Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Inflammatory mediators and immune function in different stages of systemic lupus erythematosus

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ARTICLE INFO

Original paper

Article history:
Received: November 17, 2022
Accepted: February 15, 2023
Published: February 28, 2023

Keywords:

SLE, inflammatory mediators, immune function, IL-36β, IL-36R

ABSTRACT

This study aimed to study the relationship between the expression levels of inflammatory mediators IL-36β and IL-36R and disease symptoms, laboratory indices and somatic immune function in Systemic Lupus Erythematosus (SLE) of different stages. In this research 70 patients with SLE who were treated in public hospitals from February 2020 to December 2021 were randomly divided into the stable group (n=35) and active group (n=35), and serum IL-36 was measured in the two groups β and IL-36R concentration with the standard curve of Enzyme-Linked Immunosorbent Assay (ELISA) to analyze IL-36β and IL-36R concentrations. 36β and IL-36R concentrations were analyzed in relation to the Disease activity score of systemic lupus erythematosus (SLEDAI), disease duration, typical symptoms of SLE and experimental characteristics. Results showed that the differences in IL-36β and IL-36R concentrations between the stable and active groups overall and for each disease duration group were tiny. There was no significant correlation between serum IL-36β and IL-36R concentrations and SLEDAI scores in stable and active patients, and a negative correlation between them and disease duration. Serum inflammatory mediator IL-36R concentrations were significantly higher in patients with mucosal ulcers and the difference was statistically significant. differences in IL-36β concentrations were statistically significant only for indicators of decreased erythrocyte count and IL-36R concentrations were statistically significant for indicators of decreased erythrocyte count, decreased haemoglobin and decreased lymphocytes The differences were huge and tiny in C4 decline, anti-dsDNA, and urinary routine protein. There was a significant positive correlation between IL-36β and IL-36R concentrations in patients with stable and active SLE, with correlation coefficients of 0.448 and 0.452 respectively. The differences in IL-36β and IL-36R concentrations between the stable and active groups were tiny for patients in the stable and active groups as a whole and for all disease groups. The differences in the number of each inflammatory mediator positive cells in the epidermal stratum corneum and superficial dermis between patients in the stable and active groups were tiny. In conclusion, IL-36β and IL-36R proteins in SLE patients are expressed in immune cells as well as epithelial cells of patients, indicating that these two inflammatory mediators may be one of the early signals that activate the immune system of SLE patients and trigger the immune response of patients; the onset of SLE may be associated with the inflammatory response induced by IL-36β and IL-36R.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.2.25

Introduction

The etiology of SLE is complex, and studies have shown that SLE disease is associated with immune dysfunction, as evidenced by abnormalities in T, B and macrophages and cytokine imbalance. The former has similar signaling pathways and amino acid sequence homology to the latter (1). IL-36 protein is mainly expressed in human dendritic cells, epithelial cells, etc. Its immune activation and immunomodulatory effects are stronger than those of other IL-1 proteins. IL-36α, IL-36β and IL-36γ mRNA have now been shown to be expressed at higher levels in psoriatic lesions. This suggests that two inflammatory mediator proteins, IL-36β and IL-36R, can be linked to the development of automotive immune diseases. However, the literature examining the association of two inflammatory mediator proteins, IL-36β and IL-36R, with the pathogenesis and experimental features, clinical symptoms and immune function of SLE is still quite scarce, but such an exploration would clearly inform the search for precise treatments for SLE disease (2). Therefore, this study prospectively selected SLE patients to analyse the expression of two inflammatory mediator proteins, IL-36β and IL-36R, in their serum and whole blood and their association with the disease course, immune response and clinical symptoms of SLE.

The management of autoinjury disorders like SLE is often characterised by long recovery periods and complex treatment processes, and numerous studies have been carried out by medical experts and clinicians to find better clinical approaches. (3-4). Lu X et al. analyzed the mechanism of trimethoprim polysaccharide for the therapy of SLE using network pharmacology. The results showed that the treatment mechanism of trimethoprim polysaccharide for SLE was linked to heat shock proteins Family A Member 5, heat shock proteins Family A Member 8, and

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eukaryotic translation elongation factor 1α1. While analysis of molecular dating suggests that hydronium bonding is the primary mode of interaction, this study provides a protein target for the impact of rituximab in the treatment of SLE (5). Wu S's team conducted a therapeutic trial to analyze the effect of rituximab in refractory SLE patients and the test results revealed that the severely or refractory SLE patients treated with rituximab in an observational study Additional data obtained to support this conclusion were the British Isles Lupus Evaluation Group index score, SLE disease activity index score, complement C3/C4, anti-dsDNA antibodies, and peripheral CD19+ B cells (6). A therapeutic trial was conducted by Huang X et al. to validate the effectiveness of mesenchymal stem cell transplantation for the treatment of SLE to improve the therapeutic efficiency of SLE. The trial results revealed a 26.7% reduction in treatment time and a 15.4% increase in cure rate compared to patients receiving conventional treatment (7). Shi H et al. studied a large body of literature on the high resistance of SLE to conventional therapies and found that the combination of rituximab and belimumab was more effective in patients with SLE and severely active SLE (8). Devaux M designed and conducted a treatment trial to validate the efficacy of long-term hydroxychloroquine in the treatment of SLE. The results showed that long-term treatment with hydroxychloroquine significantly improved the immune system of patients with SLE who had contracted or were contracting neo-coronary pneumonia, thus reducing the time to self-healing (9). Mai Y et al. concluded that hydroxychloroquine is active in the treatment of SLE, but it remains unclear whether an overdose of hydroxychloroquine is beneficial in improving the efficacy of SLE. The authors therefore designed and conducted a clinical trial that showed that the time to self-healing was not significantly shorter in SLE patients using hydroxychloroquine overdose compared to those using normal hydroxychloroquine treatment, indicating hydroxychloroquine overdose does not improve the therapeutic efficacy in SLE patients (10). NDL Visitación conducted an animal study in mice to determine whether *Lactobacillus fermentum* A study was carried out in mice to determine whether *L. fermentum* could protect the kidneys of female lupus mice with high blood pressure. The results showed that intake of *L. fermentum* prevented impaired renal function, in part because of its ability to decrease the production of anti-dsDNA and circulating layers of lipopolysaccharides, thereby reducing immune complex deposits, and inflammation and oxidative strain. These findings open up new possibilities for the long-term use of probiotics to prevent renal complications related to hypertensive SLE(11). Chandra T studied a 40-year-old male with SLE and found that SLE disease can induce cerebral venous sinus thrombosis, but no targeted specific therapy has been found for the time being(12). Zhang J et al. found that existing therapies for SLE suffer from inefficient treatment The team found that existing therapies for SLE have the disadvantage of being inefficient or having severe side effects and that overexpression of miR-125a may have the potential for the treatment of SLE. The maintenance team constructed a nano-delivery system based on polyethylene glycol, propylene glycol and polylysine to deliver miR-125a to splenic T cells. The results showed that miR-125a showed good therapeutic efficacy and Safety profile, which may offer a more potent therapy for SLE patients(13).

Materials and Methods

Common messages

Subjects were selected from SLE patients treated between February 2020 and December 2021 in a public hospital in China. The inclusion criteria were as follows. (i) meeting the diagnostic criteria for systemic lupus erythematosus as revised by the American Society for Rheumatology in 1997; (ii) not having any other systemic diseases; (iii) not yet using immunosuppressive drugs or glucocorticoids for SLE; and (iv) being between the ages of 18 and 60. Exclusion criteria were as follows: (i) unable to determine if they had SLE; (ii) major psychiatric illness; (iii) not accepting the study team intervention; and (iv) not having basic communication skills. Patients signed an informed consent form voluntarily and on the basis of equality, and the study was approved by the hospital ethics committee. Seventy patients were selected for the study, 25 males and 45 females, with a median age of (29.1±5.0) years. The patients were classified into a stable group (n=35) and an active group (n=35) according to the American Society of Rheumatology's 1997 modified diagnostic criteria for SLE.

Clinical data collection

General demographic information on the study population needs to be investigated and a survey conducted based on the SLE Activity Index Scale designed to find out information about the patient's disease so that they can be scored on the SLEDAI.

Specimen collection and handling methods

In the early morning 5 ml of anticoagulated blood was collected from the patient with 3 ml of non-anticoagulated peripheral blood and the serum was isolated from the non-anticoagulated blood within 30 minutes, while the anticoagulated blood was used to extract RNA, the extracted RNA needed to be stored in -80°C environment(14). After surgical extraction of the patient's skin lesion (0.5cm x 1cm, upper trunk), the sample was placed in 10% neutral formalin solution and embedded in conventional paraffin wax, followed by immunohistochemical and pathological examination.

Reagent acquisition methods

The reagents used in the study are routine and are available from most biological companies and do not require special descriptions. The auxiliary reagents used in the experiments and their preparation are listed below. Phosphate Buffered Saline (PBS) buffer: take 1 packet of PBS powder and dissolve it in 2000 ml of distilled water with a Potential of hydrogen (pH) of 7.4 and store it in a refrigerator at 4°C.(15). 10% neutral formalin solution: take 100 ml of 40% formaldehyde solution and add it to 900 ml of PBS solution with a pH of 7.4. Add to 900ml of PBS buffer at pH 7.4, mix uniformly and keep at room temp. Antigen Repair Solution: Dissolve 1 sachet of sodium citrate powder into 2000ml of distilled water to maintain pH in the range of 6.0±0.1 and keep at 4°C in a refrigerator. Primary antibody mixture: add 100 μ l of 20% sheep anti-human IL-36β and IL-36R reagents to 400 μ l of sterile PBS solution and stir well, do not prepare the solution in

advance, prepare it when used. 0.1% Diethyl pyrocarbonate (DEPC) solution: take 0.5 ml of DEPC, dissolve it in 500 ml of Stir well and cool by autoclaving, then store in a refrigerator at 4°C.

Research Methodology

Detection of IL-36β and IL-36R concentrations in serum

Remove the kit 30 minutes before the experiment, then configure the standard curve as follows. Place the standard in the environment of 6000~10000rpm and centrifuge for the 30s, use 1ml of sample dilution solution to obtain standard T8. Take another 7 centrifuge tubes numbered (T7~T1), add sample dilution 250 μ l respectively, remove T8 into T7 and mix well, then aspirate all and put into T6, and so on until diluted to T1, and set T0 as blank control. After the configuration of the standard curve, the next step is to add samples, set up the sample wells to be tested, standards, add 100 μ l of sample per well, mix them well and cover the plate membrane, with 37°C for 2 hours. The liquid is then removed, shaken dry and the first antibody working liquid, 100 μ l, is added to each well and cultured at 37°C for 1 hour. The liquid was again removed, shaken dry, and the plate was washed three times and soaked after 2 min each time. Add 100 μ l of horseradish peroxidase-labelled affinity working fluid to each well, cover the plate membrane and incubate at 37°C. The discard-wash-dry-wash-plate immersion-wash-dry operation is performed again. Add 90 μ l of substrate working solution to each well and place at 37°C for 20 min. Add 50 μ l of termination solvent was added to each well and absorbance was measured at 450 nm within 5 min.

Histopathological examination

Specimens were first dehydrated in 75%, 95%, 95%, 100%, and 100% ethanol, xylene transparent, paraffin-embedded, sectioned at a thickness of 3 μm and baked at 67°C for 2 hours. They were then subjected to conventional dewaxing to water treatment. The next step was sequential hematoxylin staining for 3 min, alcohol fractionation with 1% hydrochloric acid for 20 s, and anti-blue treatment with 1% dilute ammonia for 30 s. After each treatment, wash with steam distilled water for 1 minute, followed by eosin staining for 5 min and distilled water wash for 30 s. Dehydration was carried out using 75%, 85%, 95% and 100% ethanol concentrations for 2 min, followed by xylene transparent treatment for 2 min. Finally, the film was sealed with neutral gum.

IL-36β and IL-36R positive cells were identified by the appearance of yellow or tan-colored granules in the nucleus. Under a 400x microscope, five randomly selected fields of view for each section were selected and the amount of a positive cell in each field was computed and the mean value was used as the desired indicator.

Statistical methods

Numerical statistics were performed using SPSS 20.0 software and data of the measurement type were presented using the mean ± standard deviation form and were considered to conform to a normal distribution, using a t-test, in the experiment, all the counting features were tested by chi-square test, and displayed in the form of the number of cases or proportion of cases. The relationship between the variables was determined using Pearson correlation analysis, set at a significant level of 0.05.

Results

Comparison of general patient demographics

A total of 68 patients completed the complete experiment, including 34 in the stable group and 34 in the active group, and subsequent statistics were carried out on this group, and the general demographic statistics of this group are shown in Table 1. Comparable.

Comparison of serum levels of inflammatory mediators in patients at different stages

The statistical results of serum inflammatory mediator IL-36β and IL-36R concentration levels in the stable and active groups are shown in Table 2. The differences between the overall and each disease course group serum inflammatory mediator IL-36β and IL-36R concentration data in the stable and active groups were tiny.

The ELISA standard curves of patients' serum inflam-

Table 1. General metropolitan demographic data comparison of patients with SLE at different stages.

No.	Information items		Stable group (n=34)	Activity group (n=34)	T/ χ^2	P
#01	Age (years)	/	29.4±5.2	28.9±4.8	1.450	1.138
#02	Gender	Male	13	11	0.852	0.743
		Female	21	23		
#03	Smoking or not	Yes	15	16	0.761	0.627
		No	19	18		
#04	Drinking alcohol or not	Yes	22	20	1.038	0.851
		No	12	14		
#05	Economic level	A good living	12	10	0.886	0.709
		Middle class	17	18		
		Affluent	5	6		
#06	Body Mass Index (BMI)	/	23.5±1.7	23.6±1.8	1.859	1.483
#07	Exercise hours/ min per week	/	87.5±15.6	90.3±16.4	1.577	1.290

Table 2. Comparative serum inflammatory mediator levels between the stable and active groups.

Inflammatory mediators	Course of disease	Stable group (n=34)	Activity group (n=34)	*T*	*P*
IL-36β (pg/ml)	Less than 100d	342.1±124.0	385.7±147.2	1.304	0.197
	100d~300d	217.5±87.8	228.3±92.5	0.503	0.617
	Greater than 300d	83.2±46.1	97.6±52.0	1.187	0.239
	All	182.8±107.4	237.4±128.0	1.905	0.06
IL-36R (pg/ml)	Less than 100d	746.8±428.1	914.0±573.3	1.363	0.178
	100d~300d	573.9±342.8	637.1±356.5	0.744	0.459
	Greater than 300d	329.0±172.5	402.8±186.2	1.680	0.098
	All	513.8±267.2	648.5±379.1	1.688	0.096

matory mediators IL-36β and IL-36R are shown in Fig. 1. Subplots (a) and (b) in Fig. 1 show the ELISA standard curves of IL-36R and IL-36β respectively, the transverse axis is the OD of inflammatory mediators and the vertical axis is the concentration of inflammatory mediators (pg/ml).

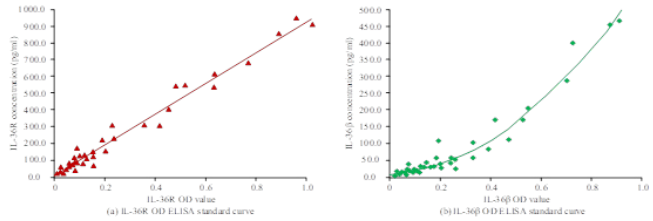

Figure 1. ELISA standard curves of serum inflammatory mediators IL-36β and IL-36R in study subjects.

Correlation of serum inflammatory mediators with disease duration and SLEDAI in patients with different stages

The results of the trend analysis of the correlation between serum inflammatory mediators and SLEDAI in patients with different stages of SLE are shown in Figure 2. as illustrated in Figure 2, no significant correlation was found between serum inflammatory mediator IL-36β and IL-36R concentrations and SLEDAI scores in patients with stable and active stages.

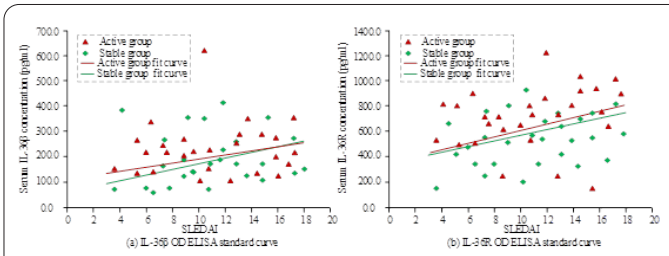

Figure 2. Analysis of the correlation between serum inflammatory mediators and SLEDAI in patients at different stages.

Figure 3 shows the results of the analysis of the correlation between serum inflammatory mediators and disease course in patients with different stages of SLE. As indicated in Figure 3, serum inflammatory mediators IL-36β and IL-36R concentrations were negatively correlated with the disease course in patients in the stable and active phases, with correlation coefficients of -0.628, -0.496 and -0.714 and -0.577, respectively.

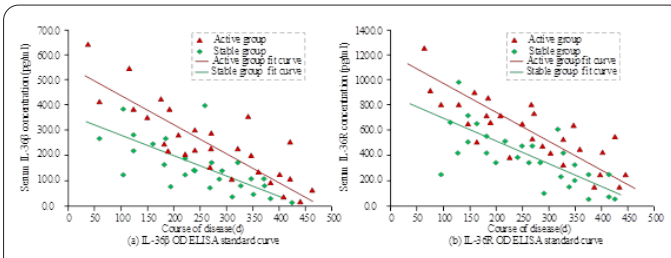

Figure 3. Analysis of the correlation between serum inflammatory mediators and disease course in patients at different stages.

The correlation between serum inflammatory mediators and screening indicators in patients

The levels of serum inflammatory mediator concentrations in subgroups of patients presenting with different indications of clinical examination symptoms are shown in Table 3. Observation of Table 3 shows that serum inflammatory mediator IL-36R concentrations are significantly

Table 3. Serum inflammatory mediator concentration levels in different groups of clinical examination symptoms.

Symptoms	With or without	Number of examples	IL-36β			IL-36R		
			Concentration (pg/ml)	*T*	*P*	Concentration (pg/ml)	*T*	*P*
Arthritis	Yes	20	230.4±152.8	0.145	0.885	643.5±329.4	0.500	0.620
	None	14	238.4±166.7			582.7±376.2		
Hair loss	Yes	6	271.45±165.0	0.647	0.522	635.0±414.2	0.241	0.811
	None	28	219.6±181.3			598.7±326.1		
Photosensitive	Yes	8	204.8±92.2	0.594	0.557	547.6±288.3	0.559	0.580
	None	26	245.2±187.5			628.1±376.9		
Butterfly-shaped red spot	Yes	24	253.7±188.6	1.040	0.306	637.7±342.9	0.666	0.510
	None	10	185.9±130.6			551.3±346.5		
Mucosal ulcers	Yes	11	253.1±237.2	0.517	0.609	816.2±376.5	2.269	0.030
	None	23	219.5±146.4			536.8±317.4		

Table 4. Serum inflammatory mediator concentration levels in different laboratory characteristic groups.

Experimental features	With or without	Number of examples	IL-36β			IL-36R		
			Concentration (pg/ml)	*T*	*P*	Concentration (pg/ml)	*T*	*P*
Decreased red blood cell count	Yes	6	357.4±186.2	2.404	0.022	974.8±436.0	3.554	0.001
	None	28	189.6±149.5			496.3±266.1		
Decreased haemoglobin	Yes	19	257.9±182.6	1.238	0.225	742.0±425.9	2.294	0.026
	None	15	184.7±155.7			469.3±198.5		
C4 decline	Yes	28	235.8±186.5	0.136	0.893	652.8±386.0	1.332	0.192
	None	6	224.5±143.9			436.2±163.8		
Anti-dsDNA	Yes	16	229.51±187.5	0.040	0.968	652.3±369.2	0.483	0.632
	None	18	227.1±163.4			591.8±366.4		
Decline in lymphocytes	Yes	8	269.4±239.2	0.844	0.405	946.3±408.6	3.853	0.001
	None	26	207.6±163.5			488.2±253.4		
Urine Protein	Yes	11	238.7±205.8	0.328	0.745	205.7±123.0	1.294	0.205
	None	23	217.5±159.2			278.1±164.7		

higher in patients with mucosal ulcers only and the difference is huge. The presence or absence of other symptoms in the patients did not affect the serum inflammatory mediator concentrations in the two selected cases.

The serum inflammatory mediator concentration levels in the subgroup of patients presenting with different laboratory characteristics are presented in Table 4. Observations Table 4, it can be seen that the differences in serum inflammatory mediator IL-36β concentrations in patients were huge only in the indicators of decreased erythrocyte count, and the differences in serum inflammatory mediator IL-36R concentrations in patients were huge in the indicators of decreased erythrocyte count, decreased haemoglobin, decreased lymphocytes The differences were huge and tiny in C4 decline, anti-dsDNA and urinary routine protein.

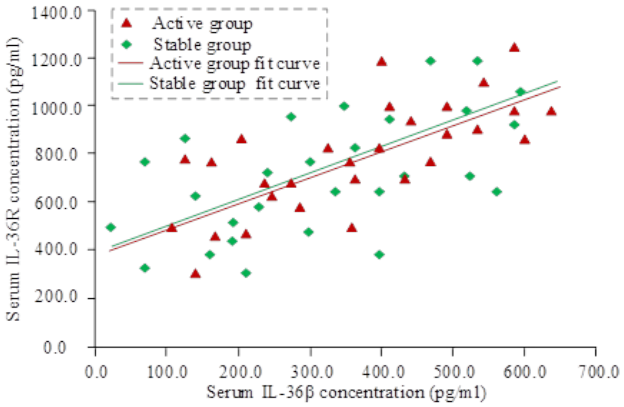

Figure 4. Statistical results of correlation between serum inflammatory mediators IL-36β and IL-36R concentrations in patients of different stages.

Correlation between various serum inflammatory mediators in patients of different stages

The statistical results of the correlation between serum inflammatory mediators IL-36β and IL-36R concentrations in stable and active patients are shown in Figure 4. Looking at Figure 4, it can be found that there is a clear positive relationship between IL-36β concentration and IL-36R concentration in stable and active SLE patients, with correlation coefficients of 0.448 and 0.452, respectively.

Serum mRNA expression levels of inflammatory mediators in the whole blood of patients with different stages

The statistical results of the mRNA concentration levels of serum inflammatory mediators IL-36β and IL-36R in the whole blood of the stable and active groups are shown in Table 5. As can be seen from Table 5, the differences in the data of serum inflammatory mediators IL-36β and IL-36R concentrations between the patients in the stable and active groups as a whole and each disease duration group were tiny. However, from the perspective of disease duration, the mRNA concentrations of IL-36β and IL-36R in patients in both the stable and active groups showed an overall decreasing trend as the disease duration increased.

Serum inflammatory mediator expression levels in skin lesions of patients with different stages

The number of serum inflammatory mediators IL-36β

Table 5. Comparison of whole blood mRNA concentrations of inflammatory mediators in the stable and active groups.

Inflammatory mediators	Course of disease	Stable group (n=34)	Activity group (n=34)	*T*	*P*
IL-36β mRNA (pg/ml)	Less than 100d	0.0494±0.0210	0.0705±0.0342	1.663	0.114
	100d~300d	0.0275±0.0133	0.0382±0.0190	1.512	0.250
	Greater than 300d	0.0136±0.0084	0.0154±0.0112	1.028	0.698
	All	0.0241±0.0152	0.0360±0.0193	1.764	0.107
mRNA of IL-36R (pg/ml)	Less than 100d	0.1193±0.0785	0.1416±0.0872	0.622	0.541
	100d~300d	0.0603±0.0438	0.0717±0.0526	0.745	0.461
	Greater than 300d	0.0298±0.0173	0.0319±0.0184	0.293	0.796
	All	0.0561±0.0324	0.0667±0.0451	1.207	0.231

Table 6. Expression levels of inflammatory mediators in different skin cells in different groups of patients.

Cell type	Inflammatory mediators	Stable group (n=34)	Activity group (n=34)	*T*	*P*
Number of epidermal keratin-positive forming cells	IL-36β	79.52±17.62	83.15±20.66	0.775	0.441
	IL-36R	85.30±17.53	88.10±16.95	0.671	0.505
Number of positive cells in superficial lymphoid tissue of the dermis	IL-36β	75.96±28.49	77.57±30.18	0.239	0.812
	IL-36R	71.51±31.92	74.70±25.45	0.457	0.649

and IL-36R positive cells were counted in the epidermal stratum corneum and superficial dermis of selected patients to obtain Table 6. Observing Table 6, it can be seen that the differences in the number of each inflammatory mediator-positive cell in the epidermal stratum corneum and superficial dermis of patients in the stable and active groups were tiny.

Discussion

SLE is an autoimmune illness marked by the development of excessive amounts of abnormal autoantibodies that affect various organs and systems throughout the body, and its main manifestation is an autoimmune inflammatory connective tissue disease. It is often thought that SLE is triggered by immune abnormalities, infections, endocrine and genetic factors, but clinically it is believed that genetic and environmental factors act together to cause the patient's own immune system to remain in a state of recurrent, chronic activation, resulting in SLE disease. In turn, the patient's autoimmune response is constantly running, leading to the accumulation of immune autoantibodies, immune complexes and other immune activities, causing an inflammatory response that leads to vascular damage. Current clinical medicine generally uses an immunological approach to test for SLE disease, as the immune response in SLE is a combination of self-generated immune tolerance and rejection of exogenous antigenic substances. Therefore, immunological indicators of SLE are tested for both endogenous and exogenous antigens. In recent years, it has been found in several studies that IL-36β protein requires binding to IL-36R for activation and that both play a key role in the induction of inflammation and immune disease. Specifically, they are directly involved in the activation of dendritic cells and helper T cells, the stimulation of pro-inflammatory factor secretion and antigen presentation. IL-36 proteins also work synergistically with many cytokines, for example, IL-36β works synergistically with IL-2 to stimulate Th1 cell differentiation, and IL-36 proteins are also regulated by Th17 factors and enhance the functional expression of Th17 factors.

As discussed above, IL-36 protein is associated with inflammation and immune function, e.g. IL-36Ra gene variants are associated with fatal pustular psoriasis or its associated skin diseases; IL-36α mRNA concentrations are significantly higher in patients with chronic kidney disease; chondrocytes or synovial fibroblasts from patients with rheumatoid arthritis express IL-36β protein. 36β protein, which will bind to IL-36R to induce the production of inflammatory molecules. This stimulates the onset of rheumatoid arthritis; IL-36 protein also has a role in regulating adaptive immunity to Mycobacterium tuberculosis infection in vitro and Th1 immunity to BCG vaccine in vivo. For SLE disease, studies have shown that IL-1 type proteins are deeply involved in the immune process of SLE, and IL-36 is an important member of IL-1 type proteins, the former being more similar to the latter in terms of protein expression and function, but the former is more effective in activating the NF-κ B signalling pathway. The IL-36β protein is also the most highly regulated of the IL-36 proteins, so this study proposes that IL-36β and IL-36R proteins will be involved in SLE pathogenesis, and experiments were conducted to test this hypothesis.

The findings showed that the concentrations of IL-36β and IL-36R proteins in the serum of SLE patients were positively correlated, and both were also negatively associated with the course of the patients' disease. This suggests that IL-36β and IL-36R proteins may function to activate adaptive or innate immune system responses during the early stages of SLE. However, the findings also showed no clear correlation between serum IL-36β and IL-36R proteins and SLEDAI in SLE patients, suggesting that the above results alone do not lead to the conclusion that "serum IL-36β and IL-36R proteins can be used as early warning indicators for the development of LSE". Dai Y et al. studied the IL -36β protein concentrations and found no statistically significant transport between them, a result that is consistent with that obtained in the present study, but the findings may be related to the following factors(16). One is the genetic variability of individuals, as the relatively limited sample size does not clearly reflect the exact relationship between the two inflammatory mediator proteins and SLEDAI; the second is that the two inflammatory mediator proteins only activate the immune system of patients in the early stages of SLE onset, while the concentrations of both in serum and whole blood have decreased substantially in the subsequent stages.

In experiments related to clinical symptoms, the team found a correlation between serum IL-36R concentrations and mucosal ulcer symptoms in SLE patients. Hsieh C Y's study also showed that IL-36R protein is mainly present in the skin and in parts of the patient's epithelial tissue (e.g. oesophagus, intestine, trachea) (17). Therefore, it can be deduced that the accumulation of immune complexes secreted by the body of SLE patients on the mucosal vessels, which makes dendritic cells activate habituated cells to secrete IL-36R and activate complement to cause vasculitis, bringing about the problem of inadequate local blood supply to the mucosa and the formation of ulcers is the reason why serum IL-36R concentrations in SLE patients correlate with the symptoms of mucosal ulcers. In addition, serum IL-36R concentrations in SLE patients were significantly associated with a decrease in erythrocytes, lymphocytes and C4 complement in the blood, specifically, IL-36R concentrations were significantly higher in patients with these experimental indicators, but serum IL-36β protein concentrations were only associated with a decrease in erythrocytes. This suggests that IL-36R has a closer influence on the secretion of immune factors in SLE patients.

The results of semi-quantitative experiments on skin lesion cells showed that IL-36β and IL-36R proteins are expressed in the nuclei of cells forming at the epidermal keratin of skin lesions and superficial dermal lymphohistiocytes in SLE patients, suggesting a role for both in the epithelial barrier. studies by Lee I also showed that IL-36 proteins are involved in the inflammatory response in psoriasis, leading to IL-36α, and IL-36β increased mRNA difficulty (18-22). However, there is a paucity of literature examining IL-36β and IL-36R protein expression in the treatment of SLE with immunosuppressants, so whether both can be used as targets for the treatment of SLE needs further study.

Based on the above discussion, it is clear that IL-36β and IL-36R proteins are expressed in immune cells as well as epithelial cells in SLE patients, suggesting that these two inflammatory mediators may be one of the early signals that activate the immune system in SLE patients and trigger an immune response in patients. This will inform the search for new ways to precisely treat SLE disease.

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