Expanded circulating peripheral helper T cells in systemic lupus erythematosus: association with disease activity and B cell differentiation

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Abstract

Objective: Peripheral helper T (T_{PH}) cells are a recently identified helper T cell subset that promotes B cell differentiation and antibody production in inflamed tissues. This study investigated circulating T_{PH} cells to determine their involvement in systemic lupus erythematosus (SLE).

Methods: Peripheral blood mononuclear cells collected from SLE patients and healthy individuals were analysed. T_{PH} cells were identified as $CD3^+CD4^+CD45RA^-CXCR5^-$ cells with a high expression of programmed cell death protein 1 (PD-1). The frequency, activation status, and subsets of T_{PH} cells were evaluated by flow cytometry. The production of interleukin (IL)-21 was assessed by intracellular staining and the association of T_{PH} cells with disease activity and B cell populations was determined.

Results: Circulating T_{PH} cells, identified as $CD3^+CD4^+CD45RA^-PD\text{-}1^{high}CXCR5^-$ cells were increased in the peripheral blood of SLE patients compared with controls. Circulating T_{PH} cells produced similar amounts of IL-21 to follicular helper T cells. The expansion and activation of T_{PH} cells were correlated with SLE disease activity. Activated T_{PH} cells, particularly Th1 type T_{PH} cells, were associated with the promotion of B cell differentiation in SLE patients.

Conclusion: The association of T_{PH} cells with disease activity suggests the involvement of extra-follicular T–B cell interactions in the pathogenesis of SLE. T_{PH} cells promote autoantibody production in aberrant lymphoid organs and therefore might be a novel therapeutic target in autoantibody-producing disorders.

Keywords

Systemic lupus erythematosus, Peripheral helper T cells, Disease activity, B cell differentiation

Key messages

- Peripheral helper T cells are associated with lupus disease activity.
- Activated Peripheral helper T cells might promote B cell differentiation and autoantibody production.
- Extra-follicular T−B cell interactions might be involved in the pathogenesis of SLE.

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the production of various autoantibodies and immune-complex-mediated tissue damage in multiple organs. The activation of autoreactive B and T cells is a hallmark of lupus pathology and their functional abnormalities may lead to autoantibody production in SLE. Autoantibodies observed in SLE patients are class-switched and somatically mutated, reflecting the involvement of effective T–B cell interactions in the generation of autoantibodies [1,2]. Follicular helper T (T_{FH}) cells were first identified in human tonsils as a $CD4^+$ helper T cell subset that helps germinal centre responses. T_{FH} cells have an important role in the class switching and affinity maturation of B cells through the production of interleukin (IL)-21, which leads to the generation of memory B cells and plasma cells [3,4]. Several groups have reported the expansion of T_{FH} cells in SLE patients [5-15] and the correlation of their increased frequency in peripheral blood with disease activity or severity.

Recently, the marked expansion of a new subset of $CD4^+$ helper T cells termed peripheral helper T (T_{PH}) cells with a capacity to promote B cell responses and antibody production was reported in the inflamed tissues of rheumatoid arthritis (RA) patients

[16]. Similar to T_{FH} cells, T_{PH} cells express high levels of program cell death protein-1 (PD-1) and produce CXCL13 and IL-21. However, T_{PH} cells lack the expression of CXCR5, which is expressed on T_{FH} cells and is critical for entrance to germinal centres in lymphoid organs. Instead, they express CCR2, CX3CR1, and CCR5, which reflects their migratory capacity to inflamed sites. The abundance of T_{PH} cells in inflamed joints in autoantibody positive RA patients suggests the importance of local sites of inflammation as regions of autoantibody production. In addition to RA, the role of the tertiary lymphoid organs has been reported in other autoimmune diseases such as Hashimoto's thyroiditis and Sjogren's syndrome [17,18]. In SLE, the aggregation of T and B cells was reported in nephritic kidneys and the local production of autoantibodies was observed in lupus models, suggesting the extra-follicular production of autoantibodies [19].

Here, we show the expansion of T_{PH} cells in the peripheral blood of patients with SLE. Their frequency was correlated with the disease activity of lupus. Furthermore, the activation status of T_{PH} cells correlated with lupus disease activity and the frequency of plasmablasts and activated switched memory B cells. Among T_{PH} cells, the expansion of activated Th1 type cells was associated with increased B cell

differentiation in SLE patients. Taken together, the activation of T_{PH} cells associated with lupus disease activity and B cell differentiation suggest the importance of extra-follicular T–B cell interactions in the pathogenesis of SLE.

Methods

Human samples

We obtained peripheral blood for flow cytometric analysis from 65 patients with SLE (57 females and 8 males, median age 41.0 years [IQR 33.0–46.5], median disease duration 11.0 years [IQR 3.5–18.0]) and 27 healthy control subjects. Healthy controls were matched to patients with SLE by sex and age. The characteristics of patients with SLE and healthy controls are shown in Table 1. The study of cell sorting and intracellular cytokine staining analysis included 9 patients with SLE (Table 2). SLE was diagnosed according to the 1997 American College of Rheumatology criteria and disease activity was measured using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). We obtained peripheral blood from patients with SLE and healthy controls after receiving informed consent in accordance with the local ethics committee guidelines of Juntendo University. This study was conducted with the

approval of the regional ethics committee at Juntendo University Hospital (No. 17-258). Informed consent was received from all patients and healthy volunteers.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density-gradient centrifugation using BD Vacutainer CPT Mononuclear Cell Preparation Tubes with sodium heparin (BD Biosciences, Franklin Lakes, NJ, USA). The cells were incubated with FcR blocking reagent (BioLegend, San Diego, CA, USA) to block the non-specific binding of antibodies. Then, cell surface staining was performed using the following monoclonal antibodies: CD38-FITC (Beckman Coulter, Indianapolis, IN, USA); CD4-APC-H7, CD180-PE, HLADR-V500 (BD Biosciences); CD185 (CXCR5)-PE/Dazzle 594, CD196 (CCR6)-PerCP/Cy5.5, CD3-Brilliant Violet 421, CD45RA-Brilliant Violet 605, CD278 (ICOS)-FITC, CD279 (PD-1)-PE, CD38-PE/Cy7, CD183 (CXCR3)-APC, CD19-PE/Dazzle 594, CD20-Alexa 700, CD27-APC/Fire 750, and IgD-Brilliant Violet 421 (BioLegend). Data were acquired on a FACS LSR Fortessa™ (BD Biosciences) and analysed by FlowJo software (FlowJo, LLC, Ashland, OR, USA).

Cell sorting

Total $CD4^+$ T cells were isolated from PBMCs by negative selection using a human $CD4^+$ T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). $CD4^+$ cells were stained with anti-PD-1-PE, CXCR5-APC, and CD45RA-BV605 antibodies (BioLegend). Dead cells were excluded by staining with Zombie NIR (BioLegend). Surface-stained $CD4^+$ cells were sorted by FACSAria Fusion (BD Biosciences) into $CD45RA^-CXCR5^-PD\text{-}1^{high}$ (T_{PH}), $CD45RA^-CXCR5^-PD\text{-}1^{int}$, $CD45RA^-CXCR5^-PD\text{-}1^-$, and $CD45RA^-CXCR5^+PD\text{-}1^{high}$ (T_{FH}) cell populations.

Intracellular cytokine staining

Sorted memory $CD4^+$ T cell subsets were cultured in 96-well V-bottom plates in RPMI 1640 medium supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 50 U/mL penicillin and 50 µg/mL streptomycin (all from Thermo Fisher Scientific, Waltham, MA, USA) and then stimulated with phorbol 12-myristate 13-acetate (PMA, 50 ng/ml) and ionomycin (1 µg/ml) for 4 hours. GolgiPlug (100 ng/ml; BD Biosciences) was added for the last 3 hours of incubation. Cells were fixed with Cytofix/Cytoperm

solution, permeabilized with Perm/Wash buffer (BD Biosciences), and then stained with anti-IL-21-BV421 antibody (BD Biosciences) or an isotype control antibody. Data were acquired on a FACS LSR Fortessa.

Statistical analysis

All data were analysed using Prism 6 software (Graph-Pad Software Inc., La Jolla, CA, USA), and differences between the groups were analysed using the Mann–Whitney *U*-test or the Kruskal-Wallis test followed by Dunn's multiple comparisons test, or the Wilcoxon's matched pairs test. The significance level was set at $p < 0.05$. Associations between two variables were analysed using Spearman's correlation.

Results

Expansion of T_{PH} cells in association with disease activity in SLE

First, we investigated the frequencies of T_{PH} cells in the peripheral blood of SLE patients by FACS. T_{PH} cells were identified as $CD3^+CD4^+CD45RA^-CXCR5^-$ cells with a high expression of PD-1 as described previously [16] (Fig. 1A). Representative data of T_{PH} cells in healthy control subjects (HC) and patients with SLE are shown in

Fig. 1B. To confirm that the T_{PH} cells we identified in SLE patients had similar characteristics to T_{PH} cells previously found in the synovium of RA patients, we assessed whether these cells produced IL-21. As shown in Fig. 1C, T_{PH} cells identified in SLE patients produced IL-21 upon activation with PMA and ionomycin similar to T_{FH} cells. These data indicated that the increased numbers of T_{PH} cells in SLE patients produced IL-21, which promotes B cell differentiation. The frequency of T_{PH} cells was increased in SLE patients (median 2.60% [IQR 1.86–4.32]) compared with healthy controls (median 1.45% [IQR 1.11–2.07]) (Fig. 1D). We assessed whether the increase in T_{PH} cells was affected by treatment (which included prednisone, hydroxychloroquine, and immunosuppressive agents); however, there was no difference in T_{PH} cell frequency among the different treatment groups (Fig. 1E).

We next examined whether the increase of T_{PH} cells in patients with SLE was correlated with disease activity. We found the frequency of T_{PH} cells was positively correlated with SLEDAI and the titre of anti-dsDNA antibodies, and negatively correlated with serum complement measured as the CH50 (Fig. 1F). After treatment, the median frequency of T_{PH} cells in patients with active disease decreased from 5.83% to 3.65% (Fig. 1G). These results indicated that T_{PH} cells were increased in SLE patients

and that their expansion was associated with disease activity.

Correlation of T_{PH} cell activation status with disease activity

Because the frequency of T_{PH} cells was associated with the enhanced production of autoantibodies, we investigated the activation status of T_{PH} cells by determining their expression of the activation markers CD38 and HLA-DR [20]. Both $CD38^+$ and HLA-DR^+ T_{PH} cells among $CD4^+$ T cells were increased in SLE patients (median 1.25% [IQR 0.78–2.29], median 0.35% [IQR 0.18–0.95], respectively) compared with HCs (median 0.50% [IQR 0.41–0.63], median 0.14% [IQR 0.11–0.20], respectively) (Fig. 2A). We further assessed whether the activation status of T_{PH} cells was correlated with disease activity and found a positive correlation between the frequency of $CD38^+$ or HLA-DR^+ T_{PH} cells with SLEDAI and anti-dsDNA antibody levels as well as a negative correlation with serum complement measured as CH50 and the number of lymphocytes (Fig. 2B). These findings suggest that the activation status of T_{PH} cells reflects disease activity in SLE patients.

Association of T_{PH} cell activation with B cell differentiation

Because T_{PH} cells were reported to help B cell differentiation, we investigated the relationship of T_{PH} cell activation with B cell populations. Plasmablasts were reported to be increased in SLE patients [21]. We identified plasmablasts as $CD19^{+}CD27^{+}CD38^{+}CD180^{-}$ cells and found their expansion in SLE patients (median for HCs 0.06% [IQR 0.04–0.10] vs median for SLE 0.14% [IQR 0.06–0.39]) (Fig. 3A). Consistent with previous studies, their frequency was positively correlated with SLEDAI and the titre of anti-dsDNA antibodies (Fig. 3A). As shown in Fig. 3B, the frequency of plasmablasts was correlated with activated T_{PH} cells. We also assessed the association of activated T_{PH} cells with other B cell populations and found a positive correlation between activated T_{PH} cells and activated switched memory B cells identified as $CD19^{+}CD20^{+}CD27^{+}$ $IgD^{-}CD38^{+}$ cells (Fig. 3B). These results indicated that activated T_{PH} cells might promote B cell differentiation in SLE patients.

T_{PH} cell subset correlation with B cell differentiation

The differential expression of CXCR3 and CCR6 has been used to separate helper T cells and T_{FH} cells into effector T cell subsets characterized by specific cytokine production such as interferon-γ (IFN-γ) producing $CXCR3^{+}CCR6^{-}$ (Th1 type)

cells, IL-4 producing $CXCR3^-CCR6^-$ (Th2 type) cells and IL-17 producing $CXCR3^-CCR6^+$ (Th17 type) cells. We assessed the Th subsets among T_{PH} cells based on their expression of these chemokine receptors. As shown in Fig. 4A, Th1 type-T_{PH} ($T_{PH}1$) and Th2 type-T_{PH} ($T_{PH}2$) cells were increased in SLE patients (median for HCs 0.87% [IQR 0.67–1.42] vs median for SLE 1.71% [IQR 1.24–2.59]; median for HCs 0.13% [IQR 0.09–0.20] vs median for SLE 0.41% [IQR 0.22–0.77], respectively). There was a tendency for increased numbers of Th17 type-T_{PH} ($T_{PH}17$) cells in SLE patients, but the difference did not reach statistical significance (Fig. 4A). We further analysed the expression of activation markers on these subsets. Activated $T_{PH}1$ cells but not $T_{PH}2$ or $T_{PH}17$ cells were increased in SLE patients (median percentage of $CD38^+$ $T_{PH}1$ cells for HCs 38.50% [IQR 28.80–47.30] vs SLE 56.25% [IQR 39.60–71.50]; median percentage of HLA-DR^+ $T_{PH}1$ cells for HCs 12.20% [IQR 8.91–17.10] vs SLE 21.65% [IQR 12.25–31.25]) (Fig. 4B). The frequency of activated $T_{PH}1$ cells was correlated with that of plasmablasts (Fig. 4C); moreover, the median frequency of $CD38^+$ $T_{PH}1$ cells decreased from 66.45% to 48.95% after treatment (Fig. 4D), suggesting that the activation of $T_{PH}1$ cells promoted B cell differentiation in SLE patients.

Discussion

In the current study, we demonstrated that T_{PH} cells, which have IL-21 producing capacity, are increased in the peripheral blood of patients with SLE, and that their expansion and activation status are associated with disease activity. Furthermore, we showed the activation status of T_{PH} cells, particularly $T_{PH}1$ cells, reflects the promotion of B cell differentiation in SLE patients.

T_{PH} cells were originally identified in the synovium of RA patients indicating they are involved in tertiary lymphoid neogenesis [16]. An increase in T_{PH} cells was recently demonstrated in the peripheral blood of Sjogren's syndrome cases [22]. In this study, we showed the expansion of T_{PH} cells in SLE patients. Here, we demonstrated that increased numbers of activated T_{PH} cells were associated with disease activity, as well as with promotion of B cell differentiation. Han et al. also reported increased frequency of circulating PD-1^{+}CXCR5^{-}CD45RO^{+}C$D4^{+}$ T cells, which was positively correlated with anti-dsDNA antibody levels in SLE patients [23]. These findings suggest that enhanced antibody production may occur in the peripheral tissues in addition to germinal centres in secondary lymphoid organs in SLE patients. Indeed, the

infiltration of B cells into the kidney was observed in patients with SLE and local autoantibody production has been shown in lupus animal models [19,24]. In the RA synovium, T cells adjacent to B cells outside lymphoid aggregates were preferentially T_{PH} cells, although both T_{PH} cells and T_{FH} cells were found adjacent to B cells in lymphoid aggregates [16]. T_{FH} cells were present in inflamed kidneys [25]. Recently, expanded $CXCR5^{-}CXCR3^{+}PD1^{high}CD4^{+}$ T cells and B cells were found in the peripheral blood of SLE patients [26]. Moreover, $CD4^{+}$ T cells with a similar phenotype to that of cells in the blood were found in the kidneys of lupus nephritis patients [26]. Further studies to address the presence of T_{PH} cells in peripheral tissues such as the kidney will be interesting.

Recently, the expansion of activated Th1 type-T_{FH} ($T_{FH}1$) cells as well as activated Th1 cells in association with the elevation of IL-12 was reported in SLE patients [27]. In the present study, we found increased numbers of activated $T_{PH}1$ cells and confirmed the expansion of activated Th1 cells in SLE patients. Numerous previous studies reported that elevated serum levels of interferon-α (IFN-α) were associated with disease activity in SLE patients [1,2]. We also reported the elevation of IFN-α associated with enhanced Toll-like receptor 7 signalling in plasmacytoid dendritic cells

in SLE patients [28]. IL-12 and IFN-α are important for the induction of Th1 cells through the activation of STAT4 [29], and thus the expansion of $T_{PH}1$ cells and Th1 cells probably reflects the cytokine environment in SLE patients. Because IFN-γ was reported to be important for the production of autoantibodies in spontaneously developed germinal centres [30], the activation of $T_{PH}1$ cells as well as T_{FH} cells might contribute to the pathogenesis of SLE.

The importance of interactions between B cells and helper T cells is well recognized for the differentiation of memory B and plasma cells. Recent studies revealed that plasma cell differentiation requires strong germinal centre responses with prolonged help by T_{FH} cells, but that T_{FH} cell help is dispensable for memory B cell differentiation [31, 32]. IL-21 produced by T_{FH} cells appears to play a key role in the process of germinal centre responses and plasma cell differentiation, but IL-21 is not essential for memory B cell generation [33, 34]. In the current study, the proportion of T_{PH} cells was strongly correlated with that of plasmablasts, but not with that of memory B cells. Therefore, T_{PH} cells may be more important in the generation of plasma cells. Indeed, Rao et al., demonstrated that T_{PH} cells promoted plasma cell differentiation from memory B cells in a co-culture system [16]. We found that the frequency of T_{PH}

cells correlated with that of activated memory B cells and plasmablasts. We also demonstrated that T_{PH} cells have the capacity to produce IL-21 to a similar level as T_{FH} cells. Thus, T_{PH} cells may have an important role in plasma cell differentiation.

In summary, we showed the expansion and activation of T_{PH} cells in the peripheral blood of SLE patients was associated with disease activity. The increase in activated T_{PH} cells, particularly $T_{PH}1$ cells, correlated with plasmablast frequency, indicating the promotion of B cell differentiation. Our findings shed light on the involvement of extra-follicular T–B cell interactions in the pathogenesis of SLE. These cells may serve as a novel therapeutic target in autoantibody-mediated disorders.

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Tables

Table 1. Characteristics of healthy control subjects (HCs) and patients with systemic lupus erythematosus (SLE)

	HCs	Patients with SLE
Number of patients	27	65
Females/males, n	22/5	57/8
Age, years	37.0 (30.0-44.0)	41.0 (33.0-46.5)
Disease duration, years	-	11.0 (3.5-18.0)
SLEDAI score	-	4.0 (2.0-7.5)
Medications		
Medication-naïve, n	-	7
Prednisone, n	-	57
Prednisone dose (mg/day)	-	7.0 (5.0-10.0)
Hydroxychloroquine, n	-	8
Immunosuppressive agent[a], n	-	31

Values are number or median (interquartile range). [a]Tacrolimus, cyclosporine,

azathioprine, mycophenolate mofetil, mizoribine

Table 2. Characteristics of patients with lupus for cell sorting and intracellular cytokine staining analysis.

	Patients with SLE
Number of patients	9
Females/males, n	7/2
Age, years	39.0 (26.5-45.0)
Disease duration, years	5.5 (0.2-18.2)
SLEDAI score	4.0 (4.0-13.0)
Medications	
Medication-naïve, n	3
Prednisone, n	5
Prednisone dose (mg/day)	7.0 (0-9.0)
Hydroxychloroquine, n	1
Immunosuppressive agent[a], n	3

Values are number or median (interquartile range). [a]Tacrolimus, azathioprine.

Figure Legends

Figure 1. Correlation between T_{PH} frequency and SLE. (**A)** T_{PH} gating strategy. (**B)** Representative profile of T_{PH} cells (%) from healthy control (HC) and SLE. (**C)** IL-21-producing T_{PH} cells and other memory $CD4^+$ T cell subsets from SLE patients after PMA/ionomycin. Each symbol represents an individual subject. Data are median/interquartile range. Whiskers indicate minimum/maximum. *$p<0.05$ (Kruskal-Wallis test). (**D)** T_{PH} percentage among $CD4^+$ T cells in HC and SLE patients. ****$p<0.0001$ (Mann–Whitney *U*-test). (**E)** T_{PH} proportions among $CD4^+$ T cells in patients receiving different medications.. (**F)** Correlation between T_{PH} frequency and clinical parameters (Spearman's correlation). (**G)** T_{PH} percentage among $CD4^+$ T cells in SLE patients with active disease, before and after treatment (n = 6). * $p<0.05$ (Wilcoxon matched-pairs signed-rank test). ns: not significant; T_{PH} : Follicular helper T; TAC: tacrolimus; MZB: mizoribine

Figure 2. Correlation of T_{PH} cell activation status with disease activity. (**A)** The percentages of $CD38^+$ or HLA-DR^+ T_{PH} cells among $CD4^+$ T cells in HC and SLE patients. Each symbol represents an individual subject. The box plot indicates the first

and third quartiles, and the middle line indicates the median. Whiskers indicate the minimum/maximum. ****p<0.0001 (Mann–Whitney *U*-test). (**B)** Correlations between the frequency of $CD38^+$ or HLA-DR^+ T_{PH} cells and clinical parameters including SLEDAI, anti-dsDNA antibody titres, CH50 and lymphocyte counts were examined using Spearman's correlation. ns: not significant; T_{PH} : Follicular helper T; TAC: tacrolimus; MZB: mizoribine

Figure 3. Association of T_{PH} cell activation with B cell differentiation. (**A)** The percentages of plasmablasts among lymphocytes. Each symbol represents an individual subject. The box plot indicates the first and third quartiles, and the middle line indicates the median. Whiskers indicate the minimum/maximum. **p<0.01 (Mann–Whitney *U*-test). Correlation of the frequency of plasmablasts with SLEDAI or anti-dsDNA antibody levels were examined using Spearman's correlation. (**B)** Correlation between the frequency of $CD38^+$ or HLA-DR^+ T_{PH} cells and the frequency of plasmablasts or $CD38^+$ switched memory B cells in SLE patients was examined using Spearman's correlation. ns: not significant; T_{PH} : Follicular helper T; TAC: tacrolimus; MZB:

mizoribine

Figure 4. T_{PH} cell subsets and correlation with B cell differentiation. (**A)** The percentages of Th subsets (among memory $CD4^+$ T cells) and T_{PH} cell subsets (among T_{PH} cells) in HC and SLE patients. Each symbol represents an individual subject. Data were expressed as the median/interquartile range. Whiskers indicate the minimum/maximum. ****p<0.0001 (Mann–Whitney *U*-test). (**B)** The percentages of $CD38^+$ or HLA-DR^+ cells among each Th and T_{PH} subset. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (Mann–Whitney *U*-test). (**C)** Correlation between the frequency of $CD38^+$ or HLA-DR^+ T_{PH} cells and plasmablasts in SLE patients was examined using Spearman's correlation. (**D)** The percentages of $CD38^+$ cells among $T_{PH}1$ cells, before and after treatment. * p<0.05 (Wilcoxon matched-pairs signed-rank test). ns: not significant; T_{PH} : Follicular helper T; TAC: tacrolimus; MZB: mizoribine

Figure 1

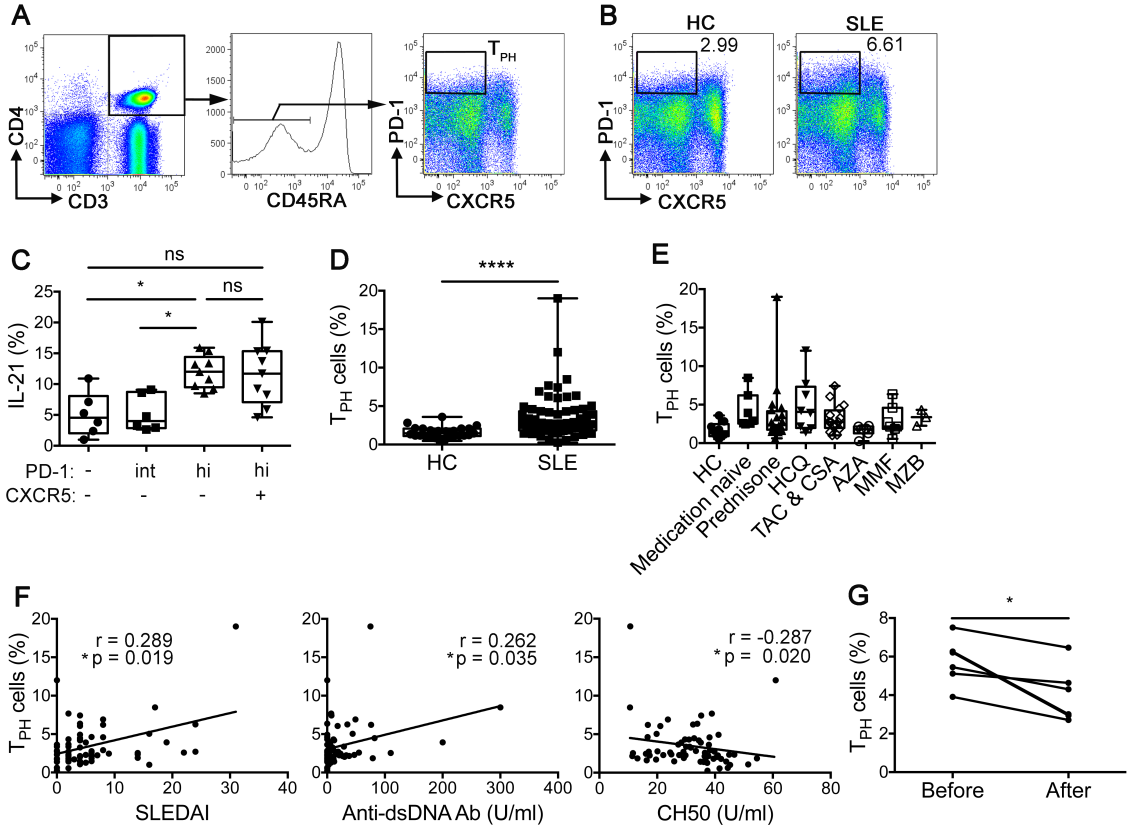

A
CD4
CD3
CD45RA
PD-1
CXCR5
T_{PH}
B
HC
SLE
2.99
6.61
PD-1
CXCR5
C
IL-21 (%)
ns
*
ns
*
PD-1: - int hi hi
CXCR5: - - - +
D
T_{PH} cells (%)
****
HC
SLE
E
T_{PH} cells (%)
HC
Medication naive
Prednisone
HCQ
TAC & CSA
AZA
MMF
MZB
F
T_{PH} cells (%)
r = 0.289
*p = 0.019
SLEDAI
r = 0.262
*p = 0.035
Anti-dsDNA Ab (U/ml)
r = -0.287
*p = 0.020
CH50 (U/ml)
G
T_{PH} cells (%)
*
Before
After

Figure 2

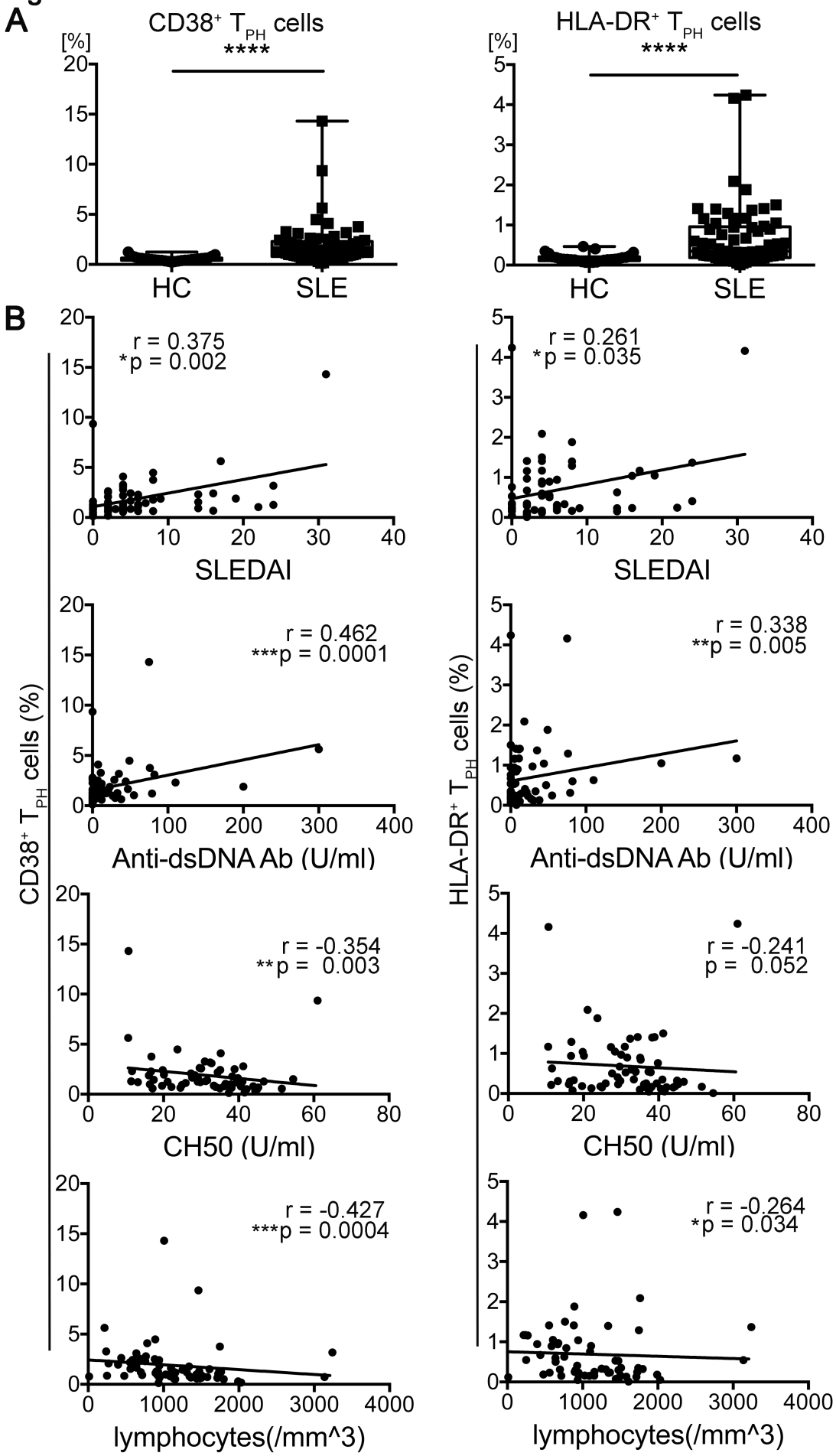

A
CD38+ TPH cells
[%]
****
HC
SLE
HLA-DR+ TPH cells
[%]
****
HC
SLE
B
r = 0.375
*p = 0.002
SLEDAI
r = 0.261
*p = 0.035
SLEDAI
CD38+ TPH cells (%)
r = 0.462
***p = 0.0001
Anti-dsDNA Ab (U/ml)
HLA-DR+ TPH cells (%)
r = 0.338
**p = 0.005
Anti-dsDNA Ab (U/ml)
r = -0.354
**p = 0.003
CH50 (U/ml)
r = -0.241
p = 0.052
CH50 (U/ml)
r = -0.427
***p = 0.0004
lymphocytes(/mm^3)
r = -0.264
*p = 0.034
lymphocytes(/mm^3)

Figure 3

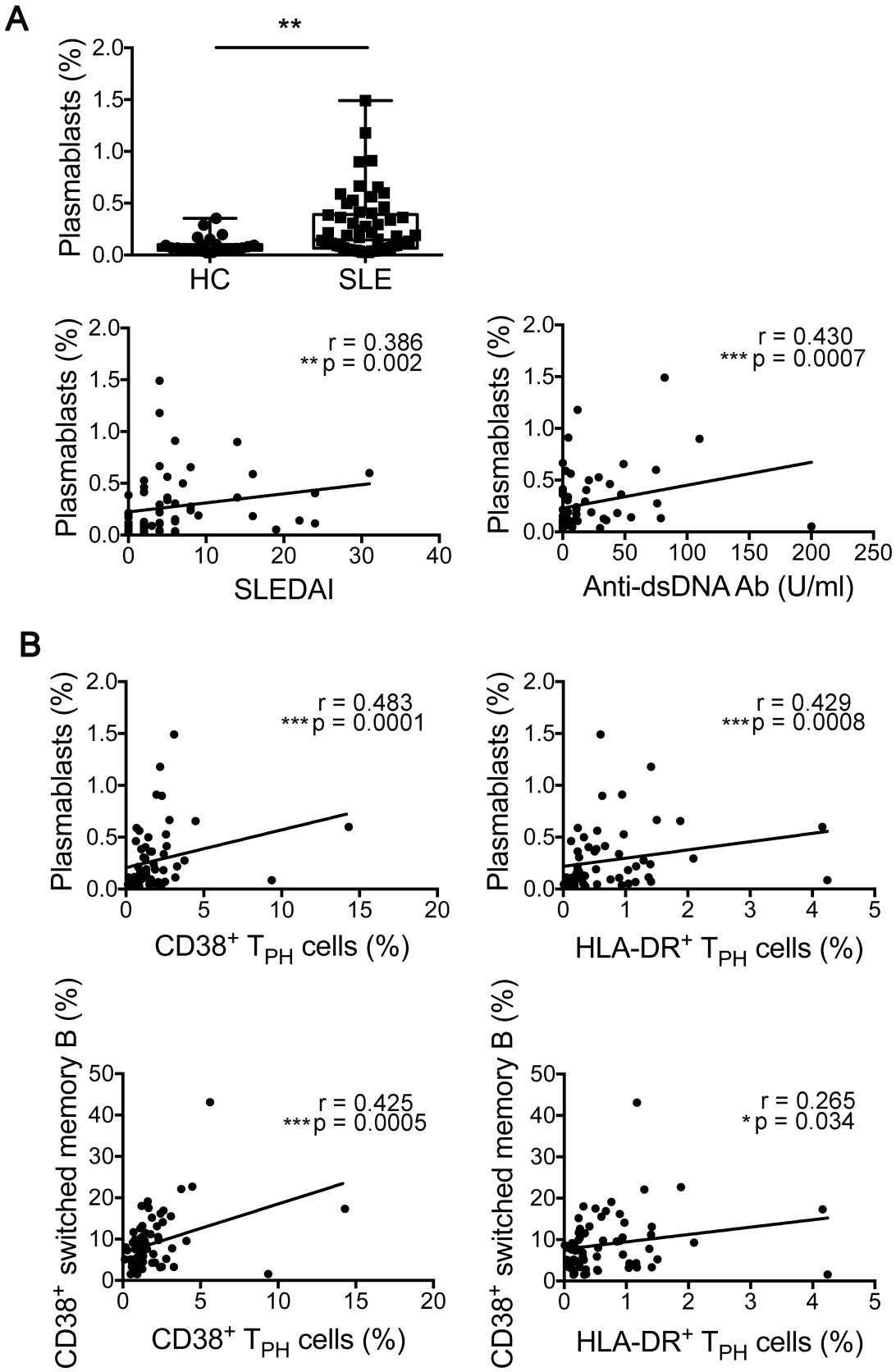
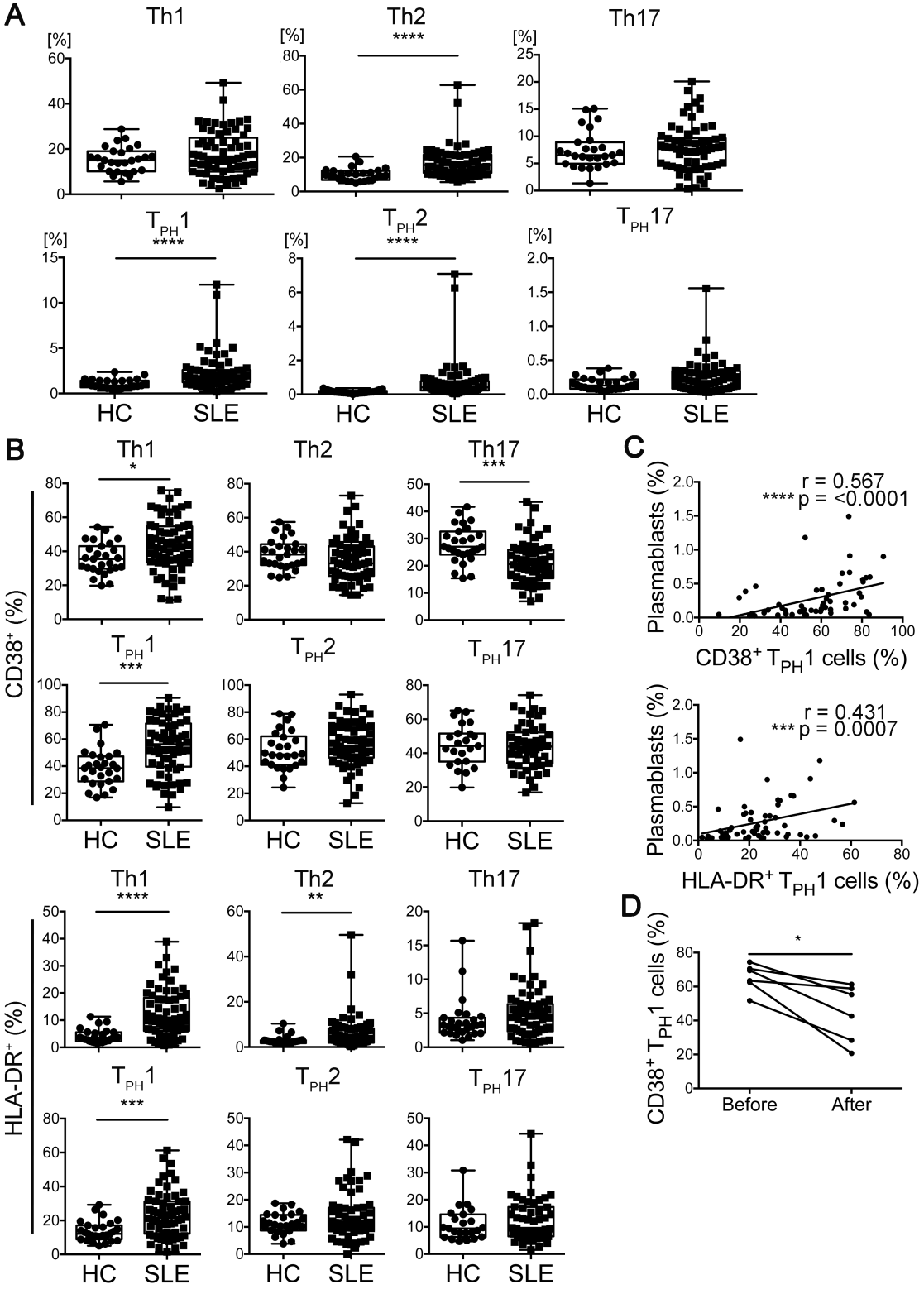

A
**
Plasmablasts (%)
2.0
1.5
1.0
0.5
0.0
HC
SLE
r = 0.386
** p = 0.002
SLEDAI
r = 0.430
*** p = 0.0007
Anti-dsDNA Ab (U/ml)
B
r = 0.483
*** p = 0.0001
CD38+ TPH cells (%)
r = 0.429
*** p = 0.0008
HLA-DR+ TPH cells (%)
CD38+ switched memory B (%)
r = 0.425
*** p = 0.0005
CD38+ TPH cells (%)
r = 0.265
* p = 0.034
HLA-DR+ TPH cells (%)

Figure 4


A
Th1
Th2
Th17
[%]
****
$T_{PH}1$
$T_{PH}2$
$T_{PH}17$
HC
SLE
B
Th1
Th2
Th17
*
***
$T_{PH}1$
$T_{PH}2$
$T_{PH}17$
$CD38^+$ (%)
Th1
Th2
Th17
****
**
***
$T_{PH}1$
$T_{PH}2$
$T_{PH}17$
$HLA\text{-}DR^+$ (%)
C
Plasmablasts (%)
r = 0.567
**** p = <0.0001
$CD38^+ T_{PH}1$ cells (%)
r = 0.431
*** p = 0.0007
$HLA\text{-}DR^+ T_{PH}1$ cells (%)
D
$CD38^+ T_{PH}1$ cells (%)
Before
After