Add-on sirolimus for the treatment of mild or moderate systemic lupus erythematosus via T lymphocyte subsets balance

Meng Ding, Lu Jin, Jinwen Zhao, Lin Yang, Shaoxin Cui, Xiaoping Wang, Jingjing He, Fei Chang, Min Shi, Jun Ma, Shuran Song, Hongtao Jin, Aijing Liu

ABSTRACT

Objective The efficacy of sirolimus in treating severe or refractory systemic lupus erythematosus (SLE) has been confirmed by small-scale clinical trials. However, few studies focused on mild or moderate SLE. Therefore, in this study we elucidated clinical efficacy of add-on sirolimus in patients with mild or moderate SLE.

Methods Data of 17 consecutive patients with SLE were retrospectively collected. SLE Disease Activity Index-2000 (SLEDAI-2K), clinical manifestation, laboratory data and peripheral T lymphocyte subsets with cytokines were collected before and 6 months after sirolimus add-on treatment. T cell subsets were detected by flow cytometry and cytokines were determined by multiplex bead-based flow fluorescent immunosassay simultaneously. Twenty healthy controls matched with age and sex were also included in our study.

Results (1) The numbers of peripheral blood lymphocytes, T cells, T helper (Th) cells, regulatory T (Treg) cells, Th1 cells, Th2 cells and Treg/Th17 ratios in patients with SLE were significantly higher, while the numbers of Th17 cells were evidently higher than those of healthy control (p<0.05). (2) After 6 months of sirolimus add-on treatment, urinary protein, pancytopenia, immunological indicators and SLEDAI-2K in patients with SLE were distinctively improved compared with those before sirolimus treatment (p<0.05). (3) The numbers of peripheral blood lymphocytes, T cells, Th cells, Treg cells, Th2 cells and the ratios of Treg/Th17 in patients with SLE after treatment were clearly higher than those before (p<0.05). (4) The levels of plasma interleukin (IL)-5, IL-6 and IL-10 in patients with SLE decreased notably, conversely the IL-4 levels increased remarkably compared with pretreatment (p<0.05).

Conclusions (1) Patients with SLE presented imbalanced T cell subsets, especially the decreased ratio of Treg/Th17. (2) Sirolimus add-on treatment ameliorated clinical involvement, serological abnormalities and disease activity without adverse reactions in patients with SLE. (3) The multi-target therapy facilitated the enhanced numbers of Treg cells, Treg/Th17 imbalance and anti-inflammatory cytokines, simultaneously, reducing inflammatory cytokines.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by the presence of varied serum autoantibodies and multisystem involvement. In recent decades, due to the continuous improvement of medical diagnostic technology with early application of glucocorticoids, antimarial drugs or immunosuppressants, the 5-year survival rate of patients with SLE has greatly improved, but the standardised mortality rate is still higher than that of healthy individuals. Additionally, the adverse effects with long-term treatment, such as infection and organs damage, also seriously plague the prognosis of the disease, while there are still unmet needs in therapy strategies. With the in-depth research on the pathogenesis of
SLE, especially in immune-competent cells and molecular pathways, the treatment plan of SLE has gradually entered the targeting era.

The key factor associated with SLE pathogenesis is the imbalance in immune regulation. The number and function of active immune cells are directly or indirectly involved in the occurrence and development of SLE. Among them, the aberrant proliferation, differentiation and function of T lymphocytes are critical in the pathogenesis of SLE. It has been confirmed that Th17 cells and Tregs are two important immune cell subsets in the peripheral blood of patients with SLE. Th17 cells lead to tissue inflammation by releasing interleukin (IL)-17, IL-21, IL-23 and tumour necrosis factor (TNF)-α, while Tregs mediate immune tolerance by releasing IL-4 and IL-10, thus further regulating immune response. Existing studies have shown the Treg/Th17 ratio reduction is closely related to SLE occurrence and disease progression. On the one hand, the abnormal expression of cytokines in patients with SLE is conducive to Th17 cells’ development, while the increase in number of Th17 cells causes the Treg/Th17 to decrease. On the other hand, when the number of Tregs or function is reduced or impaired, this leads to the dysfunction of autoreactive T lymphocytes and more Th17 cells and IL-17 in target organs, resulting in tissue damage. In addition, it was also found that the number of Tregs in patients with refractory SLE was significantly reduced compared with that of healthy individuals, while the number of Th17 cells did not significantly change, suggesting the reduction in number of Tregs may be the main cause of imbalance of Treg/Th17 in patients. Furthermore, studies have shown that low-dose IL-2 can stimulate the expansion of Tregs in vivo can significantly alleviate inflammation, inhibit autoimmune responses and reduce lupus disease activity. Th1 and Th2 cells are the most classic Th cell subsets with an important regulatory role in antibody-mediated autoimmune diseases. However, it was reported there were controversial conclusions in regard of the dominance of T1/Th2 in SLE. Collectively, therapies targeting the regulation of the balance of T cell subsets in SLE have potential clinical application prospects.

In recent years, the mammalian target of rapamycin (mTOR) pathway has gradually become the focus in the study of molecular pathways of SLE pathogenesis. Besides, sirolimus, also called rapamycin (RAPA), the specific inhibitor of the mTOR pathway, has gradually gained increasing interest in basic research and clinical trials of SLE. In 1999, sirolimus was officially approved by the Food and Drug Administration (FDA) for the antirejection treatment of kidney transplantation. Later, studies found it can reduce the anti-double-strand DNA (anti-dsDNA) antibody in serum and urine protein in MRL/lpr mice and prolong its survival. Clinical studies showed that patients suffering from SLE who did not respond or were intolerant to traditional treatment had different degrees of reduced disease activity and good tolerance when they were applied with sirolimus combined therapy. Zhao et al used a low dose of sirolimus (0.5 mg qod (quaque omni die/every other day)) and IL-2 to treat 50 patients with SLE who failed traditional treatment, and the results showed the dramatically increasing numbers of Tregs and the ratio of Treg/Th17 in patients after 12 and 24 weeks of treatment. Sirolimus combined therapy could effectively inhibit the progression of severe or refractory SLE; however, few reports focused on mild or moderate SLE. T cell subsets with the balance and the corresponding cytokines before and after treatment. Here, we aim to explore the possible immunoregulatory mechanism and clinical efficacy of add-on sirolimus in treating patients with SLE with mild or moderate disease activity from the perspective of immune cell balances and cytokines changes and provide a reference for clinical multi-target therapy.

METHODS

Patients
We respectively analysed the data of 17 consecutive Chinese patients with SLE who visited in the Second Hospital of Hebei Medical University between August 2019 and December 2020. All patients met the 2019 European League Against Rheumatism/American College of Rheumatology criteria for SLE with mild or moderate SLE Disease Activity Index-2000 (SLEDAI-2K) and were treated with add-on low dose (<2 mg/d) of sirolimus for 6 months based on routine therapy. Exclusion criteria included (1) overlap with other connective tissue disease as defined by validated criteria; (2) female patients with pregnancy or lactation; (3) severe infection, malignancy, cardiovascular and cerebrovascular diseases or other diseases; (4) <18 years or >80 years old; (5) allergic to sirolimus. Ethics approval was obtained from the ethics committee of the Second Hospital of Hebei Medical University (2019-R190) and all participants signed a written informed consent form. Twenty healthy controls matched by age, gender and body mass index were also included in the study.

Demographic, clinical and laboratory data
Profiles of demographics, disease duration, clinical manifestation, organ involvement, SLEDAI-2K and therapeutic regimens on lupus patients before and after sirolimus add-on treatment were systematically reviewed. Blood and urine routine, biochemical index, Ig, complement (C)3, C4, anti-dsDNA, values of T cell subsets and cytokines were collected simultaneously.

Phenotypic analyses of T cell subset
Phenotypic cell analyses were performed by co-staining peripheral blood mononuclear cells with fluorochrome-labelled monoclonal antibodies (mAb) to CD3, CD4, CD25, CD45, CD127, CD183 and CD196 according to standard protocols and reference. All mAb were from Beckman Coulter and Becton Dickinson, USA. Cells were acquired on a NAVIOS flow cytometer (Beckman Coulter, US) and analysed using Kaluza software.
Cytokines detection
Cytokines were determined by multiplex bead-based flow fluorescent immunoassay according to protocols. Microbeads were acquired on a BriCyte E6 flow cytometer (Mindray, China). The kit was purchased from GenScript Biotech Corporation of Qingdao, China.

Statistical analysis
Statistical analysis was performed using GraphPad Prism V.9.0. Values were presented as $\overline{X} \pm S$ or M(Q1, Q3) for normal distribution data or non-normal distribution data. T-test and Mann-Whitney test were used to identify differences between the sample values. For categorical data, the $\chi^2$ test or Fisher exact probability test was used for comparisons between two groups. P value <0.05 was considered significant for all analyses.

RESULTS
Clinical baseline data of patients with SLE
Seventeen patients (15 female, 2 male) were recruited in the study. Age was 29.76±9.78 years and the average disease duration was 15.53±25.89 months. All the patients were followed up 11.12±2.76 months after sirolimus add-on treatment. Among 17 patients, 9 (52.94%) presented with maculopapule, 2 (11.76%) complained for arthritis, 9 (52.94%) involved with haematological abnormalities (7 leucopenia and 3 thrombocytopenia), 8 (47.06%) existed renal involvement (6 proteinuria >0.5 g/24 hours, 2 haematuria) and SLEDAI-2K was 7.65±2.89. In terms of immunological data, anti-dsDNA antibody was detected (elevated in ELISA as well as positive in indirect immunofluorescence (IIF)) in 11 cases (64.71%), all cases presented with hypocomplementemia, and 11 (64.71%) with elevated IgG (figure 1). Glucocorticoid (GC), hydroxychloroquine (HCQ) and with or without immunosuppressants were applied with patients as traditional therapy for at least 3 months, sirolimus was added on with 0.5 mg qd (quaque die/every day) and 1 mg qd, consecutively (figure 1).

Comparisons of peripheral white blood cells (WBCs), lymphocytes, T cells and Th cells between healthy controls and patients with SLE
There were no significant differences between the numbers of peripheral WBCs in lupus patients and healthy controls (p=0.368), while the numbers of lymphocytes, T cells and Th cells in patients were all apparently lower than that of healthy controls (p=0.004, 0.000 and 0.000, respectively) (figure 2A and online supplemental table 1)

Comparisons of peripheral T cell subsets between healthy controls and patients with SLE
When we compared peripheral Tregs and Th17 cells between healthy controls and patients with SLE, it was found there was no significant difference in Tregs percentages (p=0.311) while notable differences existed in percentages or absolute numbers of Th17 (p=0.000 and 0.000) and numbers of Tregs between the two groups (p=0.045). Unsurprisingly, Treg/Th17 ratios in SLE were lower than those of the controls (p=0.000). See figure 2B,C and online supplemental table 1. Either the absolute numbers of Th1 or Th2 in patients were lower when compared with the health controls (p=0.000 and 0.000), while there were no differences in percentages of the two subsets nor the ratios of Th1/Th2(p=0.907, 0.069 and 0.235, respectively). See figure 2D,E and online supplemental table 1. Clinical data of patients with SLE after add-on sirolimus treatment for 6 months
The numbers of WBCs, haemoglobins and platelets after 6-month add-on sirolimus treatment in lupus patients were all notably ameliorated compared with pretreatment (p=0.036, 0.000 and 0.000, respectively). In terms of leucopenia, five lupus patients responded to therapy and recovered to the normal range and two patients had higher WBCs compared with pretreatment. With respect to renal injury, the urine protein

Figure 1 Clinical baseline data of patients with SLE (hot map showed clinical feathers, immunological indicators and dosage of sirolimus; pie chart displayed the appliance of immunosuppressants in patients with SLE). C3, complement 3; C4, complement 4; dsDNA, double-strand DNA; GC, glucocorticoid; HCQ, hydroxychloroquine; SLE, systemic lupus erythematosus.
was 0.145 (0.085, 1.898) g/24 hours, lower than the number of pretreatment (p=0.046). Among the six patients with SLE with proteinuria, there were five patients with data of 24-hour proteinuria after 6-month of add-on therapy. Two patients proved to be complete renal remission and three showed partial remission. Consequently, the increased levels of plasma albumin were observed in the treated patients. In immunological index, C3 and C4 were 0.845 (0.660, 0.910) g/L and 0.185 (0.128, 0.215) g/L, respectively, which had much improvement than pretreatment (p=0.003 and 0.000); consist with the complements, IgG and IgM levels were 13.455 (12.870, 14.037) g/L and 1.063±0.557 g/L, respectively, and lower than before (p=0.016 and 0.000). Additionally, the levels of anti-dsDNA antibodies (ELISA) decreased from 137.700 (33.282, 670.030) IU/mL to 19.620 (10.142, 49.475) IU/mL with the treatment (p=0.000); however, the titre of anti-dsDNA antibodies (IIF) did not change demonstrably (p=0.067). SLEDAI-2K decreased from 8.000 (6.000, 10.000) to 1.000 (0.000, 4.000) after treatment (p=0.000) with less dose of GCs (table 1). Of note, there were nine patients with SLEDAI-2K <4, and five patients with SLEDAI-2K ≥4 except for incomplete data in three patients. It was suggested that patients with proteinuria tend to remain active disease after treatment despite the decreased SLEDAI-2K. Patients did not complain about adverse reactions nor severe reactions during sirolimus add-on treatment, for example, hyperlipidaemia, liver damage and myelosuppression.

Figure 2 Comparisons of numbers of peripheral blood lymphocytes, T cells and T cell subsets between the SLE group and healthy control group. (A) Absolute values of peripheral blood lymphocytes, T cells and T cell subsets. (B) Absolute values of Treg cells and Th17 cells. (C) Treg/Th17 cell ratios. (D) Absolute values of Th1 cells and Th2 cells. (E) Th1/Th2 cell ratios. *p<0.05; **p<0.01; ***p<0.001. SLE, systemic lupus erythematosus; Th, T helper; Treg, regulatory T.
Changes of peripheral lymphocytes, T cells and T cell subsets in patients with SLE before and after 6-month sirolimus add-on treatment

The numbers of peripheral lymphocytes, T cells and Th cells in patients with SLE were (1.610±0.634)×10^9/L, (1.230±0.581)×10^9/L and (0.548±0.248)×10^9/L, respectively, having remarkable improvement compared with pretreatment (p=0.007, 0.008 and 0.009, respectively) (figure 3A and table 1). In terms of Treg cell and Th17 cell, no significant difference was observed in the percentage of Tregs and Th17 cells in patients with SLE before and after 6 months of treatment (p=0.891 and 0.615). The absolute number of Tregs was obviously higher than pretreatment (p=0.011), while there was no difference in the absolute value of Th17 cells (p=0.169). In addition, the Treg/Th17 ratio after treatment was increased compared with pretreatment (p=0.018). The changes of clinical data, numbers of peripheral lymphocytes, T cells and their subsets, and plasma cytokines of patients with SLE are shown in table 1.

### Table 1

Comparisons of clinical data, numbers of peripheral lymphocytes, T cells and their subsets, and plasma cytokines of patients with SLE before and after sirolimus add-on treatment

<table>
<thead>
<tr>
<th>Case (n=17)</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>T/z/χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLEDAI-2K</td>
<td>8.000 (6.000, 10.000)</td>
<td>1.000 (0.000, 4.000 )</td>
<td>−5.657</td>
<td>0.000***</td>
</tr>
<tr>
<td>WBC (10⁹/L)</td>
<td>4.355±2.611</td>
<td>5.770±2.228</td>
<td>2.290</td>
<td>0.036*</td>
</tr>
<tr>
<td>Hgb (g/L)</td>
<td>108.937±21.665</td>
<td>129.500±16.541</td>
<td>4.337</td>
<td>0.000***</td>
</tr>
<tr>
<td>Platelet (10⁹/L)</td>
<td>165.875±90.543</td>
<td>235.000±72.427</td>
<td>3.284</td>
<td>0.000***</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>36.206±8.877</td>
<td>42.227±5.241</td>
<td>5.125</td>
<td>0.000***</td>
</tr>
<tr>
<td>Proteinuria/24 hours (g)</td>
<td>1.795 (0.790, 7.700)</td>
<td>0.145 (0.085, 1.898)</td>
<td>−1.997</td>
<td>0.046*</td>
</tr>
<tr>
<td>Positive anti-dsDNA (IIF)</td>
<td>14 (84.351%)</td>
<td>9 (52.941%)</td>
<td>−3.360</td>
<td>0.067</td>
</tr>
<tr>
<td>Anti-dsDNA† (0–100 IU/mL)</td>
<td>137.700 (33.282, 670.030)</td>
<td>19.620 (10.142, 49.475 )</td>
<td>−3.258</td>
<td>0.000***</td>
</tr>
<tr>
<td>C3 (0.80–1.60 g/L)</td>
<td>0.395 (0.268, 0.638)</td>
<td>0.845 (0.660, 0.910)</td>
<td>3.721</td>
<td>0.003**</td>
</tr>
<tr>
<td>C4 (0.16–0.38 g/L)</td>
<td>0.050 (0.038, 0.083)</td>
<td>0.185 (0.128, 0.215)</td>
<td>5.832</td>
<td>0.000***</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>20.750 (15.775, 24.850)</td>
<td>13.455 (12.870, 14.037)</td>
<td>−2.417</td>
<td>0.016**</td>
</tr>
<tr>
<td>IgA (g/L)</td>
<td>3.275 (2.765, 3.945 )</td>
<td>2.740 (2.360, 3.813 )</td>
<td>−0.724</td>
<td>0.469</td>
</tr>
<tr>
<td>IgM (g/L)</td>
<td>3.069±1.114</td>
<td>1.063±0.557</td>
<td>−6.762</td>
<td>0.000***</td>
</tr>
<tr>
<td>Lymphocyte (10⁹/L)</td>
<td>1.184±0.743</td>
<td>1.610±0.634</td>
<td>3.153</td>
<td>0.007**</td>
</tr>
<tr>
<td>T cell (10⁹/L)</td>
<td>0.713±0.512</td>
<td>1.230±0.581</td>
<td>3.059</td>
<td>0.008**</td>
</tr>
<tr>
<td>Th cell (10⁹/L)</td>
<td>0.333±0.194</td>
<td>0.548±0.248</td>
<td>2.948</td>
<td>0.009**</td>
</tr>
<tr>
<td>Treg cell (%)</td>
<td>4.234±1.743</td>
<td>4.245±2.567</td>
<td>0.140</td>
<td>0.891</td>
</tr>
<tr>
<td>Th17 cell (%)</td>
<td>4.531±3.657</td>
<td>3.245±2.543</td>
<td>−0.502</td>
<td>0.615</td>
</tr>
<tr>
<td>Treg cell (10⁹/L)</td>
<td>0.028 (0.012, 0.048 )</td>
<td>0.049 (0.026, 0.067 )</td>
<td>2.889</td>
<td>0.011*</td>
</tr>
<tr>
<td>Th17 cell (10⁹/L)</td>
<td>0.088 (0.040, 0.029 )</td>
<td>0.015 (0.008, 0.033 )</td>
<td>−1.439</td>
<td>0.169</td>
</tr>
<tr>
<td>Treg/Th17</td>
<td>1.886 (0.827, 2.872 )</td>
<td>4.272 (1.743, 35.562 )</td>
<td>2.366</td>
<td>0.018*</td>
</tr>
<tr>
<td>Th1 cell (%)</td>
<td>28.436±14.572</td>
<td>24.156±14.534</td>
<td>−1.187</td>
<td>0.253</td>
</tr>
<tr>
<td>Th2 cell (%)</td>
<td>61.667±19.036</td>
<td>68.564±17.945</td>
<td>1.524</td>
<td>0.147</td>
</tr>
<tr>
<td>Th1 cell (10⁹/L)</td>
<td>0.062 (0.040, 0.107 )</td>
<td>0.094 (0.046, 0.199 )</td>
<td>1.321</td>
<td>0.205</td>
</tr>
<tr>
<td>Th2 cell (10⁹/L)</td>
<td>0.198±0.130</td>
<td>0.377±0.208</td>
<td>3.258</td>
<td>0.005**</td>
</tr>
<tr>
<td>Th1/Th2</td>
<td>0.393 (0.278, 0.772 )</td>
<td>0.304 (0.114, 0.768 )</td>
<td>−1.264</td>
<td>0.224</td>
</tr>
</tbody>
</table>

| Cytokine (pg/mL) | | | | |
|---|---|---|---|
| IL-5 | 1.909±0.516 | 1.425±0.818 | −2.221 | 0.048* |
| IL-6 | 16.126±13.577 | 8.098±4.569 | −2.040 | 0.041* |
| IL-10 | 2.950 (1.505, 6.322) | 1.510 (1.075, 1.817) | −2.794 | 0.005** |
| IL-17 | 1.939±1.644 | 1.493±0.563 | −0.415 | 0.678 |
| IL-4 | 0.780 (0.388, 1.493) | 1.520 (1.060, 2.100) | 2.337 | 0.036* |
| IL-2 | 1.220 (0.802, 2.640 ) | 1.340 (0.780, 2.440 ) | 0.511 | 0.619 |
| IFN-γ | 10.850 (1.560, 24.698 ) | 9.66 (2.895, 12.913) | −1.490 | 0.136 |
| TNF-α | 4.300 (2.152, 15.750 ) | 2.145 (0.990, 4.015 ) | −1.726 | 0.084 |
| GC dose | 8.75 (7.5, 10) | 7.5 (7.5, 8.75) | −11.000 | 0.027* |

*p<0.05; **p<0.01; ***p<0.001.

C3, complement 3; C4, complement 4; dsDNA, double-strand DNA; GC, glucocorticoid; Hgb, haemoglobin; IFN, interferon; IIF, indirect immunofluorescence; IL, interleukin; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index-2000; Th, T helper; TNF, tumour necrosis factor; Treg, regulatory T; WBC, white blood cell.
Lupus Science & Medicine


4.272 (1.743, 35.562), clearly higher than pretreatment (1.886 (0.827, 2.872), p=0.018). See figure 3B,C and table 1. Meanwhile, the absolute number of Th2 cells was (0.377±0.208) ×10^9/L after treatment, evidently higher than pretreatment (0.198±0.130) ×10^9/L, p=0.005. However, there were no distinctive differences in the percentage of Th1 cells and Th2 cells, Th1 numbers and the Th1/Th2 ratio after and before treatment (p=0.253, 0.147, 0.205 and 0.224, respectively). See figure 3D,E and table 1.

Comparisons of plasma cytokines of patients with SLE before and after sirolimus add-on treatment

Different T cell subsets release the same or different cytokines when activated. We seek to determine if the corresponding cytokines produced by T cells would be changed after sirolimus add-on treatment. We observed serum IL-5, IL-6 and IL-10 levels significantly reduced (1.425±0.818 pg/mL, 8.098±4.569 pg/mL and 1.510 (1.075, 1.817), p=0.048, 0.041 and 0.005, respectively) compared with pretreatment. In addition, serum IL-4...
levels were higher than that of pretreatment (1.520 (1.060, 2.100) pg/mL vs 0.780 (0.388, 1.493) pg/mL, p=0.036), while IL-2, IL-17, interferon (IFN)-γ and TNF-α concentrations did not remarkably change, p=0.619, 0.678, 0.136 and 0.084, respectively. See figure 4 and table 1.

DISCUSSION

mTOR is a serine/threonine protein kinase, which is divided into two complex forms, that is, mTORC1 and mTORC2, each one having a different composition. The mTOR signalling pathway is involved in the pathogenesis of SLE by regulating immune cell differentiation, releasing inflammatory cytokines, autophagy, oxidative stress and energy metabolism. Recent clinical studies have found that sirolimus, which mainly inhibits the mTORC1 pathway, does not only improve renal damage, cytopenia and skin damage in patients with SLE but also reduces the disease activity and glucocorticoid use, resulting in improved quality of life. In this study, we retrospectively analysed and compared the clinical and laboratory data of patients with mild or moderate SLE treated with add-on sirolimus of low dose for 6 months. The results showed the numbers of lymphocytes, T cells, Th cells, Tregs, Th1 cells, Th2 cells and Treg/Th17 ratios were lower, while the Th17 cell numbers were higher compared with the healthy people. After add-on treatment, the urine protein, blood system and immunological indicators of patients definitely improved. In addition, compared with pretreatment, the number of peripheral lymphocytes, T cells, Th cells, Tregs, Th2 cells and the Treg/Th17 ratios in patients with SLE were considerably increased. Simultaneously, the plasma cytokines levels, such as IL-5, IL-6 and IL-10, were decreased, while the IL-4 was distinctively increased. As shown above, the changes of numbers in peripheral lymphocytes and T cell subsets in these patients with SLE were consistent with the results of most clinical trials as compared with health controls and pretreatment.

Apoptosis is the programmed cell death regulated by genes, which has a key role in the growth and development of lymphocytes and immune tolerance. Rastin et al found mRNA levels of FasL and caspase-8, which are known as apoptosis-inducing genes, were definitely higher than those in healthy people, indicating the decrease in lymphocytes number in patients with SLE was related to the increased expression of apoptosis-regulating genes. Previous studies have also found overactivated NOD-like receptor thermal protein domain associated protein 3 (NLRP3) activated caspase-1 pathway in patients with SLE, leading to lymphocyte pyroptosis and promoting the disease development. The energy form required for cell proliferation and apoptosis is ATP, which is mainly provided by cellular glycolysis and oxidative phosphorylation (OXPHOS). Mitochondria are the main site of cellular OXPHOS, and ATP synthesis is driven by an electrochemical gradient across the inner mitochondrial membrane maintained by the electron transport chain and membrane potential. Abnormal apoptotic T cells from lupus showed persistently elevated mitochondrial membrane potential and mitochondrial hyperpolarisation (MHP), depletion of ATP and glutathione, increased Ca2+ influx and mitochondrial dysfunction, exerting a crucial role in the pathogenesis of SLE. In their clinical trial, Fernandez et al showed RAPA rescued the restoration of Ca2+ influx by modulating Ca2+ flux without affecting T cell MHP. In addition, a clinical study in 2009 revealed that mTOR inhibitors increased the number of Th cells in patients with SLE, consistent with the results of our study. The underlying mechanism includes mTOR inhibitors, which can inhibit the expression of small G protein family in Th cells, including HRES-1/Rab4 and Rab5A. These, in turn, further inhibit the overactivation of lymphomes and rescue the loss.

Different cell subsets and their secreted cytokines have different regulatory roles. Th cells differentiate into different subsets under the action of different cytokines, transforming growth factor (TGF)-β induces Th cells to differentiate into Tregs, while IL-6 combined with TGF-β promotes Th cells to differentiate into Th17 cells. A large number of studies have shown that due to the decrease in number of Tregs and increase in number of Th17 cells, Treg/Th17 ratio decreases, which negatively correlates with SLE disease activity. In this study, the number of Tregs in the patients was lower than that of healthy individuals, while the absolute value of Th17 cells was higher. Therefore, the Treg/Th17 ratio was also significantly lower compared with healthy individuals, and the Treg/Th17 balance was tilted towards Th17. Among the changes in the above ratio, the change in the number of Th17 cells was more significant. Previous studies have confirmed that abnormal metabolisms of T cells, such as enhanced glycolysis, lipid synthesis and glutamine decomposition, with high activation of mTOR, are all conducive to the differentiation and function of Th17 cells, which is the main reason for the imbalance of Treg/Th17 in patients with SLE.

Kato et al showed the mTORC1 activity of T cells in patients with SLE increases to inhibit the expression of
Foxp3 and promotes the reduction and/or dysfunction of Tregs. However, when the mTOR pathway is inhibited, the expression of Foxp3 is enhanced, promoting the differentiation of Tregs and is beneficial to the recovery of immune tolerance. Kurebayashi et al confirmed sirolimus combines with FKBP12, an intracellular immunosuppressive protein, to form an immunosuppressive compound, which combines with mTORC1 to inhibit the mTOR pathway, reduce the expression of RORγt, inhibit the differentiation of Th17 cells and increase the Foxp3 transcriptional activity to promote the proliferation and differentiation of Treg. Delgoffe et al inhibited the differentiation of Th17 and Th1 cells by selective knockout of Rheb in T cells from wild-type mice and the loss of mTORC1 activity. Compared with other Th cells, Th17 cells’ differentiation is more dependent on glycolysis. Hypoxia-inducible factor (HIF)-1α is a key factor for regulating the downstream glycolytic response of mTORC1, while sirolimus reduces glycolysis by blocking HIF-1α, thereby inhibiting the differentiation of Th17 cells. The above results certified sirolimus could regulate the proliferation, differentiation and function of Treg and Th17 cells by inhibiting the mTORC1 pathway.

In this study, the absolute value of Tregs in patients with SLE after treatment with sirolimus was obviously higher, while the absolute value of Th17 cells did not significantly differ from pretreatment; Yet, Th17 cells still could be observed to be decreased tendency, and the Treg/Th17 ratio was significantly higher than pretreatment, suggesting sirolimus was more effective in restoring numbers of Tregs than reducing Th17 cell numbers, thus contributing to the increase of Treg/Th17 ratio, which is consistent with the previous study.

Different immunomodulatory drugs have a different effect on the development and function of T cell subsets. Janyst et al conducted in vitro studies examining the effects and mechanisms of different drugs on Tregs, finding sirolimus and prednisone had a stronger ability to promote the expression of Foxp3 in Th cells than several other immunomodulators. In vitro experiments and the SLE mice model showed HCQ reduced number of Th17 cells, increased the number of Treg and restored the Treg/Th17 balance by inhibiting abnormal activation of autophagy, thereby restoring immune tolerance. Myco-phenolate mofetil was observed to inhibit the differentiation of Th17 cells by inhibiting the expression of T-cell Ig mucin 1 molecule, promoting Tregs differentiation and tilting Treg/Th17 balance to Treg. The above results were mostly obtained by in vitro or in vivo experiments. More clinical studies need to further verify the effects of antirheumatic drugs on different T cell subsets.

The imbalance of the Th1/Th2 has important clinical significance in the pathogenesis of SLE, though there are many controversies about whether type I or type II Th cell subsets dominate. Gu et al found the Th1 cells and Th1/Th2 ratio of patients with SLE in the active phase were distincitively higher than the stable phase and healthy individuals, while the number of Th2 cells was lower. The balance of Th1/Th2 was tilted towards Th1, and there was no significant difference in Th1, Th2 cells and Th1/Th2 ratio between patients in the stable phase and healthy people. Jiang et al indicated the inclination of Th1/Th2 balance to Th2 was an important feature of the pathogenesis of SLE. Compared with the healthy individuals, the number of Th1 cells and the Th1/Th2 ratio in patients with SLE were considerably lower, and the Th2 cells were clearly increased. Besides, the level of IFN-γ secreted by Th1 cells was lower, and the IL-4 level secreted by Th2 cells was higher than in healthy individuals. Yusoff et al suggested that Th1 and Th2 cells in patients with SLE were higher compared with healthy people. In this study, the numbers of Th1 and Th2 cells in patients with SLE were lower, while the Th1/Th2 ratio observed increased tendency despite the lack of statistical difference when compared with the healthy people. After treatment with sirolimus, with the increase in the number of Th2 cells, the Th1/Th2 balance in patients gradually showed a trend of being dominated by Th1. Delgoffe et al inhibited the differentiation of Th1 cells and promoted the phosphorylation of STAT6 and the expression of the transcription factor GATA-3, a key transcription factor of Th2 cells, by selective knockout of Rheb in T cells of a wild-type mouse and loss of mTORC1 activity, which enhanced the differentiation of Th2 cells. The above results indicated that sirolimus could inhibit the mTORC1 pathway and promote Th2 cell differentiation, which is consistent with the results of this study.

Sirolimus also had a different effect on cytokines secreted by different Th cell subsets. Here, we found after 6 months of treatment with sirolimus, the plasma levels of IL-5, IL-6 and IL-10 in patients with SLE were considerably lower, while the IL-4 level was higher than pretreatment. There was no obvious difference in IL-2, IL-17, IFN-γ and TNF-α before and after treatment.

IL-5 and IL-6 are the main proinflammatory factors secreted by Th2 cells. They aggravate immune-mediated inflammatory response in SLE via inducing the activation of B cells and promoting the differentiation of Th to Th17 cells. Sirolimus reduces their levels through the mTORC1–S6 kinase 1 pathway, thereby reducing immune-mediated organ damage in patients with SLE. The level of IL-10 in patients with SLE after treatment was lower, which was inconsistent with our expected results. Many clinical studies have shown plasma IL-10 in SLE is evidently higher than that of healthy individuals, which is positively correlated with disease activity. IL-10 also declines with the decreased disease activity in patients with SLE after treatment, which is consistent with this study. As mentioned above, cytokines produced by Tregs include IL-10 and TGF-β; however, Tregs are not the only immune cells that secrete IL-10, as activated Th2 cells, B cells and monocytes also promote IL-10 expression. In addition, the mTOR pathway is highly expressed in Th cells and correlates with susceptibility genes of SLE in a dose-dependent manner, while B cell hyperactivity may be associated with the autocrine and paracrine
effects of IL-10. Also, the secreted cytokines can react against multiple immune cells, forming intricate regulatory networks involved in different pathophysiological processes between immune cells and cytokines. IL-4 is mainly expressed by Th2 cells and can counteract Th2 cells to promote the proliferation of Th2 and inhibit the release of TNF-α and IL-8. Delgoffe et al confirmed that inhibiting mTORC1 activity increased the STAT6 phosphorylation and GATA-3 expression and promoted the differentiation of Th to Th2 cells, thus promoting the IL-4 secretion. In this study, the number of Th2 cells in lupus patients increased after treatment coupled with the increased IL-4, according to the above findings.

In our study, although there were no significant differences in plasma IL-17 and TNF-α levels in patients, they both showed a downward trend compared with pretreatment, followed by disease remission and the improvement of organ damage and immunological indicators, which might be related to the small sample size and short follow-up time. Cytokines produced by Th1 cells, including IFN-γ and IL-2, have a leading role in cellular immune responses. IFN-γ promotes the production of Igs by B cells, which further activates the production of proinflammatory factors, leading to or aggravating autoimmune diseases. Song et al showed that after 3 weeks of sirolimus treatment, the kidney injury and immunological indicators of SLE mice were significantly improved, Th1 cell numbers in the peripheral blood, spleen and kidney were significantly reduced, and the IFN-γ releasing was also significantly reduced. Although there was no significant difference in plasma IFN-γ levels of patients with SLE after sirolimus treatment, our data indicated that IFN-γ levels had a downward trend. IL-2 is a key factor involved in normal T cell activation, which can inhibit the occurrence and development of SLE by regulating Tregs to induce immune tolerance. A previous study has found that sirolimus promotes IL-2 secretion by inhibiting the differentiation of memory T cells into their corresponding T cells, further promoting the phosphorylation of STAT5 and the differentiation of Th cells to Treg. However, the plasma IL-2 levels in patients with SLE of the study showed a slightly increased trend, and there was also no significant difference in the number of Th1 cells as well as IFN-γ levels before and after treatment with sirolimus.

Based on the traditional therapy, the add-on treatment with low-dose sirolimus has a certain clinical effect on patients with SLE, mainly through renal and blood system involvement without obvious adverse effects. Meanwhile, this approach somewhat alleviates the patients’ economic burden. It was true that patients with SLE with low disease activity could have perceptions of fatigue and pain; however, we did not include those items into the parameters for evaluation because of the alikeness of chronic fatigue syndrome caused by the COVID-19 infection during the global epidemic period. But, of course, the long-term clinical efficacy of sirolimus in treating SLE and the effect of sirolimus on T cell subsets and cytokines need to be further evaluated and confirmed by prospective clinical studies with larger sample sizes and traditional therapy control group.

Author affiliations
Department of Rheumatology and Immunology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China
Department of Clinical Laboratory, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China
Hebei Key Laboratory of Laboratory Medicine, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China
Department of Anatomy, Hebei Medical University, Shijiazhuang, Hebei, China
Hebei Research Center for Stem Cell Medical Translational Engineering, Shijiazhuang, Hebei, China

Acknowledgements We thank Professor Jingyi Yang, Department of Hematology of The Second Hospital of Hebei Medical University, for the skill in flow cytometry.

Contributors AL and HJ designed the study protocol. MD, LJ, JZ, SC and LY collected and analysed clinical data of the patients. XW, JH, SS and FC conducted laboratory data analysis. MD, LJ and JZ drafted the manuscript and LY and SC contributed to the drafting of the review. AL, HJ, JM and MS revised the manuscript critically for important intellectual content. AL acted as the guarantor in the manuscript. All authors approved the final version of the article. All authors had access to all the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Funding This work was supported by S&T Program of Hebei (19277721D), National Science Foundation of Hebei Province (H2019206367, H2022206264,) and Government Foundation of Excellent Clinical Medicine Talent Program of Hebei (303-2021-58-18).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by 2019-R190. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Meng Ding http://orcid.org/0009-0009-8452-1279
Lu Jin http://orcid.org/0009-0004-3282-233X
Aijing Liu http://orcid.org/0000-0002-5762-5592

REFERENCES


