

Case report for recurrent and new-onset SLE patients treated by high-dose glucocorticoid therapy

Characteristics of peripheral TCR beta chain CDR3 repertoires

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Abstract

Rationale: High-dose glucocorticoid therapy has been widely applied in clinical practice in systemic lupus erythematosus (SLE) patients, but less is known about the changes of T cells, especially the T cell receptor (TCR) repertoires, during the treatment. The aim of this paper is to describe the changes of TCR that recurrent and new-onset SLE patients treated by high-dose glucocorticoid therapy.

Patient concerns: Drugs of clinical treatment of SLE mainly include glucocorticoid, immunosuppressive agents, nonsteroidal anti-inflammatory drugs and B cell targeted drugs, etc, but the clinical symptoms were in remission and recurrent of onset patients with SLE.

Diagnoses: Refer to the diagnostic criteria for SLE in 2011 by the American society of rheumatology.

Interventions: All patients were treated with High-dose glucocorticoid therapy and surveyed the TCR repertoires at 3 monitoring moments (before treatment, one month after treatment, and 3 months after treatment) to analyze the relationship between the characteristics of TCR repertoire and the highdose glucocorticoid therapy.

Outcomes: We found that high-dose glucocorticoid therapy resulted in clinical symptom remission, as well as change of diversity, highly expanded clones (HEC), usage of TCR beta chain variable gene (TRBV)/TCR beta chain joining gene (TRBJ), and overlapped sequences of TCR beta chain complementarity determining region 3 (CDR3) repertoires. This suggests that the effect of high-dose glucocorticoids on TCR repertoires is closely related to individual autoimmune T cells.

Lessons: In this study, we have shown that we could evaluate the effect of therapy, the pathogenesis, and the prognosis for the patients with SLE by monitoring the TCR CDR3 repertoires. It could afford a new method to find the therapeutic target of SLE.

Abbreviations: CDR3 = complementarity determining region 3, HEC = highly expanded clones, HTS = high-throughput sequencing, SLE = systemic lupus erythematosus, TCR = T cell receptor, *TRBJ* = TCR beta chain joining gene, *TRBV* = TCR beta chain variable gene.

Keywords: high-dose glucocorticoid therapy, high throughput sequencing, SLE, TCR beta chain CDR3 repertoire

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JY, BS, and LM contributed equally to this work and should be considered cofirst authors.

Authorship: XY designed the research and wrote the paper; JY, BS, and LM performed the research and analyzed data; CL, SS, RM, and YQ performed the high-dose glucocorticoid therapy and collected the peripheral blood samples of 2 SLE patients. All authors read and approved the final manuscript.

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1. Introduction

Systemic lupus erythematosus (SLE) is mainly manifested as chronic inflammation of connective tissue and multiple organ damage, which is the result of immune complex deposition caused by destruction of immune tolerance and the activation of B cells. The majority of B cells have T cell-dependence, and T cells play an important role in the initiation, maintenance, and organ damage of SLE.^[1] Currently, multiple T cell subsets (include Th2, Th1, Th17, Treg, and Tfh, etc.) were closely related to the occurrence and development of SLE.^[2–4] In the early days, Holbrook et al^[5] found that TCR beta chain CDR3 repertoire had phenomenon of TRBV family bias usage from peripheral T cells of SLE patients.^[6] Recently, Thapa et al^[7] used Illumina HTS technology to monitor the TCR beta chain CDR3 repertoire of peripheral blood, indicating that the diversity of SLE patient's TCR CDR3 repertoire was significantly less than that of healthy controls, while there was no significant difference between the majority of SLE patients with progression and remission compared to healthy controls. Sui et al^[8] analyzed TCR beta chain CDR3 repertoire of peripheral blood between SLE patients and healthy controls by Illumina HTS technology, indicating SLE patients had significantly higher degree of clonal expansion; Public CDR3 sequences were found among SLE patients. In a previous study, we found that certain TRBVs were preferentially used in SLE patients, and TCR beta CDR3 sequences had a common motif among SLE patients.^[9] Meanwhile, we sequenced TCR beta CDR3 repertoire of thymus and spleen from MRL/lpr mouse at different stages of disease, indicating that in the composition of CDR3, repertoires were significantly different between different stages.^[10] It is unclear what the inhibitory effects and therapeutic effects of drugs are on SLE patients T cell CDR3 repertoire. Dynamic characteristics of peripheral T cells CDR3 repertoire of SLE patients before and after the treatment in disease progression, therapeutic effect, and prognosis are still waiting to be investigated.

Currently, drugs of clinical treatment of SLE mainly include glucocorticoid, immunosuppressive agents, nonsteroidal anti-inflammatory drugs and B cell targeted drugs, etc. Of these, the shock therapy of glucocorticoid is especially applicable to SLE patients with acute stage; high-dose glucocorticoid treatment can universally provide quick control and remission for disease activity.^[11] However, no matter long-term oral use or short-term therapy with intravenous high-dose glucocorticoid would cause irreversible different degree damage of the body. At present, there is an unclear relationship between the diversity and clonal characteristic of peripheral T cell repertoire of SLE patients and count changes over time of white blood cell and lymphocyte after high-dose glucocorticoid therapy. Therefore, we used Illumina HTS technology to analyze the composition and characteristics of peripheral TCR beta CDR3 repertoire from SLE patients before and after short-term glucocorticoid treatment, and investigate possible relationship between TCR beta CDR3 repertoire and therapeutic effect, and pathology mechanisms induced by T cells in SLE and potential therapeutic targets.

2. Materials and methods

2.1. Subjects and preparation of sample

Under the principle of informed consent of the ethics committee of Zunyi hospital and patients, among patients with SLE from the department of nephropathy and rheumatology of the hospital affiliated to Zunyi Medical College, 1 recurrent SLE patient (Patient 1, P1) and 1 new-onset SLE patient (Patient 2, P2) with

high-dose glucocorticoid therapy (≥ 500 mg) were screened out as activity volunteers to participate in this study (without concurrent infection, tumors or other autoimmune diseases, etc.). Their peripheral blood was sampled for testing before treatment (P1–0 and P2–0), 1 month after treatment (P1–1 and P2–1), and 3 months after treatment (P1–3 and P2–3): First, the full automatic blood cell analyzer assembly line (XE-5000; Sysmex, Japan) was used to test leukocyte and lymphocyte counts of P1 and P2. Second, EURO Blot (according to standard operating process of the factory) was applied to test antinuclear antibody level change of P1 and P2. Third, the density gradient centrifugation (Ficoll lymphocyte separation medium; Beijing Solarbio Science & Technology Co., Ltd, China, Cat. No. P8610) was adopted to separate peripheral blood mononuclear cells (PBMCs) of each sample and the QIA amp DNA Mini-Kit (Cat.No.51304; QIAGEN, TELLGEN Life Science Co. Ltd., Germany) was employed to extract genomic DNA of each sample.

2.2. High-throughput technique sequencing human TCR beta chain CDR3 repertoire

Preliminary identification on genomic DNA of each sample was conducted with agarose gel electrophoresis (AGE). Six genomic DNA samples were stored in QIA safe DNA tube (QIAGEN) and sent to Adaptive Biotechnologies Corp (Seattle, WA) for sequencing. Before HTS, the concentration and purity of genomic DNA was confirmed for TCR beta chain CDR3 sequencing (<http://www.immunoseq.com>). Multiplex PCR system was designed to amplify rearranged human TCR beta chain CDR3 from genomic DNA (ImmunoSEQ assay). The TCR beta chain CDR3 sequences were performed using the Illumina Genome Analyzer.^[12,13] Amplification and sequencing were completed by Adaptive Biotechnologies ImmunoSEQ platform.

2.3. Screening and analysis of the TCR beta chain CDR3 repertoire

The raw sequences with FASTA format were analyzed using the analyzer tool set and IMGT/High V-QUEST (version 1.3.1) (<http://www.imgt.org>). IMGT summary file was used to filter sequence according to the following principle: No results; Unknown; Warnings; Unproductive; V gene was pseudogene; AA of 104 position was non-cysteine (Cysteine, C) or AA of 118 position was non-Phenylalanine (Phenylalanine, F). V-region identity < 85%. The contrastive analysis of main composition characteristics of the TCR beta chain CDR3 repertoire was carried out: Diversity and clonal expansion of the CDR3 repertoire; Composition and distribution of high frequency CDR3; Usage and pairing of *TRBV* and *TRBJ* gene families in the CDR3 repertoire; General length distribution and amino acid usage of the CDR3 repertoire; and Overlap ratios and characteristics of the CDR3 repertoire. The analysis and storage of sequences were completed in Excel (version 2010) (Microsoft Corp, Redmond, WA) and graph was completed by Prism 5 software (GraphPad, La Jolla). The formula of inverse Simpson index ($1/D_s$) was $1/D_s = 1/[1 - \sum (N_i(N_i - 1))/(N(N - 1))]$, where N_i was defined as the frequency of gene, and N was defined as the total number of genes.^[14–16]

3. Results

3.1. Leukocyte/lymphocyte counts and antinuclear antibody level

Leukocyte and lymphocyte counts of P1 and P2 at 3 monitoring moments before and after treatment: (Supplement Table 4, <http://>

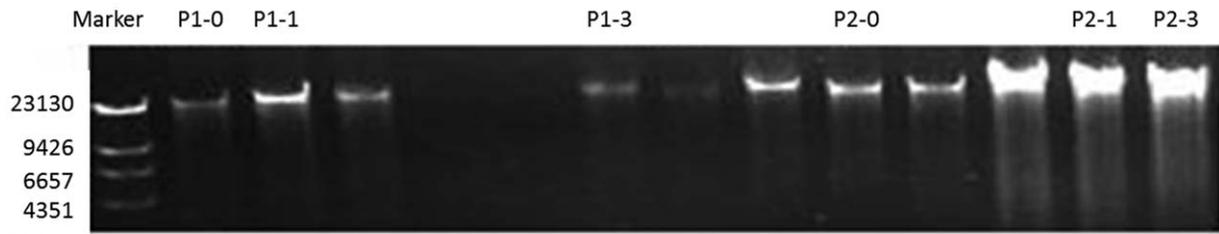


Figure 1. Analysis of the purity and concentration of genome DNA by 1% agarose gel and ultraviolet spectrophotometry at 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy.

links.lww.com/MD/B991). Changed antinuclear antibody levels of P1 and P2 at three monitoring moments before and after treatment: (Supplement Table 5, <http://links.lww.com/MD/B991>).

3.2. Concentrations and purity of genomic DNA and TCR beta CDR3 repertoire sequence of samples

In each sample of P1 and P2 at 3 monitoring moments, clear strips of genomic DNA in PBMCs appeared in the predicted positions on the agarose gel electrophoretogram (Fig. 1) and by Adaptive Biotechnologies ImmunoSEQ tests; each sample met requirements of CDR3 database foundation and the Illumina HTS

CDR3 repertoire. The total T cells TCR beta chain CDR3 Raw sequences and Analyzed sequences of P1 and P2 were basically the same (Table 1).

3.3. Diversity and clonal expansion characteristics of the TCR beta CDR3 repertoire

At 3 monitoring points, the unique and total proportions of P1 reduced gradually: P1-0=4.666%, P1-1=3.063%, P1-3=1.717%; the unique and total proportions of P2 raised gradually: P2-0=2.467%, P2-1=3.426%, P2-3=4.050% (Table 1). TCR beta chain CDR3 repertoires of P1 (P1-0: 1/DS=393.0113; P1-1: 1/DS=423.7155; P1-3: 1/DS=346.8453) before and after

Table 1

The total and unique TCR-β CDR3 AA sequences at 3 time-points of P1 and P2 with high-dose glucocorticoid treatment.

Patients no.	Raw sequences			Analyzed sequences	
	Total	Unique	Total	Unique (aa)	Unique/Total
P1-0	884,760	43,600	731,401	34,130	4.666%
P1-1	1,539,362	50,169	1,268,445	38,849	3.063%
P1-3	1,654,320	30,303	1,371,381	23,553	1.717%
P2-0	1,219,887	32,373	994,323	24,527	2.467%
P2-1	811,161	29,776	660,616	22,632	3.426%
P2-3	979,612	42,372	794,783	32,190	4.050%

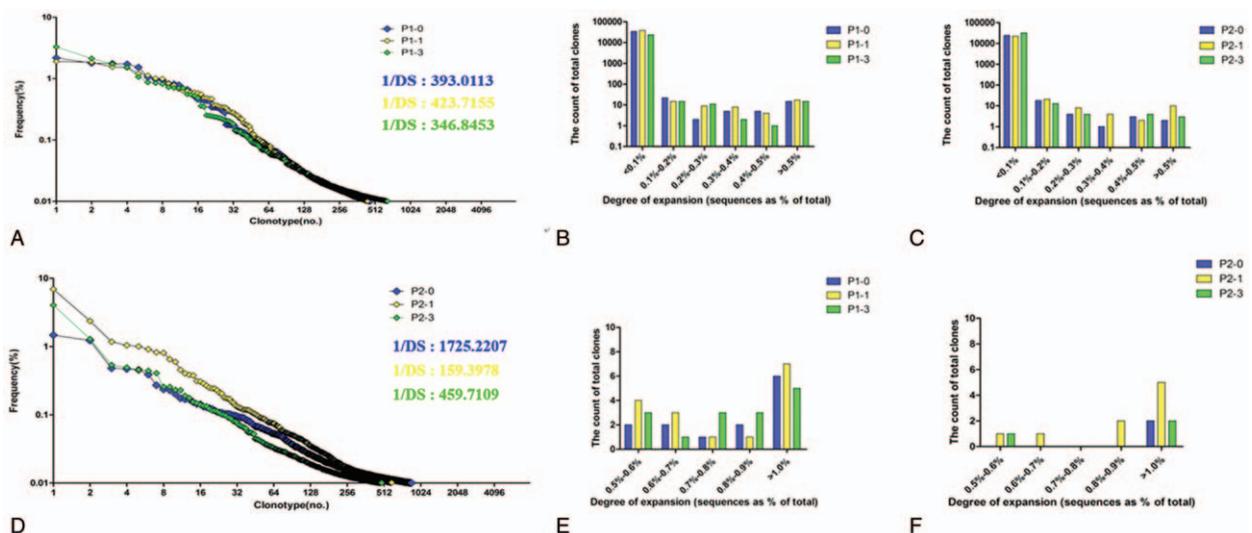


Figure 2. Clonotype distribution and expansion reads of TCR beta CDR3 sequences at 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy. (A and D) Clonotype distribution plots of TCR beta CDR3 sequences at 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy. (B and C) Degree of expansion of TCR beta CDR3 total clones and (E and F) TCR beta CDR3 highly expanded clones (frequency >0.5%) at 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy.

Table 2
The number and frequency of TCR-β CDR3 AA sequences at 3 time points of P1 and P2 with high-dose glucocorticoid treatment.

Frequency	P1-0	P1-1	P1-3	P2-0	P2-1	P2-3
<0.1%	34,081	38,796	23,509	24,499	22,587	32,166
0.1–0.2%	22	15	15	18	21	13
0.2–0.3%	2	9	11	4	8	4
0.3–0.4%	5	8	2	1	4	0
0.4–0.5%	5	4	1	3	2	4
>0.5	15	17	15	2	10	3

treatment did not change obviously (Fig. 2A). One month after treatment, TCR beta chain CDR3 repertoires 1/DS of P2 decreased significantly (before treatment: 1/DS=1725.2207), while 3 months later, they (1/DS=495.71086) began to recover (Fig. 2D).

Sequences <0.1% dominated the total clonal expansion frequencies of TCR beta chain CDR3 sequences, which were less than 0.1% dominated the total clonal expansion frequencies of TCR beta chain CDR3 repertoires of P1 and P2 and there was no obvious change at 3 monitoring moments (Fig. 2B, C). However, at 3 monitoring moments, the total HEC rates of TCR beta chain CDR3 repertoires of P1 were obviously higher than those of P2: P1-0=0.0440%, P1-1=0.0440%, P1-3=0.0637%; P2-0=0.0082%, P2-1=0.0442%, P2-3=0.0093% (Table 2). In P1 and P2, HECs whose clonal expansion frequencies were greater than 0.5% increased significantly in the first month after treatment (Fig. 2E, F).

3.4. Analysis of HECs whose clonal expansion frequencies were greater than 0.5% in TCR beta chain CDR3 repertoires of P1 and P2 at 3 monitoring moments

HEC CDR3 of P1 and P2 had different frequencies before treatment. At 3 monitoring moments, the total sequences of P1 that were greater than 0.5% were higher than those of P2. HECs CDR3 of both P1 and P2 might come from rearrangement of one or a number of different TRBV families. For TRBJ usage, HECs of P1 and P2, which was greater than 0.5%, were basically the same at 3 monitoring moments. However, at 3 monitoring moments, HECs of P1, which was greater than 0.5%, mainly used TRBD1, while that of P2 mainly used TRBD2. Multiple HECs CDR3 of P1 and P2 were expressed continuously, but

dynamic change of their frequencies appeared before and after treatment. New HEC CDR3, which was greater than 0.5%, appeared in both P1 and P2 after treatment (Table 2).

3.5. Usage and pairing of TRBV and TRBJ, entire CDR3 length distribution, and AA usage in TCR beta chain CDR3 repertoires of P1 and P2 at 3 monitoring moments

Usage and pairing of TRBV and TRBJ were dynamic changed of P1 and P2 in the first and third months after treatment compared with those before treatment (Fig. 3A, C). TRBJ family usage (Fig. 3B, D) and pairing with TRBV of P1 and P2 had no obvious change (Supplement Figure 1, <http://links.lww.com/MD/B991> and Supplement Figure 2, <http://links.lww.com/MD/B991>) at 3 monitoring moments. However, the top 5 TRBV of high-frequency usage of P1 at each monitoring moment were with common TRBV2 and TRBV19 families, while the top 5 TRBV of high-frequency usage of P2 at each monitoring moment were with common TRBV2, TRBV28, and TRBV20-1 families. The top 5 TRBJ of high-frequency usage of P1 and P2 at each monitoring moment were with common TRBV1-1, TRBV1-2, TRBV2-1, and TRBJ2-7 (Tables 3 and 4). There were no obvious changes of total CDR3 lengths (Fig. 4A, B) and aa usage (Fig. 4C, D) of P1 and P2 at 3 monitoring moments.

3.6. Overlap ratios and characteristics of TCR beta chain CDR3 repertoires of P1 and P2 at 3 monitoring moments

The average ratio of overlapping Unique CDR3 between P1 and P2 at any monitoring moments was about 1.8%, and the average ratio of overlapping Total CDR3 was about 6.2% (Table 5). The frequency was very lower of the top 15 overlap TCR beta CDR3

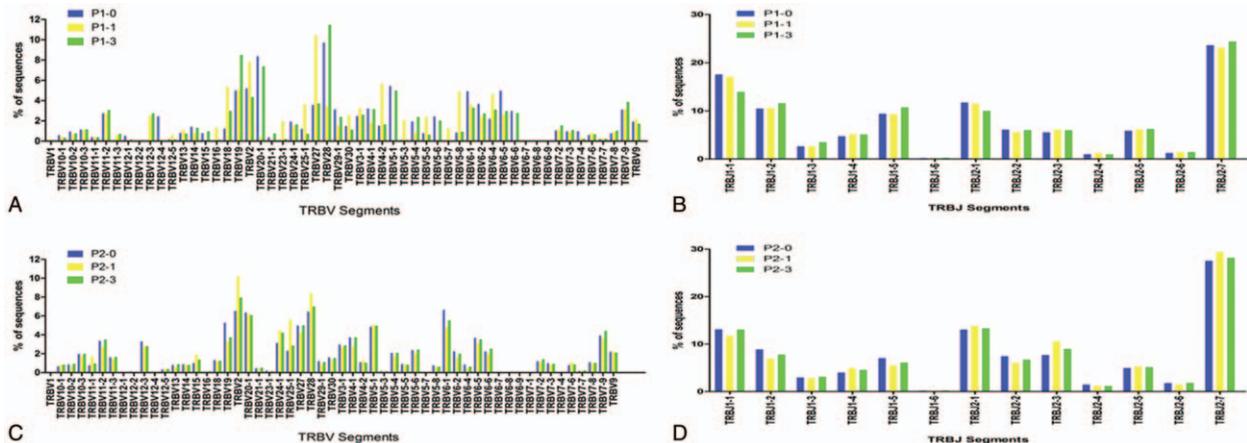


Figure 3. TRBV gene families (A and C) and TRBJ gene families (B and D) usage of TCR beta CDR3 repertoire at 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy.

Table 3

Composition characteristics of the top 5 TCR-β CDR3 AA sequences (frequency >0.5%) at 3 time-points of P1 and P2 with high-dose glucocorticoid treatment (Note. P2-0 only has 2 CDR3 sequences >0.5%; P2-3 only has 3 CDR3 sequences >0.5%).

Sample	TRBV genes	TRBJ genes	TRBD genes	CDR3 sequences (Amino acid)	Frequency (%)
P1-0	BV28	BJ1-5	BD1	CASRTVSNQPQHF	2.18%
	BV6-1/6-2/6-5/6-6	BJ2-7	BD1	CASSYNHEQYF	1.79%
	BV6-1/6-2/6-4/6-5/6-6/10-3	BJ2-7	BD1	CASSPRTSYEQYF	1.76%
	BV6-1/6-4	BJ1-1	BD1	CASSVVGNTAEFF	1.72%
P1-1	BV20-1	BJ2-1	BD2	CSALGLAGGQVYNEQFF	1.53%
	BV2/6-1/6-2/6-4/6-5/6-6/10-3	BJ1-1	BD1	CASSVVGNTAEFF	1.91%
	BV28	BJ1-5	BD1	CASRTVSNQPQHF	1.84%
	BV6-1/6-2/6-5/6-6	BJ2-7	BD1	CASSYNHEQYF	1.55%
	BV6-1/6-2/6-4/6-5/6-6/12-3	BJ1-1	BD1	CASNKQGSTEAFF	1.51%
P1-3	BV19	BJ2-5	BD1	CASSWDVQETQYF	1.21%
	BV6-1/6-5/27/28	BJ1-5	BD1	CASRTVSNQPQHF	3.29%
	BV6-1/6-2/6-4/6-5/6-6/9	BJ1-1	BD1	CASSVVGNTAEFF	2.13%
	BV19	BJ2-5	BD1	CASSWDVQETQYF	1.73%
	BV6-1/6-2/6-4/6-5/6-6	BJ2-7	BD1	CASSYNHEQYF	1.52%
P2-0	BV6-1/6-2/6-4/6-5/6-6	BJ1-1	BD1	CASNKQGSTEAFF	1.06%
	BV2/6-5/27	BJ2-7	BD2	CASSPRTSYEQYF	1.47%
P2-1	BV19	BJ1-2	BD2	CASSIGNYGYTF	1.23%
	BV2/5-4/7-9/12-3/27	BJ2-7	BD2	CASSPRTSYEQYF	6.95%
P2-3	BV24-1	BJ2-3	BD1/BD2	CATSELSTDTQYF	2.37%
	BV11-1	BJ2-7	BD2	CASSTEGGGFSLAGVYEYF	1.18%
	BV28	BJ1-4	BD1	CASRGDGTATNEKLF	1.04%
	BV5-1/11-3	BJ2-1	BD2	CASSSGTSGTYNEQFF	1.01%
	BV2/9/12-3/13/7	BJ2-7	BD2	CASSPRTSYEQYF	4.05%
P2-3	BV24-1	BJ2-3	BD1/BD2	CATSELSTDTQYF	1.29%
	BV28	BJ2-3	BD1	CASSLEQGAGNTDTQYF	0.54%

aa sequences between P1 and P2 at any monitoring moments (Supplement Table 1, 2, and 3, <http://links.lww.com/MD/B991>). The average ratio of overlapping Unique CDR3 of P1 between any 2 of 3 monitoring moments was about 10% (Table 6, Fig. 5A), but the average ratio of overlapping Total CDR3 was about 40% (Table 6, Fig. 5B). The average ratio of overlapping Unique CDR3 of P2 between any 2 of 3 monitoring moments was about 5% (Table 7, Fig. 5C), but the average ratio of overlapping Total CDR3 was about 23% (Table 7, Fig. 5D). Unique CDR3 (1476 pieces) of P1 overlapping at all 3 monitoring moments was considerably higher than Unique CDR3 (559 pieces) of P2. As for top 15 CDR3 of P1 and P2 whose frequencies were greater than 0.1%, most frequencies reduced constantly 1 and 3 months after treatment. However, after treatment, the rate of CDR3 of P1 whose frequencies were greater than 0.1% was obviously higher than that of P2 (Fig. 5E, F). The number of Overlapped Unique

CDR3 of P1 and P2 among 3 monitoring moments is summarized in Tables 5 and 6.

4. Discussion

T cells play an important role in the occurrence and development of SLE; high-dose glucocorticoid therapy has been widely applied in clinical practice in SLE patients, but there was no method or technology to evaluate the prognosis and the effect to T cells. This study constantly monitored the level of anti-nuclear antibodies and the SLEDAI score in a recurrent SLE patient (P1) and in a new-onset SLE patient (P2), collected the peripheral blood at 3 monitoring moments: before treatment, 1 month, and 3 months after the high-dose glucocorticoid therapy. In our prophase research, we have surveyed the B cell receptor (BCR) repertoires at these 3 monitoring moments^[17]; in this study, we surveyed the

Table 4

The top 5 TRBV and TRBJ usage in TCR-β CDR3 AA sequences at 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy.

Gene	P1-0		P1-1		P1-3		P2-0		P2-1		P2-3	
TRBV	BV28	9.70%	BV27	10.43%	BV28	11.43%	BV6-1	6.64%	BV2	10.17%	BV2	7.92%
	BV20-1	8.37%	BV2	7.79%	BV19	8.46%	BV2	6.52%	BV28	8.37%	BV28	8.37%
	BV5-1	5.42%	BV4-2	5.62%	BV20-1	7.34%	BV28	6.44%	BV20-1	6.20%	BV20-1	6.04%
	BV2	5.18%	BV18	5.32%	BV5-1	4.96%	BV20-1	6.34%	BV25-1	5.58%	BV6-1	5.50%
	BV19	4.98%	BV19	4.99%	BV2	4.31%	BV19	5.26%	BV5-1	5.03%	BV5-1	4.92%
TRBJ	BJ2-7	23.62%	BJ2-7	23.08%	BJ2-7	24.38%	BJ2-7	27.54%	BJ2-7	29.43%	BJ2-7	28.17%
	BJ1-1	17.54%	BJ1-1	17.08%	BJ1-1	13.93%	BJ1-1	13.12%	BJ2-1	13.82%	BJ2-1	13.31%
	BJ2-1	11.73%	BJ2-1	11.50%	BJ1-2	11.58%	BJ2-1	13.04%	BJ1-1	11.74%	BJ1-1	13.05%
	BJ1-2	10.47%	BJ1-2	10.58%	BJ1-5	10.71%	BJ1-2	8.86%	BJ2-3	10.56%	BJ2-3	8.99%
	BJ1-5	9.40%	BJ1-5	9.29%	BJ2-1	10.00%	BJ2-3	7.68%	BJ1-2	6.97%	BJ1-2	7.78%

T=total CDR3 AA sequences, U=unique CDR3 AA sequences.

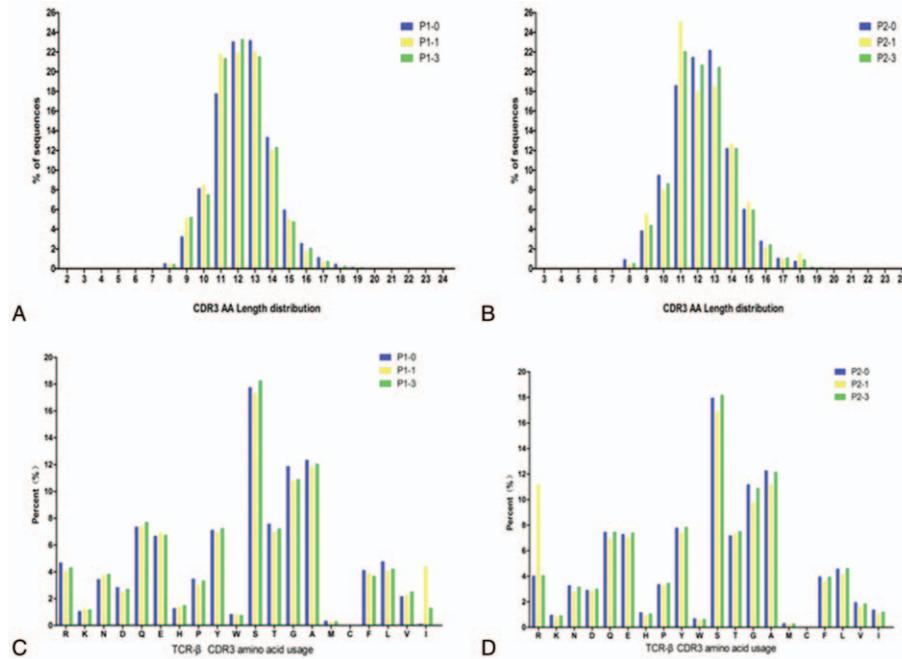


Figure 4. CDR3 region length distribution (A and B) and AA usage (C and D) of TCR beta CDR3 repertoire at 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy.

Table 5

The overlap TCR-β CDR3 AA sequences among the 3 time-points of P1 and P2.

P1&P2	P1-0 34130 ^U /731401 ^T	P1-1 38849 ^U /1268445 ^T	P1-3 23553 ^U /1371381 ^T
P2-0 24527 ^U /994323 ^T	493 ^U (1.44%) (2.01%) 58164 ^T (7.95%) (5.85%)	542 ^U (1.40%) (2.21%) 62834 (4.95%) (6.32%)	367 ^U (1.56%) (1.50%) 47713 ^T (3.48%) (4.80%)
P2-1 22632 ^U /660616 ^T	479 ^U (1.40%) (2.12%) 56283 ^T (7.70%) (8.52%)	518 ^U (1.33%) (2.29%) 60963 ^T (4.81%) (9.23%)	322 ^U (1.37%) (1.42%) 22310 ^T (1.63%) (3.38%)
P2-3 32190 ^U /794783 ^T	640 (1.88%) (1.99%) 65680 ^T (8.98%) (8.26%)	688 ^U (1.77%) (2.14%) 70351 ^T (5.55%) (8.85%)	467 ^U (1.98%) (1.45%) 55276 ^T (4.03%) (6.95%)

T=total CDR3 AA sequences, U=unique CDR3 AA sequences.

TCR repertoires at these 3 monitoring moments to analyze the relationship between the characteristics of TCR repertoire and the high-dose glucocorticoid therapy; then, we analyzed the potential application value of TCR repertoire monitoring to evaluate the treatment outcome and prognosis of patients with SLE; we also discussed the potential application value of individual autoimmune T cells in pathogenesis and therapeutic target of SLE. In the 2 patients, the total numbers and percentage of peripheral blood white cells and lymphocytes were within normal limits, the numbers of peripheral blood white cells and lymphocytes were reduced at the first month after therapy, and were within normal limits at the third month after therapy. After the high-dose glucocorticoid therapy, the levels of anti-nuclear

antibodies and the SLEDAI scores were reduced both in P1 and P2, and the clinical symptom was remission; these indicate that the patients' autoimmunity was inhibited after high-dose glucocorticoid therapy.

The peripheral blood TCR beta chain CDR3 repertoires of the 2 patients were surveyed by HTS at 3 monitoring moments; we got about 1,000,000 total productive sequences from each sample; the percentage of unique productive CDR3 sequences was about 5% for each sample. Similarly, Warren et al^[18] got about 10 million productive sequences from peripheral blood of health volunteers, and the percentage of unique productive CDR3 sequences was about 5% in total productive CDR3 sequences. The study about multiple sclerosis patients' TCR

Table 6

The overlap TCR-β CDR3 AA sequences among the 3 time-points of P1 treated with high-dose glucocorticoid therapy.

Patient 1	P1-0	P1-1	P1-3
P1-0	34130 ^U /731401 ^T	3851 (9.91%)/547947 (43.20%)	2392 (10.16%)/516223 (37.64%)
P1-1	3851 (11.28%)/310958 (42.52%)	38849 ^U /1268445 ^T	2798 (11.88%)/538879 (39.29%)
P1-3	2392 (7.01%)/278117 (38.03%)	2798 (7.20%)/516116 (40.69%)	23553 ^U /1371381 ^T

T=total CDR3 AA sequences, U=unique CDR3 AA sequences.

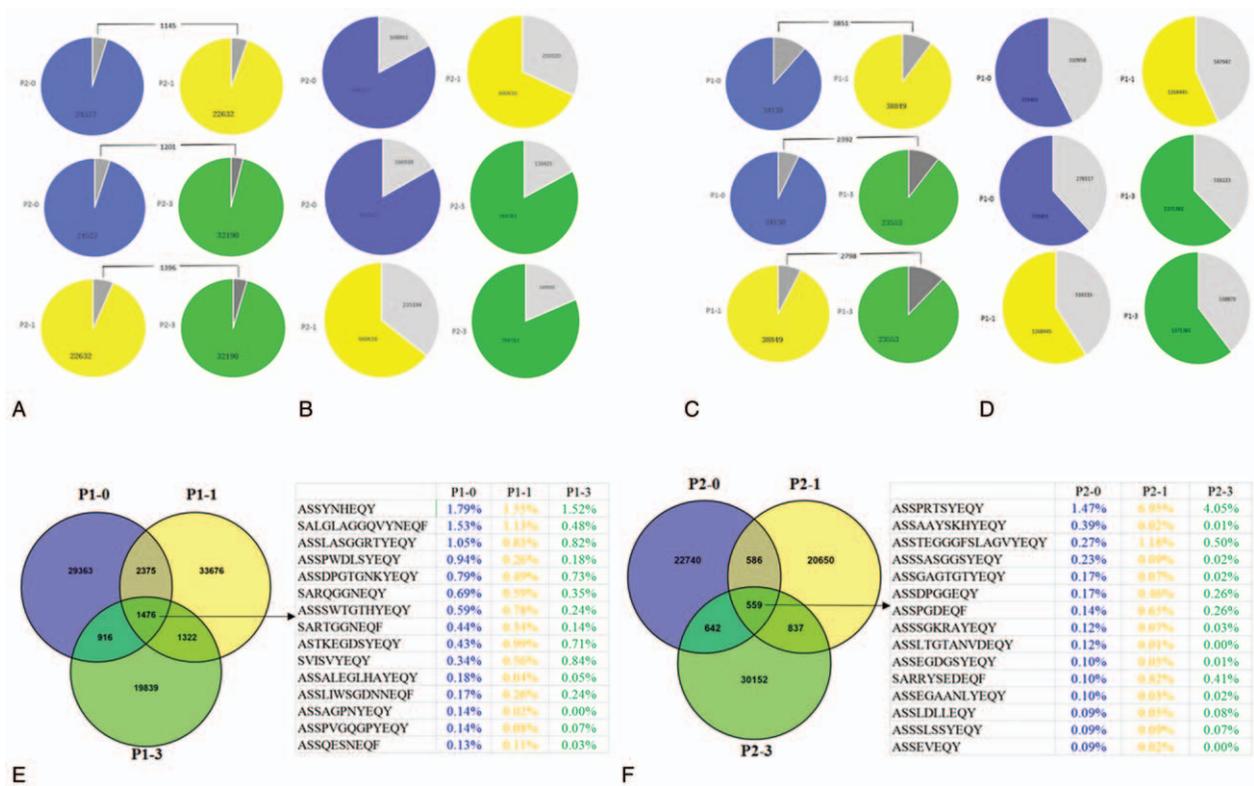


Figure 5. The unique (A and C) and total (B and D) TCR beta CDR3 AA overlap sequences between the 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy. (E and F) The distribution of top 15 common overlap TCR beta CDR3 AA sequences 3 time-points of P1 and P2 treated with high-dose glucocorticoid therapy.

repertoires reported by Andreas Lossius et al^[19] mentioned that the numbers of unique productive CDR3 sequences in peripheral blood were about 50,000. The numbers of total CDR3 sequences and of unique CDR3 sequences in our study are consistent with the data in the studies which were reported; we could analyze the diversity and clonality of the TCR repertoires and analyze the relationship between the repertoires and the stage of disease.

The diversity of T cells in peripheral blood of patients with SLE is closely related to the stage of SLE (active stage or remission stage); Luo et al^[20] found the diversity of TCR CDR3 were reduced in active stage of SLE by CDR3 spectratype. The percentage of unique sequences in total sequences and inverse simpson index (1/DS) are used to evaluate the diversity of TCR CDR3 repertoires, the higher percentage of unique sequences means the higher diversity, and the greater of inverse simpson index is, the higher diversity have. The diversity among samples could be quantitatively compared by inverse simpson index.^[21,22] In this study, we found that the variation tendency of diversity of TCR CDR3 repertoires was not the same in 3 monitoring moments. In P1, the percentage of unique sequences in total

sequences was deduced after therapy, but there were no changes of inverse simpson index. This indicated that P1 showed tolerance to high-dose glucocorticoid therapy; this method did not influence the T cells and had worse curative effect. In P2, the percentage of unique sequences in total sequences was increased after therapy, but the inverse simpson index was reduced at the first month after therapy; this may related with the low clone T cells were inhibited by glucocorticoid therapy. The numbers of peripheral blood white cells and lymphocytes were much less and the diversity of TCR repertoires were much less than those who within normal limits. The inverse simpson index of P2 at the third month after therapy was back to 495.71; this indicated that the low tolerance to glucocorticoid and showed good curative effect. The difference between the TCR/BCR repertoires before and after high-dose glucocorticoid therapy in P1 and P2 suggests that different patients with SLE have different t/b autoimmunity.^[17]

We surveyed the TCR CDR3 repertoires at 3 monitoring moments; most of them were low clone expanded (Frequency <0.1%). But we found that the HEC^[23] in P1 was much higher than the HEC in P2; we also found that the clone with expanded

Table 7
The overlap TCR-β CDR3 AA sequences among the 3 time-points of P2 treated with high-dose glucocorticoid therapy.

Patient2	P2-0	P2-1	P2-3
P2-0	24527 ^U /994323 ^T	1145 (5.06%)/210320 (31.84%)	1201 (3.73%)/138425 (17.42%)
P2-1	1145 (4.67%)/168892 (16.99%)	22632 ^U /660616 ^T	1396 (4.34%)/149930 (18.86%)
P2-3	1201 (4.90%)/166938 (16.79%)	1396 (6.17%)/235194 (35.60%)	32190 ^U /794783 ^T

NT = total CDR3 AA sequences, U = unique CDR3 AA sequences.

clones >0.5% in P1 were higher than those in P2 at 3 monitoring moments. This indicated that there were more autoreactive T cells in P1 than in P2, and there may be stronger autoimmunity in P1. The numbers of HEC (>0.5%) clones were increased at the first month after therapy in P1 and P2; these suggested that the clonality and distribution of T cells may be influenced by high-dose glucocorticoid therapy.

We also analyzed the characteristics of HECs (>0.5%) at 3 monitoring moments in P1 and P2. The HEC sequences in P1 used TRBD1, while most HEC sequences in P2 used TRBD2; this may be associated with the individual autoimmunity in patients with SLE. We also found that the HEC sequences in P1 and P2 have the same CDR3 sequences but with different TRBV families; the sequences with the same CDR3 recognize the same epitope, and there may be different t cells (the same CDR3 region with different TRBV) reactive to 1 epitope. Luo et al^[20] found high consist motif (GGX) in CDR3 region in the clone expanded sequences in the peripheral blood from patients with SLE by CDR3 spectratype. There were consistent motif SSG, GQG, and VRG in kidneys in patients with lupus nephritis,^[24] and the LXG motif in shin lesions from patients with SLE.^[25] These motifs may be public CDR3 sequences associated with self-antigen. Zang et al^[26] found that high-frequency TCR CDR3 motif was related in the pathogenesis of autoimmune disease with anti-RNP. In this study, there were consist sequences with similar motif (EQY and EQF etc.) in 3 monitoring moments in P1 and P2, and suggested that these HEC sequences may vary from autoimmunity memory t cells and closely related to the occurrence and development of SLE; these t cells may have potential application value in study of the individual auto-reactive t cell in pathogenesis and therapeutic target of SLE.

We analyzed the TRBV usage in repertoires of peripheral blood from P1 and P2. TRBV2 and TRBV19 were the top 5 TRBV families in all the 3 monitoring moments in P1; TRBV2, TRBV28, and TRBV20-1 were the top 5 TRBV families in all the 3 monitoring moments in P2. The advantage usage of TRBV2 in patients with SLE is consistent with Luo et al.^[20] There were several TRBV families usages that were inhibited by glucocorticoid therapy or caused by some new t cells occurrence. Then, we analyzed that the TRBV-TRBJ was similar in 3 monitoring moments, and TRBV1-1, TRBV1-2, TRBV2-1, and TRBJ2-7 were the top 5 TRBJ families in 3 monitoring moments in P1 and P2. Ndifon et al^[27] contribute the advantage usage of TRBJ families to gene sites in DNA, such as *TRBD* genes. The advantage usage of TRBJ families in P1 and P2 may be related to public T cells in patients with SLE. The length of CDR3 and usage of aa in 3 monitoring moments in P1 and P2 was similar, and suggested the total CDR3 length and aa were not significant influenced by high-dose glucocorticoid therapy in patients with SLE.

The percentage of overlapped unique CDR3 sequences was about 1.8% on average in 3 monitoring moments in P1 and P2; The percentage of overlapped total CDR3 sequences was about 6.2% on average. These were consistent with research by Harlan Robins.^[28] The percentage of overlapping sequences in patients with SLE was similar with healthy volunteers. Sui et al^[8] also detected the overlapping sequences among patients with SLE; the percentage was similar to the percent among healthy volunteers. Our prophase research found that the percentage of overlapping TCR sequences among MRL/lpr mice was about 2%.^[10] The percentage of overlapping TCR sequences among patients with SLE was not higher than among healthy volunteers, which suggested that the high diversity autoimmune t cells in patients with SLE.

The percentage of overlapped unique CDR3 sequences was about 10% on average in multiple comparison in 3 monitoring moments in P1, and the percentage of overlapped total CDR3 sequences was much higher, about 40% on average, in multiple comparison. The percentage of overlapped unique CDR3 sequences was about 5% on average in multiple comparison in 3 monitoring moments in P2, and the percentage of overlapped total CDR3 sequences was much higher, about 23% on average, in multiple comparison. The number of overlapped unique sequences in all 3 monitoring moments in P1 was 1476; this was much higher than that in P2 (559). Then, we analyzed the top 15 overlapped HECs (>0.1%), at the first month and the third month after therapy; the frequency of most of HECs was reduced by the stage of disease, but the frequency of HECs in P1 (recurrent patient) was higher than in P2 (new-onset patient), suggested that the clinical symptoms were in remission, but the overlapping sequences consisted of patients; this may be related to the individual autoimmune T cells. The high-dose glucocorticoid therapy may do not inhibit the auto-reactive t cells, and the prognosis may be worse. In P2, the overlapped sequences were lower after high-dose glucocorticoid therapy, and suggested that this therapy do inhibits the auto-reactive t cells; the prognosis may be good. Venturi et al^[29] reported that there were higher overlap rates in memory t cells than that in naive t cells. The high overlapped sequences in 3 monitoring moments in P1 and P2 may be from memory t cells. And the change of overlapped sequences showed different trend in P1 and in P2, and indicated that there were much more consist autoimmune memory t cells in P1 than that in P2. The frequency of HECs (>0.1%) at 3 monitoring moments may be used to evaluate the individual autoimmune t cells and to evaluate the prognosis of patients with SLE, and may have potential application value of individual autoimmune t cells in pathogenesis and therapeutic target of SLE.

In a word, the clinical symptoms were in remission in recurrent and onset patients with SLE, but the change of diversity, HEC, the usage of TRBV/TRBJ, and overlapped sequences of TCR beta chain CDR3 repertoires were different between these 2 patients. This suggests the effect of high-dose glucocorticoids on TCR repertoires is closely related to individual autoimmune T cells. To monitor the TCR CDR3 repertoires could evaluate the effect of therapy, the pathogenesis, and the prognosis for the patients with SLE. It could afford a new method to find the therapeutic target of SLE.

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