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*Original Article*

# Correlation of latent membrane protein (LMP)-1, LMP-2A and early antigen B-cell epitope peptides of Epstein-Barr virus with risk and disease conditions of systemic lupus erythematosus

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**Abstract:** This study aimed to predict B-cell epitopes of Epstein-Barr virus (EBV) latent membrane protein (LMP)-1, LMP-2A and early antigen (EA) related to systemic lupus erythematosus (SLE) self-antigens, and to investigate the association of according B-cell epitope peptides (EPs) indirect levels in serum with risk and clinical features of SLE. Fifty-nine SLE patients and 58 health controls (HCs) were enrolled in this study. EBV LMP-1, LMP-2A and EA B-cell epitopes were predicted by analysis of amino acid sequence, secondary structure, transmembrane domains, surface properties and comparison between the epitopes and SLE self-antigens. Selected EPs were combined and purified by a bio-tech company. Serum levels of EPs were detected in SLE patients and HCs using indirect enzyme-linked immuno sorbent assay (ELISA). Four EBV LMP-1, LMP-2A and EA B-cell epitopes peptides (EP1-4) were selected by prediction to be combined and purified. EP1-4 indirect levels were all increased in SLE patients compared with HCs (all $P<0.001$), and ROC curve showed a great diagnostic value of combined EP1-4 levels for SLE with AUC=0.976, 95% CI=0.950-1.000. EP1, EP2 and EP4 levels were found to be correlated with higher immune globulins and less renal dysfunction. In addition, EP1 level was positively associated with SLE disease activity index (SLEDAI) score ($P=0.039$). This study revealed that EBV LMP-1, LMP-2A and EA B-cell EPs were correlated with the risk and disease severity of SLE, which shed light on a novel mechanism of EBV in SLE etiology.

**Keywords:** Systemic lupus erythematosus (SLE), Epstein-Barr virus (EBV), latent membrane protein (LMP)-1, LMP-2A, early antigen (EA), B-cell epitope, epitope peptide (EP)

## Introduction

Systemic lupus erythematosus (SLE), with increasing incidence and prevalence worldwide due to more mild cases diagnosed and improved survival, affects approximately 2.8 to 5.2 per 10000 populations [1]. Comprehensive understanding in the pathogenesis of SLE etiology has been hampered resulting from the disease heterogeneity, which presents with a broad clinical phenotype of systemic autoimmunity such as arthritis, rash, nephropathy and so on [2]. Despite of the ambiguous etiology, SLE is widely considered to be hinged on loss of tolerance and sustained autoantibody production as well as apoptotic cell production and disposal of apoptotic material [3].

Epstein-Barr virus (EBV), one of the most important triggers as infectious factors for various immunological diseases including SLE, is found to infect more SLE patients compared with age-matched controls [4]. And the antibodies against EBV antigens are reported to elevated in SLE patients as well [5]. EBV latent membrane protein (LMP)-1, LMP-2A, and early antigen (EA) are observed to play important roles in SLE etiology through assisting infected naïve B-cells in the germinal center (GC) process and gaining access to the memory B-cell tool, which

**Table 1.** Characteristics of SLE patients and HCs

| Items | SLE patients (N=59) | HCs (N=58) | *P* value |
|---|---|---|---|
| Age (years) | 37.3±12.7 | 36.2±6.8 | 0.556 |
| Gender (Female n/%) | 50 (85%) | 47 (81%) | 0.594 |
| Disease duration (years) | 8.0 (4.0-10.8) | - | - |
| Neurological disorder (n/%) | 3 (5%) | - | - |
| Renal involvement (n/%) | 23 (39%) | - | - |
| Arthritis (n/%) | 13 (22%) | - | - |
| Myocarditis (n/%) | 3 (5%) | - | - |
| Alopecia (n/%) | 3 (5%) | - | - |
| Erythra (n/%) | 16 (27%) | - | - |
| Ulcer (n/%) | 4 (7%) | - | - |
| Pleurisy (n/%) | 1 (2%) | - | - |
| vasculitis (n/%) | 1 (2%) | - | - |
| Fever (n/%) | 17 (29%) | - | - |
| Thrombocytopenia (n/%) | 8 (14%) | - | - |
| Leukopenia (n/%) | 17 (29%) | - | - |
| Hematuria (n/%) | 31 (53%) | - | - |
| Proteinuria (n/%) | 28 (47%) | - | - |
| Cylindruria (n/%) | 26 (44%) | - | - |
| CRP (mg/L) | 4.01 (1.64-17.00) | - | - |
| ESR (mm/h) | 33.08 (8.45-46.21) | - | - |
| IgG (mg/mL) | 14.45 (10.50-20.48) | - | - |
| IgA (mg/mL) | 2.33 (1.50-3.89) | - | - |
| IgM (mg/mL) | 0.94 (0.55-1.51) | - | - |
| ANA Positive (n/%) | 55 (93%) | - | - |
| Anti dsDNA Positive (n/%) | 29 (49%) | - | - |
| Anti SSA Positive (n/%) | 31 (53%) | - | - |
| Anti SSB Positive (n/%) | 13 (22%) | - | - |
| Anti Sm Positive (n/%) | 17 (29%) | - | - |
| Anti u1RNP Positive (n/%) | 26 (44%) | - | - |
| Anti Ro52 Positive (n/%) | 34 (58%) | - | - |
| Anti SCL70 Positive (n/%) | 2 (3%) | - | - |
| Anti RibP Positive (n/%) | 31 (53%) | - | - |
| SLEDAI Score | 5.00 (1.25-8.00) | - | - |

Data was presented as mean ± SD, median (1/4-3/4 quarter) or count (%). Comparison was determined by Student test or Chi-square test. P<0.05 was considered significant.

are potential targets for EBV vaccine [6, 7]. Thus, identification of EBV LMP-1, LMP-2A and EA B-cell epitopes related to SLE self-antigens is of great need to further understand the mechanism of EBV in development and progression of SLE.

This study aimed to predict B-cell epitopes of EBV LMP-1, LMP-2A and EA related to SLE self-antigens, and to investigate the association of according B-cell epitope peptides (EPs) indirect levels in serum with risk and clinical features of SLE.

## Materials and methods

### *Participants*

A total of 59 SLE patients were consecutively recruited in this study from Feb. 2016 to Oct. 2016 at Department of Rheumatology in the First Affiliated Hospital of Wenzhou Medical University. All patients were diagnosed with SLE according to 1982 American College of Rheumatology (ACR) criteria for the classification of SLE. Fifty-eight age and gender matched health controls (HCs) were also enrolled in the same duration at Department of Physical Examination as well, while HCs with history of rheumatoid diseases, severe infection, malignant tumors and severe hepatic or renal dysfunction were excluded.

All participants (SLE patients and HCs) provided written informed consents, and the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University approved this study.

### *Assessments*

Data of demographic, clinical and biological features of SLE patients was collected at the time of enrollment as follows: (1) Demographic data including age, gender; (2) Clinical data including disease duration, neurological disorder, renal involvement, arthritis, myocarditis, alopecia, erythra, ulcer, pleurisy, vasculitis, fever, thrombocytopenia, leukopenia, hematuria, pyuria, proteinuria and cylindruria; (3) Biological features including CRP, ESR, IgG, IgA, IgM, ANA, anti dsDNA, anti SSA, anti SSB, anti Sm, anti u1RNP, anti Ro52, anti SCL70 and anti RibP. In the meanwhile, SLE disease activity index (SLEDAI) score was assessed

**Table 2.** B-cell epitopes prediction by various methods

| EBV proteins | Locations of predicted epitopes |
|---|---|
| LMP-1 | 9-18, 95-116, 189-226, 230-244, 253-278, 293-308, 321-331 |
| LMP-2A | 13-18, 20-33, 39-64, 67-82, 90-92, 95-109, 173-179, 201-205, 415-421, 483-494 |
| EA | 62-76, 113-125, 149-162, 178-196, 230-242, 307-357, 379-398 |

**Table 3.** 4 B-cell epitopes selected by comparison with SLE pathogenic antigens

| Name | Parameters | Amino acid sequence |
|---|---|---|
| EP1 | LMP-1 (229-238) | LVSGAGDGPP |
| | SmB | LVSMTVEGPP |
| EP2 | LMP-2A (156-163) | GL ALS LLL |
| | Ro | GMALALAV |
| EP3 | EA (114-121) | YKRPQGC S |
| | Ro | YKQRNGWS |
| EP4 | EA (315-323) | PRVQPLGTG |
| | SmB | PTQYPPGRG |

by two rheumatoid specialists to evaluate the disease severity of SLE.

*Prediction of B-cell epitopes*

Amino acid sequences of EBV LMP-1, LMP-2A and EA were retrieved from UniProKB Protein Database in Swiss Institute of Bioinformatics (http://www.uniprot.org/). The secondary structure prediction of the EBV LMP-1, LMP-2A and EA was analyzed by Protean module in DNAStar software. Transmembrane domains were identified using TMpred method on EXPASY Internet Server (http://www.expasy.ch/tools). And surface properties of EBV LMP-1, LMP-2A and EA proteins were analyzed as well. The accessibility, flexibility, hydrophilicity, antigenicity and polarity were assessed by Emini method, Karplus-Schulz method, Hoppy & Woods method, Jameson-Wolf method and Zimmerman method, respectively.

According to the results of above analysis, B-cell epitopes of EBV LMP-1, LMP-2A and EA with high accessibility, flexibility, strong antigenicity as well as good hydrophilicity were identified. And BLAST module in NTI8.0 Vector Software was used to compare the amino acid sequences of EBV LMP-1, LMP-2A and EA B-cell epitopes and SLE self-antigens (Sm B, Sm D, Sm E, rRNP and Ro) in order to subsequently determine the B-cell epitopes with high homology to SLE self-antigens.

*Combination and purification of epitope peptide*

Selected SLE related EBV LMP-1, LMP-2A and EA B-cell EPs were combined by a third party company (Zexiyuan Bio-Tech Company, Beijing, China), and then purified by Delta 600 HPLC. Mass spectrometer was used to measure molecular weight of peptides. EPs with purification above 95% were subsequently stored at -20°C for further experiment.

*Sample collection and Indirect ELISA analysis*

Serum samples were obtained from all SLE patients and HCs. 100 μL EPs (10 μg/mL) were coated in each microplate. 1:50 diluent serum samples were used as primary antibody, while 1:10000 diluent goat anti-human IgG-HRP were applied to carry out indirect ELISA. The absorbance (OD) was subsequently measured at 450 nm by using a Bio-Tek ELISA microplate reader. All samples were independently analyzed in triplicate, and the mean value of ODs was used for analysis in this study.

*Statistics*

SPSS 21.0 Software (IBM, USA) was used for statistics. Data was presented as mean ± standard deviation, median and 1/4-3/4 quarter, or count and percentage. Comparison between two groups was determined by t test, Wilcoxon rank sum test or Chi-square test. Receiver Operating Characteristic (ROC) curve was drawn for evaluating the predictive value of selected EPs for SLE risk. Spearman test was used to analyze the correlation of EPs with clinical and biochemical features. $P$ value <0.05 was considered significant.

## Results

*Patient characteristics*

59 SLE patients with age 37.3±12.7 years (85% females) were enrolled. The median disease duration was 8.0 (range 4.0-10.8) years and

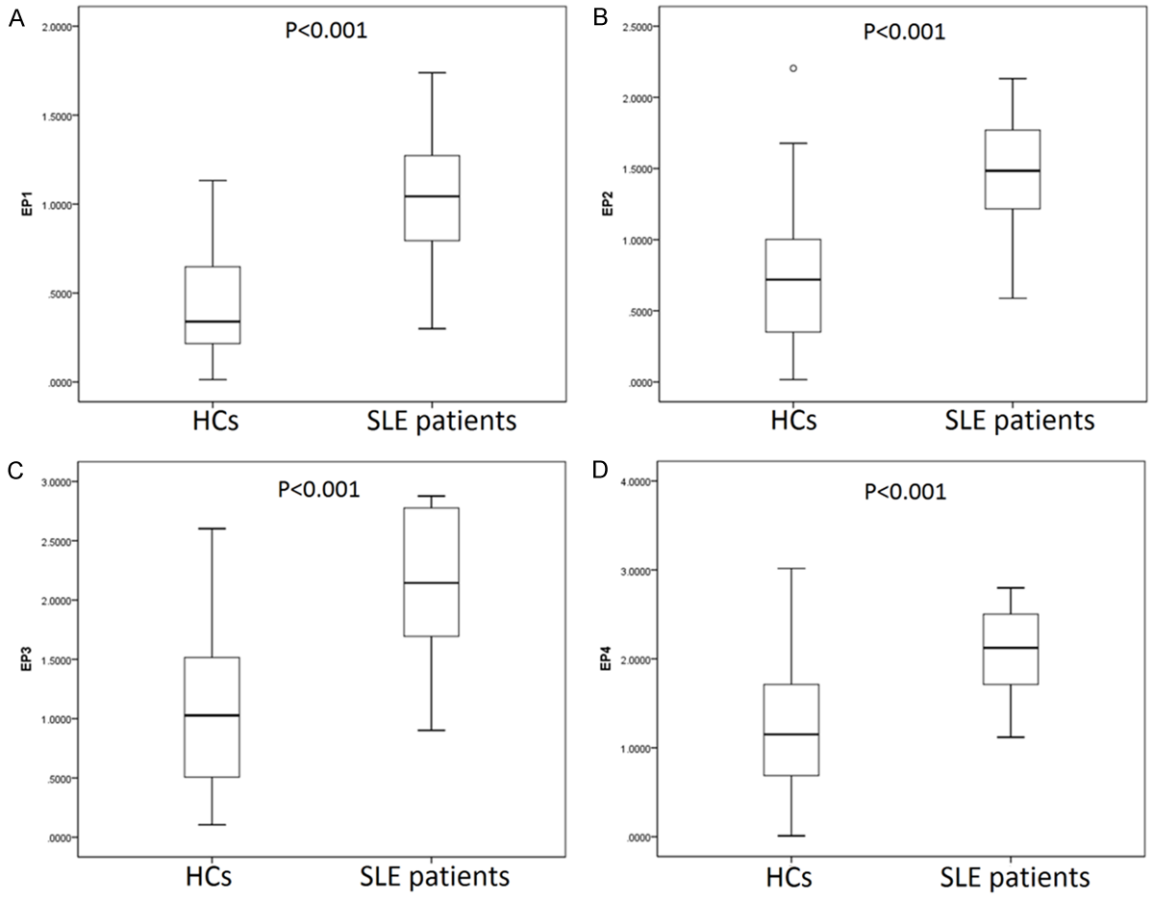


**Figure 1.** EP1-4 indirect levels in SLE patients and HCs. EP1-4 levels were all dramatically increased in SLE patients compared with HCs (A-D). Comparison was determined by Wilcoxon rank sum test, P<0.05 was considered significant.

the median SLEDAI score was 5.00 (range 1.25-8.00). The other clinical and biochemical features were presented in **Table 1**. 58 HCs with matched age (36.2±6.8 years, P=0.556) and gender (81% female, P=0.594) were also recruited.

*SLE related B-cell epitopes prediction*

Followed by analysis of secondary structure, transmembrane domains, surface properties of EBV LMP-1, LMP-2A and EA protein, 7 regions for LMP-1, 10 regions for LMP-2A and 7 regions for EA were predicted as candidate B-cell epitopes which were presented in **Table 2**. B-cell epitopes related to SLE were further analyzed by comparing the amino acid sequences of epitopes with SLE self-antigens (Sm B, Sm D, Sm E, rRNP and Ro), and four B-cell epitopes were selected. Subsequently, the corresponding EPs were combined and purified (named as EP1, EP2, EP3 and EP4) for further determination which were shown in **Table 3**.

*Indirect level of EP 1-4 in SLE patients and HCs*

Serum EP1 indirect level was dramatically increased in SLE patients (1.028 (range 0.790-1.279)) compared with HCs (0.340 (range 0.215-0.655)), P<0.001 (**Figure 1A**). The trends were the same in EP2, EP3 and EP4 levels between SLE patients and HCs as follows (**Figure 1B-D**): EP2, 1.484 (1.216-1.787) *vs.* 0.719 (0.350-1.005), P<0.001; EP3, 2.144 (1.681-2.780) *vs.* 1.027 (0.501-1.517), P<0.001; EP4, 2.124 (1.711-2.504) *vs.* 1.151 (0.675-1.729), P<0.001.

In order to further investigate the value of serum EP1-4 levels in predicting SLE risk, ROC curves were drawn (**Figure 2**) and we found that EP1-4 levels were all good predictors for SLE susceptibility with AUC as follows: EP1, AUC: 0.897, 95% CI 0.843-0.951; EP2, AUC: 0.904, 95% CI 0.848-0.960; EP3, AUC: 0.890, 95% CI 0.834-0.946; EP4, AUC: 0.848, 95% CI 0.777-

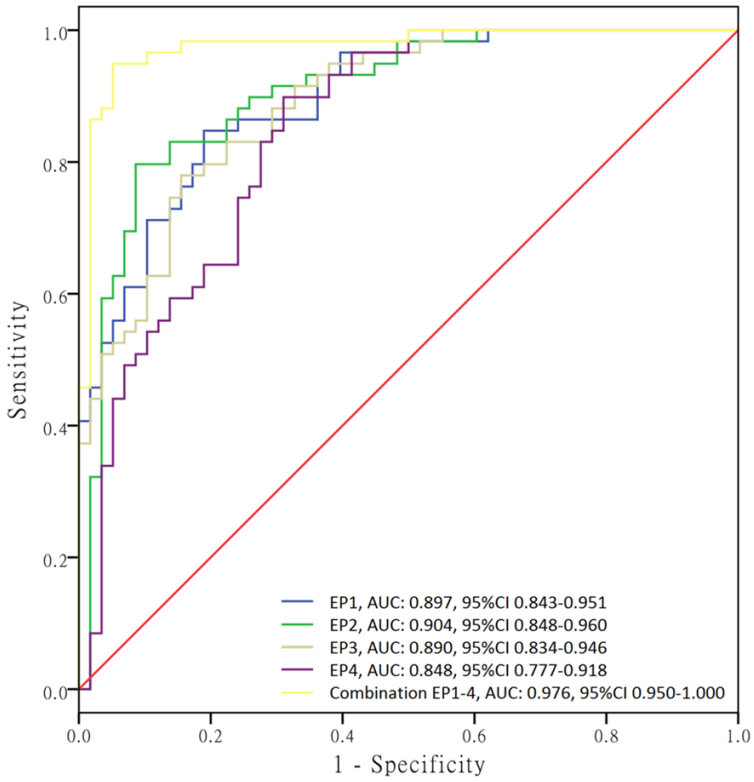


**Figure 2.** ROC curve analysis of EP1-4 levels for SLE diagnosis. All EP1-4 levels had a good predictive value for SLE risk with AUC range 0.848 to 0.904, while combined together, EP1-4 levels appeared a remarkably great value in diagnosis of SLE with AUC 0.976, 95% CI 0.950-1.000. Analysis was performed using ROC curve analysis.

0.918. And when combining EP1-4 levels together, the diagnostic value for SLE risk was even higher with AUC: 0.976, 95% CI 0.950-1.000.

*Correlation of EP1-4 levels with clinical and biochemical features in SLE patients*

As presented in **Table 4**, EP1 indirect level was observed to be positively correlated with ALT (P=0.013), IgG (P<0.001) and IgA (P<0.001), while negatively associated with renal involvement (P=0.004), proteinuria (P=0.002), hematuria (P=0.013), Scr (P=0.009) and 24 h proteinuria (P<0.001). EP2 indirect level was disclosed to be positively correlated IgG (P<0.001), LgA (P=0.001) and IgM (P=0.003), but negatively associated with Scr (P=0.048) and 24 h proteinuria (P=0.003). As to EP3, only lupus anticoagulant was found to be positively associated with EP3 indirect level (P=0.027). In addition, EP4 indirect level was associated with higher IgA (P=0.034), less fever (P=0.038), decreased proteinuria (P=0.022), less 24 h proteinuria (P=0.005) and lower Scr (P=0.028).

*Association of EP1-4 levels with disease severity according to SLEDAI score*

Furthermore, we explored the value of EP1-4 levels in disease severity management. As shown in **Figure 3**, EP1 level was negatively correlated with SLEDAI score (P=0.039), but no association of EP2-4 levels with SLEDAI score was observed (P=0.847, P=0.831 and P=0.129, respectively).

## Discussion

In this study, we predicted four EBV LMP-1, LMP-2A and EA B-cell epitope related to SLE by analyzing amino acid sequence, secondary structure, transmembrane domains, surface properties and homology between the epitopes and SLE self-antigens, and combined corresponding EPs (EP1-4). Indirect ELISA was performed to determine the serum level of EP1-4, and we found EP1-4 levels were all increased in SLE patients compared with HCs, and ROC curve showed a great diagnostic value of combining EP1-4 levels for predicting SLE risk. EP1 level was observed to be positively associated with ALT, LgG and LgA, while negatively correlated with renal dysfunctions. EP2 level was found to be associated with higher LgG, LgA and LgM and less renal dysfunctions. EP4 level was correlated with elevated LgA, less fever, and less renal dysfunctions. In addition, only EP1 level was negatively correlated with SLEDAI score, while no association of EP2-4 levels with SLEDAI score was found.

SLE, as a multifactorial disease resulting from genetic susceptibility, environmental effects, and disturbances in both innate and adaptive immunity, is characterized by disturbances in apoptotic cell clearance, cytokines, B-cell

**Table 4.** Correlation of EP 1-4 with clinical and biochemical features of SLE patients

| Parameters | Items | EP1 | EP2 | EP3 | EP4 |
|---|---|---|---|---|---|
| Age | r | 0.203 | -0.054 | 0.035 | 0.014 |
| | *P* value | 0.124 | 0.687 | 0.791 | 0.918 |
| Gender-Female | r | 0.111 | 0.000 | -0.021 | 0.105 |
| | *P* value | 0.404 | 1.000 | 0.876 | 0.428 |
| Disease duration | r | -0.094 | -0.217 | 0.064 | -0.096 |
| | *P* value | 0.477 | 0.099 | 0.630 | 0.469 |
| Neurological disorder | r | 0.059 | 0.063 | -0.014 | -0.136 |
| | *P* value | 0.658 | 0.633 | 0.919 | 0.305 |
| Renal involvement | r | -0.371** | -0.184 | -0.033 | -0.222 |
| | *P* value | 0.004 | 0.164 | 0.806 | 0.090 |
| Arthritis | r | 0.026 | 0.187 | 0.002 | 0.007 |
| | *P* value | 0.843 | 0.155 | 0.986 | 0.957 |
| Myocarditis | r | 0.109 | -0.050 | 0.091 | 0.063 |
| | *P* value | 0.412 | 0.708 | 0.495 | 0.633 |
| Alopecia | r | -0.181 | 0.045 | -0.150 | -0.009 |
| | *P* value | 0.170 | 0.733 | 0.258 | 0.946 |
| Erythra | r | -0.038 | 0.004 | 0.077 | -0.009 |
| | *P* value | 0.775 | 0.973 | 0.561 | 0.946 |
| Ulcer | r | 0.224 | 0.191 | 0.071 | 0.122 |
| | *P* value | 0.092 | 0.151 | 0.596 | 0.362 |
| Pleurisy | r | 0.062 | -0.031 | 0.177 | 0.093 |
| | *P* value | 0.643 | 0.817 | 0.179 | 0.486 |
| vasculitis | r | -0.210 | 0.051 | -0.115 | -0.036 |
| | *P* value | 0.114 | 0.701 | 0.391 | 0.791 |
| Fever | r | 0.059 | -0.084 | -0.048 | -0.270* |
| | *P* value | 0.655 | 0.529 | 0.716 | 0.038 |
| Thrombocytopenia | r | 0.154 | 0.180 | 0.055 | 0.134 |
| | *P* value | 0.244 | 0.172 | 0.678 | 0.313 |
| Leukopenia | r | -0.090 | -0.101 | -0.182 | -0.207 |
| | *P* value | 0.497 | 0.446 | 0.167 | 0.116 |
| Proteinuria | r | -0.404** | -0.226 | -0.159 | -0.300* |
| | *P* value | 0.002 | 0.088 | 0.232 | 0.022 |
| Hematuria | r | -0.323* | -0.113 | -0.186 | -0.158 |
| | *P* value | 0.013 | 0.400 | 0.163 | 0.236 |
| Cylindruria | r | -0.244 | -0.089 | 0.035 | -0.024 |
| | *P* value | 0.070 | 0.516 | 0.795 | 0.859 |
| ESR | r | 0.204 | 0.022 | -0.027 | 0.019 |
| | *P* value | 0.142 | 0.875 | 0.848 | 0.890 |
| ALT | r | 0.322* | 0.190 | 0.001 | 0.012 |
| | *P* value | 0.013 | 0.149 | 0.996 | 0.926 |
| AST | r | 0.235 | 0.048 | 0.067 | -0.041 |
| | *P* value | 0.073 | 0.719 | 0.613 | 0.759 |
| Scr | r | -0.336** | -0.259* | -0.048 | -0.286* |
| | *P* value | 0.009 | 0.048 | 0.720 | 0.028 |
| TC | r | -0.126 | 0.003 | 0.168 | 0.090 |
| | *P* value | 0.351 | 0.981 | 0.213 | 0.506 |

immunity and T-cell signaling [8]. Environment factors such as ultraviolet light, infection agents are common triggers of SLE, among which EBV is suggested to be one of the most important incentives of SLE [9]. And virtually all SLE patients, no matter adults or pediatrics, are EBV seropositive [10]. Although The mechanism of EBV infection in SLE is still obscure, it's widely considered that the following factors might contribute to the consequence: (1) infection and immortalization of autoreactive B-cells, T-cells and NK cells; (2) exacerbated inflammation by innate immune responses; (3) activation of human endogenous retroviruses (HERVs) related to autoimmunity; (4) cross-reactivity between microbial peptides and similar self-peptides caused by molecular mimicry; (5) augmenting autoimmunity through bystander activation such as promoting activation of autoreactive lymphocytes; (6) progressed autoimmunity through autoreactive T-cells escaping the negative selection allowed by dual T-cell receptor (TCRs) [11, 12].

Molecular mimicry, characterized by cross-reactivity of B-cells, T cells and antibodies resulting from sequential and/or structural similarities between virus and antigens which was firstly proposed at 1983, is one of the most critical causes of virus inducing autoimmunity thus leads to various autoimmune diseases including SLE, RA, MS and so on [13]. Autoantibodies against epitopes on SLE SmB and SmD are disclosed to illuminate cross-reactivity with various domains of EBV EBNA-1, and EBNA-1 motif PPPGRRP immunized mice and rabbits presented lupus-like autoimmune disease [14, 15]. These indicate that based on the mechanism of molecular mimicry, EBV infection acts as cru-

| | | | | | |
|---|---|---|---|---|---|
| TG | r | -0.029 | -0.111 | -0.121 | -0.050 |
| | *P* value | 0.830 | 0.412 | 0.372 | 0.714 |
| Lupus anticoagulant | r | -0.140 | 0.214 | 0.330* | -0.010 |
| | *P* value | 0.361 | 0.158 | 0.027 | 0.948 |
| IgG | r | 0.528** | 0.547** | -0.026 | 0.202 |
| | *P* value | <0.001 | <0.001 | 0.843 | 0.125 |
| IgA | r | 0.567** | 0.435** | -0.090 | 0.277* |
| | *P* value | <0.001 | 0.001 | 0.497 | 0.034 |
| IgM | r | 0.210 | 0.380** | 0.004 | 0.041 |
| | *P* value | 0.111 | 0.003 | 0.977 | 0.756 |
| CRP | r | -0.011 | 0.056 | -0.144 | -0.170 |
| | *P* value | 0.941 | 0.694 | 0.307 | 0.229 |
| 24 h proteinura | r | -0.598** | -0.468** | -0.107 | -0.437** |
| | *P* value | <0.001 | 0.003 | 0.517 | 0.005 |
| ANA | r | 0.146 | 0.028 | -0.111 | 0.012 |
| | *P* value | 0.268 | 0.835 | 0.403 | 0.929 |
| Anti-dsDNA | r | -0.145 | -0.032 | -0.163 | -0.153 |
| | *P* value | 0.272 | 0.811 | 0.217 | 0.246 |
| Anti-SSA | r | 0.092 | 0.090 | 0.038 | 0.042 |
| | *P* value | 0.493 | 0.503 | 0.776 | 0.752 |
| Anti-SSB | r | 0.127 | 0.137 | 0.074 | 0.164 |
| | *P* value | 0.341 | 0.305 | 0.580 | 0.218 |
| Anti-Sm | r | 0.029 | 0.042 | 0.033 | 0.033 |
| | *P* value | 0.830 | 0.754 | 0.804 | 0.804 |
| Antiu1RNP | r | 0.234 | 0.014 | 0.115 | 0.172 |
| | *P* value | 0.077 | 0.914 | 0.390 | 0.197 |
| Anti-Ro52 | r | 0.153 | -0.025 | -0.157 | -0.010 |
| | *P* value | 0.253 | 0.852 | 0.240 | 0.938 |
| Anti-SCL70 | r | -0.023 | -0.040 | 0.017 | -0.034 |
| | *P* value | 0.866 | 0.768 | 0.900 | 0.801 |
| Anti-RibP | r | 0.144 | 0.022 | -0.059 | 0.070 |
| | *P* value | 0.278 | 0.869 | 0.658 | 0.600 |

Data was presented as r and *P* value. Analysis was carried out by Spearman test. *P<0.05, **P<0.01.

cial role in SLE pathogenesis by cross-reactivity between EBV and SLE self-antigens [8]. However, few studies on cross-reactivity of EBV LMP-1, LMP-2A and EA with self-antigens of SLE are illuminated, which might explain a novel mechanism on how EBV infection inducing SLE.

EBV affects B-cells by binding viral envelop glycoprotein 350 to complement receptor 2, CD21 [16]. And in EBV latent state of infection of naïve B-cells, most viral promoters (approximately 80) are silenced, while 9 genes are possible to be expressed including: nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B and EBNA-3C), leader protein (LP) and LMPs (LMP-1, LMP-2A and LMP-2B) (latency III) [17]. While infected cells enter the GC, the viral transcription is changed to only secrete EBNA-1, LMP-1 and LMP-2 (latency II) [7, 18]. And in vitro studies, LMP-1 is disclosed to be a signal that normally results from CD40 signal transduction pathway activated by $CD4^+$ T-cells, and LMP-2A mimics a constitutively activated B-cell receptor. Subsequently, LMP-1 and LMP-2A induce infected B-cells into GC process and assist EBV to entry the memory B-cell pool (latency 0/I) [6, 7, 19]. EA, as an EBV lytic cycle antigen, is localized both in the cytoplasm and in the nucleus of infected cells. EBV EA binds dsDNA without sequence specificity and is essential for polymerase to replicate viral genome [20]. These indicate the critical role of LMP-1, LMP-2A and EA in etiology of EBV infection. Thus investigating EBV LMP-1, LMP-2A and EA novel B-cell epitopes is essential to further understanding the pathogenesis of EBV in SLE. In this study, we predicted four SLE related EBV LMP-1, LMP-2A and EA B-cell epitopes by various analyzing methods and the homological analysis between EBV epitopes with SLE self-antigens, and subsequently combined the corresponding EPs.

In order to explore the value of selected B-cell EPs in SLE susceptibility, we determined the serum levels of EP1-4 in both SLE patients and HCs by indirect ELISA, and we found EP1-4 levels were all elevated in SLE patients and combining the four EPs levels together was of great value in diagnosis of SLE, which suggested EBV might play an important role in SLE pathogenesis through cross-activity between these selected EBV epitopes with SLE self-antigens. What's more, EP1, EP2 and EP4 indirect levels were found to be correlated with immunoglobulins and renal functions, which indicated that these B-cell epitopes may be used as disease management biomarkers to some extents.

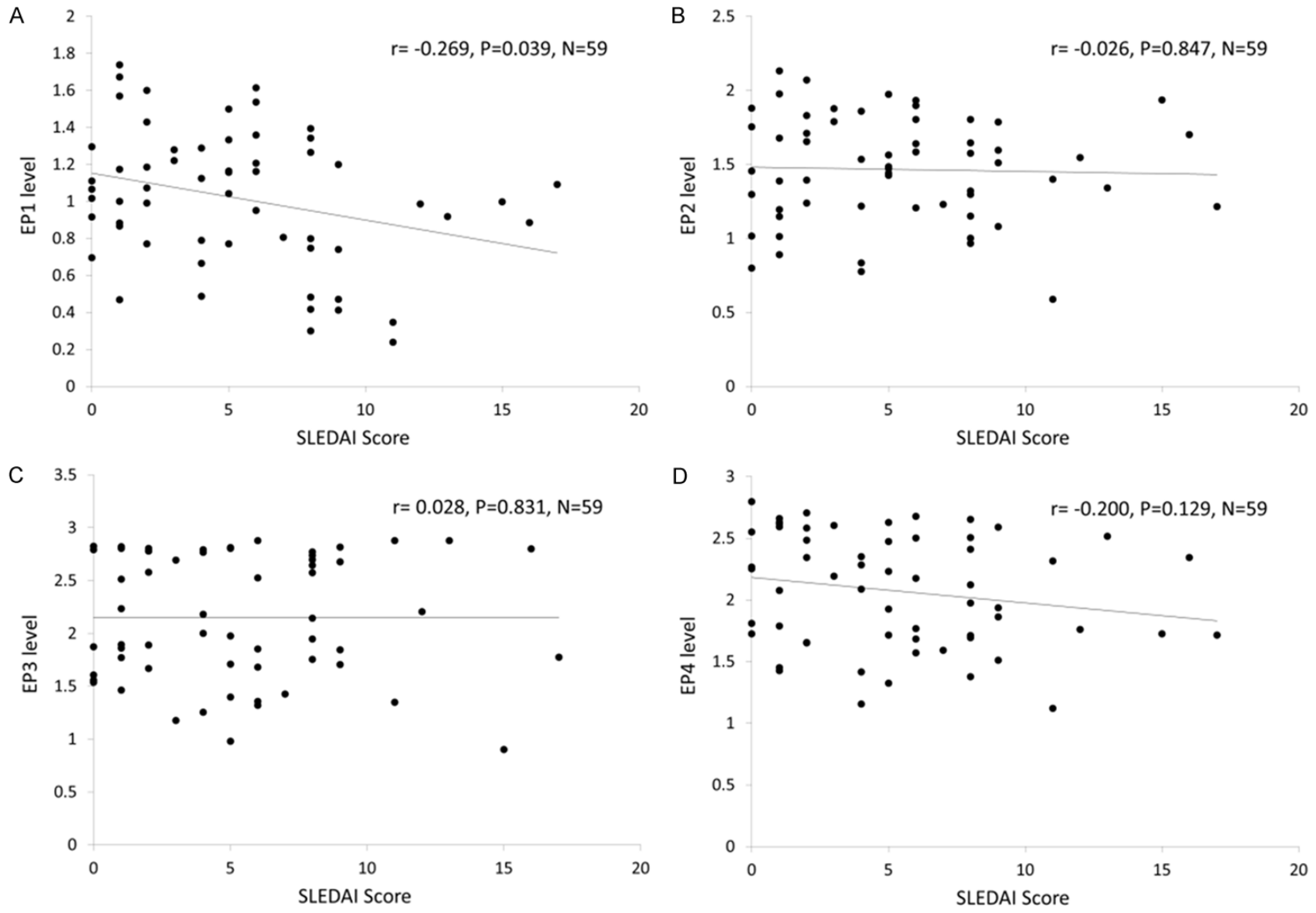


**Figure 3.** Correlation of EP1-4 levels with SLEDAI score. EP1 level was negatively correlated with SLEDAI score (A), while no association was disclosed in EP2-4 with SLEDAI score (B-D). Correlation was analyzed by Spearman test.

In conclusion, this study revealed that EBV LMP-1, LMP-2A and EA B-cell EPs were correlated with risk and disease severity of SLE, which shed light on a novel mechanism of EBV in SLE etiology.

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## Disclosure of conflict of interest

None.

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## References

[1] Lisnevskaia L, Murphy G and Isenberg D. Systemic lupus erythematosus. Lancet 2014; 384: 1878-1888.

[2] Tsokos GC, Lo MS, Reis PC and Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. Nat Rev Rheumatol 2016; 12: 716-730.

[3] Bengtsson AA and Ronnblom L. Systemic lupus erythematosus: still a challenge for physicians. J Intern Med 2017; 281: 52-64.

[4] Murata T, Sato Y and Kimura H. Modes of infection and oncogenesis by the Epstein-Barr virus. Rev Med Virol 2014; 24: 242-253.

[5] Draborg AH, Jorgensen JM, Muller H, Nielsen CT, Jacobsen S, Iversen LV, Theander E, Nielsen LP, Houen G and Duus K. Epstein-Barr virus early antigen diffuse (EBV-EA/D)-directed immunoglobulin A antibodies in systemic lupus erythematosus patients. Scand J Rheumatol 2012; 41: 280-289.

[6] Chen YP, Zhang WN, Chen L, Tang LL, Mao YP, Li WF, Liu X, Zhou GQ, Sun Y, Kang TB, Zeng MS, Liu N and Ma J. Effect of latent membrane protein 1 expression on overall survival in Epstein-Barr virus-associated cancers: a literature-based meta-analysis. Oncotarget 2015; 6: 29311-29323.

[7] Lossius A, Johansen JN, Torkildsen O, Vartdal F and Holmoy T. Epstein-Barr virus in systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis-association and causation. Viruses 2012; 4: 3701-3730.

[8] Wahren-Herlenius M and Dorner T. Immunopathogenic mechanisms of systemic autoimmune disease. Lancet 2013; 382: 819-831.

[9] Hanlon P, Avenell A, Aucott L and Vickers MA. Systematic review and meta-analysis of the sero-epidemiological association between Epstein-Barr virus and systemic lupus erythematosus. Arthritis Res Ther 2014; 16: R3.

[10] Taylor GS, Long HM, Brooks JM, Rickinson AB and Hislop AD. The immunology of Epstein-Barr virus-induced disease. Annu Rev Immunol 2015; 33: 787-821.

[11] Draborg AH, Duus K and Houen G. Epstein-Barr virus and systemic lupus erythematosus. Clin Dev Immunol 2012; 2012: 370516.

[12] Gross AJ, Hochberg D, Rand WM and Thorley-Lawson DA. EBV and systemic lupus erythematosus: a new perspective. J Immunol 2005; 174: 6599-6607.

[13] Munz C, Lunemann JD, Getts MT and Miller SD. Antiviral immune responses: triggers of or triggered by autoimmunity? Nat Rev Immunol 2009; 9: 246-258.

[14] Poole BD, Gross T, Maier S, Harley JB and James JA. Lupus-like autoantibody development in rabbits and mice after immunization with EBNA-1 fragments. J Autoimmun 2008; 31: 362-371.

[15] James JA and Harley JB. Linear epitope mapping of an Sm B/B' polypeptide. J Immunol 1992; 148: 2074-2079.

[16] Yang CF, Yang GD, Huang TJ, Li R, Chu QQ, Xu L, Wang MS, Cai MD, Zhong L, Wei HJ, Huang HB, Huang JL, Qian CN and Huang BJ. EB-virus latent membrane protein 1 potentiates the stemness of nasopharyngeal carcinoma via preferential activation of PI3K/AKT pathway by a positive feedback loop. Oncogene 2016; 35: 3419-3431.

[17] Thorley-Lawson DA. Epstein-Barr virus: exploiting the immune system. Nat Rev Immunol 2001; 1: 75-82.

[18] Graham JP, Arcipowski KM and Bishop GA. Differential B-lymphocyte regulation by CD40 and its viral mimic, latent membrane protein 1. Immunol Rev 2010; 237: 226-248.

[19] Rancan C, Schirrmann L, Huls C, Zeidler R and Moosmann A. Latent membrane protein LMP2A impairs recognition of EBV-infected cells by CD8+ T cells. PLoS Pathog 2015; 11: e1004906.

[20] Kayamba V, Monze M, Asombang AW, Zyambo K and Kelly P. Serological response to Epstein-Barr virus early antigen is associated with gastric cancer and human immunodeficiency virus infection in Zambian adults: a case-control study. Pan Afr Med J 2016; 23: 45.