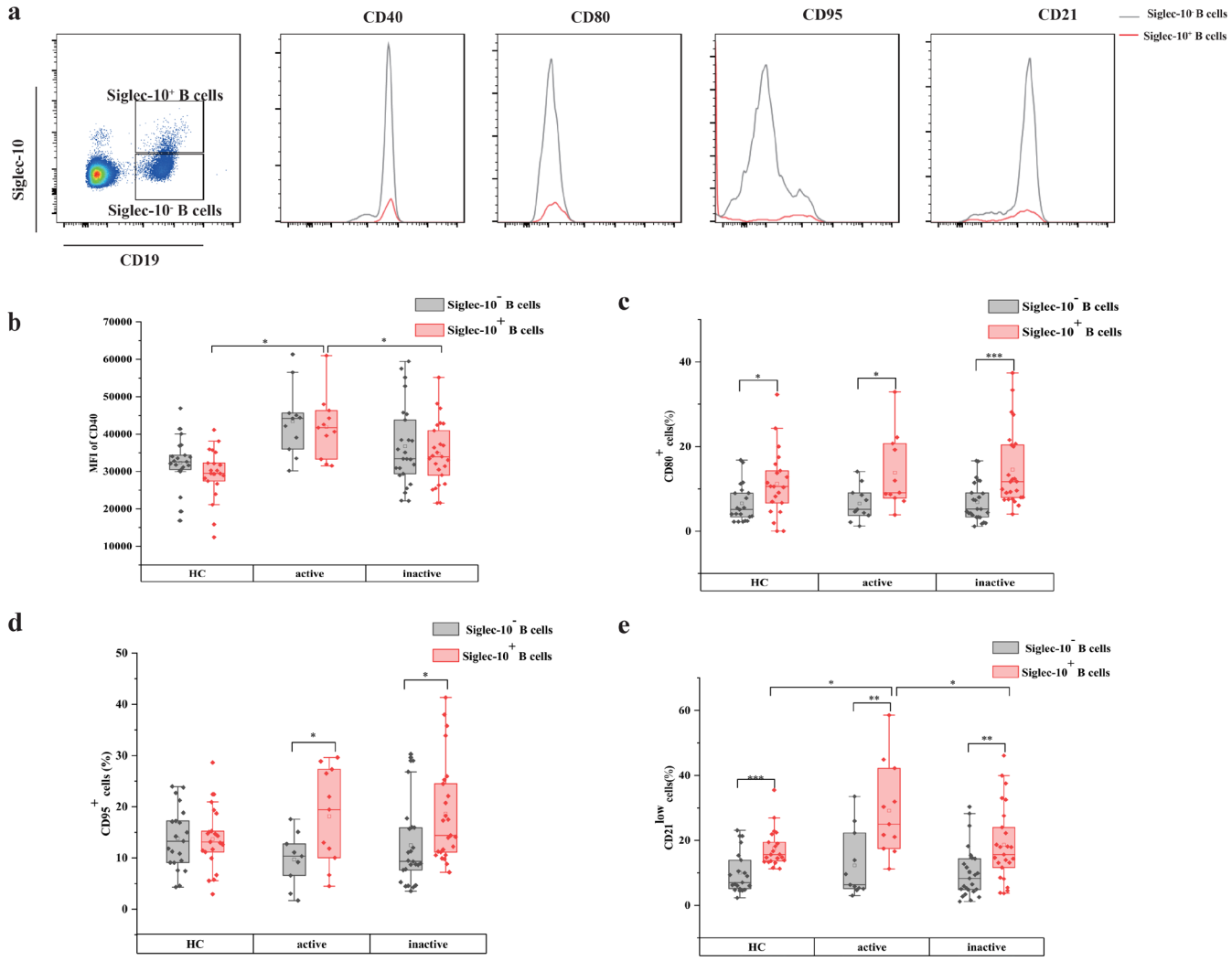

Fig. 3. The phenotypes of the indicated cell surface markers, CD40, CD80, CD95, and CD21^{low}, were evaluated in both the Siglec-10$^+$ and Siglec-10$^-$ B-cell subpopulations.
a. A representative histogram demonstrating the MFI of CD40 and the proportions of CD80, CD95, and CD21^{low} cells in the Siglec-10$^+$ and Siglec-10$^-$ B-cell subpopulations. Siglec-10$^+$ and Siglec-10$^-$ B cells are represented by red and gray rectangles, respectively.
b-e. Comparative analysis of CD40 expression and the proportions of CD80, CD95, and CD21^{low} cells among Siglec-10$^+$ and Siglec-10$^-$ B cells from HCs, active SLE patients, and inactive SLE patients.
The bars represent the medians with interquartile ranges. *p*-values were determined by the Mann-Whitney U-test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.
HCs: healthy controls; SLE: systemic lupus erythematosus; DN: double negative; MFI: median fluorescent intensity.

4°C in the dark. Subsequently, the PBMCs were washed again with Perm/Wash buffer and resuspended in a volume of 200 µl of Perm/Wash buffer for analysis on a flow cytometer.

Statistical analysis

The results are expressed as medians with corresponding interquartile ranges (25th and 75th percentiles) or as indicated. The statistical analysis was conducted using SPSS software v. 22, developed by SPSS Inc. in Chicago, IL, USA. Descriptive analysis, including calculations of averages, proportions, or rates, was performed. The normality of the data was assessed using the Shapiro-Wilk test, while the homogeneity of variance was evaluated using the Levene test. When comparing mean differences between groups, either Student's t-test or the Mann-Whitney U-test was used depending on the situation. Correlation differences were assessed using either Pearson or Spearman analysis. A *p*-value <0.05 was considered to indicate statistical significance.

Results

Diverse composition of Siglec-10$^+$ B-cell subpopulations

The composition of different Siglec-10$^+$ and Siglec-10$^-$ B-cell subpopulations, including non-switched, switched, naive, and DN B cells, was examined in the peripheral blood of SLE patients and HCs (Fig. 1a). The percentage of naive B cells was significantly decreased in Siglec-10$^+$ B cells compared to their Siglec-10$^-$ counterparts in HCs [28.75% (21.97-35.22%) *vs.* 57.77% (49.41-62.49%), $p<0.001$]. However, these differences were not detected in active SLE patients [70.94% (60.97-75.97%) *vs.*73.37% (58.53-81.36%), $p=0.61$] or inactive SLE patients [63.84% (28.22-75.87%) *vs.* 52.39% (44.72-78.40%), $p=0.25$] (Fig. 1b). A lower percentage

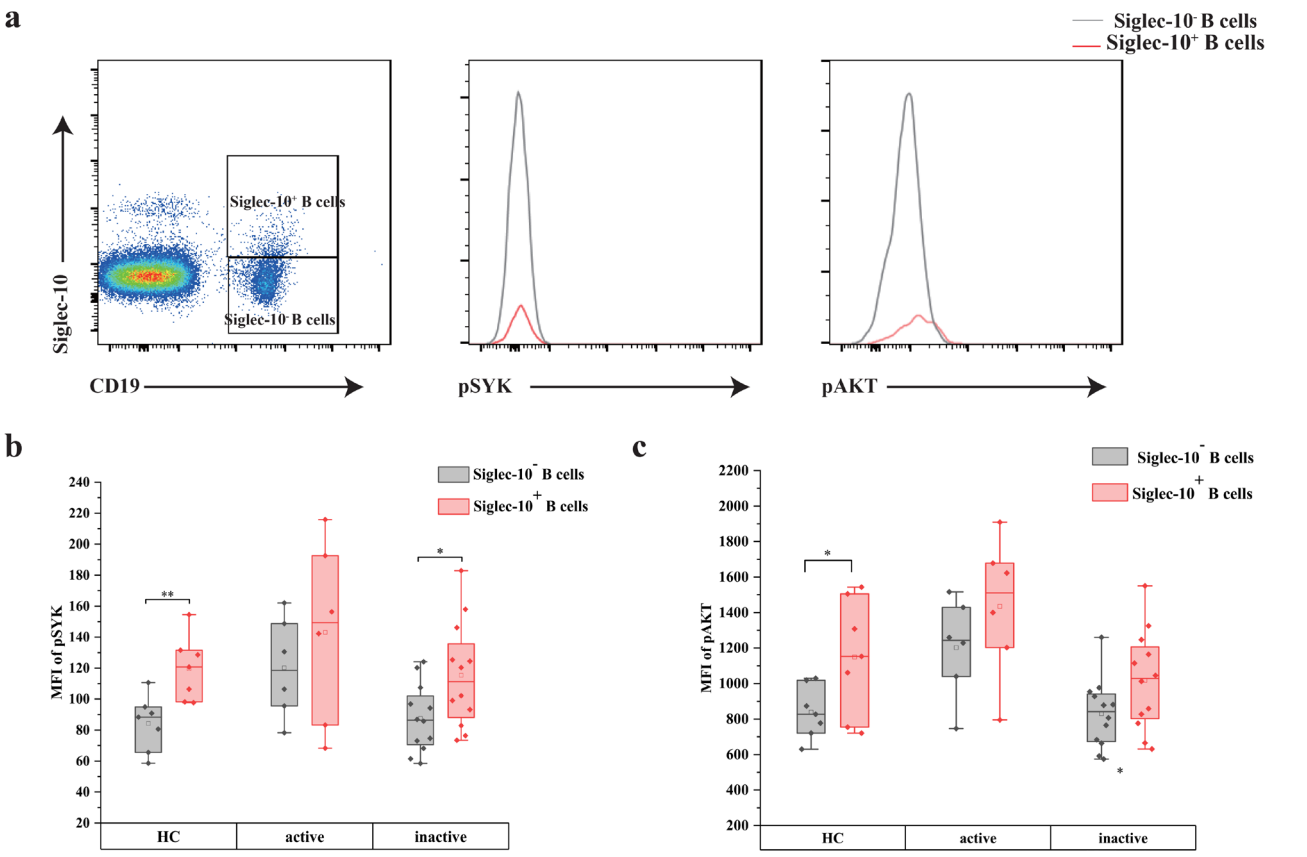

Fig. 4. B-cell receptor responsiveness in Siglec-10$^+$ and Siglec-10$^-$ B cells.
a. Kinetics of SYK and AKT phosphorylation in Siglec-10$^+$ and Siglec-10$^-$ B cells from HCs, active SLE patients, and inactive SLE patients 5 minutes after stimulation with an antibody against the B cell receptor.
b. The MFI of pSYK and pAKT expression in Siglec-10$^+$ and Siglec-10$^-$ B cells for HCs, active SLE patients, and inactive SLE patients.
The bars represent the medians with interquartile ranges. *p*-values were determined by the Mann-Whitney U-test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.
HCs: healthy controls; SLE: systemic lupus erythematosus; MFI: median fluorescent intensity; pSYK: phosphorylated SYK; pAKT: phosphorylated AKT.

of DN B cells and a higher percentage of non-switched and switched B cells were found among than among their Siglec10$^-$ counterparts in both HCs and inactive SLE patients. In active SLE patients, there was a higher proportion of non-switched B cells among Siglec-10$^+$ B cells than among Siglec-10$^-$ B cells, while the proportions of DN B cells and switched B cells were similar (Fig. 1c-e). We further observed that, compared with HCs, both active SLE patients and inactive SLE patients had a higher proportion of naive B cells and DN Siglec-10$^+$ B cells and a lower proportion of non-switched B cells and switched Siglec-10$^+$ B cells. Therefore, there is a significant difference in the proportion of Siglec10$^+$ naive B cells between SLE patients and HCs.

The expression of Siglec-10 on different B-cell subpopulations

Moreover, the mean fluorescence intensity (MFI) of Siglec-10 was analysed for distinct B-cell subpopulations in HCs and patients with SLE. The expression of Siglec-10 on naive B cells was higher in active SLE patients than HCs and inactive SLE patients [1881 (1571–2107) *vs.* 1286 (1068-1591) *vs.* 1607 (1192–1810), $p=0.002$, $p=0.04$] (Fig. 2a-b). Moreover, the expression of Siglec-10 on non-switched B cells (r= -0.30, $p=0.02$) and naive B cells (r= -0.34, $p=0.008$) was negatively correlated with the proportion of switched B cells, and the expression of Siglec-10 on DN B cells (r= -0.38, $p=0.003$) was negatively correlated with the proportion of DN B cells (Fig. 2c).

Common phenotype of Siglec-10$^+$ B cells

To compare the characteristic phenotypic features of Siglec-10$^+$ B cells, we assessed the MFI of CD40 and the percentages of CD80$^+$, CD95$^+$, and CD21^{low} Siglec-10$^+$ B cells in patients with SLE and HCs (Fig. 3a). Our results revealed that the MFI of CD40 [41723(33288-46299) *vs.* 29497 (27083-33693) *vs.* 33953(27842–41686), $p<0.001$, $p=0.045$] (Fig. 3b) and the percentage of CD21^{low} [25 (17.48–42.22) *vs.* 15.64 (13.52–20.67) *vs.* 15.64(10.07-25.80), $p=0.01$, $p=0.04$] (Fig. 3e) in Siglec-10$^+$ B cells was higher in active SLE patients than in HCs and inactive SLE patients. We compared the phenotypes of Siglec-10$^+$ B cells and their Siglec-10$^-$counterparts in both SLE patients and HCs. More Siglec-10$^+$ B cells expressed CD80$^+$ and CD21^{low} than their Siglec10 counterparts in both the active SLE patients, inactive SLE patients, and HCs. Moreover, we observed that the proportion of CD95$^+$Siglec10$^+$ cells was higher than that of CD95$^+$Siglec10$^-$ cells in both the active SLE and inactive SLE patients, while this difference was not found in HCs.

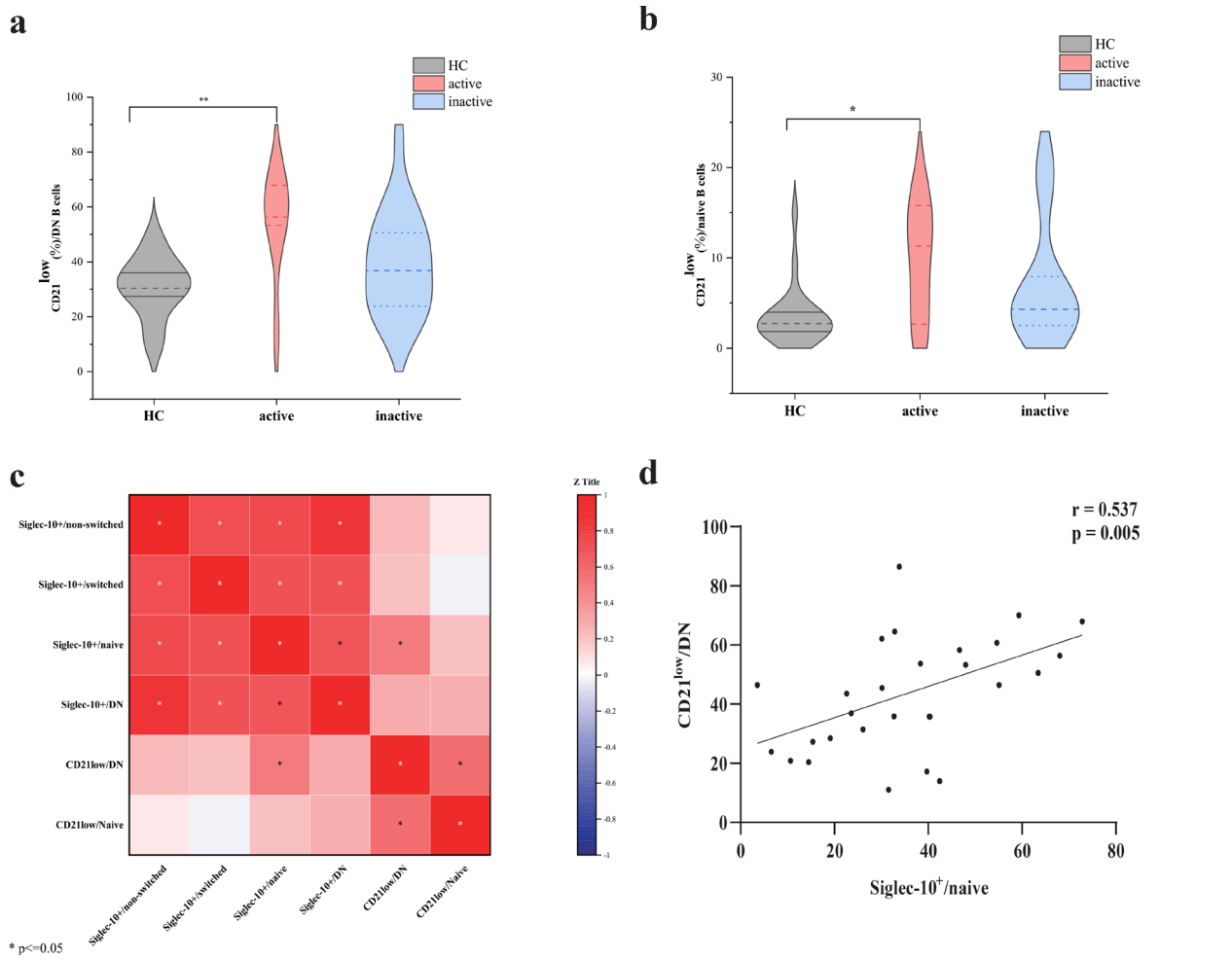

Fig. 5. The correlation between Siglec-10$^+$/naive B cells and CD21^{low}/DN B cells.
a. Proportions of CD21^{low}/DN B cells in HCs, active SLE patients, and inactive SLE patients. **b.** Proportions of CD21^{low}/naive B cells in HCs, active SLE patients, and inactive SLE patients. **c.** Correlation matrix of Siglec-10$^+$ B-cell subsets correlated with CD21^{low}/DN B cells and CD21^{low}/naive B cells. **d.** The proportion of Siglec-10$^+$/naive B cells was positively correlated with that of CD21^{low}/DN B cells.
The bars represent the medians with interquartile ranges. *p*-values were determined by the Mann-Whitney U-test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Spearman's correlation coefficient (r) was utilised as a metric to calculate the correlations among the numerical data.
HCs: healthy controls; SLE: systemic lupus erythematosus; DN: double negative.

Siglec-10$^+$ B cells exhibit an increased signalling capacity following BCR stimulation

We further analysed the phosphorylation levels of signalling molecules after BCR engagement in both Siglec-10$^+$ and Siglec-10$^-$ B cells. In HCs, the levels of pSYK (120.7 [98.2–131.5] *vs*. 88.4 [65.60–94.90], p=0.004) and pAKT (1153 [754.3–1506] *vs*. 827.1 [721.1–1018], p=0.04) were significantly higher in Siglec-10$^+$ B cells than in Siglec-10$^-$ B cells. However, in active SLE patients, the levels of pSYK (149.3 [79.55–198.4] *vs*. 118.5 [91.28–152.1], p=0.59) and pAKT (1511 [1101–1736] *vs*. 1244 [966.9–1451], p=0.31) were comparable between Siglec-10$^+$ and Siglec-10$^-$ B cells. In inactive SLE patients, the expression of pSYK (111.3 [85.48–140.9] *vs*. 86.30 [69.4-104.8], p=0.03) was significantly higher than that in HCs, but the expression of pAKT was similar between Siglec-10$^+$ and Siglec-10$^-$ B cells (Fig. 4a-c).

The proportion of Siglec-10$^+$/naive B cells is positively correlated with the proportion of CD21^{low}/DN B cells

More recently, expansions of DN2 (IgD$^-$CD27$^-$CD21^{low}) and IgD$^+$CD27$^-$CD21^{low} activated naive B cells have been reported to be associated with the formation of autoantibodies (7). In our study, we assessed whether there was a difference in the proportions of CD21^{low} /DN B cells and CD21^{low} /naive B cells. Our findings revealed a significant increase in the proportions of CD21^{low}/DN B cells [56.39(53.33–67.92) *vs*. 30.43(26.20–37.70), p=0.003] and CD21^{low}/naive B cells [11.30(2.67–15.79) *vs*. 2.73(1.72–4.03), p=0.03] in active SLE patients compared to HCs. Additionally, the proportions of CD21^{low}/DN B cells and CD21^{low}/naive B cells were greater in active SLE patients than in inactive SLE patients, although these differences were not statistically significant (Fig. 5a-b). Furthermore, we examined whether Siglec-10 expression on B subsets correlated with the CD21^{low}/DN B cells and CD21^{low}/naive B cells. Our results demonstrated a significant correlation between the proportions of Siglec-10$^+$/naive B cells and CD21^{low}/ DN B cells (r=0.537, p=0.005) (Fig. 5c-d).

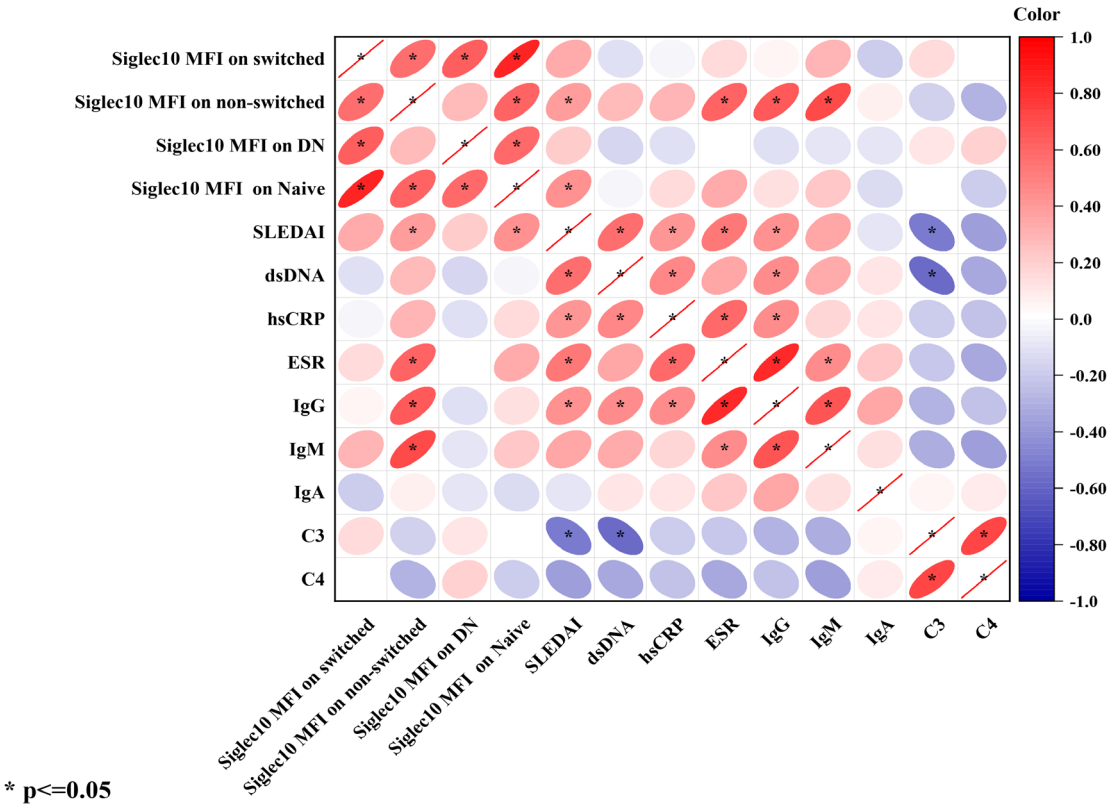

Fig. 6. Correlation matrix of Siglec-10 expression on B-cell subsets and clinical parameters from SLE patients**.** Only significant Spearman's correlations are presented in this matrix.
SLE: systemic lupus erythematosus; HC, healthy control; SLEDAI-2K, systemic lupus erythematosus disease activity index 2000; anti-dsDNA antibody, anti-double stranded DNA antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; C3, complement 3; C4, complement 4; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein. *p<0.05

The expression of Siglec-10 on B-cell subsets is correlated with various clinical parameters

There was a significant correlation between the expression of Siglec-10 on naive B cells and SLEDAI-2K score (r=0.421, p=0.036). Additionally, we observed strong correlations between Siglec-10 expression in non-switched B-cell subsets and SLEDAI-2K score (r=0.398, p=0.049), ESR (r=0.611, p=0.001), IgG (r=0.578, p<0.001), and IgM levels (r=0.565, p<0.001) (Fig. 6). Our data suggest that evaluating Siglec-10 expression in B-cell subsets could be useful as a biomarker for assessing disease activity in SLE patients.

Discussion

In this study, we explored the differences in the composition, phenotype and signalling processes of Siglec-10$^+$ B cells among HCs, active SLE patients, and inactive SLE patients. Our results indicated elevated levels of Siglec-10 on CD27$^-$IgD$^+$ naive B cells in active SLE patients compared to HCs and individuals with inactive SLE. The elevated levels of Siglec-10 on naive B cells were positively correlated with autoreactive specificities of CD21^{low}/DN B cells and SLEDAI-2K scores. Compared to those of Siglec-10$^+$ B cells in in HCs and inactive SLE patients, the phenotypic characteristics of Siglec-10$^+$ B cells in active SLE patients included elevated levels of CD40 and a greater percentage of CD21^{low} cells. Therefore, the results indicate that monitoring the proportion of Siglec-10$^+$/naive B cells may contribute to the assessment of disease progression in SLE.

We observed that, compared with their Siglec-10$^-$ counterparts, Siglec-10$^+$ B cells were composed of significantly fewer naive B cells in HCs. In contrast, these differences were not found in active SLE patients or inactive SLE patients. The study also revealed an increase in the MFI of Siglec-10 on naive B cells in active SLE patients compared to HCs and inactive SLE patients. We hypothesise that the elevated levels of Siglec-10 on naive B cells may indicate heightened chronic antigen stimulation in patients with more severe disease. Additionally, we identified a significant correlation between Siglec-10 expression on naive B cells and the SLEDAI-2K score. Kartik Bhamidipati reported that the level of CD52 (a Siglec-10 ligand) was significantly higher on B cells and that the level of soluble CD52 was higher in the plasma of SLE patients than in that of healthy controls. Moreover, CD52 expression was found to be positively correlated with SLE disease activity (18) Given their predominant inhibitory effects on B-cell activation, we hypothesise that the up-regulation of Siglec-10 may serve as a negative feedback mechanism aimed at limiting the hyperactive responses of naive B cells. We further observed that, compared with HCs, both active SLE patients and inactive SLE patients had a greater proportion of naive and DN B cells and a lower proportion of non-

switched Siglec-10+ B cells. The present findings indicate that the upregulation of Siglec-10 serves as an autoregulatory mechanism, that restricts the responsiveness of B cells to antigen stimulation in SLE.

The phenotypic features of Siglec-10+ B cells in active SLE patients included a significantly greater MFI of CD40. Additionally, our results indicate that Siglec-10+ B cells exhibit higher levels of pSYK and pAKT in active SLE patients than in healthy controls. CD40, a member of the TNF receptor superfamily, acts on human B lymphocytes to promote B cell activation and proliferation. The interaction between CD40, which is expressed on B cells, and its binding partner CD40L, which is predominantly expressed on activated CD4+ T cells, was demonstrated to be crucial for germinal centre (GC) responses and isotype class switching in the T-dependent antigen response (24, 25). Siglec-10 functions as a coinhibitory receptor by harbouring an ITIM in its cytoplasmic domain. Upon BCR stimulation, tyrosine phosphorylation occurs, leading to the recruitment of SH2 family phosphatases such as SHP-1, SHP-2, and PTPN6, which in turn inhibit signal transduction (16). One study reported that SHP-1 deficiency affects the calcium response elicited by B-cell antigen receptors and hinders CD40-induced proliferation (26). Furthermore, SHP-1 plays a critical role in regulating the activities of SYK in B cells. Maintaining an equilibrium between these enzymes is vital for normal B-cell development and function (27). Our study showed expression elevated levels of Siglec-10 on naive B cells. Moreover, the expression of Siglec-10 on naive B cells and non-switched B cells was negatively correlated with the proportion of switched B cells. Thus, we hypothesise that activation of the CD40-CD40L axis or BCR signalling results in the upregulation of Siglec-10, which in turn restricts BCR activation, the germinal centre (GC) responses and isotype class switching.

The percentage of CD21low cells among Siglec-10+ B cells was greater in active SLE patients than in both HCs and inactive SLE patients. Additionally, we found an elevated percentage of CD95+Siglec-10+ B cells compared to their CD95+Siglec-10- counterparts in SLE patients, which was not observed in HCs. Complement receptor 2 (CD21) forms a complex with CD19 and CD81, serving as coreceptor for BCR (28). An accumulating population of circulating CD21low B cells has been reported in patients with SLE (29). Fas (CD95) is a transmembrane receptor involved in the maintenance of tolerance and immune homeostasis and is a marker of apoptosis induced by cell activation (30). Age-associated B cells (ABCs) are a subset of B cells defined as CD19+CD21-CD11c+ cells that continuously expand with age and in autoimmune and infectious diseases (31-33). In humans, ABCs primarily consist of DN B cells. Data from murine models of autoimmunity implicate ABCs/DN in the development of autoimmune disorders (34). A previous study showed that, compared with those of CD21pos B cells, naive-like and memory-like CD21low B-cell subsets exhibit higher MFI values for Siglec10, CD95, FcRL4, CD11c, and CD32 and elevated phosphorylation of the signalling molecules SYK, AKT and PLCΓ2 downstream of the BCR (35). The findings suggest that Siglec-10+ B cells possess certain characteristics of ABCs/DN cells.

We next evaluated the correlation between Siglec-10+ B cells and CD21low B-cell subsets. First, we found an increased percentage of CD21low/DN B cells and CD21low /naive B cells in active SLE patients compared to HCs. In addition, the level of Siglec-10 expression on naive B cells was positively correlated with that CD21low/DN B cells. Previous research has indicated that both "activated naive B cells" (aNAV) resembling naive B cells expressing low levels of CD21 and double-negative "DN2" cells, which are closely linked to CD21low CD27- B cells, exhibit autoreactivity and generate increased amounts of self-antibodies in SLE (6, 7, 29, 36). The literature reports revealed a significant increase in the levels of DN2 and aNAV B cells in SLE patients. These findings indicate a connection between Siglec-10 expression on naive B cells and autoreactive B cells. Additionally, we observed strong correlations between Siglec-10 expression in non-switched B-cell subsets and the SLEDAI-2K, IgG, and IgM scores. Notably, Siglec-10 may play a critical role in regulating naive B cells, non-switched B cells, and CD21low/DN B cells, with the goal of limiting hyperactive B cell responses.

Our data suggest that evaluating Siglec-10 expression on naive B cells could be useful as a biomarker for assessing disease activity in SLE patients. We speculate that the increase in the expression level inhibitory receptor Siglec-10 serves as a regulatory feedback mechanism for B-cell activation in subsets of B cells. However, future studies should investigate the role of Siglec-10 on naive B cells in the pathogenesis of SLE, including its effect on plasma cell differentiation, autoantibody production, proinflammatory cytokine secretion, and T-cell induction.

Conclusions

In this study, we explored the differences in the composition, phenotype, and involved signalling processes of Siglec10+ B cells among HCs, active SLE patients, and inactive SLE patients. Our results indicated elevated levels of Siglec-10 on CD27-IgD+ naive B cells in active SLE patients compared to HCs and individuals with inactive SLE. Elevated levels of Siglec-10 on naive B cells were positively correlated with autoreactive specificities of CD21low/DN B cells and SLEDAI-2K scores. The phenotypic characteristics of Siglec-10+ B cells in active SLE patients included elevated levels of CD40 and a greater percentage of CD21low cells than in HCs and inactive SLE patients. The results indicate that the upregulation of Siglec-10+/naive B cells may function as a feedback mechanism to regulate B cell hyperreactivity. Monitoring the proportion of Siglec-10+/naive B cells may contribute to the evaluation of disease progression in SLE.

Acknowledgments

All the participants, both patients and healthy controls, are acknowledged for their valuable contributions to this study. The authors sincerely thank Prof. Baojun Zhang for his guidance and support with the Flow cytometry analysis.

References

1. SIEGEL CH, SAMMARITANO LR: Systemic lupus erythematosus: a review. *JAMA* 2024; 33: 1480-91. http://dx.doi.org/10.1001/jama.2024.2315
2. SCHILIRÒ D, SILVAGNI E, CIRIBÈ B *et al*.: Systemic lupus erythematosus: one year in review 2024. *Clin Exp Rheumatol* 2024; 42: 583-92. https://doi.org/10.55563/clinexprheumatol/mnvmvo
3. ODENDAHL M, JACOBI A, HANSEN A *et al*.: Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J Immunol* 2000; 165: 5970-9. http://doi.org/10.4049/jimmunol.165.10.5970
4. KARRAR S, CUNNINGHAME GRAHAM DS: Abnormal B cell development in systemic lupus erythematosus: what the genetics tell us. *Arthritis Rheumatol* 2018; 70: 496-507. http://doi.org/10.1002/art.40396
5. ZHU L, YIN ZJ, JU BM *et al*.: Altered frequencies of memory B cells in new-onset systemic lupus erythematosus patients. *Clin Rheumatol*, 2018; 37: 205-12. http://doi.org/10.1007/s10067-017-3877-1
6. WEI C, ANOLIK J, CAPPIONE A *et al*.: A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J Immunol* 2007; 178: 6624-33. http://doi.org/10.4049/jimmunol.178.10.6624
7. JENKS SA, CASHMAN KS, ZUMAQUERO E *et al*: Distinct effector B cells induced by unregulated toll-like receptor 7 contribute to pathogenic responses in systemic lupus erythematosus. *Immunity* 2020; 52: 203. http://doi.org/10.1016/j.immuni.2019.12.005
8. HURTADO C, ROJAS-GUALDRON DF, URREGO R *et al*.: Altered B cell phenotype and CD27+ memory B cells are associated with clinical features and environmental exposure in Colombian systemic lupus erythematosus patients. *Front Med (Lausanne)*, 2022; 9: 950452. http://doi.org/10.3389/fmed.2022.950452
9. SACHINIDIS A, XANTHOPOULOS K, GARYFALLOS A: Age-associated B cells (ABCs) in the prognosis, diagnosis and therapy of systemic lupus erythematosus (SLE). *Mediterr J Rheumatol* 2020; 31: 311-8. http://doi.org/10.31138/mjr.31.3.311
10. MA S, WANG C, MAO X, HAO Y: B cells dysfunction associated with aging and autoimmune diseases. *Front Immunol* 2019; 10: 318. http://doi.org/10.3389/fimmu.2019.00318
11. FLEISCHER SJ, GIESECKE C, MEI HE, LIPSKY PE, DARIDON C, DORNER T: Increased frequency of a unique spleen tyrosine kinase bright memory B cells population in systemic lupus erythematosus. *Arthritis Rheumatol* 2014; 66: 3424-35. http://doi.org/10.1002/art.38854
12. JOURDAN M, ROBERT N, CREN M *et al*.: Characterization of human FCRL4-positive B cells. *PLoS One* 2017; 12: e0179793. http://doi.org/10.1371/journal.pone.0179793
13. GJERTSSON I, MCGRATH S, GRIMSTAD K *et al*.: A close-up on the expanding landscape of CD21-/low B cells in humans. *Clin Exp Immunol* 2022; 210: 217-29. http://doi.org/10.1093/cei/uxac103
14. LIU Y, ZHANG Z, KANG Z *et al*.: Interleukin 4-driven reversal of self-reactive B cell anergy contributes to the pathogenesis of systemic lupus erythematosus. *Ann Rheum Dis* 2023; 82: 1444-54. http://doi.org/10.1136/ard-2023-224453
15. LI N, ZHANG W, WAN T *et al*.: Cloning and characterization of Siglec-10, a novel sialic acid binding member of the Ig superfamily, from human dendritic cells. *J Biol Chem* 2001; 276: 28106-12. http://doi.org/10.1074/jbc.M100467200
16. WHITNEY G, WANG SL, CHANG H *et al*.: A new siglec family member, siglec-10, is expressed in cells of the immune system and has signaling properties similar to CD33. *Eur J Biochem* 2001; 268: 6083-96. https://doi.org/10.1046/j.0014-2956.2001.02543.x
17. YIN SS, GAO FH: Molecular mechanism of tumor cell immune escape mediated by CD24/Siglec-10. *Front Immunol* 2020; 11. https://doi.org/10.3389/fimmu.2020.01324
18. BHAMIDIPATI K, SILBERSTEIN JL, CHAICHIAN Y *et al*.: CD52 Is elevated on B cells of SLE patients and regulates B Cell function. *Front Immunol* 2021; 11. https://doi.org/10.3389/fimmu.2020.626820
19. KIVI E, ELIMA K, AALTO K *et al*.: Human Siglec-10 can bind to vascular adhesion protein-1 and serves as its substrate. *Blood* 2009; 114: 5385-92. http://doi.org/10.1182/blood-2009-04-219253
20. JU B, WANG J, MO L *et al*.: Elevated CD19(+) Siglec-10(+) B cell levels are correlated with systemic lupus erythematosus disease activity. *Int Immunopharmacol* 2022; 102: 108403. http://doi.org/10.1016/j.intimp.2021.108403
21. ARINGER M, COSTENBADER K, DAIKH D *et al*.: 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. *Arthritis Rheumatol* 2019; 71: 1400-12. http://doi.org/10.1002/art.40930
22. GLADMAN DD, IBANEZ D, UROWITZ MB: Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002; 29: 288-91
23. MACHALINSKI B, HONCZARENKO M, GONTAREWICZ A, RATAJCZAK MZ: Isolation of human mononuclear cells from bone marrow, peripheral blood and cord blood using Ficoll-Paque (Pharmacia) and Gradisol L (Polfa). Comparative study. *Pol Arch Med Wewn* 1998; 99: 15-23
24. HOKAZONO Y, ADACHI T, WABL M, TADA N, AMAGASA T, TSUBATA T: Inhibitory coreceptors activated by antigens but not by anti-Ig heavy chain antibodies install requirement of costimulation through CD40 for survival and proliferation of B cells. *J Immunol* 2003; 171: 1835-43. http://doi.org/10.4049/jimmunol.171.4.1835
25. KARNELL JL, RIEDER SA, ETTINGER R, KOLBECK R: Targeting the CD40-CD40L pathway in autoimmune diseases: Humoral immunity and beyond. *Adv Drug Deliv Rev* 2019; 141: 92-103. http://doi.org/10.1016/j.addr.2018.12.005
26. PAO LI, LAM KP, HENDERSON JM *et al*.: B cell-specific deletion of protein-tyrosine phosphatase Shp1 promotes B-1a cell development and causes systemic autoimmunity. *Immunity* 2007; 27: 35-48. http://doi.org/10.1016/j.immuni.2007.04.016
27. ALSADEQ A, HOBEIKA E, MEDGYESI D, KLÄSENER K, RETH M: The role of the syk/shp-1 kinase-phosphatase equilibrium in b cell development and signaling. *J Immunol* 2014; 193: 268-76. http://doi.org/10.4049/jimmunol.1203040
28. FEARON DT, CARTER RH: The CD19/CR2/TAPA-1 complex of B lymphocytes: linking natural to acquired immunity. *Annu Rev Immunol* 1995; 13: 127-49. http://doi.org/10.1146/annurev.iy.13.040195.001015
29. WEHR C, EIBEL H, MASILAMANI M *et al*.: A new CD21low B cell population in the peripheral blood of patients with SLE. *Clin Immunol* 2004; 113: 161-71. http://doi.org/10.1016/j.clim.2004.05.010
30. BENSON RJ, HOSTAGER BS, BISHOP GA: Rapid CD40-mediated rescue from CD95-induced apoptosis requires TNFR-associated factor-6 and PI3K. *Eur J Immunol* 2006; 36: 2535-43. http://doi.org/10.1002/eji.200535483
31. HAO Y, O'NEILL P, NARADIKIAN MS, SCHOLZ JL, CANCRO MP: A B-cell subset uniquely responsive to innate stimuli accumulates in aged mice. *Blood* 2011; 118: 1294-304. http://doi.org/10.1182/blood-2011-01-330530
32. RUBTSOV AV, RUBTSOVA K, FISCHER A *et al*.: Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c(+) B-cell population is important for the development of autoimmunity. *Blood* 2011; 118: 1305-15. http://doi.org/10.1182/blood-2011-01-331462
33. RUBTSOVA K, RUBTSOV AV, HALEMANO K *et al*.: T Cell production of IFNgamma in response to TLR7/IL-12 stimulates optimal B Cell responses to viruses. *PLoS One* 2016; 11: e0166322. http://doi.org/10.1371/journal.pone.0166322
34. SACHINIDIS A, TRACHANA M, TAPARKOU A *et al*.: Investigating the role of t-bet(+) B cells (abcs/dn) in the immunopathogenesis of systemic lupus erythematosus. *Mediterr J Rheumatol* 2023; 34: 117-20. http://doi.org/10.31138/mjr.34.1.117
35. FREUDENHAMMER M, VOLL RE, BINDER SC, KELLER B, WARNATZ K: Naive- and memory-like cd21(low) B cell subsets share core phenotypic and signaling characteristics in systemic autoimmune disorders. *J Immunol* 2020; 205: 2016-25. http://doi.org/10.4049/jimmunol.2000343
36. JACOBI AM, REITER K, MACKAY M *et al*.: Activated memory B cell subsets correlate with disease activity in systemic lupus erythematosus: delineation by expression of CD27, IgD, and CD95. *Arthritis Rheum* 2008; 58: 1762-73. http://doi.org/10.1002/art.23498