Imbalance between Th17 and regulatory T cells in patients with systemic lupus erythematosus combined with EBV/CMV viraemia

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Abstract

Objective

Infection is one of the leading causes of morbidity and mortality in patients with systemic lupus erythematosus (SLE). Excessive use of glucocorticoids, disease-modifying anti-rheumatic drugs (DMARDs) and immune disturbances associated with lupus itself lead to reduced immune function with consequent increases in opportunistic infections, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV). A recent study showed that an imbalance between T helper 17 (Th17) cells and regulatory T (Treg) cells is a major cause of autoimmune disease. However, the relationship between Th17/Treg imbalance and SLE combined with EBV and/or CMV is unknown. Here, we investigated lymphocyte subsets, especially CD4⁺ T cells, in patients with SLE combined with EBV/CMV viraemia.

Methods

The clinical records of 36 SLE patients with EBV and/or CMV viraemia (SLE infection group), hospitalised at the Second Hospital of Shanxi Medical University, were analysed. As controls, we selected 20 healthy subjects (healthy control group), 30 SLE patients without infection (SLE non-infection group), and 20 patients with other non-SLE connective tissue diseases with EBV/CMV viraemia (non-SLE infection group), the controls were age-matched with the SLE infection group. The absolute numbers of lymphocytes and CD4⁺ T cells in peripheral blood were examined by flow cytometry.

Results

There were significant decreases in Th17 and Treg levels in the SLE infection group compared to the SLE non-infection group and non-SLE infection group. Similarly, the absolute numbers of Th17 ($p=0.003$) and Treg ($p<0.001$) cells in the SLE infection group were markedly decreased compared to the healthy controls, although the difference in Th17/Treg cell ratio was not significant. The absolute number of Treg cells ($p=0.001$) was decreased in the SLE non-infection group compared to the healthy controls, leading to a higher Th17/Treg cell ratio in the former group ($p=0.018$). There was no significant difference in the absolute number of Th17 cells between the SLE non-infection group and healthy controls.

Conclusion

The monitoring of lymphocytes and CD4⁺ T cell subsets, especially Th17 and Treg cells, may be helpful for identifying EBV/CMV infection in SLE patients. The results presented here suggest that, in addition to Treg, Th17 may also be crucial in the Th17/Treg imbalance seen in patients with SLE combined with EBV and/or CMV viraemia. A decrease in Th17 cells may be an important feature of EBV and/or CMV infection in SLE. Appropriate immunomodulatory therapy for CD4⁺ T cell subsets based on antiviral therapy may be beneficial for SLE patients with EBV and/or CMV viraemia.

Key words

regulatory T cell, Th17 cell, systemic lupus erythematosus, EBV, CMV viraemia

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Introduction

Systemic lupus erythematosus (SLE) is a heterogeneous inflammatory multisystem autoimmune disorder, characterised by the production of a multitude of autoantibodies and immune complex deposition, which can affect the skin, joints, central nervous system and kidneys (1). Studies of SLE have shown that it is essential to also consider its comorbidities. Infection is a common comorbidity that can be especially hazardous to SLE patients with a compromised immune system, and is a major cause of mortality in SLE (2). The use of glucocorticoids and immunosuppressive agents in SLE patients impairs humoral and cellular immunity, thus reducing the body's resistance to pathogens and increasing the incidence of opportunistic infections, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV) viraemia. Human CMV and EBV viraemia are emerging problems that are not commonly seen in SLE patients, but can severely damage the immune system.

EBV and CMV belong to the human herpesvirus (HHV) family, the members of which are ubiquitous dsDNA viruses that infect the majority of adults worldwide (3). EBV infection is known to occur during childhood without any symptoms. However, it causes infectious mononucleosis in adults, which is characterised by skin rash, arthralgia, renal disorders, cytopoenia, pharyngitis and lymphadenopathy. EBV is extremely efficient at establishing a lifelong infection in human B cells and has been reported to be associated with the pathogenesis of autoimmune diseases, such as SLE, and malignant tumours, such as lymphoma and nasopharyngeal carcinoma. After primary infection, EBV usually enters the latent state as a consequence of the host's immune response. Under certain conditions, however, EBV can reactivate and switch back to the lytic cycle, exacerbating any immune imbalance. EBV infection causes T cells to produce increased amounts of interferons (IFNs), which are proinflammatory cytokines crucial for systemic autoimmunity (4). CMV can infect several types of cells, including epithelial cells, haematopoietic cells and connective tissue. CMV infection of endothelial cells and haematopoietic cells leads to systemic spread of the infection, with CMV pneumonia, gastrointestinal infections, central nervous system infections and CMV retinitis being common (5).

CMV and EBV have been suggested to play roles in autoimmunity, and may trigger a cascade of inflammatory factors, thus aggravating immune disorders (6-7). In recent years, it has been found that the disorder of immunity and humoral immunity, as well as the number and functional abnormalities of T lymphocyte subgroup are associsted with SLE. As well as the imbalance between T helper 1 (Th1) cells and T helper 2 (Th2) cells, an imbalance between regulatory T (Treg) cells and T helper 17 (Th17) cells was also found. Pro-inflammatory Th17 cells contribute to the induction and propagation of inflammation. Treg cells are a subset of $CD4^+$ T lymphocytes expressing the cytokine IL-2 receptor α-chain (CD25), which inhibit effector T cells and play essential roles in immune homeostasis (8). Peripheral Tregs in humans are usually identified by their high levels of CD25 membrane expression and intracellular forkhead box P3 (Foxp3) expression. Foxp3 is a transcription factor that is essential for the development, stability and function of Tregs. Studies of Tregs have revolutionised our understanding of immune control mechanisms and immune-mediated diseases, and opened a pathway to the development of a new generation of therapies involving modulation of the number and function of Treg cells.

Although there have been reports on the role of Th17 and Treg cells in SLE patients, there have been few studies of SLE patients with EBV and/or CMV viraemia. Human CMV and EBV are emerging problems that are not commonly seen in SLE infection. In cases of severe infection, the focus is often on anti-infection treatment, with little emphasis placed on immune function. Studying changes in $CD4^+$ T cell subsets may provide new insight for the treatment of such patients. Immunomodulatory and antiviral therapy for SLE patients with EBV and/or CMV

viraemia targeting CD4+ T cell subsets may be beneficial.

Materials and methods

Patients

A total of 36 SLE patients with EBV and/or CMV viraemia, consisting of 23 patients with EBV viraemia, 7 patients with CMV viraemia, and 6 patients having both EBV and CMV viraemia, along with 30 SLE patients without infection diagnosed in accordance with the 1997 American College of Rheumatology (ACR) SLE diagnostic standard, were enrolled in this study. Twenty patients with other non-SLE connective tissue diseases with EBV/CMV viraemia (non-SLE infection group), including eight patients with primary Sjögren's syndrome (pSS) according to the ACR criteria, eleven patients who fulfilled the ACR criteria for rheumatoid arthritis (RA), and one patient with Behçet's disease (BD) according to the International Study Group Criteria for BD. Patients with the following conditions were excluded: (1) tumours; (2) other autoimmune diseases; (3) pregnancy; and (4) other serious pathogen infections. All patients were hospitalised at the Department of Rheumatology, Second Hospital of Shanxi Medical University, from October 2014 to June 2019. We selected 20 healthy subjects as controls (healthy control group) who were age-matched with the SLE infection group. Data on the clinical and serological parameters of these patients were collected retrospectively, including white blood cells (WBCs), lymphocytes, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), immunoglobulin (Ig) G, IgA, IgM, complement C3, complement C4, anti-double-stranded DNA (anti-dsDNA) and anti-Sm. Peripheral lymphocytes and CD4+ T cells were detected before antiviral therapy. In this study, EBV-DNA and CMV-DNA levels >$5x10^2$ IU/ml were considered to indicate infection with EBV and CMV, respectively. And this study was approved by the Ethics Committee of the Second Hospital of Shanxi Medical University.

Statistical analysis

Data with a normal distribution and homogeneity of variance are presented as mean ± standard deviation and were compared by independent-samples *t* test, while data without a normal distribution are presented as the median (interquartile range) and were compared using the Wilcoxon's rank sum test. In all analyses, $p<0.05$ was taken to indicate statistical significance. All statistical analyses were performed using SPSS software (v. 22.0; SPSS Inc., Chicago, IL, USA).

Table I. Clinical characteristics of the SLE infection group and SLE non-infection group.

Clinical characteristics	SLE infection group	SLE non-infection group	*p*-value
Age (years)	42.58 ± 16.54	39.67 ± 16.94	0.483
Gender			
Male, *n* (%)	7 (19.4%)	2 (5.7%)	-
Female, *n* (%)	29 (80.6%)	28 (93.3%)	-
SLEDAI score	11.9 (8.0–15)	8.80 (7.0–10.25)	0.007
Fever (%)	24 (66.7%)	6 (20%)	-
Alopecia (%)	4 (11.1%)	7 (23.3%)	-
Proteinuria (%)	16 (44.4%)	16 (53.3%)	-
Arthritis (%)	5 (13.9%)	10 (33.3%)	-
Neuropsychiatric (%)	13 (36.1%)	2 (6.7%)	-
Mucosa ulcer (%)	7 (19.4%)	4 (13.3%)	-
Low C3/C4 (%)	25 (69.4%)	22 (73.3%)	-
Anti-dsDNApositive (%)	9 (25%)	12 (40%)	-
Anti-Sm-positive (%)	10 (27.8%)	4 (13.3%)	-
Anti-SSA-positive (%)	15 (41.7%)	14 (46.7)	-
Anti-SSB-positive (%)	4 (11.1%)	4 (13.3%)	-
Leukocytopenia (%)	16 (44.4%)	4 (13.3%)	-
Lymphocytopenia (%)	23 (63.9%)	9 (30%)	-
Thrombocytopenia (%)	10 (27.8%)	5 (16.6%)	-

*7 patients had CMV viraemia, 23 patients had EBV viraemia, and 6 patients had combined CMV and EBV viraemia. All data are reported as (n, %) except for age and SLEDAI score.
Age is expressed as the mean ± standard deviation. SLEDAI score is expressed as the median and 25th and 75th percentiles.
SLE: systemic lupus erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; C3: complement C3; C4: complement C4; Anti-dsDNA: anti-double-stranded DNA.

Results

Comparison of demographic and clinical characteristics and laboratory data between the SLE infection group and SLE non-infection group

The demographic and clinical characteristics and laboratory data of the SLE infection and SLE non-infection groups are listed in Tables I and II. The SLE infection group consisted of 29 women and 7 men, while the SLE non-infection group consisted of 28 women and 2 men. The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores were compared between the SLE infection group and SLE non-infection group, and the results indicated a positive correlation between EBV/CMV viraemia and SLEDAI score, with a significantly higher SLEDAI score in the SLE infection group than the SLE non-infection group [11.9 (8.0–15) *vs*. 8.80 (7.0–10.25), respectively, $p=0.007$]. In addition, comparison of laboratory findings between the two groups indicated that the lymphocyte count was significantly lower in the SLE infection group than the SLE non-infection group [0.82 (0.56–1.15) *vs*. 1.25 (0.95–1.87), respectively, $p<0.001$]. The levels of CRP, serum glutamic oxaloacetic transaminase (also known as aspartate aminotransferase; AST) and glutamate-pyruvate transaminase (also known as alanine transaminase; ALT) were lower in the SLE infection group than the SLE non-infection group. However, the mean ESR, complement C3, complement C4 and Ig levels were similar between the SLE infection group and the SLE non-infection group.

Absolute Treg ($CD4^+CD25^+Foxp3^+$) cell count was decreased in SLE patients (SLE non-infection group) compared to healthy controls

The Treg cell count was significantly lower in the SLE non-infection group than the healthy control group [15.91 (9.52–23.17) *vs*. 27.60 (17.44–37.43),

Table II. Comparison of laboratory results between the SLE infection group and SLE non-infection group.

	SLE infection group (n=36)	SLE non-infection group (n=30)	*p*-value
WBC (*10^9/L)[b]	3.75 (2.42–7.22)	5.15 (4.19–8.05)	0.073
LY (*10^9/L)[b]	0.82 (0.56–1.15)	1.25 (0.95–1.87)	0.000
N (*10^9/L)[b]	2.20 (1.59–5.76)	3.56 (2.48–5.53)	0.392
MO (*10^9/L)[a]	0.32 ± 0.23	0.49 ± 0.23	0.001
ESR (mm/h)[b]	40.00 (24.00–73.25)	59.00 (35.25–67.00)	0.652
CRP (mg/L)[b]	5.93 (2.56–12.04)	3.35 (2.28–6.11)	0.011
C3 (g/L)[a]	0.42 ± 0.23	0.56 ± 0.31	0.369
C4 (g/L)[b]	0.10 (0.04–0.14)	0.11 (0.04–0.16)	0.911
IgG (g/L)[b]	12.10 (5.53–21.50)	14.40 (10.02–20.48)	0.437
IgM (g/L)[b]	0.81 (006–1.99)	0.75 (0.55–1.20)	0.900
IgA (g/L)[a]	2.23 ± 1.97	2.84 ± 1.14	0.114
ALT (U/L)[b]	31.00 (16.98–48.68)	14.25 (10.58–23.58)	0.000
AST (U/L)[b]	31.75 (25.98–60.28)	19.35 (14.90–25.15)	0.000
Bun (nmol/L)[b]	4.15 (3.23–9.98)	5.37 (4.68–6.30)	0.498
Cr (μmol/l)[b]	53.00 (47.25–74.50)	66.00 (50.25–96.75)	0.088

[a]Results are expressed as the mean ± standard deviation.
[b]Results are expressed as the median and 25th and 75th percentiles.
The independent-samples *t* test was used for analysis of quantitative variables with normal distributions. Wilcoxon's rank sum test was used for analysis of quantitative variables with a non-normal distribution.
SLE: systemic lupus erythematosus; WBC: white blood cells; N: neutrophils; MO: mononuclear cells. LY: lymphocytes; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; C3: complement C3; C4: complement C4; Ig: immunoglobulin; ALT: alanine transaminase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; Cr: creatinine.

respectively, p=0.001]. In addition, among the CD4+ T cell subsets, the Th2 cell count was also reduced in the SLE non-infection group compared to the healthy control group [2.76 (1.90–6.53) *vs*. 7.25 (4.90–11.82), respectively, p=0.002], while there were no significant differences between the two groups in Th17 cell count [5.47 (2.72–9.29) *vs*. 6.04 (4.04–10.12), respectively, p=0.586] or Th1 cell count [83.04 (33.94–147.98) *vs*. 102.66 (80.87–179.59), respectively, p=0.068]. We also compared the absolute counts of total T cells, total B cells, natural killer (NK) cells, CD4+ T cells and CD8+ T cells between the SLE non-infection group and the healthy control group. The results indicated that the absolute NK cell and CD4+ T cell counts were significantly decreased in the SLE non-infection group compared to the healthy controls [100.44 (43.73–161.23) *vs*. 377.56 (232.31–552.52), respectively, p<0.001; 436.18 (259.51–726.48) *vs*.

Table III. Absolute lymphocyte and CD4+ T cell subset counts (cells/μL) in the SLE infection group (**A**), SLE non-infection group (**B**) and healthy control group (**C**).

Cell count cells/μL	SLE infection group (A) (n=36)	SLE non-infection group (B) (n=30)	Healthy control group (C) (n=20)	*p*-value A *vs*. B	*p*-value A *vs*. C	*p*-value B *vs*. C
Total T	440.96 255.13–731.97	996.59 785.53–1620.85	1173.25 1032.26–1399.13	0.000	0.000	0.137
Total B	102.56 57.63–194.48	168.14 66.48–288.45	165.63 136.70–227.97	0.157	0.015	0.921
NK	53.00 20.95–196.30	100.44 43.73–161.23	377.56 232.31–552.52	0.005	0.000	0.000
CD4+	199.79 104.29–320.86	436.18 259.51–726.48	588.04 519.72–678.96	0.000	0.000	0.031
CD8+	212.07 152.12–433.00	554.72 348.36–678.10	474.7 401.80–554.10	0.000	0.000	0.342
Th1	17.87 9.51–50.27	83.04 33.94–147.98	102.66 80.87–179.59	0.000	0.000	0.068
Th2	2.21 1.26–5.38	2.76 1.90–6.53	7.25 4.90–11.82	0.051	0.000	0.002
Th17	2.06 0.99–6.16	5.47 2.72–9.29	6.04 4.04–10.12	0.004	0.003	0.586
Treg	6.66 4.34–16.68	15.91 9.52–23.17	27.60 17.44–37.43	0.009	0.000	0.001
Th1/Th2	10.64 4.89–14.70	22.29 14.40–37.42	16.55 10.98–25.75	0.000	0.147	0.154
Th17/Treg	0.31 0.16–0.56	0.37 0.22–0.57	0.23 0.13–0.34	0.409	0.442	0.018

Results are expressed as the median and 25th and 75th percentiles.
Statistics: Wilcoxon's rank sum test.
SLE: systemic lupus erythematosus; Total T: total T cells; Total B: total T cells; NK: natural killer T cells; CD4+: CD4+ T cells; CD8+: CD8+ T cells; Th1: T helper 1 cells; Th2: T helper 2 cells; Th17: T helper 17 cells; Treg: regulatory T cells; Th1/Th2: T helper 1 cell/ T helper 2 cell ratio; Th17/Treg: T helper 17 cell/regulatory T cell ratio.

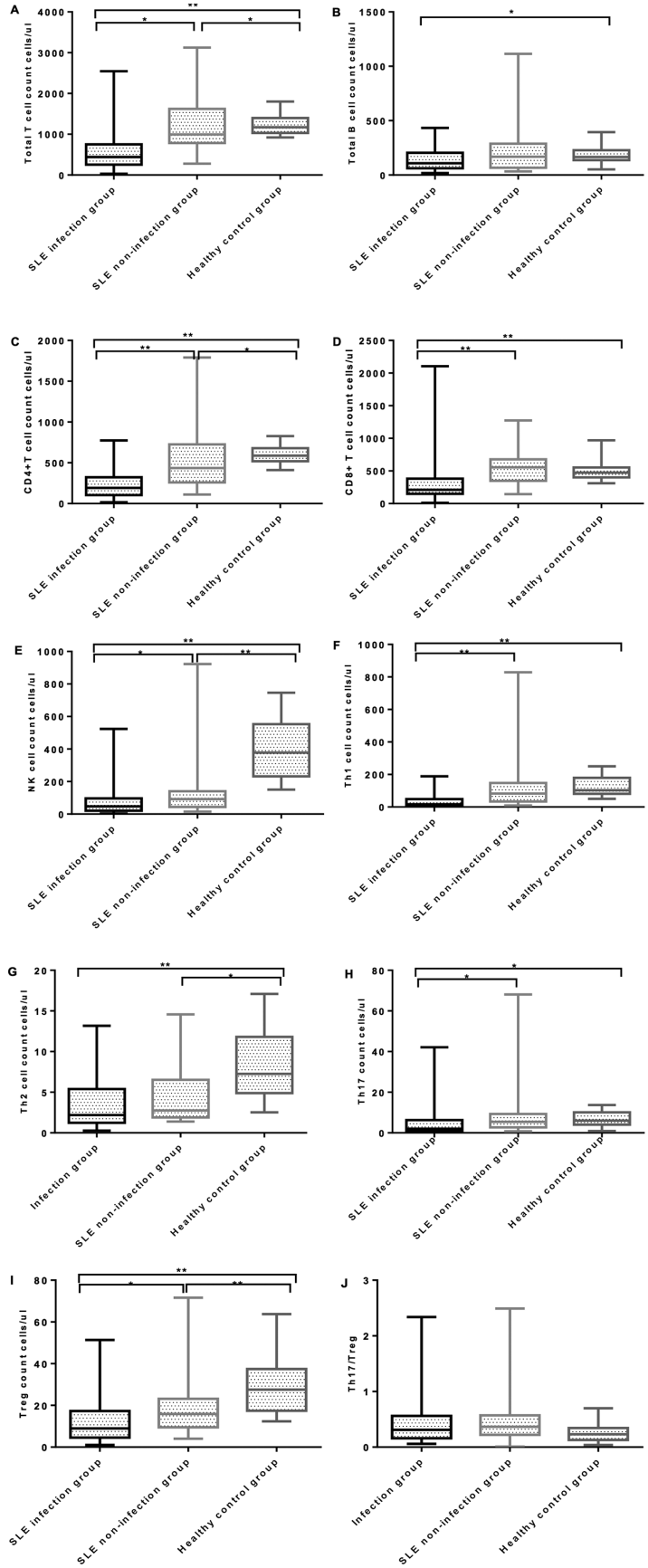

Fig. 1. Comparison of absolute lymphocyte and CD4+ T cell subset counts among the systemic lupus erythematosus (SLE) infection group (n=36), SLE non-infection group (n=30) and healthy control group (n=20). *p<0.05, **p<0.001.

588.04 (519.072–678.96), respectively, p=0.031]. However, there were no differences between the two groups in total T cell count [996.59 (785.53–1620.85) *vs*. 1173.25 (1032.26–1399.13), respectively, p=0.137], total B cell count [168.14 (66.48–288.45 *vs*. 165.63 (136.70–227.97), respectively, p=0.921] or CD8+ T cell count [554.72 (348.36–678.10) *vs*. 474.7 (401.80–554.10), respectively, p=0.342] (Table III, Fig. 1).

Absolute Th17 and Treg cell counts were lower in SLE patients with EBV/CMV viraemia than healthy controls

Next, we compared the Th17 and Treg cell counts between the SLE patients with EBV/CMV viraemia and the healthy controls. As shown in Table III and Fig. 1, unlike the SLE non-infection group, the absolute counts of Th17 and Treg cells were significantly decreased in the SLE infection group compared to the healthy control group [2.06 (0.99–6.16) *vs*. 6.04 (4.04–10.12), respectively, p=0.003; 6.66 (4.34–16.68) *vs*. 27.60 (17.44–37.43), respectively, p<0.001]. We also compared the counts of lymphocytes and other CD4+ T subpopulations between the two groups, and the results indicated that Th1 cells [17.87 (9.51–50.27) *vs*. 102.66 (80.87–179.59), respectively, p<0.001] and Th2 cells [2.21 (1.26–5.38) *vs*. 7.25 (4.90–11.82), respectively, p<0.001], total T cells [440.96 (255.13–731.97) *vs*. 1173.25 (1032.26–1399.13), respectively, p<0.001], total B cells [102.56 (57.63–194.48) *vs*. 165.63 (136.70–227.97), respectively, p=0.015], NK cells [53.00 (20.95–196.30) *vs*. 377.56 (232.31–552.52), respectively, p<0.001], CD4+ T cells [199.79 (104.29–320.86) *vs*. 588.04 (519.72–678.96), respectively, p<0.001] and CD8+ T cells [212.07 (152.12–433.00) *vs*. 474.70 (401.80–554.10), respectively, p<0.001] were significantly reduced in the SLE infection group compared to the healthy control group. These observations suggested that EBV and/or CMV infection exacerbated SLE, including via cellular immunity and humoral immunity, and thus contributed to disease progression (Table III, Fig. 1).

Table IV. Absolute lymphocyte and CD4+ T cell subset counts (cells/μL) in the SLE infection group (**A**), non-SLE infection group (**B**) and healthy control group (**C**).

Cell count cells/μL	SLE infection group (A) (n=36)	Non-SLE infection group[a] (B) (n=20)	Healthy control group (C) (n=20)	*p*-value A *vs*. B	*p*-value A *vs*. C	*p*-value B *vs*. C
Total T	440.96 255.13–731.97	1084 653.70–1911.00	1173.25 1032.26 – 1399.13	0.000	0.000	0.465
Total B	102.56 57.63–194.48	118.70 70.04–166.16	165.63 136.70 – 227.97	0.986	0.015	0.014
NK	53.00 20.95–196.30	114.30 61.72–220.63	377.56 232.31 – 552.52	0.010	0.000	0.000
CD4+T	199.79 104.29–320.86	533.31 401.72–721.25	588.04 519.72 – 678.96	0.000	0.000	0.402
CD8+T	212.07 152.12–433.00	376.00 182.13–925.25	474.7 401.80 – 554.10	0.034	0.000	0.516
Th1	17.87 9.51–50.27	51.68 25.96–157.84	102.66 80.87 – 179.59	0.004	0.000	0.010
Th2	2.21 1.26–5.38	6.18 1.67–17.45	7.25 4.90 – 11.82	0.013	0.000	0.534
Th17	2.06 0.99–6.16	5.05 2.13–11.00	6.04 4.04 – 10.12	0.011	0.003	0.715
Treg	6.66 4.34–16.68	20.91 13.30–67.36	27.60 17.44 – 37.43	0.001	0.000	0.766
Th1/Th2	10.64 4.89–14.70	13.43 3.59–27.81	16.55 10.98 – 25.75	0.419	0.147	0.304
Th17/Treg	0.31 0.16–0.56	0.18 0.095–0.48	0.23 0.13 – 0.34	0.150	0.442	0.725

Results are expressed as the median and 25th and 75th percentiles.
Statistics: Wilcoxon's rank sum test.
SLE: systemic lupus erythematosus; Total T: total T cells; Total B: total T cells; NK: natural killer T cells; CD4+: CD4+ T cells; CD8+: CD8+ T cells; Th1: T helper 1 cells; Th2: T helper 2 cells; Th17: T helper 17 cells; Treg: regulatory T cells; Th1/Th2: T helper 1 cell/ T helper 2 cell ratio; Th17/Treg: T helper 17 cell/regulatory T cell ratio.
[a]The non-SLE infection group consisted of 8 patients with primary Sjögren's syndrome (pSS), 11 patients with rheumatoid arthritis and 1 patient with Behçet's disease.

Absolute Th17 and Treg cell counts were lower in the SLE with EBV/CMV viraemia group than in the SLE non-infection group

CMV and EBV viraemia are emerging complications of immunosuppressive therapy in SLE patients, and an imbalance between Th17 and Treg cells has been shown to play a major role in the pathogenesis of SLE. However, there have been few previous studies regarding the roles of Th17 and Treg in SLE patients with EBV and/or CMV viraemia. Therefore, we compared the Th17 and Treg cell counts between 36 SLE patients with EBV/CMV viraemia (SLE infection group) and 30 SLE patients without infection (SLE non-infection group). The results indicated that Th17 and Treg cell counts were both significantly lower in the SLE infection group than the SLE non-infection group [2.06 (0.99–6.16) *vs*. 5.47 (2.72–9.29), respectively, p=0.004; 6.66 (4.34–16.68) *vs*. 15.91 (9.52–23.17), respectively, p=0.009]. Furthermore, comparison of the absolute Th1 and Th2 cell counts between the SLE infection and SLE non-infection groups indicated that only the Th1 cell count was reduced in the SLE infection group compared to the SLE non-infection group [17.87 (9.51–50.27) *vs*. 83.04 (33.94–147.98), respectively, p<0.001]. The absolute Th2 cell count was similar between the two groups [2.21 (1.26–5.38) *vs*. 2.76 (1.90–6.53), respectively, p=0.051]. The absolute total T cell count (p<0.001), NK cell count (P=0.005), CD4+ T cell count (p<0.001) and CD8+ T cell count (p<0.001) were significantly lower in the SLE infection group than the SLE non-infection group. In contrast, there was no significant difference in absolute total B cell count between the two groups [102.56 (57.63–194.48) *vs*. 168.14 (66.48–288.45), respectively, p=0.157] (Table III, Fig. 1).

There were no differences in absolute Th17 and Treg cell counts between non-SLE connective tissue disease patients and healthy controls

As discussed above, the Th17 and Treg cell counts were lower in the SLE infection group than the SLE non-infection group. To determine whether these results were applied only to patients with SLE, we compared the absolute counts of peripheral blood lymphocytes and CD4+ subsets between 20 patients with connective tissue diseases other than SLE combined with EBV and/or CMV viraemia (non-SLE infection group) and healthy controls (healthy control group). The non-SLE infection group consisted of 8 patients with pSS, 11 patients with RA and 1 patient with BD. There were no significant differences between the non-SLE infection group and the healthy control group in the absolute Th17 cell count [5.05 (2.13–11.00) *vs*. 6.04 (4.04–10.12), re-

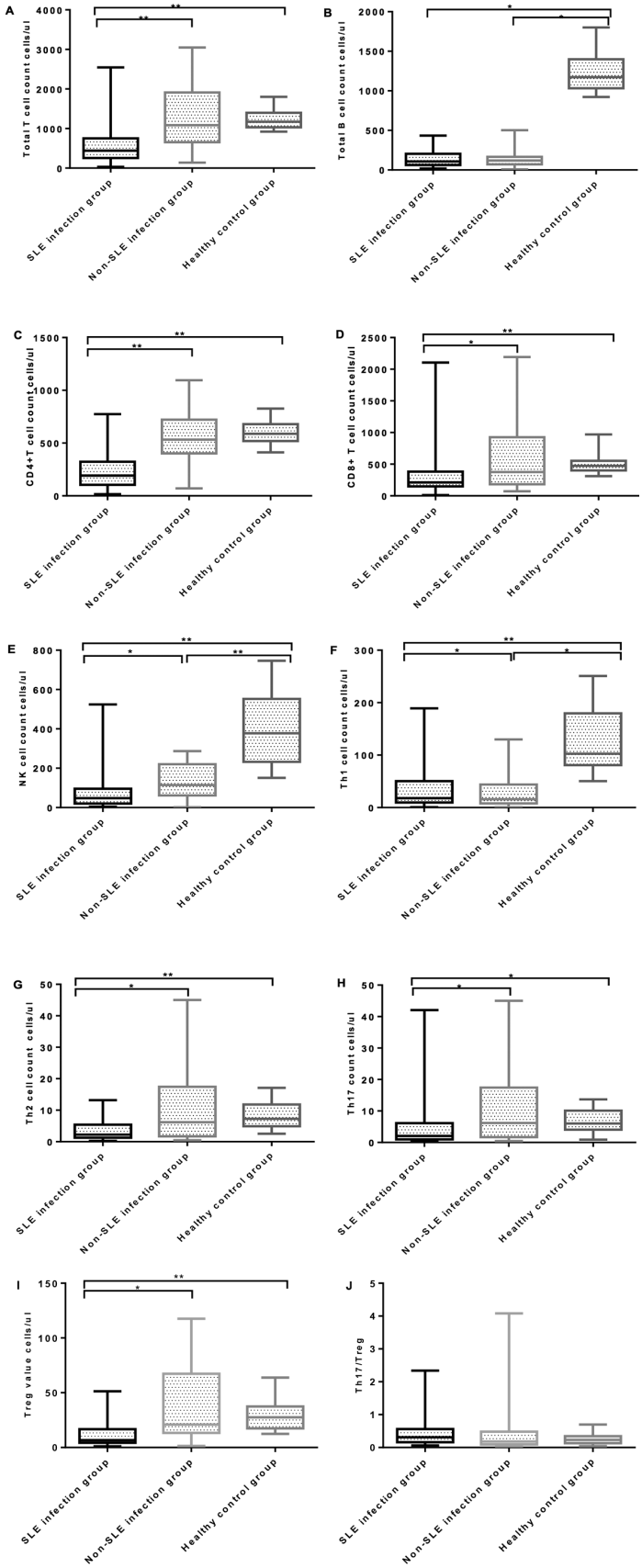

Fig. 2. Comparison of absolute lymphocyte and CD4+ T cell subset counts among the SLE infection group (n=36), non-SLE infection group (n=20) and healthy control group (n=20). *p<0.05,**p<0.001. The non-SLE infection group consisted of 8 patients with primary Sjögren's syndrome (pSS), 11 patients with rheumatoid arthritis and 1 patient with Behçet's disease.

spectively, p=0.715] or Treg cell count [20.91 (13.30–67.36) *vs*. 27.60 (17.44–37.43), respectively, p=0.766]. We also examined lymphocytes and other CD4+ T subpopulations, and found a significant difference between the two groups only in the absolute count of total B cells (p=0.014), NK cells (p=0.000) and Th1 cells (p=0.010) (Table IV, Fig. 2).

Absolute Th17 and Treg cell counts were significantly lower in the SLE infection group than the non-SLE infection group

Patients with SLE are more susceptible to opportunistic infections than those with other connective tissue diseases. We compared the absolute counts of lymphocytes and CD4+ T cell subsets between SLE patients with EBV/CMV viraemia (SLE infection group) and patients with connective tissue diseases other than SLE with EBV/CMV viraemia (non-SLE infection group). There was no significant differences in B cell count between the SLE infection group and the non-SLE infection group. However, the counts of total T cells p=0.000), NK cells (p=0.010), CD4+ T cells p=0.000), CD8+ T cells p=0.034), Th1 cells p=0.004), Th17 cells p=0.011) and Treg cells p=0.001) were significantly lower in the SLE infection group than the non-SLE infection group (Table IV, Fig. 2).

There were no differences in IFN-γ level between the SLE infection group and SLE non-infection group

Cytokine IFN-γ levels were determined in 14 patients in the SLE infection group and 25 patients in the SLE non-infection group. The level of IFN-γ was lower in the SLE infection group than the SLE non-infection group [4.22 (2.08–13.20 *vs*. 5.53 (4.08–8.63), respectively, p=0.327], but the difference was not statistically significant (Fig. 3).

Discussion

In this study, we investigated hospitalised SLE patients seen at our centre and explored the caracteristics of lymphocytes and CD4+ T cell subsets, especially Th17/Treg cells, in SLE patients with and without EBV/CMV infection. Our observations indicated that the im-

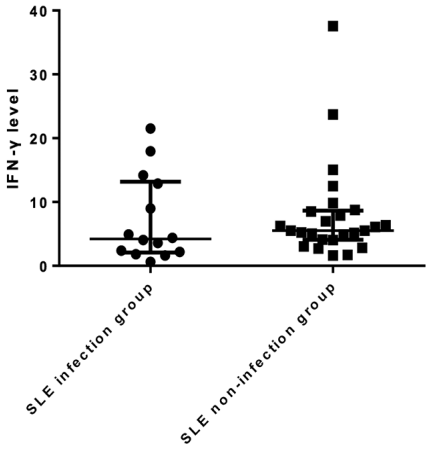

Fig. 3. Comparison of interferon (IFN)-γ levels between the SLE infection group (n=14) and SLE non-infection group (n=25).

balance between Th17 and Treg cells plays an important role in SLE. In addition, our findings suggested that Th17 cells may play a much larger role in the imbalance in the Th17/Treg cell ratio seen in SLE patients with EBV and/or CMV viraemia compared to those without comorbid viral infection. Our results may have significant clinical implications with respect to the roles of Th17 and Treg cells in the diagnosis and treatment of SLE patients with EBV/CMV viraemia.

SLE occurs due to a failure of immune tolerance mechanisms to prevent the expansion of autoreactive T cells. The immune disturbances associated with lupus itself and the use of immunosuppressive drugs are both important risk factors for infections in SLE patients, but the underlying mechanisms remain unclear. The associations of CMV and EBV infection with SLE have long been a matter of debate. Many studies have demonstrated associations between EBV and/or CMV and SLE (9–11), and it has been suggested that they may induce or promote the development of SLE in genetically predisposed individuals. However, there have been few previous studies regarding changes in peripheral lymphocyte and $CD4^+$ T cell subsets in SLE patients with EBV and/or CMV infection. The occurrence of EBV/CMV viraemia in SLE may be related not only to treatment with immunosuppressive agents, but also to the immune disorder underlying the disease. However, it is unclear which immune disorder in SLE patients is most closely related to EBV/CMV infection. Severe infection may stimulate systemic inflammatory response syndrome (SIRS), which in turn stimulates the immune response and induces disease activity. The occurrence of SIRS shows interindividual variation, and does not proceed linearly from immune activation to immunosuppression. In the early stages of the disease, the pro-inflammatory response and anti-inflammatory response can coexist. Therefore, it is very important to evaluate the immune status of patients with severe infection. During the treatment of severe infection, a great deal of attention is often paid to anti-infection treatment, while evaluation of immune function is neglected. A better understanding of the role of $CD4^+$ subsets in cases of SLE with EBV/CMV virus infection may provide a basis for improved treatments. This study was performed to determine the immune conditions in SLE patients with EBV and/or CMV viraemia. We proactively monitored changes in $CD4^+$ T cell subsets, especially Th17 and Treg cells, in SLE patients with EBV and/or CMV viraemia, to predict the severity of infection and adjust the therapeutic schedule appropriately.

$CD4^+$ T cells regulate or assist other lymphocytes. Impaired T cell proliferation and abnormal cytokine production have also been shown to play roles in the pathogenesis of SLE. Treg cells are a subset of $CD4^+$ T lymphocytes that inhibit effector T cells and inflammation. These cells are responsible for the maintenance of self-tolerance, and have been used in the treatment of tumours, infections, inflammatory diseases and type I diabetes. Treg cells can actively downregulate the activation/proliferation of self-reactive T cells. Th17 cells are another subset of $CD4^+$ T cells, characterised by the production of IL-17A, IL-17F and IL-22 (12). Pro-inflammatory Th17 cells contribute to the induction and propagation of inflammation. An imbalance of the Th17/Treg ratio in favour of pro-inflammatory Th17 cells is thought to exacerbate autoimmune disorders. Indeed, many groups have reported an imbalance in the Th17/Treg ratio in SLE patients, and the Treg cell count was shown to be reduced in the peripheral blood of SLE patients (13-15). New treatment strategies targeting the Th17/Treg ratio in may be beneficial (16). Although there have been many studies of Th17 and Treg cells in SLE patients, there have been few such studies in SLE patients with EBV and/or CMV viraemia without antiviral treatment.

This study was performed in a population consisting of 36 SLE patients with EBV and/or CMV viraemia (SLE infection group), 30 SLE patients without infection (SLE non-infection group) 20 patients with other non-SLE connective tissue diseases with EBV/CMV viraemia (non-SLE infection group), and 20 healthy controls (healthy control group). The absolute counts of Th17 and Treg cells in the SLE infection group were lower than those in healthy controls. Compared to the SLE non-infection group, the absolute counts of pro-inflammatory Th17 and Treg cells were also obviously decreased in the SLE infection group. The Treg cell count was lower in the SLE non-infection group than healthy controls, but there was no significant difference in Th17 cell count between these two groups, suggesting that a reduction in Treg cells may be responsible for the imbalance in the Th17/Treg ratio observed in SLE patients without infection, although Th17 cells may also play a vital role in SLE patients with EBV and/or CMV viraemia. Th17 cells, which have long been considered pro-inflammatory cells, were not elevated as expected in patients with SLE combined with EBV and/or CMV viraemia. Many studies have shown that dysfunction of IL-17-producing Th17 cells is associated with SLE. Lee *et al*. reported that IL-17 deficiency enhanced Treg differentiation and IL-10 production by effector T cells in Roquinsan/san mice. Furthermore, IL-17 deficiency was reported to reduce the severity of SLE by increasing the number of Treg cells and the production of IL-10 (17). Yang *et al*. also reported that patients with active SLE exhibit an increased proportion of Th17 cells relative to Treg cells compared to healthy individuals (18). These results suggest that IL-17 is a

promising target for the development of novel therapeutic regimens for SLE. However, in the present study, the absolute Th17 count in SLE patients without viral infection was similar to that in the healthy controls. Moreover, Th17 cell counts were markedly decreased in the SLE infection group. We believe that this may have been related to the relatively low disease activity in our SLE non-infection control group. However, the reduction in Th17 cell count seen in the SLE infection group was more important. To some extent, our results suggested that the decrease in Th17 cell count is closely related to EBV and/or CMV infection in SLE. Thus, clinicians should be alert to the occurrence of EBV/CMV viraemia in SLE patients showing a reduction in Th17 cell count.

To determine whether the results outlined above were confined to patients with SLE, we also evaluated the same parameters in a group of 20 patients with other non-SLE connective tissue diseases with EBV/CMV viraemia, consisting of 8 patients with pSS, 11 patients with RA and 1 patient with BD. There was no difference in the absolute Th17 or Treg cell count between these patients and the healthy controls. However, the Th17 and Treg cell counts in the SLE infection group were significantly reduced in comparison to the non-SLE infection group. These results suggested that an imbalance in the Th17/Treg ratio may play a crucial role in SLE with EBV and/or CMV viraemia. Appropriate immunomodulatory therapy to address the imbalance between Th17 and Treg cells based on antiviral therapy may be beneficial in SLE patients with EBV and/or CMV infection.

The IFNs comprise a group of multifunctional cytokines produced by monocytes and lymphocytes, which have a wide spectrum of antiviral activities, affect cell growth and differentiation, and regulate immune function and other biological activities. INF-I may have a more important antiviral role than IFN-II (INF-γ). However, due to the retrospective nature of this study, some subjects were not tested for INF-I. Moreover, INF-γ is more closely related to immune regulation. However, in our study, there was no significant difference in INF-γ level between the SLE infection group and the SLE non-infection group. This may have been due to the relatively small sample size, or may have been related to the complex interactions among different cytokines.

Infection is one of the leading causes of morbidity and mortality in SLE patients. Therefore, there have been many studies on the risk factors of infection in SLE patients (19-22). However, most of these were general studies, and no specific pathogens were examined. However, it is known different infections show heterogeneous effects. EBV and CMV, as opportunistic infections in SLE, are not uncommon and seriously affect the immune system, but there have been few studies of EBV/CMV infection in SLE patients. Wu *et al*. examined the risk factors for infection in SLE patients, and detected IgG, complement C3, complement C4 and anti-ds-DNA in 117 SLE patients with infection and 61 SLE patients without infection. Their study showed that the $CD4^+$ T cell count and $CD4^+/CD8^+$ ratio were lower in SLE patients with infection compared to SLE patients without infection, and the IgG concentration was significantly lower in SLE patients with infection than in those without infection (22). Consistent with those findings, we found that the $CD4^+$ T cell count was also decreased in SLE patients with EBV/CMV viraemia. In addition, the absolute total T cell, NK cell and $CD8^+$ T cell counts were also reduced in these patients. In our study, however, the complement C3/C4 and Ig levels in SLE patients with EBV/CMV viraemia were similar to those in the non-infection group. These results suggested that the decreases in total T/B and NK cell counts were closely related to EBV and/or CMV infection in SLE. We also found that the absolute counts of both Th1 and Th2 cells were lower in the SLE infection group than healthy controls, while only the Th1 cell count was lower in the SLE infection group compared to the SLE non-infection group. These observations suggest that Th1 cells also play vital roles in SLE with EBV and/or CMV viraemia.

The lymphocyte count was significantly lower in our SLE infection group than the SLE non-infection group. Although the pathogenesis of lymphopenia in SLE patients is still unclear, anti-lymphocyte antibodies and apoptosis may play vital roles (23-24). In our study, the SLE patients were prone to lymphocytopenia, and the SLE infection group had lower lymphocyte counts than the SLE non-infection group. This suggested that EBV/CMV infection may cause immunosuppression in SLE patients, resulting in a further reduction in the lymphocyte count. In conclusion, lymphocytopenia in SLE patients may be a sign of immunosuppression after EBV and/or CMV infection, and clinicians should be alert to the lymphocytopenia caused by EBV and CMV infection.

This study indicated that it is important to pay attention to changes in lymphocytes and $CD4^+$ subsets while controlling the primary disease in SLE patients with EBV/CMV infection. This study had some limitations, including the fact that all patients were drawn from one medical centre and the number of SLE patients included in the study was relatively small. In addition, we did not dynamically monitor changes in lymphocytes or $CD4^+$ T cell subsets during EBV/CMV infection, and we were unable to quantify the subjects' medication use. The detailed mechanism underlying the imbalance between Th17 and Treg cells should be examined further, in addition to the changes in cytokines over the course of the disease. Further studies are required to determine the role of Th17 cells in SLE with EBV/CMV infection.

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