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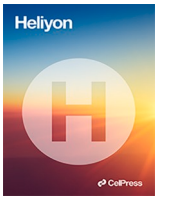

Research article

Circulating TIGIT$^{\pm}$PD1^{+}TPH, TIGIT $^{\pm}$ PD1^{+}TFH cells are elevated and their predicting role in systemic lupus erythematosus

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ABSTRACT

It is well established that increased peripheral helper T cells (TPH) and follicular helper T cells (TFH) was found in systemic lupus erythematosus (SLE) patients. However, the expression patterns and immunomodulatory roles of TIGIT and PD1 on TPH/TFH in SLE are poorly understood. The expression patterns of TIGIT and PD1 on TPH and TFH cells were examined using flow cytometry and their expression patterns in SLE patients were then further evaluated for their correlation with auto-antibodies, disease activity and severity, B cell differentiation. Logistic regression was used to analyze the risk factors. And the receiver operating characteristic curves and logistic regression model were created to evaluate the predicting role in SLE. TIGIT$^{\pm}$PD1^{+}TPH, TIGIT$^{\pm}$PD1^{+}TFH cells in the peripheral blood of SLE patients were upregulated, whereas TIGIT^{+}PD1^{-}TFH was downregulated. TIGIT $^{\pm}$ PD1^{+}TPH, TIGIT $^{\pm}$ PD1^{+}TFH cells positively correlated with auto-antibodies production, disease activity and severity, whereas TIGIT^{+}PD1^{-}TFH cells negatively correlated. TIGIT $^{\pm}$ PD1^{+}TPH, TIGIT^{-}PD1^{+}TFH were positively correlated with the frequency of plasmablasts. Furthermore, higher TIGIT^{+}PD1^{+}TPH and TIGIT^{+}PD1^{+}TFH were shown to be risk factors for SLE, whereas TIGIT^{+}PD1^{-}TFH was found to be a protective factor, according to logistic regression analysis. A further logistic regression model showed that combination of TPH/TFH and routine blood indicators may has potential predicting value for SLE, with AUC of 0.957. The increased TIGIT $^{\pm}$ PD1^{+}TPH, increased TIGIT $^{\pm}$ PD1^{+}TFH, decreased TIGIT^{+}PD1^{-}TFH correlates with disease severity and activity, may boost our comprehending of the role of TIGIT and PD1 on TPH/TFH in SLE, and a logistic regression model

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based on combination of TPH/TFH and routine blood indicators shows prominent value for predicting SLE.

Article headings

TIGIT and PD1 expression on TPH/TFH in SLE.

Significance paragraph

It is well-known that increased peripheral helper T cells (TPH) and follicular helper T cells (TFH) was found in patients with systemic lupus erythematosus (SLE). However, the expression patterns and immunomodulatory roles of TIGIT and PD1 on TPH/TFH in SLE are poorly understood. This is the first report on the expression intensity and pattern of TIGIT and PD1 on TPH/TFH in SLE. Additionally, our research established correlation between TIGIT$^{\pm}$PD1^{+}TPH, TIGIT$^{\pm}$PD1^{+}TFH, TIGIT^{+}PD1^{-}TFH and disease activity and severity in SLE, which might improve our understanding of the role of TIGIT and PD1 on TPH/TFH in SLE. Moreover, combination of TPH/TFH and routine blood indicators may has potential predicting value for SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is a disease of broad autoimmunity characterized by producing a variety of pathogenic autoantibodies and immune-complex-mediated multiple organs and systems damage [1]. Despite overall improvement in the care of SLE patients and an increase in survival rates of SLE patients, its prognosis remains unsatisfactory. Though, there have been many researches trying to illuminate the pathogenesis of SLE, the precise etiopathogenesis remains unclear, which is the reason of diagnosis, treatment and prognosis remains unsatisfactory in SLE [1,2]. Thus, the research of precise etiopathogenesis, early and accurate diagnosis and proper treatment are urgently requisite, which could dramatically change the unpredictable course of SLE and improve the long-term survival.

The auto-reactive B and T cells activation is a mark of lupus pathology and their abnormal functions might contribute to autoantibodies production in SLE, which thought to play a pivotal role in the pathogenesis of SLE [3]. It is widely recognized that follicular helper T (TFH) cells, a subset of helper T cells that express C-X-C motif chemokine receptor type 5 (CXCR5), possess the capacity to facilitate the B cell-mediated immunoreaction and regulate the formation of germinal centre in the secondary lymphoid organs through IL-21 production. Several evidences have demonstrated SLE patients had upregulated TFH cells [4–6] and their rising percentage in peripheral blood correlated with disease activity and severity. Although many studies have shown that some co-stimulatory molecules can be expressed on TFH cells, the most well-researched and established one is programmed death 1 (PD-1) [7]. T-cell immunoreceptor with Ig and immunoreceptor tyro-sine-based inhibitory domains (TIGIT) is a newly discovered negative costimulatory molecule that can be expressed in TFH cells in recent years, and data suggest the TIGIT-positive TFH cells promote strong B-cell help and ensuing immunoglobulin G production [8]. In addition, TIGIT and PD-1 can be used to characterize the phenotype and function of TFH subsets in healthy volunteer donors and in a group of chronically transfused sickle cell alloimmunization patients. However, the co-expression of TIGIT and PD1 on TFH in SLE patients and the predictive role in SLE are unknown.

It has been reported that another subset of CD4^{+}Th cells, called peripheral helper T (TPH) cells with a capacity to promoted B cell differentiation and antibody production were observed in SLE [9–11] and other autoimmune disease [12]. TPH cells in the RA synovium express PD-1 and inducible TIGIT, as well as IL-21 and C-X-C motif chemokine 13, similar to TFH cells, but they do not have CXCR5 [13]. However, the expression of TIGIT on TPH, the co-expression of TIGIT and PD1 on TPH in SLE patients, and their role in the pathogenesis and predicting of SLE are unknown.

It is generally accepted that patients with SLE have greater quantities of follicular helper T cells (TFH) and peripheral helper T cells (TPH). However, the expression patterns and immunomodulatory roles of TIGIT and PD1 on TPH/TFH in SLE are poorly understood. This is the first report on the expression intensity and pattern of TIGIT and PD1 on TPH/TFH in SLE. Additionally, our research established correlation between TIGIT$^{\pm}$PD1^{+}TPH, TIGIT$^{\pm}$PD1^{+}TFH, TIGIT^{+}PD1^{-}TFH and disease activity and severity in SLE, which might improve our understanding of the role of TIGIT and PD1 on TPH/TFH in SLE. Moreover, combination of TPH/TFH and routine blood indicators may has potential predicting value for SLE.

2. Materials and methods

2.1. Patient variables

Subject patients (n = 82) for the present study were consecutively recruited between November 2017 and September 2019 at the First Affiliated Hospital of Nanchang University. Diagnosis of SLE was performed according to the revised American College of Rheumatology criteria for SLE [14]. Those SLE patients accompanied by other disorder were excluded. Among them, 24 patients were new-onset and the subjects not yet use immunosuppressants and/or corticosteroids before enrollment. Other subjects were re-visiting SLE receiving drug. Then, 9 SLE subjects were administered with immunosuppressants and/or corticosteroids for at least one week according to therapeutic schedules. Disease activity was assessed by the SLE disease activity index (SLEDAI) [15]. The information on

SLEDAI, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white and red blood cell counts (WBC, RBC), hemoglobin (HGB), hematocrit (HCT), platelet count (PLT), lymphocyte counts (L) and percentages (L%), monocyte counts (M) and percentages (M %), neutrophil counts (N) and percentages (N%), neutrophil-to-lymphocyte ratio (NLR), derived neutrophil-lymphocyte ratio (dNLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), systemic immune-inflammation index (SII), immunoglobulin G (IgG), complement 3 (C3), complement 3 (C4), antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-nuclear ribonucleoprotein/Smith antibody (anti-nRNP/Sm), anti-ribosome RNP antibody (anti-RIB-P), anti-Smith antibody (anti-Sm), anti-sjögren syndrome A antigen antibody (anti-SSA), anti-sjögren syndrome B antigen antibody (anti-SSB), anti-nucleosome (ANUA) and clinical symptoms was collected. In addition, 48 matched healthy controls (HC) without diagnosis of other chronic and serious illness were enrolled from the First Affiliated Hospital of Nanchang University during the same period. The demographic characteristics of 82 SLE subjects and 48 matched HC were presented in Table 1. All study protocols complied with the principles outlined in the Declaration of Helsinki and were approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University ((2022) CDYFYYLK(06-005)).

2.2. Collection of PBMCs and flow cytometry analysis

To isolate PBMC for analyses, fasting blood (≈3 ml) was collected from the elbow vein into EDTA-coated and the PBMC then isolated using previously-reported protocols [16]. cell concentrations were determinated of in each isolated sample, followed by analyzed immediately for the frequency and phenotype of TPH cells, TFH cells and plasmablasts, using flow cytometry. The antibodies for flow cytometry were used: phycoerythrin-Texas Red (ECD)-conjugated anti-CD4 (SFCI12T4D11 clone, BD Biosciences, San Diego, CA, USA), phycoerythrin-Cyanin 7 (PC7)-conjugated anti-CXCR5 (MU5UBEE clone, e Bioscience, San Diego, CA, USA), phycoerythrin

Table 1
Clinical details and conventional indexs of subjects with SLE and HC.

Categories	SLE (n = 82)	HC (n = 48)
Females, % (n/total n)	91.46 (75/82)	89.58(43/48)
Age, years, (mean ± S.D.)	37.28 ± 12.88	41.23 ± 10.19
SLEDAI score, (mean ± S.D.)	8.52 ± 5.12	
ANA (+,>1:1000), % (n/total n)	44.68 (21/47)	–
Anti-dsDNA (IU/mL), (mean ± S.D.)	156.13 ± 274.60	–
Anti-ENA		–
Anti-Sm, % (n/total n)	28.81(17/59)	–
Anti-rRNP/Sm, % (n/total n)	54.24 (32/59)	–
Anti-RIB-P, % (n/total n)	28.81 (17/59)	–
ANUA, % (n/total n)	33.90 (20/59)	–
Anti-SSA, % (n/total n)	62.71 (37/59)	–
Anti-SSB, % (n/total n)	18.64 (11/59)	–
C3 (g/L), (mean ± S.D.)	0.61 ± 0.25	–
C4 (g/L), (mean ± S.D.)	0.14 ± 0.08	–
IgG (g/L), (mean ± S.D.)	17.23 ± 9.83	–
ESR (mm/h), (mean ± S.D.)	49.45 ± 37.02	–
CRP (mg/L), (mean ± S.D.)	19.09 ± 36.69	–
WBC (10^9/L), (mean ± S.D.)	6.14 ± 2.99	5.78 ± 1.30
RBC (10^{12}/L), (mean ± S.D.)	3.83 ± 0.86*	4.55 ± 0.28
HGB (g/L), (mean ± S.D.)	110.26 ± 24.74*	136.78 ± 9.43
HCT (L/L), (mean ± S.D.)	0.34 ± 0.07*	0.42 ± 0.03
PLT (10^9/L), (mean ± S.D.)	200.73 ± 98.06*	254.94 ± 50.88
L (10^9/L), (mean ± S.D.)	1.32 ± 0.81*	1.99 ± 0.46
L %, (mean ± S.D.)	23.24 ± 10.75*	34.85 ± 5.86
M (10^9/L), (mean ± S.D.)	0.44 ± 0.20*	0.36 ± 0.11
M%, (mean ± S.D.)	7.82 ± 3.15*	6.14 ± 1.38
N (10^9/L), (mean ± S.D.)	4.34 ± 2.69	3.30 ± 0.93
N%, (mean ± S.D.)	67.93 ± 12.38*	56.62 ± 5.96
NLR, (mean ± S.D.)	4.68 ± 6.31*	1.70 ± 0.50
dNLR, (mean ± S.D.)	2.99 ± 2.95*	1.35 ± 0.35
PLR, (mean ± S.D.)	195.03 ± 129.51*	133.42 ± 34.63
LMR, (mean ± S.D.)	3.49 ± 2.65*	5.98 ± 1.76
SII, (mean ± S.D.)	887.04 ± 1001.55*	432.21 ± 140.35
Clinical features		
Fever, % (n/total n)	4.88(4/82)	–
Cutaneous manifestations, % (n/total n)	29.27(24/82)	–
Arthritis, % (n/total n)	36.59(30/82)	–
Effusion, % (n/total n)	19.51(16/82)	–
Alopecia, % (n/total n)	7.32(6/82)	–
RI, % (n/total n)	45.12(37/82)	–
Ulcer, % (n/total n)	3.66(3/82)	–

According to the normality, the *t*-test or the nonparametric Mann-Whitney test was used to analyze the data. $P < 0.05$ was considered as statistically significant.

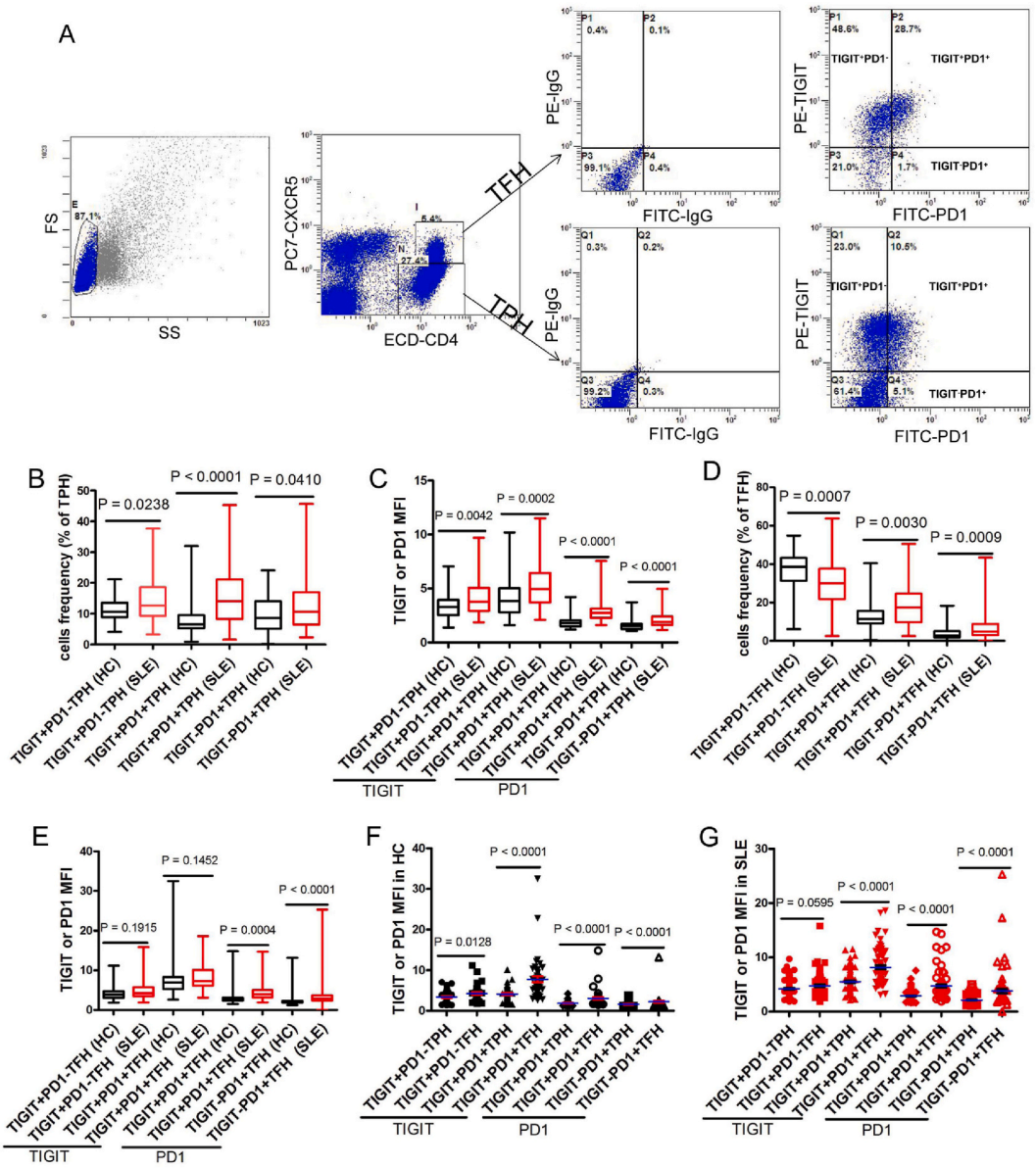

Fig. 1. TIGIT$^{\pm}$PD1^{+}TPH and TIGIT$^{\pm}$PD1^{+}TFH cells in the peripheral blood of SLE patients and HC

(A) Example of gating of TPH/TFH cells with different expression of TIGIT and PD-1 by flow cytometry analysis. (B) The frequency of TIGIT^{+}PD1^{-}TPH, TIGIT^{+}PD1^{+}TPH and TIGIT^{-}PD1^{+}TPH in the peripheral blood of SLE patients and HC (n = 82 SLE, n = 48 HC; n = 1 experiment). (C) The MFI for TIGIT on TIGIT^{+}PD1^{-}TPH, TIGIT^{+}PD1^{+}TPH and the MFI for PD1 on TIGIT^{+}PD1^{+}TPH, TIGIT^{-}PD1^{+}TPH in the peripheral blood of SLE patients and HC (n = 82 SLE, n = 48 HC; n = 1 experiment). (D) The frequency of TIGIT^{+}PD1^{-}TFH, TIGIT^{+}PD1^{+}TFH and TIGIT^{-}PD1^{+}TFH in the peripheral blood of SLE patients and HC (n = 72 SLE, n = 47 HC; n = 1 experiment). (E) The MFI for TIGIT on TIGIT^{+}PD1^{-}TFH, TIGIT^{+}PD1^{+}TFH and PD1 on TIGIT^{+}PD1^{+}TFH, TIGIT^{-}PD1^{+}TFH in the peripheral blood of SLE patients and HC (n = 72 SLE, n = 47 HC; n = 1 experiment). (F) The MFI for TIGIT on TPH (TIGIT^{+}PD1^{-}, TIGIT^{+}PD1^{+}) and TFH (TIGIT^{+}PD1^{-}, TIGIT^{+}PD1^{+}) in HC (n = 48 TPH, n = 47 TFH; n = 1 experiment), the MFI for PD1 on TPH (TIGIT^{+}PD1^{+}, TIGIT^{-}PD1^{+}) and TFH (TIGIT^{+}PD1^{+}, TIGIT^{-}PD1^{+}) in HC (n = 48 TPH, n = 47 TFH; n = 1 experiment). (G)The MFI for TIGIT on TPH (TIGIT^{+}PD1^{-}, TIGIT^{+}PD1^{+}) and TFH (TIGIT^{+}PD1^{-}, TIGIT^{+}PD1^{+}) in SLE (n = 82 TPH, n = 72 TFH; n = 1 experiment), the MFI for PD1 on TPH (TIGIT^{+}PD1^{+}, TIGIT^{-}PD1^{+}) and TFH (TIGIT^{+}PD1^{+}, TIGIT^{-}PD1^{+}) in SLE (n = 82 TPH, n = 72 TFH; n = 1 experiment). Data were acquired by flow cytometry in (A–G). (B–E) depict Box whiskers: Min to Max, (F–G) depict mean ± SEM. Significance determined by Mann-Whitney *U* test comparing HC versus SLE in (B), the MFI for PD1 (C), TIGIT^{+}PD1^{+}TFH/TIGIT^{-}PD1^{+}TFH (D), TIGIT on TIGIT^{+}PD1^{+}TFH/PD1 on TIGIT^{-}PD1^{+}TFH(E) and Student's t-test (unpaired *t*-test) comparing HC versus SLE in the MFI for TIGIT (C), TIGIT^{+}PD1^{-}TFH (D), TIGIT on TIGIT^{+}PD1^{-}TFH/PD1 on TIGIT^{+}PD1^{+}TFH(E). Significance determined by Mann-Whitney *U* test comparing TPH versus TFH in (F–G).

(PE)-conjugated anti-TIGIT (MBSA43 clone, e Bioscience, San Diego, CA, USA), fluorescein isothiocyanate (FITC)-conjugated anti-PD1 (MIH clone, e Bioscience, San Diego, CA, USA). Briefly, 50 μL of fresh PBMCs (5×10^5) were incubated simultaneously with 5 μL ECD-conjugated anti-CD4, 5 μL PC7-conjugated anti-CXCR5, 5 μL PE-conjugated anti-TIGIT and 5 μL FITC-conjugated anti-PD1 for 30 min in the dark on ice. PBMC incubated with PE- and FITC- conjugated mouse IgG were used as isotype controls. Subsequently, After careful staining, cells were analyzed with a CYTOMICS FC 500 flow cytometer and CXP software programs (Beckman Coulter Inc., Brea, CA, USA).

2.3. Statistical analysis

All data are expressed in terms of means ± SE. For the analyses, a Student's *t*-test (unpaired *t*-test) or a Mann-Whitney *U* test was performed to identify statistical differences among groups according to the normality. The paired *t*-test was performed to evaluate the TIGIT/PD1 changes on TPH/TFH in treatment. The Spearman method was used for correlation analysis. Univariate and multivariate analysis were used for risk factors analysis. Receiver operating characteristic (ROC) curves were builded to predict the diagnostic value for TIGIT/PD1 expression on TPH/TFH and routine blood indicators. Binary logistic regression was used to calculate the probability of combination indicators. Based on probability of logistic regression, ROC curve analysis also used to explore the predict value of combination of TIGIT/PD1 expression on TPH/TFH and routine blood indicators. SPSS v.17.0 (SPSS Inc., Chicago, IL) and Prism v.5.0 (GraphPad, San Diego, CA) software were used for data analysis. P-value ≤ 0.05 was considered as statistically significant.

3. Results

3.1. $TIGIT^{\pm}PD1^{+}TPH$ and $TIGIT^{\pm}PD1^{+}TFH$ cells in the peripheral blood of SLE patients were increased

Firstly, we explored the frequencies of TPH cells and TFH cells in the peripheral blood of SLE patients by flow cytometry analysis. TPH cells were termed as $CXCR5^{-}CD4^{+}$cells with expression of PD-1 or TIGIT and TFH cells were termed as $CXCR5^{+}CD4^{+}$cells with expression of PD-1 or TIGIT as described previously [5]. As shown in Fig. 1A, we identified the $TIGIT^{+}PD1^{-}CXCR5^{-}CD4^{+}$T cells ($TIGIT^{+}PD1^{-}$TPH), $TIGIT^{+}PD1^{+}CXCR5^{-}CD4^{+}$T cells ($TIGIT^{+}PD1^{+}$TPH), $TIGIT^{-}PD1^{+}CXCR5^{-}CD4^{+}$T cells ($TIGIT^{-}PD1^{+}$TPH) and $TIGIT^{+}PD1^{-}CXCR5^{+}CD4^{+}$T cells ($TIGIT^{+}PD1^{-}$TFH), $TIGIT^{+}PD1^{+}CXCR5^{+}CD4^{+}$T cells ($TIGIT^{+}PD1^{+}$TFH), $TIGIT^{-}PD1^{+}CXCR5^{+}CD4^{+}$T cells ($TIGIT^{-}PD1^{+}$TFH) in SLE patients and HC. Compared with HC, SLE patients demonstrated increased frequencies of $TIGIT^{+}PD1^{-}$TPH ($P = 0.0238$) (Fig. 1B), $TIGIT^{+}PD1^{+}$TPH ($P < 0.0001$) (Fig. 1B), $TIGIT^{-}PD1^{+}$TPH ($P = 0.0410$) (Fig. 1B). The mean fluorescence intensity (MFI) for TIGIT and PD1 expression on these TPH were significantly higher as compared with HC (all $P < 0.0100$) (Fig. 1C). And, the frequencies of $TIGIT^{+}PD1^{+}$TFH ($P = 0.0030$) (Fig. 1D), $TIGIT^{-}PD1^{+}$TFH ($P = 0.0009$) (Fig. 1D) were noticeably higher, while the frequency of $TIGIT^{+}PD1^{-}$TFH was clearly lower in SLE patients than HC ($P = 0.0007$) (Fig. 1D). The MFI for PD1 expression on these TFH were substantially higher as compared with HC (all $P < 0.0010$) (Fig. 1E), but the MFI for TIGIT expression on these TFH did not differ

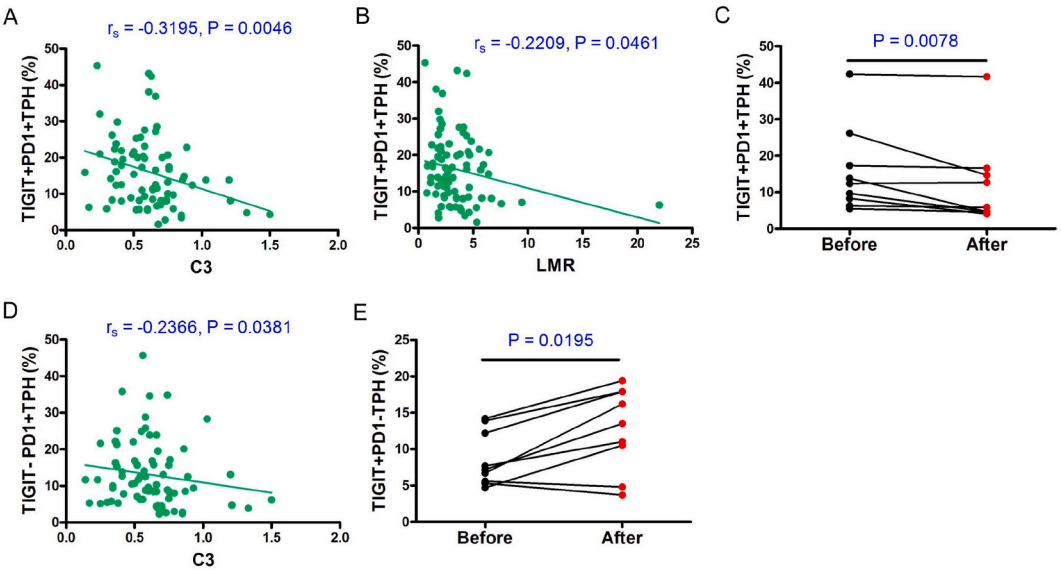

Fig. 2. $TIGIT^{\pm}PD1^{+}$TPH cells were associated with disease activity index in SLE patients
(A-B) Correlation of (A) the frequency of $TIGIT^{+}PD1^{+}$TPH and (B) the frequency of $TIGIT^{-}PD1^{+}$TPH with C3 (n = 77C3; n = 1 experiment). (C) Correlation of the frequency of $TIGIT^{+}PD1^{+}$TPH with LMR (n = 82 LMR; n = 1 experiment). (D–E) Association of (D) the frequency of $TIGIT^{+}PD1^{+}$TPH and (E) the frequency of $TIGIT^{+}PD1^{-}$TPH with treatment (n = 9 treatment; n = 1 experiment). Data were acquired by flow cytometry in (A–E). Symbols represent individual donors in (A–E). Spearman correlation coefficient (r_s) shown in (A–C). Significance determined by paired *t*-test comparing before treatment versus after treatment in (D–E).

between the two groups (all P > 0.0500) (Fig. 1E). Interestingly, the MFI for TIGIT and PD1 expression on these TFH were considerably higher as compared with these TPH cells in HC (all P < 0.0500) (Fig. 1F) and SLE patients (all P < 0.0001) (Fig. 1G), in addition to the MFI for TIGIT expression between TIGIT^{+}PD1^{-}TFH and TIGIT^{+}PD1^{-}TPH in SLE patients (P = 0.0595) (Fig. 1G).

3.2. TIGIT$^{\pm}$PD1^{+}TPH and TIGIT$^{\pm}$PD1^{+}TFH cells were associated with disease activity index in SLE patients

The clinical indicators related to disease activity index (ESR, CRP, IgG, C3, C4, treatment and clinical symptoms) were collected and the SLEDAI of all SLE patients were figured up, and the relevance between this data set and these TPH and TFH were analyzed. As shown in Fig. 2, there was a moderate correlation between the frequency of TIGIT^{+}PD1^{+}TPH and C3 ($r_s = -0.3195$, P = 0.0046) (Fig. 2A), there was a weak correlation between the frequency of TIGIT^{+}PD1^{+}TPH and LMR ($r_s = -0.2209$, P = 0.0461) (Fig. 2B), and the frequency of TIGIT^{+}PD1^{+}TPH in SLE patients with treatment was decreased than those prior to treatment (P = 0.0078) (Fig. 2C), there was only a weak correlation between the frequency of TIGIT^{-}PD1^{+}TPH and C3 ($r_s = -0.2366$, P = 0.0381) (Fig. 2D), whereas the frequency of TIGIT^{+}PD1^{-}TPH increased following treatment (P = 0.0195) (Fig. 2E). Moreover, there was a moderate or weak correlation between the MFI for PD1 expression on TIGIT^{+}PD1^{+}TPH and disease activity index in SLE patients including C3, CRP, RBC, HGB, HCT, LMR, renal involvement, treatment (supplement Fig. 1A-1H), the MFI for TIGIT expression on TIGIT^{+}PD1^{+}TPH were associated with C3, anti-SSB (supplement Fig. 1I and 1J), there was a weak correlation between the MFI for PD1 expression on TIGIT^{-}PD1^{+}TPH and disease activity index in SLE patients including CRP, RBC, renal involvement (supplement Fig. 1K-1M).

The relevance between clinical indicators and TFH subgroup based on TIGIT and PD1 expression showed that there was a weak correlation between the frequency of TIGIT^{+}PD1^{+}TFH and WBC ($r_s = -0.2653$, P = 0.0243) (Fig. 3A), the frequency of TIGIT^{+}PD1^{+}TFH was significantly increased in SLE patients with positive anti-rRNP/Sm (P = 0.0189) (Fig. 3B). There was a weak correlation between the frequency of TIGIT^{-}PD1^{+}TFH and with C3 ($r_s = -0.2843$, P = 0.0188) (Fig. 3C), the frequency of

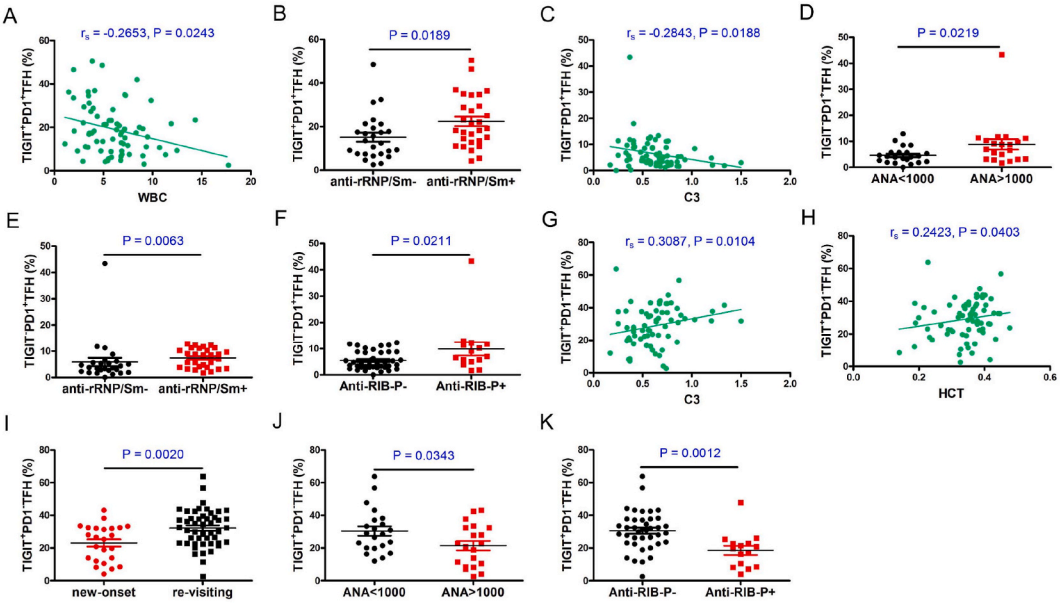

Fig. 3. TIGIT$^{\pm}$PD1^{+}TFH cells were associated with disease activity index in SLE patients
(A) Correlation of the frequency of TIGIT^{-}PD1^{+}TFH with C3 (n = 68C3; n = 1 experiment). (B) Correlation of the frequency of TIGIT^{+}PD1^{+}TFH with WBC (n = 72 WBC; n = 1 experiment). (C) The frequency of TIGIT^{-}PD1^{+}TFH was significantly increased in SLE patients with ANA titer >1000 than ANA titer <1000 (n = 22 ANA titer<1000, n = 20 ANA titer>1000; n = 1 experiment). (D–E) The frequency of (D)TIGIT^{+}PD1^{+}TFH and (E) TIGIT^{-}PD1^{+}TFH in SLE patients were different between positive anti-rRNP/Sm and negative anti-rRNP/Sm (n = 29 positive anti-rRNP/Sm, n = 26 negative anti-rRNP/Sm; n = 1 experiment). (F) The frequency of TIGIT^{-}PD1^{+}TFH was significantly increased in SLE patients with positive anti-RIB-P (n = 15 positive anti-RIB-P, n = 40 negative anti-RIB-P; n = 1 experiment). (G) Correlation of the frequency of TIGIT^{+}PD1^{-}TFH with C3 (n = 68C3; n = 1 experiment). (H) Correlation of the frequency of TIGIT^{+}PD1^{-}TFH with HCT (n = 72 HCT; n = 1 experiment). (I) The frequency of TIGIT^{+}PD1^{-}TFH was significantly decreased in new-onset than re-visiting SLE patients (n = 24 new-onset, n = 48 re-visiting; n = 1 experiment). (J) The frequency of TIGIT^{+}PD1^{-}TFH was significantly decreased in SLE patients with ANA titer = 1000 than ANA titer <1000 (n = 22 ANA titer<1000, n = 20 ANA titer = 1000; n = 1 experiment). K: The frequency of TIGIT^{+}PD1^{-}TFH was significantly decreased in SLE patients with positive anti-RIB-P (n = 15 positive anti-RIB-P, n = 40 negative anti-RIB-P; n = 1 experiment). Data were acquired by flow cytometry in (A–K). (C–F, I–K) depict mean ± SEM. Symbols represent individual donors in (A–K). Spearman correlation coefficient (r_s) shown in (A-B, G-H). Significance determined by Mann-Whitney *U* test comparing ANA titer<1000 versus = 1000 in (C), comparing positive anti-rRNP/Sm versus negative anti-rRNP/Sm in (E), comparing positive anti-RIB-P versus negative anti-RIB-P in (F). Significance determined by Student's t-test (unpaired *t*-test) comparing positive anti-rRNP/Sm versus negative anti-rRNP/Sm in (D), comparing new-onset versus re-visiting SLE patients in (I), comparing ANA titer<1000 versus = 1000 in (J), comparing positive anti-RIB-P versus negative anti-RIB-P in (K).

TIGIT^{-}PD1^{+}TFH was significantly increased in SLE patients with ANA titer = 1000 (P = 0.0219) (Fig. 3D), positive anti-rRNP/Sm (P = 0.0063) (Fig. 3E), positive anti-RIB-P (P = 0.0211) (Fig. 3F), while there was a moderate correlation between the frequency of TIGIT^{+}PD1^{-}TFH and C3 (r_s = 0.3087, P = 0.0104) (Fig. 3G), there was a weak correlation between the frequency of TIGIT^{+}PD1^{-}TFH and HCT (r_s = 0.2423, P = 0.0403) (Fig. 3H), the frequency of TIGIT^{+}PD1^{-}TFH was significantly decreased in new-onset SLE patients (P = 0.0020) (Fig. 3I), SLE patients with ANA titer = 1000 (P = 0.0343) (Fig. 3J) and SLE patients with positive anti-RIB-P (P = 0.0012) (Fig. 3K). Moreover, there was a moderate or weak correlation between the MFI of PD1 expression on TIGIT^{+}PD1^{+}TFH and disease activity index in SLE patients including C3, ESR, CRP, SLEDAI, RBC, HGB, HCT, NLR, LMR, treatment, anti-dsDNA (supplement Fig. 2A-2K), and there was a moderate or weak correlation between the MFI of PD1 expression on TIGIT^{-}PD1^{+}TFH were associated with CRP, HGB, HCT (supplement Fig. 2L-2N).

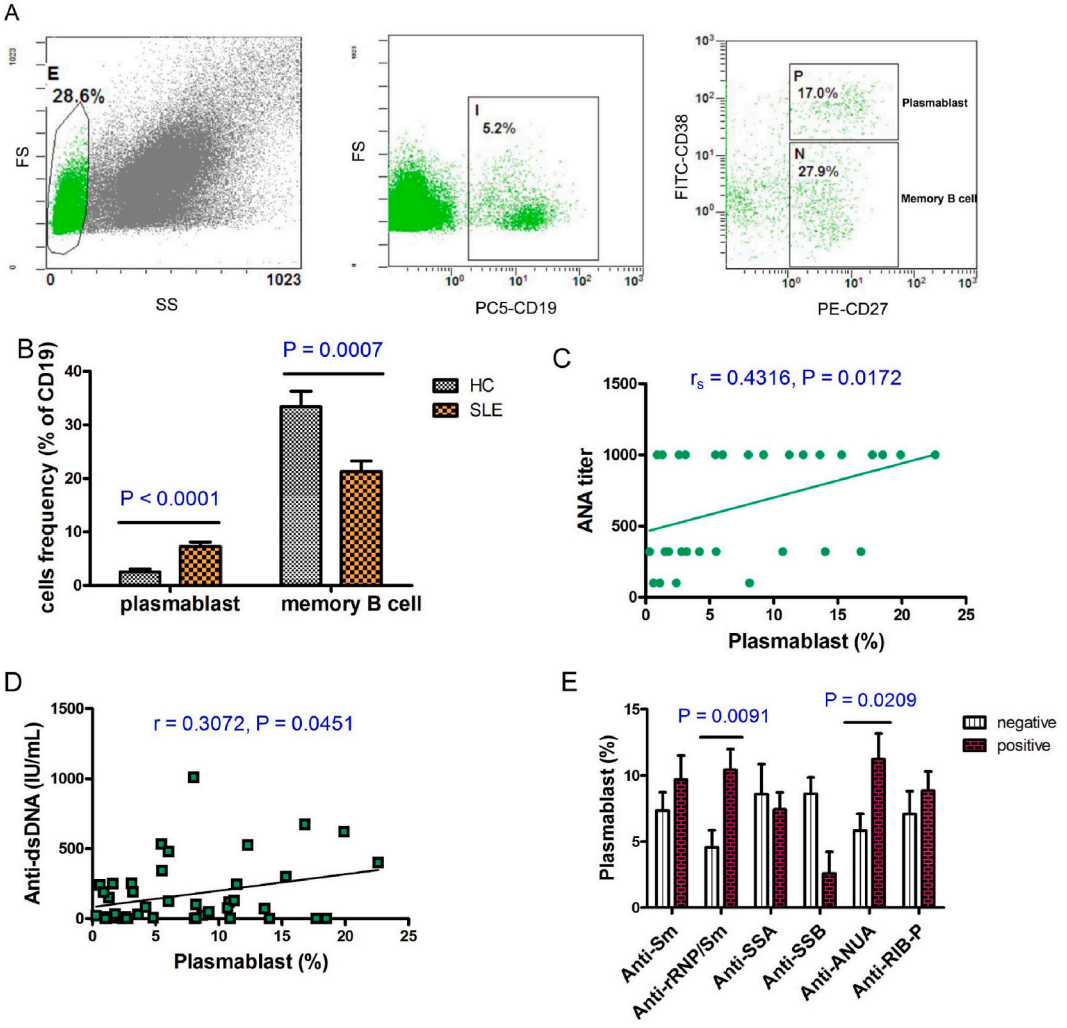

Fig. 4. Association of B cell differentiation with autoantibodies production

(A) Example of gating of plasmablasts and memory B cells by flow cytometry analysis. (B) The frequency of plasmablasts was significantly increased and the frequency of memory B cells was significantly decreased in SLE patients than HC(n = 47 SLE, n = 26 HC; n = 1 experiment). C: correlation of the frequency of plasmablasts with ANA titer (n = 30 ANA titer; n = 1 experiment). D: The frequency of plasmablasts was correlated with anti-dsDNA (n = 43 anti-dsDNA; n = 1 experiment). E: The frequency of plasmablasts was significantly increased in SLE patients with positive anti-rRNP/Sm, anti-ANUA and no difference between positive anti-Sm, anti-SSA, anti-SSB, anti-RIB-P (n = 18 positive anti-rRNP/Sm, n = 15 negative anti-rRNP/Sm, n = 12 positive anti-ANUA, n = 21 negative anti-ANUA, n = 7 positive anti-Sm, n = 26 negative anti-Sm, n = 20 positive anti-SSA, n = 13 negative anti-SSA, n = 4 positive anti-SSB, n = 29 negative anti-SSB, n = 14 positive anti-RIB-P, n = 19 negative anti-RIB-P; n = 1 experiment). Data were acquired by flow cytometry in (A–E). (B, E) depict mean ± SEM. Symbols represent individual donors in (C–E). Significance determined by Mann-Whitney *U* test comparing SLE versus HC in (B). Significance determined by Student's t-test (unpaired *t*-test) comparing positive antibody versus negative antibody in (E). Spearman correlation coefficient (r_s) shown in (C–D).

3.3. Association of TIGIT$^{\pm}$PD1^{+}TPH and TIGIT$^{\pm}$PD1^{+}TFH cells with B cell differentiation

We identified plasmablasts and memory B cells as CD19^{+}CD27^{+}CD38^{+} cells and CD19^{+}CD27^{+}CD38^{-} cells respectively (Fig. 4A). As shown in Fig. 4B, the frequency of plasmablasts was significantly increased ($P < 0.0001$) and the frequency of memory B cells was significantly decreased in SLE patients ($P = 0.0007$). Because plasmablasts were confirmed to help autoantibodies production, we explored the correlation of plasmablasts with ANA, anti-dsDNA and anti-ENA. And data showed that there was a moderate correlation between the frequency of plasmablasts and ANA titer ($r_s = 0.4316$, $P = 0.0172$) (Fig. 4C), anti-dsDNA ($r = 0.3072$, $P = 0.0451$) (Fig. 4D), the frequency of plasmablasts was associated with anti-rRNP/Sm ($P = 0.0054$) (Fig. 4E), anti-ANUA ($P = 0.0148$) (Fig. 4E).

Since evidence from previous report have confirmed that TPH and TFH cells could promote B cell differentiation, we investigated the relation of TIGIT$^{\pm}$PD1^{+}TPH and TIGIT$^{\pm}$PD1^{+}TFH cells with B cell populations. As shown in Fig. 5, there was a moderate correlation between the frequency of TIGIT^{+}PD1^{+}TPH ($r_s = 0.3070$, $P = 0.0358$) (Fig. 5A), TIGIT^{-}PD1^{+}TPH ($r_s = 0.4467$, $P = 0.0016$) (Fig. 5B), TIGIT^{-}PD1^{+}TFH ($r_s = 0.5711$, $P < 0.0001$) (Fig. 5C) and the frequency of plasmablasts, whereas no correlation was found between the frequencies of TIGIT^{+}PD1^{-}TPH ($r_s = -0.1241$, $P = 0.4060$) (Fig. 5D), TIGIT^{+}PD1^{+}TFH ($r_s = 0.2116$, $P = 0.1629$) (Fig. 5E), TIGIT^{+}PD1^{-}TFH ($r_s = -0.2290$, $P = 0.1302$) (Fig. 5F) between the frequency of plasmablasts. Moreover, there was a moderate correlation between the MFI for PD1 expression on TIGIT^{+}PD1^{+}TPH, TIGIT^{-}PD1^{+}TPH, TIGIT^{+}PD1^{+}TFH, TIGIT^{-}PD1^{+}TFH and the frequency of plasmablasts (supplement Fig. 3).

3.4. TIGIT $^{+}$ PD1^{+}TPH, TIGIT^{+}PD1^{+}TFH were risk factors and TIGIT^{+}PD1^{-}TFH was protective factor for SLE

The above data manifested that the frequencies of TIGIT^{+}PD1^{-}TPH, TIGIT^{+}PD1^{+}TPH, TIGIT^{-}PD1^{+}TPH in SLE patients were increased and the frequencies of TIGIT^{+}PD1^{+}TPH, TIGIT^{-}PD1^{+}TPH were associated with disease activity and treatment. Thus, we investigated whether the frequencies of TIGIT^{+}PD1^{-}TPH, TIGIT^{+}PD1^{+}TPH, TIGIT^{-}PD1^{+}TPH were risk factors for SLE using univariate analyses and multivariate analyses. Firstly, univariate analyses was performed, and then the indicators with P value lower than 0.05 from univariate analyses were selected as indicators for multivariate analyses. As shown in Table 2, based on univariate analyses and multivariate analyses, we found that the increased frequency of TIGIT^{+}PD1^{+}TPH was a risk factor for SLE ($P = 0.0010$), whereas the frequencies of TIGIT^{+}PD1^{-}TPH, TIGIT^{-}PD1^{+}TPH are not ($P > 0.0500$).

Moreover, TFH cells can be divided into three groups (TIGIT^{+}PD1^{-}, TIGIT^{+}PD1^{+} and TIGIT^{-}PD1^{+}) based on the expression of TIGIT and PD1. Considering that the increased frequency of TIGIT^{+}PD1^{+}TFH was positively associated with disease activity, auto-antibody production and the decreased frequency of TIGIT^{+}PD1^{-}TFH was negatively associated with disease activity, auto-antibody production, the univariate analyses and multivariate analyses were also used to explore whether the frequencies of TIGIT^{+}PD1^{-}TFH, TIGIT^{+}PD1^{+}TFH, TIGIT^{-}PD1^{+}TFH were risk factors for SLE. As shown in Table 2, based on univariate analyses and multivariate

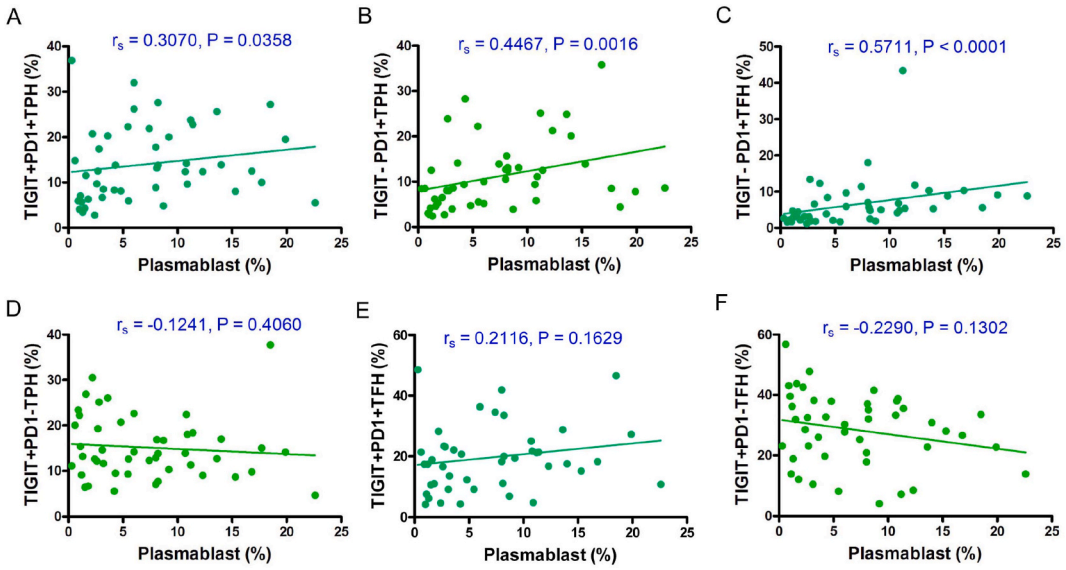

Fig. 5. Association of TIGIT$^{\pm}$PD1^{+}TPH and TIGIT $^{\pm}$ PD1^{+}TFH cells with B cell differentiation
(A) Correlation of the frequency of TIGIT^{+}PD1^{+}TPH with the frequency of plasmablasts (n = 47 plasmablasts; n = 1 experiment). (B) Correlation of the frequency of TIGIT^{-}PD1^{+}TPH with the frequency of plasmablasts (n = 47 plasmablasts; n = 1 experiment). (C) Correlation of the frequency of TIGIT^{-}PD1^{+}TFH with the frequency of plasmablasts (n = 45 plasmablasts; n = 1 experiment). (D) Correlation of the frequency of TIGIT^{+}PD1^{-}TPH with the frequency of plasmablasts (n = 47 plasmablasts; n = 1 experiment). (E) Correlation of the frequency of TIGIT^{+}PD1^{+}TFH with the frequency of plasmablasts (n = 45 plasmablasts; n = 1 experiment). (F) Correlation of the frequency of TIGIT^{+}PD1^{-}TFH with the frequency of plasmablasts (n = 45 plasmablasts; n = 1 experiment). Data were acquired by flow cytometry in (A–F). Symbols represent individual donors in (A–F). Spearman correlation coefficient (r_s) shown in (A–F).

Table 2
Univariate and multivariate analyses of risk factors associated with SLE.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
TPH						
$TIGIT^+PD1^-TPH$ (%)	1.097	1.020–1.179	0.0130			
$TIGIT^+PD1^+TPH$ (%)	1.222	1.120–1.334	<0.0001	1.219	1.090–1.364	0.0010
$TIGIT^-PD1^+TPH$ (%)	1.069	1.012–1.130	0.0180			
TFH						
$TIGIT^+PD1^-TFH$ (%)	0.943	0.909–0.978	0.0010	0.948	0.908–0.990	0.0150
$TIGIT^+PD1^+TFH$ (%)	1.072	1.024–1.122	0.0030	1.071	1.015–1.130	0.0120
$TIGIT^-PD1^+TFH$ (%)	1.196	1.054–1.357	0.0060			

CI, confidence interval; OR, odds ratio.

analyses, the results demonstrated that the frequency of $TIGIT^+PD1^-TFH$ was a protective factor for SLE ($P = 0.0150$), the frequency of $TIGIT^+PD1^+TFH$ was a risk factor for SLE ($P = 0.0120$), whereas the frequency of $TIGIT^-PD1^+TFH$ was not ($P = 0.7800$).

3.5. Predictive value of combination of TPH, TFH and routine blood indicators in SLE patients

The effect of these indexs with statistic difference on distinguishing between SLE patients and HC was further investigated. We used the Prism v.5.0 for Statistical Computing to generate ROC curves and then obtain AUC, *P*-value, ninety-five percent confidence intervals (95% CI), Sensitivity, Specificity, Cut-off. The data showed that four indexs (the frequency of $TIGIT^+PD1^+TPH$, PD1 MFI on $TIGIT^+PD1^+TPH$, PD1 MFI on $TIGIT^+PD1^+TFH$ and PD1 MFI on $TIGIT^-PD1^+TFH$) had potential in predicting SLE patients from HC, with AUC higher than 0.800 (Table 3). Considering our limited number of patients, we used the four indicators with AUC higher than 0.8 for binary logistic regression to assess the cumulative performances of the four indicators in distinguishing SLE patients from HC. Based on probability of binary logistic regression on the frequency of $TIGIT^+PD1^+TPH$, PD1 MFI on $TIGIT^+PD1^+TPH$, PD1 MFI on $TIGIT^+PD1^+TFH$ and PD1 MFI on $TIGIT^-PD1^+TFH$, we established ROC curves to explore the predictive value and showed that the combination of the frequency of $TIGIT^+PD1^+TPH$, PD1 MFI on $TIGIT^+PD1^+TPH$, PD1 MFI on $TIGIT^+PD1^+TFH$ and PD1 MFI on $TIGIT^-PD1^+TFH$ could provide the better predicted accuracy with the AUC of 0.900 (95% CI: 0.837–0.962, $P < 0.0001$, sensitivity: 80.56%, specificity: 91.49%) (Fig. 6A), which was superior to any other single indicator (Table 3).

As shown in Table 1, routine blood indicators including RBC, HGB, HCT, PLT, L, L%, M, M%, N%, NLR, dNLR, PLR, LMR, SII were all remarkably different between SLE patients and HC. Then ROC curves were builded to predict the diagnostic value for these

Table 3
ROC curve analysis of indicators with TPH, TFH and routine blood.

Variables	AUC	*P*-value	95% CI	Sensitivity	Specificity	Cut-off
TPH indicator						
$TIGIT^+PD1^-TPH$ (%)	0.619	0.0237	0.524–0.715	57.32	68.75	>12.15
$TIGIT^+PD1^+TPH$ (%)	0.802	<0.0001	0.726–0.877	60.98	95.83	>12.35
$TIGIT^-PD1^+TPH$ (%)	0.608	0.0409	0.509–0.706	28.05	91.67	>16.25
TIGIT MFI on $TIGIT^+PD1^-TPH$	0.641	0.0077	0.543–0.738	57.32	70.83	>3.66
TIGIT MFI on $TIGIT^+PD1^+TPH$	0.694	0.0002	0.600–0.787	89.02	41.67	>3.36
PD1 MFI on $TIGIT^+PD1^+TPH$	0.880	<0.0001	0.816–0.944	71.95	93.75	>2.39
PD1 MFI on $TIGIT^-PD1^+TPH$	0.764	<0.0001	0.678–0.851	68.29	75.00	>1.70
TFH indicator						
$TIGIT^+PD1^-TFH$ (%)	0.700	0.0002	0.603–0.797	65.28	72.34	<33.95
$TIGIT^+PD1^+TFH$ (%)	0.662	0.0030	0.563–0.760	56.94	85.11	>16.50
$TIGIT^-PD1^+TFH$ (%)	0.680	0.0009	0.583–0.777	79.17	51.06	>2.80
PD1 MFI on $TIGIT^+PD1^+TFH$	0.830	<0.0001	0.750–0.911	86.11	72.34	>3.03
PD1 MFI on $TIGIT^-PD1^+TFH$	0.859	<0.0001	0.790–0.929	70.83	95.74	>2.51
Routine blood indicator						
RBC (10^{12}/L)	0.783	<0.0001	0.706–0.861	71.95	83.33	<4.29
HGB (g/L)	0.868	<0.0001	0.806–0.930	73.17	95.83	<124.50
HCT (L/L)	0.862	<0.0001	0.780–0.925	73.17	89.58	<0.38
PLT (10^9/L)	0.702	0.0001	0.614–0.790	65.85	72.92	<227.50
L (10^9/L)	0.811	<0.0001	0.737–0.885	71.95	87.5	<1.45
L%	0.814	<0.0001	0.743–0.885	63.41	93.75	<27.20
M (10^9/L)	0.612	0.0334	0.517–0.707	26.83	100.00	>0.56
M%	0.672	0.0011	0.581–0.763	47.56	89.58	>7.85
N%	0.771	<0.0001	0.693–0.850	63.41	91.67	>63.90
NLR	0.795	<0.0001	0.720–0.869	62.20	93.75	>2.34
dNLR	0.772	<0.0001	0.693–0.850	62.20	93.75	>1.82
PLR	0.654	0.0035	0.560–0.747	56.10	89.58	>173.90
LMR	0.866	<0.0001	0.805–0.927	79.27	83.33	<4.53
SII	0.625	0.0179	0.531–0.719	40.24	95.83	>679.90

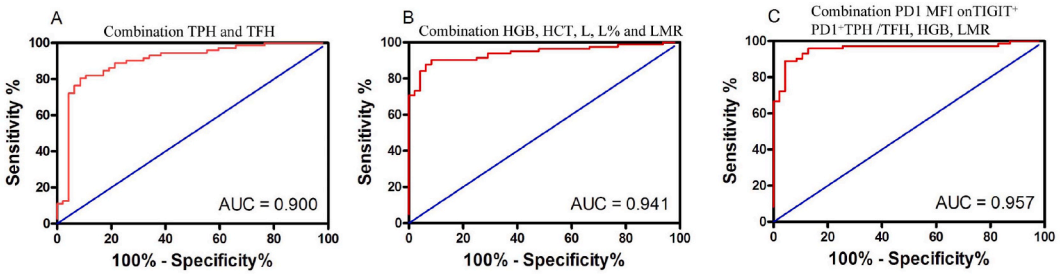

Fig. 6. Predictive value of combination of TPH, TFH and routine blood indicators in SLE patients
(A) The AUC separating SLE patients from the HC was found for combination the frequency of TIGIT$^+$PD1$^+$TPH, PD1 MFI on TIGIT$^+$PD1$^+$TPH, PD1 MFI on TIGIT$^+$PD1$^+$TFH and PD1 MFI on TIGIT$^-$PD1$^+$TFH (n = 72 SLE, n = 47 HC; n = 1 experiment). (B) The AUC separating SLE patients from the HC was found for combination HGB, HCT, L, L% and LMR (n = 82 SLE, n = 48 HC; n = 1 experiment). (C) The AUC separating SLE patients from the HC was found for combination the PD1 MFI on TIGIT$^+$PD1$^+$TPH, PD1 MFI on TIGIT$^+$PD1$^+$TFH, HGB, and LMR (n = 72 SLE, n = 47 HC; n = 1 experiment). Data were acquired by ROC in (A–C).

indicators by obtaining AUC, *P*-value, ninety-five percent confidence intervals (95% CI), Sensitivity, Specificity, Cut-off. Five indicators, including HGB, HCT, L, L% and LMR, had potential in distinguishing SLE patients and HC, with AUC higher than 0.800 (Table 3). We used the five routine blood indicators with AUC higher than 0.8 for binary logistic regression to assess the cumulative performances of the five indicators in distinguishing SLE patients from HC. Based on probability of binary logistic regression on HGB, HCT, L, L% and LMR, we established ROC curves to explore the predictive value and showed that the combination of the five routine blood indicators (HGB, HCT, L, L%, LMR) have better predicted accuracy with the AUC of 0.941 (95% CI: 0.901–0.981, $P < 0.0001$, sensitivity: 90.24%, specificity: 91.67%) (Fig. 6B).

Then, to evaluate the cumulative performances of indicators with TPH, TFH and routine blood in discriminating SLE patients from HC, and with a view to the less number of subjects, we picked all parameters with AUC >0.8 for further univariable and multivariable analyses. Based on the multivariate analysis, PD1 MFI on TIGIT$^+$PD1$^+$TPH, PD1 MFI on TIGIT$^+$PD1$^+$TFH, HGB and LMR were selected as variables for the predictive model (Table 4). We established ROC curves to explore the predictive value and showed that the combination of PD1 MFI on TIGIT$^+$PD1$^+$TPH, PD1 MFI on TIGIT$^+$PD1$^+$TFH, HGB and LMR could provide the better predicted accuracy with the AUC of 0.957 (95% CI: 0.919–0.994, $P < 0.0001$, sensitivity: 88.89%, specificity: 95.74%) (Fig. 6C).

4. Discussion

Although CD4$^+$ T cells are termed as central elements in specific autoimmune diseases, it stills challenging to define the pivotal functions of CD4$^+$ T cells in SLE chronic immune-mediated damage to the whole system [17,18]. It is well known that costimulatory molecules play an important role in the regulation of CD4$^+$T cell function and can can mediate the occurrence and progression of SLE. TIGIT, a co-inhibitory molecule, is newly identified on T cells, which binds to CD155 and CD112 ligands, leading to effect change of T cells. TIGIT exerts immunosuppressive effects by competing with CD226 for the same CD155 ligand and the expression pattern of CD226 and TIGIT on FoxP3$^+$Tregs could affect the function of FoxP3$^+$Tregs [19]. Evidence from Lie M et al. [20] and our previous research [21] have indicated that TIGIT expression on CD4$^+$ T cells was significantly elevated in patients with SLE and highly correlated with the activity of the disease. And, many researches from patients and animal level have demonstrated that the percentages of PD1 on CD4$^+$ T cells in SLE was increased, and the level of PD1 on CD4$^+$ T cells may contribute to the pathogenesis of SLE [22,23]. Moreover, Guo Q et al. showed that the IM-MNPs specifically targeted SLE CD4$^+$ T cells and agonist PD-1/TIGIT signaling to inhibit effector T cell function while enhancing the immunosuppressive function of regulatory T cells, and the IM-MNPs displayed significant therapeutic efficacy in ameliorating lupus nephritis [24]. Based on these data, we hypothesize that TIGIT and PD1 are

Table 4
Univariable and multivariable analysis.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
TIGIT$^+$PD1$^+$TPH (%)	1.222	1.120–1.334	<0.0001			
PD1 MFI on TIGIT$^+$PD1$^+$TPH	17.056	6.025–48.286	<0.0001	26.403	4.752–146.713	<0.0001
PD1 MFI on TIGIT$^+$PD1$^+$TFH	1.784	1.239–2.571	0.0020	0.627	0.439–0.896	0.0100
PD1 MFI on TIGIT$^-$PD1$^+$TFH	2.414	1.372–4.247	0.0020			
HGB (g/L)	0.897	0.860–0.936	<0.0001	0.869	0.816–0.926	<0.0001
HCT (L/L)	2.860E16	1.257E10–6.507E22	<0.0001			
L (10^9/L)	0.267	0.146–0.488	<0.0001			
L%	0.862	0.814–0.914	<0.0001			
LMR	0.575	0.459–0.720	<0.0001	0.565	0.414–0.770	<0.0001

CI, confidence interval; OR, odds ratio.

crucial for the onset and progression of SLE.

According to CXCR5 level, $CD4^+$T cells could be divided into $CXCR5^-CD4^+$T peripheral helper (TPH) cells and $CXCR5^+CD4^+$T (TFH) cells, which was reported could promote B cell responses and antibody production [25,26]. TIGIT and PD1, the negative T cell costimulatory molecules that maintains peripheral tolerance, express on TFH cells and involve in its function [8,26]. And, with the coordination of CD226, TIGIT can regulate the differentiation and maturation of human TFH cells [27]. Godefroy and colleagues [8] firstly termed $PD1^{+/-}TIGIT^+$TFH and demonstrated $PD1^{+/-}TIGIT^+$TFH can produce induce the differentiation of B cells into plasmablasts and promote immunoglobulin G production than $PD1^-TIGIT^-$TFH, and blocking antibody of TIGIT abrogated the B-cell help properties, which suggest expression of the TIGIT not only represents a novel circulating T follicular helper biomarker, but is also functional and promotes strong B-cell help and ensuing immunoglobulin G production. Moreover, Bocharnikov et al. [9] already shown RNA-seq data that SLE patients have higher PD1, ICOS, HLA-DR, TIGIT expression in TPH from SLE and they demonstrated that expanded $ICOS^+PD\text{-}1^+CXCR5^-$ TPH, $HLA\text{-}DR^+PD\text{-}1^+CXCR5^-$ TPH, $HLA\text{-}DR^-PD\text{-}1^+CXCR5^-$ TPH cells in the blood of SLE patients by AMP mass cytometry data, whereas no data about $TIGIT^+PD1^+CXCR5^-$TPH cells. Thus, we characterize and group TPH, TFH cells by PD-1, TIGIT expression and investigate the levels of $PD1^+TIGIT^+$TFH/TPH, $PD1^+TIGIT^-$TFH/TPH, $PD1^-TIGIT^+$TFH/TPH in SLE and their clinical significance.

We have herein manifested that the peripheral blood of SLE patients is characterized by an upregulated frequency of the recently defined $PD1^+CXCR5^-CD4^+$ (TPH) and $PD1^+CXCR5^+CD4^+$ (TFH) cell population [4,5,9–11]. In addition, SLE patients demonstrated augmented $TIGIT^\pm PD1^+$TFH cells and depressed $TIGIT^+PD1^-$TFH cells, which were explored in patients with sickle cell disease (SCD) [8]. This contrasts with data reported by Godefroy et al. [8], who observed no significant difference in the percentage of $TIGIT^\pm PD1^+$TFH, $TIGIT^+PD1^-$TFH cells subsets between alloimmunized or non-alloimmunized SCD patients. These findings suggested that distinct disease types have distinct TIGIT and PD1 expression patterns on TFH cells. The study of the expression patterns of TIGIT and PD1 on TFH cells has certain significance for understanding the role of $TIGIT^\pm PD1^{+/-}$TFH in diseases. Also, elevated frequencies of $TIGIT^\pm PD1^+$TPH cells, $TIGIT^+PD1^-$TPH cells were present in the peripheral blood of patients with SLE, which were investigated for the first time.

Evidence for the correlation of TPH cells with SLE disease status from many study have been demonstrated [9,10,28]. Remarkably, we observed the frequency of $TIGIT^+PD1^+$TPH cells was significantly associated with C3, LMR, treatment, and the frequency of $TIGIT^-PD1^+$TPH cells was significantly associated with C3, whereas the frequency of $TIGIT^+PD1^-$TPH cells increased following treatment. These results indicated that the frequencies of $TIGIT^\pm PD1^+$TPH cells positively correlated with disease activity and severity, but the frequency of $TIGIT^+PD1^-$TPH cells negatively correlated with disease severity. Moreover, the PD1 MFI on $TIGIT^\pm PD1^+$TPH cells was significantly associated with C3, CRP, SLEDAI, RBC, HGB, HCT, LMR, RI, treatment, anti-Sm, which was identical to concept showed by Makiyama et al. [11], who observed the expression level of PD1 on TPH was associated its function and pathopoiesis in SLE. Furthermore, the TIGIT MFI on TIGIT + PD1+TPH cells was substantially correlated with C3, anti-SSB, suggesting that both the expression level of PD1 and TIGIT on TIGIT + PD1+TPH cells is involved in the pathogenic effect.

As well as the data about $TIGIT^\pm PD1^+$TPH cells, the augmented $TIGIT^\pm PD1^+$TFH cells in SLE were positively associated with autoantibodies production, disease activity and severity, which were consistent with the results about $PD1^+$TPH/TFH cells from others [4,5,9–11,29–32]. It is surprising that the depressed $TIGIT^+PD1^-$TFH cells positively correlated with C3, HCT, and the $TIGIT^+PD1^-$TFH cells in SLE patients with new-onset, ANA = 1000, positive anti-RIB-P was significantly decreased. These results indicate $TIGIT^+PD1^-$TFH cells was negatively associated with autoantibodies production, disease activity and severity, which was inconsistent with the results from Godefroy et al. [8], showing $TIGIT^+PD1^-$TFH cells promote immunoglobulin G production in healthy control. These results suggested that different expression patterns of TIGIT and PD1 on TFH cells and the function of these cell subpopulations in different disease were different.

The previous study demonstrate that $CD4^+CXCR5^+$T (TFH), $CD4^+CXCR5^-$ T(TPH) in SLE patients could exhibit B-cell help capacity and promote IgG production, and activated $CD4^+CXCR5^+$T (TFH) that up-regulated the expression of ICOS exhibited higher B-cell help capacity than activated $CD4^+CXCR5^-$ T(TPH), suggesting the expression of costimulatory molecules on these cells could affect their function [33]. The results in the present study showed that the frequencies of $TIGIT^+PD1^+$TPH, $TIGIT^-PD1^+$TPH, $TIGIT^-PD1^+$TFH and the PD1 MFI on these cells were positively correlated with the frequency of plasmablasts, whereas no correlation was found between the frequencies of $TIGIT^+PD1^-$TPH, $TIGIT^+PD1^+$TFH, $TIGIT^+PD1^-$TFH and the frequency of plasmablasts, which indicating the expression intensity and pattern of TIGIT and PD1 on TPH/TFH adjusted their function. Moreover, the results of multivariate logistic regression about the expression pattern of TIGIT and PD1 on TPH/TFH manifested $TIGIT^+PD1^+$TPH, $TIGIT^+PD1^+$TFH were risk factors and $TIGIT^+PD1^-$TFH was protective factor for SLE, consistent with the correlation between $TIGIT^+PD1^+$TPH, $TIGIT^+PD1^+$TFH, $TIGIT^+PD1^-$TFH and disease activity and severity. However, the future investigation about the function of $TIGIT^+PD1^-$TFH in SLE should be explore.

The aforementioned results demonstrated that the expression intensity and pattern of TIGIT and PD1 on TPH/TFH were associated with disease activity and severity. Thus, we further investigated whether the differential expression of these TPH/TFH could discriminate SLE patients from HC and our results showed that combination of the frequency of $TIGIT^+PD1^+$TPH, PD1 MFI on $TIGIT^+PD1^+$TPH, PD1 MFI on $TIGIT^+PD1^+$TFH and PD1 MFI on $TIGIT^-PD1^+$TFH could provide the better predicted accuracy with the AUC of 0.900. Presently, many studies demonstrated that routine laboratory markers including routine blood indicators cloud be used for predicting disease and disease severity [34,35]. Considering the routine blood indicators in SLE are abnormal and the convenience of routine blood test, we explored the predicting value of these abnormal routine blood indicators and found combination of HGB, HCT, L, L%, LMR showed better predicted accuracy with the AUC of 0.941. Furthermore, the predicting value of combination of pathogenicity indexs and routine blood indicators was evaluated and the results showed that combination of the PD1 MFI on $TIGIT^+PD1^+$TPH, PD1 MFI on $TIGIT^+PD1^+$TFH, HGB, LMR could provide the best predicted accuracy with the AUC of 0.957.

However, there are several limitations in the present study. Firstly, the expression profile of characteristic proteins in these TPH/TFH cell subpopulations is unclear and should be explored in future. And, functional assay including generating differentiated B cells in coculture assay and promoting cytokine secretion should be investigated. Secondly, SLE is a heterogeneous disease, so there is some heterogeneity in its data, the samples were collected from only one institution, which may lead to a certain risk of bias. Multicenter clinical studies with a larger sample size remain needed, which may reduce differences caused by statistical data processing limitations in research methods. Thirdly, due to PD1 MFI on TIGIT^{+}PD1^{+}TPH/TFH, HGB, LMR also obviously abnormal in other autoimmune diseases, this study only used HC as the control group may result in the predictive combination model based on PD1 MFI on TIGIT^{+}PD1^{+}TPH/TFH, HGB, LMR will have a limited value in real clinical practice. Moreover, follow-up studies with other autoimmune diseases as disease control are needed.

5. Conclusions

This is the first report on the expression intensity and pattern of TIGIT and PD1 on TPH/TFH in SLE. In addition, our data built correlation between TIGIT$^{\pm}$PD1^{+}TPH, TIGIT$^{\pm}$PD1^{+}TFH, TIGIT^{+}PD1^{-}TFH and SLE activity and severity, which might improve the comprehending of the effect of TIGIT and PD1 on TPH/TFH in SLE. Moreover, combination of TPH/TFH and routine blood indicators may has potential predicting value for SLE.

Ethics declarations

This study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University, with the approval number: (2022)CDYFYYLK(06-005).

Informed consent was not required for this study because this study approved to exempt informed consent.

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Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

CRediT authorship contribution statement

Qing Luo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Qiuyun Xiao:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Lu Zhang:** Methodology, Formal analysis, Data curation. **Biqi Fu:** Investigation, Formal analysis, Data curation. **Xue Li:** Investigation, Data curation. **Zikun Huang:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Junming Li:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27687.

References

[1] A. Fanouriakis, N. Tziolos, G. Bertsias, D.T. Boumpas, Update omicronn the diagnosis and management of systemic lupus erythematosus, Ann. Rheum. Dis. 80 (1) (2021) 14–25, https://doi.org/10.1136/annrheumdis-2020-218272.

[2] M. Piga, L. Arnaud, The main challenges in systemic lupus erythematosus: where do we stand? J. Clin. Med. 10 (2) (2021) 243, https://doi.org/10.3390/jcm10020243.

[3] S. Wang, J.Y. Wang, V. Kumar, J.L. Karnell, B. Naiman, P.S. Gross, et al., IL-21 drives expansion and plasma cell differentiation of autoreactive CD11chiTbet + B cells in SLE, Nat. Commun. 9 (1) (2018) 1758, https://doi.org/10.1038/s41467-018-03750-7.

[4] S.J. Kim, K. Lee, B. Diamond, Follicular helper T cells in systemic lupus erythematosus, Front. Immunol. 9 (2018) 1793, https://doi.org/10.3389/fimmu.2018.01793.

[5] C.E. Faliti, R. Gualtierotti, E. Rottoli, M. Gerosa, L. Perruzza, A. Romagnani, et al., P2X7 receptor restrains pathogenic Tfh cell generation in systemic lupus erythematosus, J. Exp. Med. 216 (2) (2019) 317–336, https://doi.org/10.1084/jem.20171976.

[6] X. Zeng, M. Zheng, T. Liu, A. Bahabayi, R. Kang, Q. Xu, et al., Changes in the expression of T-cell factor-1 in follicular helper T cells reflect the condition of systemic lupus erythematosus patients, Int. Immunopharm. 108 (2022) 108877, https://doi.org/10.1016/j.intimp.2022.108877 dol:.

[7] D.A. Rao, T cells that help B cells in chronically inflamed tissues, Front. Immunol. 9 (2018) 1924, https://doi.org/10.3389/fimmu.2018.01924.

[8] E. Godefroy, H. Zhong, P. Pham, D. Friedman, K. Yazdanbakhsh, TIGIT-positive circulating follicular helper T cells display robust B-cell help functions: potential role in sickle cell alloimmunization, Haematologica 100 (11) (2015) 1415–1425, https://doi.org/10.3324/haematol.2015.132738.

[9] A.V. Bocharnikov, J. Keegan, V.S. Wacleche, Y. Cao, C.Y. Fonseka, G. Wang, et al., PD-1hiCXCR5- T peripheral helper cells promote B cell responses in lupus via MAF and IL-21, JCI Insight 4 (20) (2019) e130062, https://doi.org/10.1172/jci.insight.130062.

[10] J. Lin, Y. Yu, J. Ma, C. Ren, W. Chen, PD-1+CXCR5-CD4+T cells are correlated with the severity of systemic lupus erythematosus, Rheumatology 58 (12) (2019) 2188–2192, https://doi.org/10.1093/rheumatology/kez228.

[11] A. Makiyama, A. Chiba, D. Noto, G. Murayama, K. Yamaji, N. Tamura, et al., Expanded circulating peripheral helper T cells in systemic lupus erythematosus: association with disease activity and B cell differentiation, Rheumatology 58 (10) (2019) 1861–1869, https://doi.org/10.1093/rheumatology/kez077.

[12] Y. Long, C. Xia, Y. Sun, Y. Ma, L. Xu, Y. Song, et al., Increased circulating PD-1hiCXCR5- peripheral helper T cells are associated with disease severity of active ulcerative colitis patients, Immunol. Lett. 233 (2021) 2–10, https://doi.org/10.1016/j.imlet.2021.03.001.

[13] D.A. Rao, M.F. Gurish, J.L. Marshall, K. Slowikowski, C.Y. Fonseka, Y. Liu, et al., Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis, Nature 542 (7639) (2017) 110–114, https://doi.org/10.1038/nature20810.

[14] M.C. Hochberg, Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus, Arthritis Rheum. 40 (9) (1997) 1725, https://doi.org/10.1002/art.1780400928.

[15] C. Bombardier, D.D. Gladman, M.B. Urowitz, D. Caron, C.H. Chang, Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE, Arthritis Rheum. 35 (6) (2010) 630–640, https://doi.org/10.1002/art.1780350606.

[16] Q. Luo, L. Zhang, X. Li, B. Fu, T. Guo, Z. Huang, J. Li, Identification of circular RNA hsa_circ_0044235 and hsa_circ_0068367 as novel biomarkers for systemic lupus erythematosus, Int. J. Mol. Med. 44 (4) (2019) 1462–1472, https://doi.org/10.3892/ijmm.2019.4302.

[17] H. Ueno, The IL-12-STAT4 axis in the pathogenesis of human systemic lupus erythematosus, Eur. J. Immunol. 50 (1) (2020) 10–16, https://doi.org/10.1002/eji.201948134.

[18] L.S. Ji, X.H. Sun, X. Zhang, Z.H. Zhou, Z. Yu, X.J. Zhu, et al., Mechanism of follicular helper T cell differentiation regulated by transcription factors, J Immunol Res 2020 (2020) 1826587, https://doi.org/10.1155/2020/1826587.

[19] Long Y, Wang C, Xia C, Li X, Fan C, Zhao X, et al. Recovery of CD226-TIGIT+FoxP3+ and CD226-TIGIT-FoxP3+ regulatory T cells contributes to clinical remission from active stage in ulcerative colitis patients. Immunol. Lett.:30-39. doi: 10.1016/j.imlet.2019.12.007.

[20] L. Mao, H. Hou, S. Wu, Y. Zhou, J. Wang, J. Yu, et al., TIGIT signalling pathway negatively regulates CD4+ T-cell responses in systemic lupus erythematosus, Immunology 151 (3) (2017) 280–290, https://doi.org/10.1111/imm.12715.

[21] Q. Luo, J. Ye, L. Zeng, X. Li, L. Fang, B. Ju, et al., Elevated expression of TIGIT on CD3+CD4+ T cells correlates with disease activity in systemic lupus erythematosus, Allergy Asthma Clin. Immunol. 13 (2017) 15, https://doi.org/10.1186/s13223-017-0188-7.

[22] Y. Miyazaki, K. Tsumiyama, T. Yamane, M. Ito, S. Shiozawa, Expansion of PD-1-positive effector CD4 T cells in an experimental model of SLE: contribution to the self-organized criticality theory, Kobe J. Med. Sci. 59 (2) (2013) E64–E71. PMID: 23756664.

[23] S. Dolff, D. Quandt, T. Feldkamp, C. Jun, A. Mitchell, F. Hua, et al., Increased percentages of PD-1 on CD4+ T cells is associated with higher INF-γ production and altered IL-17 production in patients with systemic lupus erythematosus, Scand. J. Rheumatol. 43 (4) (2014) 307–313, https://doi.org/10.3109/03009742.2013.869830.

[24] Q. Guo, C. Chen, Z. Wu, W. Zhang, L. Wang, J. Yu, et al., Engineered PD-1/TIGIT dual-activating cell-membrane nanoparticles with dexamethasone act synergistically to shape the effector T cell/Treg balance and alleviate systemic lupus erythematosus, Biomaterials 285 (2022) 121517, https://doi.org/10.1016/j.biomaterials.2022.121517.

[25] Y. Wen, B. Yang, J. Lu, J. Zhang, H. Yang, J. Li, Imbalance of circulating CD4(+)CXCR5(+)FOXP3(+) Tfr-like cells and CD4(+)CXCR5(+)FOXP3(-) Tfh-like cells in myasthenia gravis, Neurosci. Lett. 630 (2016) 176–182, https://doi.org/10.1016/j.neulet.2016.07.049.

[26] S. Caielli, D.T. Veiga, P. Balasubramanian, S. Athale, B. Domic, E. Murat, et al., A CD4(+) T cell population expanded in lupus blood provides B cell help through interleukin-10 and succinate, Nat. Med. 25 (1) (2019) 75–81, https://doi.org/10.1038/s41591-018-0254-9.

[27] M. Yasutomi, A.F. Christiaansen, N. Imai, N. Martin-Orozco, C.V. Forst, G. Chen, et al., CD226 and TIGIT cooperate in the differentiation and maturation of human tfh cells, Front. Immunol. 13 (2022) 840457, https://doi.org/10.3389/fimmu.2022.840457.

[28] Q. Jiang, J. Wang, H. Jiang, W. Li, Y. Sun, Y. Shan, et al., Competitive binding of transcription factors underlies flexibility of T peripheral helper cells and T follicular helper cells in SLE, Rheumatology 61 (11) (2022) 4547–4557,, https://doi.org/10.1093/rheumatology/keac112.

[29] P. Fortea-Gordo, L. Nuño, A. Villalba, D. Peiteado, I. Monjo, P. Sánchez-Mateos, et al., Two populations of circulating PD-1hiCD4 T cells with distinct B cell helping capacity are elevated in early rheumatoid arthritis, Rheumatology 58 (9) (2019) 1662–1673, https://doi.org/10.1093/rheumatology/kez169.

[30] I. Ekman, E. Ihantola, T. Viisanen, D.A. Rao, K. Näntö-Salonen, M. Knip, et al., Circulating CXCR5−PD-1hi peripheral T helper cells are associated with progression to type 1 diabetes, Diabetologia 62 (9) (2019) 1681–1688, https://doi.org/10.1007/s00125-019-4936-8.

[31] L. Zhang, H. Li, H. Ren, P. Hu, Circulating PD-1hiCXCR5+CD4+ T cells are associated with a decrease in hepatitis B surface antigen levels in patients with chronic hepatitis B who are receiving peginterferon-α therapy, Mol. Immunol. 103 (2018) 270–278, https://doi.org/10.1016/j.molimm.2018.10.011.

[32] N. Simpson, P.A. Gatenby, A. Wilson, S. Malik, D.A. Fulcher, S.G. Tangye, et al., Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus, Arthritis Rheum. 62 (1) (2010 Jan) 234–244.

[33] H. Xu, J. Liu, X. Cui, Y. Zuo, Z. Zhang, Y. Li, et al., Increased frequency of circulating follicular helper T cells in lupus patients is associated with autoantibody production in a CD40L-dependent manner, Cell. Immunol. 295 (1) (2015) 46–51, https://doi.org/10.1016/j.cellimm.2015.01.014.

[34] G.X. Tang, S.T. Tong, X. Yuan, Q. Lin, Y. Luo, H. Song, et al., Using routine laboratory markers and immunological indicators for predicting pneumocystis jiroveci pneumonia in immunocompromised patients, Front. Immunol. 12 (2021) 652383, https://doi.org/10.3389/fimmu.2021.652383.

[35] G.H. Xue, X. Gan, Z.Q. Wu, D. Xie, Y. Xiong, L. Hua, et al., Novel serological biomarkers for inflammation in predicting disease severity in patients with COVID-19, Int. Immunopharm. 89 (Pt A) (2020) 107065, https://doi.org/10.1016/j.intimp.2020.107065.