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Alterations of Lymphocyte Subpopulations in Childhood-Onset Systemic Lupus Erythematosus: role of Th17 Cells as a marker for treatment response

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Table 1 and 2 are available in the Supplementary Files section.

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Alterations of Lymphocyte Subpopulations in Childhood-Onset Systemic Lupus Erythematosus: role of Th17 Cells as a marker for treatment response

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Key Words: Lymphocyte Subpopulations, Systemic Lupus Erythematosus, Th17, IL-12, children

Abbreviations

cSLE, childhood-onset systemic lupus erythematosus; HCs, healthy controls; cLLDAS, childhood lupus low disease activity state; IL, interleukin; EULAR, European League Against Rheumatism; ACR, American College of Rheumatology; SLEDAI, SLE Disease Activity Index; CNS, central nervous system, C3, complement component 3; C4, complement component 4; dsDNA ouble-stranded DNA; CRP, C-reactive protein; sIL-2R, soluble interleukin-2 receptor; WBC, white blood cell count; Hb, hemoglobin; Plt, platelet count; SLICC,

Systemic Lupus International Collaborating Clinics; PBMC, Peripheral blood mononuclear cell; IFN, interferon; TNF, tumor necrosis factor-alpha; IP-10, interferon-gamma-inducible protein 10; IL-1ra, IL-1 receptor antagonist: IQRs, interquartile ranges; LN, lupus nephritis; DNT, double negative T cells; pLN, proliferative LN; PSL, predonisolone;

Abstract

Background

Alterations in lymphocyte subpopulations have been implicated in the pathogenesis of Childhood-onset systemic lupus erythematosus (cSLE), however, their association with clinical outcomes remains poorly understood. This study aimed to clarify the alterations of lymphocyte subpopulations and their associations with organ involvement and prognosis in cSLE

Methods

This study included 21 patients with cSLE and nine age-matched healthy controls (HCs). Lymphocyte subpopulations were assessed using flow cytometry, and Th17-related cytokines were measured using multiplex bead-based immunoassays. Correlations between lymphocyte subpopulations, cytokines, and clinical parameters were evaluated.

Results

cSLE patients demonstrated significant alterations in lymphocyte subpopulations compared with HCs, including decreased γδT cells, NK cells, and dendritic cells, and increased regulatory T cells and plasmablasts. Skin manifestations were associated with decreased double-negative T cells and naïve B cells, while severe lupus nephritis correlated with increased memory B cells and $CD8^+$ T cells.

Th17 cells were positively correlated with time to achieve Childhood Lupus Low Disease Activity State (cLLDAS). Interleukin (IL)-12 (p70) levels were negatively correlated with time to achieve cLLDAS.

Conclusion

Alterations in lymphocyte subpopulations and Th17-related cytokines, in particular, increased Th17 cells and decreased IL-12 may contribute to the pathogenesis of refractory cSLE characterized by resistance to therapy.

Introduction

Childhood-onset systemic lupus erythematosus (cSLE) is known to have higher disease activity and more pronounced organ damage and treatment resistance than adult-onset SLE [1]. Immunological abnormalities are deeply involved in the pathogenesis of the disease, and the role of lymphocyte subpopulations and inflammatory cytokines in particular has attracted attention [2,3].

Th17 cells are a subpopulation of CD4+ T cells and produce mainly interleukin (IL)-17 (IL-17A and IL-17F). It has been shown that this group of cells secrete pro-inflammatory cytokines; and contribute to the pathogenesis of autoimmune and chronic inflammatory diseases [4-6]. In SLE, Th17 cells and the cytokines they produce also play a central role in the development of disease activity and organ damage [7-9]. However, the role of Th17 in cSLE remains unclear, as well as how the unique immunological background of children differs from the adult form and how it affects organ damage and prognosis.

In this study, we analyzed in detail the lymphocyte subpopulations and Th17-associated cytokines in patients with cSLE and evaluated their impact on organ damage and prognosis. In addition, by focusing on immunological differences between cSLE patients and healthy controls (HC), we aimed to identify cSLE-

specific immune responses and provide insight into improving disease management and treatment strategies.

Patients and methods

Study Population and Sample Collection

This study included 21 patients with cSLE, diagnosed at our institution between January 2017 and February 2024. All patients met the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) Classification Criteria for Systemic Lupus Erythematosus. Whole blood and serum samples were collected at the time of disease onset, which coincided with the diagnosis. Additionally, nine age-matched healthy controls (HCs) were included, using data from a previously reported dataset [10].

Clinical and Laboratory Data Collection

Background information, disease activity, and treatment history, including medications, were retrospectively collected from medical records. Disease activity was assessed using the SLE Disease Activity Index 2000 (SLEDAI-2K).

Clinical data included detailed evaluations of organ involvement, such as renal, central nervous system (CNS), musculoskeletal, dermatological, and

hematological manifestations. These data were used to assess disease severity and organ-specific damage. Organ involvement was identified based on clinical presentation and supported by laboratory findings, imaging studies, and biopsy results where available.

Laboratory data collected included serum levels of IgG, complement components (C3, C4, and CH50), anti-double-stranded DNA antibodies (anti-dsDNA IgG), and inflammatory markers such as C-reactive protein (CRP), ferritin, haptoglobin, and soluble interleukin-2 receptor (sIL-2R). Complete blood counts, including white blood cell count (WBC), lymphocyte count, hemoglobin (Hb), and platelet count (Plt), were also recorded.

Prognostic factors included the time (weeks) required to achieve Childhood Lupus Low Disease Activity State (cLLDAS). The cLLDAS criteria were defined based on Smith et al. (2023) and included the following:

1. SLEDAI-2K ≤ 4 with no major organ involvement and no new disease activity.
2. Daily prednisolone dose ≤ 0.15 mg/kg/day.
3. Stable immunosuppressive therapy at maintenance doses, if applicable.
4. Physician Global Assessment (PGA) <1 on a 0–3 visual analog scale.
5. No significant organ damage, as defined by the Systemic Lupus

International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index.

The time to cLLDAS was defined as the duration from the initiation of induction therapy to the first instance when all criteria were satisfied. Flare-ups were defined as instances requiring treatment escalation or addition. Information on the duration of systemic glucocorticoid therapy (prednisolone), including periods with and without prednisolone administration, was also collected.

Ethics Statement

This study was approved by the Institutional Review Board of Institute of Science Tokyo, and all specimens were used after obtaining informed consent from the patients. The committee's reference number is M2020-062. Informed consent was obtained from all individual patients and their parents in the study.

Immunophenotyping Analysis

Peripheral blood mononuclear cell (PBMC) subpopulations

Peripheral blood mononuclear cell (PBMC) subpopulations were measured using a BD LSR Fortessa flow cytometer (Becton Dickinson, Franklin Lakes, NJ). The cytometer settings and panel designs were based on the methods described

in the previous report [10]. We designed six panels (PBMC, T1, T2, T3, B, and DC). Gating strategy for each panel is shown in Supplement Figure S1-S4.

The evaluated parameters in both cSLE and HC were categorized by panels as follows: PBMC panel includes percentages of lymphocytes (% of all peripheral blood mononuclear cells), T cells (% of lymphocytes), B cells (% of lymphocytes), Helper T cells (% of $CD3^+$), Cytotoxic T cells (% of $CD3^+$), Naïve Th cells (% of $CD3^+CD4^+$), Naïve Tc cells (% of $CD3^+CD8^+$), Memory B cells (% of $CD19^+$), and NK cells (% of lymphocytes). T1 panel includes invariant NKT(iNKT) cells (% of $CD3^+$), Recent thymic emigrants (RTE, % of $CD3^+CD4^+CD45RA^+$), $CD4^+$ central memory T cells (% of $CD3^+CD4^+CD45RO^+$), $CD4^+$ effector memory T cells (% of $CD3^+CD4^+CD45RO^+$), $CD8^+$ central memory T cells (% of $CD3^+CD8^+CD45RO^+$), and $CD8^+$ effector memory T cells (% of $CD3^+CD8^+CD45RO^+$). T2 panel includes αβT cells (% of $CD3^+$), γδT cells (% of $CD3^+$), Double negative T cells (DNT, % of $CD3^+TCR\alpha\beta^+$), and Regulatory T cells (Treg, % of $CD3^+CD4^+$). T3 panel includes Th1 cells (% of $CD3^+CD4^+CD45RO^+$), Th2 cells (% of $CD3^+CD4^+CD45RO^+$), Th17 cells (% of $CD3^+CD4^+CD45RO^+$), Follicular helper T cells (Tfh, % of $CD3^+CD4^+$), and Activated T cells (% of $CD3^+CD4^+$). B panel includes Plasmablasts (% of $CD19^+$), Transitional B cells (% of $CD19^+$), $CD27^+$ IgD^+ memory B cells (IgM memory B, % of $CD19^+$), $CD27^+$ IgD^- memory B cells

(switched memory B, % of $CD19^+$), IgG^+ memory B cells (% of $CD19^+$), and IgA^+ memory B cells (% of $CD19^+$). DC panel includes Plasmacytoid dendritic cells (pDC, % of $lineage^-$ $HLD\text{-}DR^+$) and Myeloid dendritic cells (mDC, % of $lineage^-$ $HLD\text{-}DR^+$). In addition, in cSLE, Naive B cells (% of $CD19^+$), Memory CD4 (% of $CD3^+CD4^+$), and Memory CD8 (% of $CD3^+CD8^+$) were also evaluated.

Measurement of serum cytokine concentrations

Th17 related cytokines were measured using the Bio-Plex Pro Human Cytokine Assay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Serum samples were obtained from 16 out of 21 patients with cSLE at disease onset. The cytokine panel included interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-10, IL-12 (p70), IL-17A, IL-17F, and IL-23, as well as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interferon-gamma-inducible protein 10 (IP-10), and IL-1 receptor antagonist (IL-1ra). Cytokine concentrations were quantified using a multiplex bead-based immunoassay system, and results were analyzed using Bio-Plex Manager software.

Statistical Analysis

Data analysis and visualization were performed using GraphPad Prism (version 9.5.1, GraphPad Software Inc., La Jolla, California, USA). Demographic, clinical, and laboratory parameters were expressed as medians with interquartile ranges (IQRs) and percentages, where applicable. The Mann-Whitney U test was performed to compare continuous variables between two independent groups. Correlations between variables were assessed using Spearman's correlation coefficient, with statistical significance defined as a two-sided p-value < 0.05, and correlations were considered significant if $|r| > 0.5$.

Results

Patient characteristics

Background, laboratory findings, clinical course, and medications used in the 21 cSLE patients are shown in Table 1. The median age of cSLE patients was 12.6 (7 to 16) years, which was similar to the 7 to 19 year group in the HC. Nineteen cases (90.5%) were girls, accounting for most subjects. The ethnicity of all patients was Asian. The median observation period was 158.7 (IRQ 109.0 to 263.2) weeks. The median SLEDAI-2K at onset was 16 (IRQ 2.5 to 21.0). The time required to achieve cLLDAS, PSL use duration, and PSL free duration were 31.1 (25.9-42.4) weeks, 65.9 (55.0-131.4) weeks, and 76.0 (12.0-120.0) weeks,

respectively. Laboratory data, organ involvements, number of relapses, and drugs used during the observation period are shown in Table 1. The median number of Total types of medications was 4 (3 to 5) types. Of the 21 patients, 20 were untreated at the time of specimen collection, but only one patient had received prednisolone (PSL).

Alterations of Lymphocyte subpopulations in cSLE

First, the proportions of lymphocyte subpopulations in cSLE and age-matched HC were compared (Table 1). As a result, the percentage of memory B cells, NK cells, iNKT cells, γδT cells, pDC, and mDC was significantly lower in cSLE than in HC, while Treg, αβT cells, activated T cells, and plasmablasts were significantly higher in cSLE than in HC (Figure 1A, 1B).

Correlation between organ involvements and lymphocyte subpopulations in cSLE

Then, the relationship between the lymphocyte subpopulations of cSLE and the presence or absence of each organ involvement was comprehensively evaluated using Spearman's correlation coefficient, and the median proportion of each lymphocyte subpopulation was compared according to the presence or

absence of organ involvement. As a result, a specific lymphocyte subpopulation was found to be associated with skin manifestation and lupus nephritis (LN) (Figure 2).

Regarding skin manifestations, the proportion of double negative T cells (DNT) and naïve B cells was significantly lower in cSLE with rash (as determined by SLEDAI at the time of sample collection) than in cSLE without rash (DNT 1.865% vs. 1.130%, $p = 0.0294$, Naïve B 13.50 % vs. 7.77%, $p = 0.0288$) (Figure 2A, 2B). Similarly, in cSLE with mucosal ulcers (as determined by SLEDAI at the time of sample collection), the % of DNT was significantly lower than in cSLE without mucosal ulcers (DNT 1.77% vs. 1.04%, $p = 0.0415$) (Figure 2C). Furthermore, the proportion of γδT cells was significantly lower in patients with chilblain lupus erythematosus (γδT 3.86% vs. 2.20%, $p = 0.0433$) (Figure 2D).

For lupus nephritis, the lymphocyte subpopulations were compared in the following groups: non-LN (negative for proteinuria and hematuria, and no renal biopsy performed), non-proliferative LN (non-pLN) (Class I or II on renal biopsy), and proliferative LN (pLN) (Class III or IV on renal biopsy). As a result, the percentage of $CD4^+$ T cells was significantly lower in pLN than in non-pLN (non-pLN vs. pLN = 61.20 % vs. 44.65 %, $p = 0.0062$) (Figure 2E), and the percentage of $CD8^+$ T cells was significantly higher (non-pLN vs. pLN = 48.75 % vs. 32.45 %,

p = 0.0011) (Figure 2F). Moreover, the percentage of memory B cells was significantly higher in pLN than in both non-pLN and non-LN (non-pLN 1.82%, non-LN 2.51%, pLN 3.54%; non-LN vs. pLN, p = 0.0451; non-pLN vs. pLN, p = 0.0463) (Figure 2G), and it was found that there was a relationship between the severity of LN and these lymphocyte subpopulations.

There was no significant association with each lymphocyte subpopulation with respect to other organ involvement.

Correlation between disease activity and lymphocyte subpopulations in cSLE

SLEDAI-2K at the time of onset and the correlation with each lymphocyte subpopulation were evaluated (Figure 3). SLEDAI-2K showed a significant negative correlation with the percentage of Th1 (r = -0.6340, p = 00076) (Figure 3A) and a positive correlation with Th2 (r = 0.5955, p = 0.0134) (Figure 3B). The correlation evaluation with laboratory data showed a significant correlation with IgM (r = 0.5924, p = 0.0047) (Figure 3C).

Correlation between prognostic parameters and lymphocyte subpopulations in cSLE

Then, correlations between prognostic parameters and each lymphocyte subpopulation were evaluated (Figure 4).

The time to achieve cLLDAS was significantly correlated with the percentage of Th17 (r = 0.5956, p = 0.0274) (Figure 4A). The number of relapses was not significantly correlated with any of the lymphocyte subpopulations. The time to achieve cLLDAS was not related to the number of relapses but was correlated with maxPSL (mg/kg/day) (Figure 4B) and duration of PSL use, and was thought to reflect severe cases or refractory courses. A positive correlation was observed between maxPSL (mg/kg/day) and the percentage of memory B cells (Figure 4C), and a negative correlation was observed between maxPSL (mg/kg/day) and the percentage of transitional B cells (Figure 4D). There was no significant difference between the two groups in terms of the presence or absence of relapse in any of the lymphocyte subpopulations.

Based on the correlation between the time to achieve cLLDAS and the percentage of Th17 cells, the association of Th17 with clinical refractoriness was suspected. Therefore, serum cytokines (IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 (p70), IL-17A, IL-17F, IL-23, IFN-γ, TNF-α, IP-10 and IL-1ra) that are involved in the differentiation and maintenance of Th17 cells were measured, and the correlation between Th17 cells and time to achieve cLLDAS was evaluated. As a result, the

time to achieve cLLDAS was significantly negatively correlated only with IL-12 (p70) (r = -0.5841, p = 0.0302) (Figure 4E). Th17 was significantly correlated with IL-6 (r = 0.7395, p = 0.080) (Figure 4F), IFN-γ (r = 0.8531, p = 0.0008) (Figure 4G), and IL-1ra (r = 0.7273, p = 0.0096) (Figure 4H). No significant correlation was observed in other combinations.

Discussion

In this study, the characteristics of lymphocyte subpopulations in cSLE patients and their relationship with organ involvement and prognosis were examined. By comparing these results with previously reported results for adults and children, new insights were obtained into the immune responses and clinical significance specific to cSLE.

The results of our study were consistent with the previously reported significant decrease in γδT cells and NK cells in cSLE compared to HC [11]. On the other hand, although the same report also described a significant decrease in $CD4^+$ T cells and a significant increase in $CD8^+$ T cells, our study observed similar trends of $CD4^+$ T cell decrease and $CD8^+$ T cell increase without reaching statistical significance (Figure 1B). The lack of statistical significance for $CD4^+$ and $CD8^+$ T cell differences in our study may be attributed to the relatively small

sample size, which limits the statistical power to detect subtle differences. In addition, while there was no significant difference in Tregs in the same report [11], Tregs were significantly increased in this study. In adults, there have also been some reports of an increase in Tregs similar to that in this study [12]; and it is considered that the difference in measurement methods may have had an effect. The increase in plasmablasts was also reported in another pediatric study [13], and the same results were also observed in this study. Although there are no previous reports of a decrease in iNKT cells in children, there have been reports of a decrease in adults with SLE [14,15], which was consistent with the results of our study.

In our study, both pDCs and mDCs were reduced. Both pediatric and adult patients with SLE have been reported to have a decrease in mDCs compared to HC [13,16,17]. In contrast, there are various reports on pDC, including a report of a decrease in children [13] and a report of an increase in adults [17], but there is also a report of a decrease during the active phase [16]. Our study was performed on samples taken during the active phase at the time of onset, so our result was consistent with previous work.

In this study, the correlations were found between skin symptoms such as rash and DNT cells, a decrease in naive B cells, and chilblain lupus

erythematosus and a decrease in γδT cells. On the other hand, a previous report in children reported an association between mucosal ulcers and high levels of γδT and Nk cells [11]. However, multiple reports have shown that while γδT cells in the peripheral blood in adults with SLE are decreased, they are increased in the skin and subcutaneous tissue, and are associated with disease activity [18-20], which is considered to support the association with skin symptoms. In previous reports, DNT has been reported to proliferate in SLE [21] and to be involved in nephritis by infiltrating renal tissue [22]; however, on the other hand, it has also been reported to be related to SLE disease activity [23]. It is possible that the results of our study reflected the disease activity.

In a previous report on nephritis in children [11], a comparison of the non-LN group and the LN group showed that the proportion of Tregs was lower in the LN group, and there was no significant difference in $CD4^+$ and $CD8^+$ T cells or $CD19^+$ B cells. In this study, the subjects were divided based on biopsy-confirmed non-proliferative LN and pLN, and our results showed low $CD4^+$T and high $CD8^+$T and memory B cell levels with pLN, confirming the relationship between LN severity and lymphocyte subpopulation in more detail. On the other hand, in adults, an increase in $CD8^+$ T cells has been reported as an immunological feature of LN patients [24, 25], strongly suggesting that cytotoxic T cells ($CD8^+$) may contribute

to the pathogenesis of nephritis in pediatric SLE as in adult SLE.

In this study, the correlation between SLEDAI-2K at onset and lymphocyte subpopulation was evaluated, indicating that disease activity is associated with the percentage of Th1 and Th2 cells. More specifically, SLEDAI-2K showed a significant negative correlation with Th1 cells, while it showed a significant positive correlation with Th2 cells. These results suggest that changes in Th1/Th2 balance in cSLE patients may reflect disease activity.

Previous reports have suggested that the Th1/Th2 ratio is significantly decreased in SLE patients, especially those with high disease activity [26–28], which may amplify disease activity through hyperactivation of humoral immunity and autoantibody production, The results of our study are were consistent with these existing findings.

In addition, another possible mechanism by which increased Th2 cells may increase disease activity is through an enhanced humoral immune response. Th2 cells are responsible for activating B cells and inducing class switches to promote autoantibody production and immune complex formation [29]. While IgM may be relatively decreased because Th2 cells induce B cell class switch through IL-4 production, etc., SLEDAI-2K was significantly correlated with IgM in the present study. For example, IgM anti-cardiolipin and IgM anti-dsDNA are elevated in SLE

without renal involvement [30], suggesting that certain IgMs are inversely associated with disease activity, while the association between total serum IgM and disease activity in SLE has not been reported. Therefore, it is still debatable whether an increase in total IgM simply indicates the activation of liquid immunity.

Moreover, previous reports have shown that age and gender influence the Th1/Th2 imbalance. Younger cSLE patients and cSLE complicated by renal dysfunction tend to have a higher percentage of Th1 cells, while older patients and males have a higher percentage of Th2 cells [28]. In fact, in a study of lymphocyte subpopulation in adults with SLE, the percentage of Th1 was reported to be 20.2 ± 9.4% [12], and the Th1 of 26.8 ± 15.4% in cSLE patients in this study was higher than that of adults. However, the decrease in Th1 percentage in response to activity suggests that the same immunologic pathology is present in children as in adults.

Our results suggest the significance of SLEDAI-2K as an indicator of disease activity that reflects changes in Th1/Th2 balance and liquid immune hyperactivity. In particular, Th2 cell dominance may be a central mechanism of disease activity.

It is noteworthy that in this study, Th17 cells showed a significant positive correlation with time to achieve cLLDAS. Although Th17-related conditions are also emerging as new therapeutic targets, reports on the role of Th17 cells in

pediatric SLE are very limited. Our results suggest that Th17 cells may play an important role in treatment resistance and disease progression in cSLE.

According to a previous report, Th17 cells produce IL-17A and IL-17F, which increase disease activity by amplifying inflammation and tissue damage in autoimmune diseases [4]. It has also been reported that an increased Th17/Treg ratio strongly correlates with disease activity and severity in refractory SLE [8,31]. The fact that the present study confirmed that patients with a higher percentage of Th17 cells tended to take longer to achieve cLLDAS was consistent with these findings. On the other hand, the fact that Th17 cells did not directly correlate with SLEDAI-2K, indicating a refractory course of therapy rather than simply high disease activity, is noteworthy for predicting prognosis and determining treatment strategies.

Furthermore, the percentage of Th17 cells and IL-1ra were positively correlated in the present study. Our result was in contrast to previous findings showing that IL-1ra usually has a Th17 cell-suppressive effect [32,33], indicating that the feedback mechanism in the inflammatory response may not be fully functional. While IL-1ra production is induced as a response to the inflammatory environment, other cytokines such as IL-6 and IL-23 may promote Th17 cell maintenance.

The finding of a positive correlation between Th17 cells and IFN-γ also provides a new perspective that complements the existing mechanism by which IL-12 suppresses Th17 cells. In a murine model of autoimmune disease, IL-12 has been shown to produce IFN-γ *via* Th1 cells and suppress Th17 cells [34]. However, Th1 and Th17 cells may be activated in parallel in the inflammatory environment of SLE. Furthermore, it has been reported that some Th17 cells change into pathogenic Th17 and produce IFN-γ along with IL-17, and it seems that our results of the present study also support this finding. Such cell lineage crosstalk and abnormal inflammatory networks likely contribute to the pathological progression of cSLE.

It is also important to note that IL-12 (p70) showed a significant negative correlation with time to PSL 0.15 mg/kg/day. IL-12 may promote Th1 cell differentiation and contribute to Th17 cell suppression [34], and its low level suggests that the Th1/Th17 balance is disrupted and Th17 cell activation is dominant. These results provide important clues to understanding the immunological mechanisms underlying treatment resistance in cSLE.

The results of our study demonstrate the importance of the interaction of Th17 cells with IL-1Ra, IL-12 (p70), and IFN-γ in the pathogenesis of cSLE. These findings suggest the potential for therapeutic strategies targeting Th17 cells and

related pathways, and additional research could optimize treatment and improve prognosis.

Several limitations exist in this study. First, our study was conducted with a small sample size. In addition, the analysis was based on data at the time of disease onset and did not assess changes over time or immunological changes associated with disease progression, which was another issue. Furthermore, the cross-sectional study design makes it difficult to clearly demonstrate the temporal causal effects of lymphocyte subpopulation and cytokine variation on organ involvement and prognosis. In addition, since the present study only analyzed lymphocyte subpopulation and cytokines in peripheral blood, the immune response at the site of disease (e.g., skin or kidney) was not clarified, and data reflecting organ-specific pathogenesis are lacking. In inflammatory diseases, immune cell kinetics and cytokine profiles in peripheral blood and lesion localization may not always coincide, and it is important to analyze cell kinetics and cytokine profiles in lesion localization in future studies to examine the relationship with peripheral blood.

Conclusion

This study demonstrated that alterations in lymphocyte subpopulation and

Th17-related cytokines in cSLE patients may be closely related to the pathogenesis and prognosis of the disease. In particular, our study suggests that the increase in Th17 cells and the decrease in IL-12 may contribute to the pathogenesis of refractory cSLE, characterized by resistance to therapy. Furthermore, based on the association between organ involvement and specific lymphocyte subpopulations, it was confirmed that the immunological background in cSLE reflects organ-specific pathogenesis. These findings indicate that lymphocyte subpopulation and Th17-related cytokines may be used as prognostic predictors and new therapeutic targets in cSLE. Personalized treatment utilizing these immunological indicators is expected to contribute to improved disease management in the future.

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Conflict of interest statement

The authors declare no conflicts of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author, [MS], upon reasonable request.

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Tables and Figures

Table 1. Patient characteristics

Table 2 . Lymphocyte subpopulations of cSLE and HCs

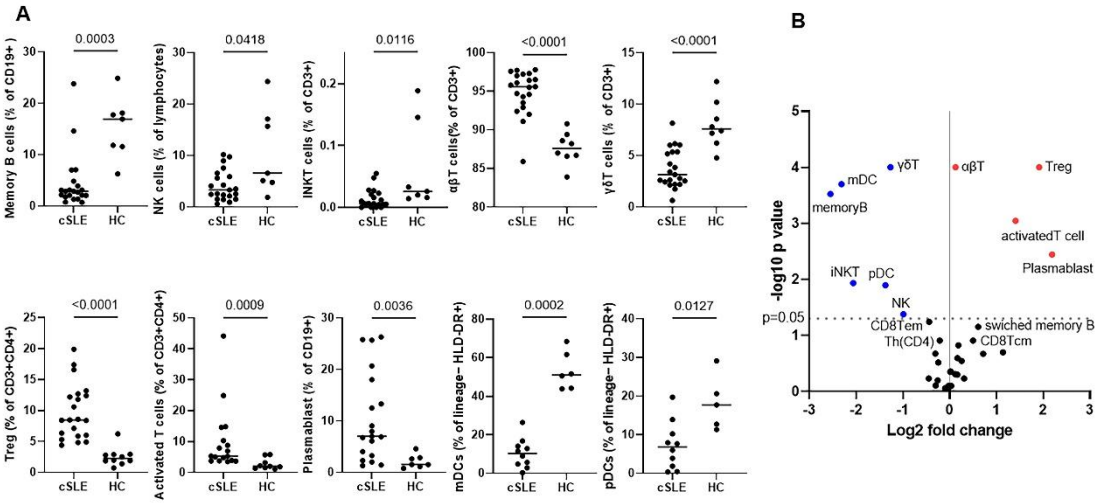


Figure1

Figure 1. Lymphocyte subpopulations of childhood-onset SLE(cSLE) and healthy control (HCs)

Peripheral blood mononuclear cell (PBMC) subpopulations were analyzed by flow cytometry. Data represent comparisons of 21 patients with childhood-onset systemic lupus erythematosus (cSLE) and nine age-matched healthy controls (HCs). Percentages of each subset are plotted, with horizontal lines indicating median values for each group. Memory B cells, natural killer (NK) cells, invariant natural killer T (iNKT) cells, γδT cells, plasmacytoid dendritic cells (pDC), and myeloid dendritic cells (mDC) were significantly lower in cSLE patients compared to HCs ($P < 0.05$), while Treg cells, αβT cells, activated T cells, and plasmablasts were significantly higher in cSLE patients ($P < 0.05$) (A). Statistical significance

was determined using a two-tailed Mann-Whitney U test. Volcano plot comparing cSLE patients and HCs (B). Positive fold changes indicate cellular populations increased in patients, while negative fold changes indicate decreases in patients. The vertical axis represents -log10 p-values, with the area above the dotted line ($p = 0.05$) indicating statistically significant differences ($p < 0.05$).

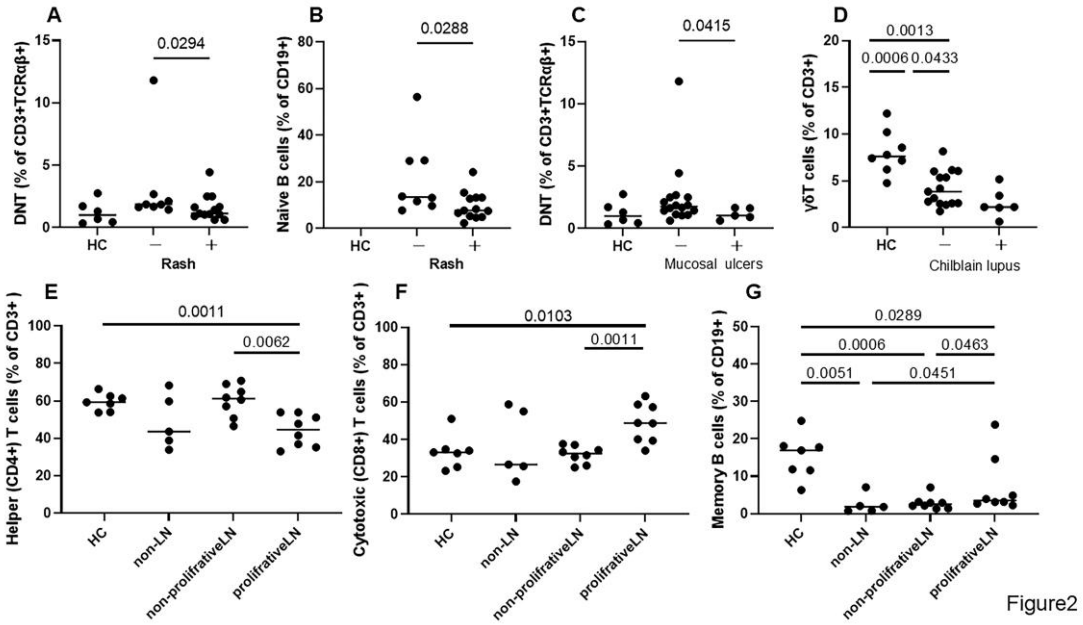


Figure2

Figure 2. Organ involvements and lymphocyte subpopulations in cSLE

Comparison of lymphocyte subpopulations among healthy controls (HCs) and cSLE patients with or without specific skin and mucosal manifestations. The proportions of double-negative T cells (DNT) and naïve B cells were significantly lower in patients with rash than those without (A,B). Similarly, DNT proportions were lower in patients with mucosal ulcers (C), and γδT cell proportions were

lower in those with chilblain lupus erythematosus (D). Comparison of lymphocyte subpopulations among HCs and cSLE patients stratified by lupus nephritis (LN) severity: non-LN, non-proliferative LN (non-pLN), and proliferative LN (pLN). CD4+ T cell proportions were significantly lower, and CD8+ T cell proportions were significantly higher in pLN compared to non-pLN (E,F). Memory B cell proportions were significantly higher in pLN compared to non-pLN and non-LN (G), indicating a relationship between LN severity and these lymphocyte subpopulations. Horizontal lines represent median values. Statistical significance was determined using the Mann-Whitney U test, and only p-values < 0.05 are shown.

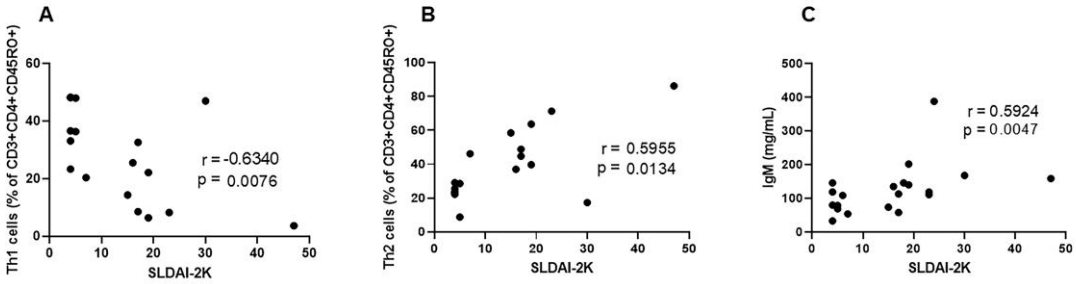


Figure3

Figure 3. Lymphocyte subpopulations and disease activity in cSLE

Correlation analysis between the Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2K) scores, lymphocyte subpopulations, and laboratory data in cSLE patients. (A) Negative correlation between SLEDAI-2K scores and Th1 cell percentage. (B) Positive correlation between SLEDAI-2K scores and Th2 cell percentage (r = 0.5955, p = 0.0134). (C) Positive correlation between SLEDAI-2K scores and serum IgM levels (r = 0.5924, p = 0.0047). Spearman's correlation coefficient was used, with significance defined as a two-sided p-value < 0.05 and |r| > 0.5.

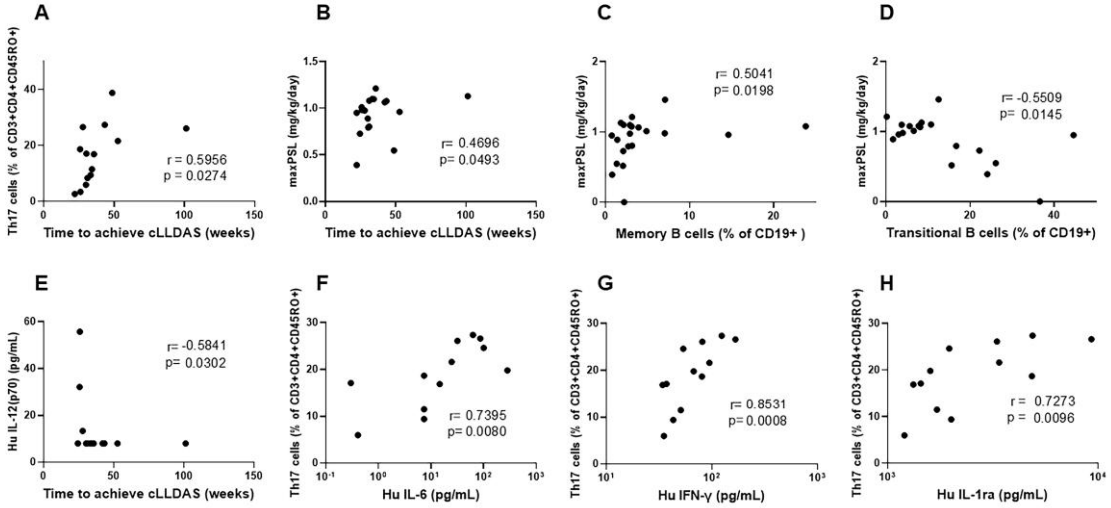


Figure4

Figure 4. Associations between prognostic parameters and Th17 cells/Th17-related cytokines

Correlations between the time to achieve clinical lupus low disease activity state

(cLLDAS), lymphocyte subpopulations, Th17-related cytokines, and prednisolone (PSL) usage in cSLE patients.

(A) The time to achieve cLLDAS showed a significant positive correlation with Th17 cell percentages. (B) The time to achieve cLLDAS was associated with maxPSL (mg/kg/day) and the duration of PSL use. (C, D) MaxPSL was positively correlated with memory B cell percentages and negatively correlated with transitional B cell percentages. (E-H) The time to achieve cLLDAS was negatively correlated with IL-12 (p70) levels. Th17 cell percentages were positively correlated with IL-6, IFN-γ, and IL-1ra.

Correlations were assessed using Spearman's correlation coefficient, with significance defined as $p < 0.05$.

Supplementary Files

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