Low Level of Serum Interleukin 27 in Patients With Systemic Lupus Erythematosus

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Abstract: T helper 17 (T_H17) cells are beginning to be implicated in the pathogenesis of systemic lupus erythematosus (SLE). Recent studies have shown that interleukin 27 (IL-27) controls the development of T_H17. However, whether IL-27 plays a role in the development of SLE is still unclear. In the present work, we investigated the serum IL-27 level in SLE and its relations to disease activity. Fifty-six patients with SLE and 30 healthy volunteers were recruited. Serum IL-27 levels were detected by enzyme-linked immunosorbent assay. The clinical and laboratory parameters were collected from medical records or by questionnaire. The serum IL-27 level in SLE patients was significantly lower than that in healthy controls ($P < 0.001$). Compared with SLE patients without nephritis, patients with nephritis had a significantly decreased serum IL-27 level ($P < 0.05$). However, there was no significant difference between less active and more active SLE ($P > 0.05$). Correlation analysis between serum IL-27 levels and SLE disease activity index showed no association ($P > 0.05$). In summary, a decrease in serum IL-27 level in patients with SLE suggested that this cytokine might be implicated in the pathomechanism of this disease.

Key Words: IL-27, T helper 17 cells, systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a systemic autoimmune disease, characterized by production of multiple autoantibodies, complement activation, and immune-complex deposition, causing tissue and organ damage. The etiology and pathogenetic mechanisms of SLE are still unclear. More than 1 mechanism could contribute to this disease. Activated autoreactive T helper (T_H) cells have been shown to be implicated in the pathogenesis of SLE, which assist B cells to differentiate and produce pathogenic autoantibodies.[1]

At present, T_H17 cells are beginning to be implicated in the pathogenesis of SLE. T helper 17 cells are a novel subset of T_H cells that selectively secrete several proinflammatory cytokines, including interleukin 17 (IL-17) and IL-22. Increased levels of IL-17 and a decreased level of IL-22 have been detected in the serum of SLE patients in some studies.[2–6] Although the exact role of IL-17 in SLE has not been fully elucidated, IL-17 was recently shown to be critical for the formation of autoreactive germinal centers in BXD2 mice, a strain that develops a lupuslike syndrome.[7] This effect was believed to be secondary to the ability of IL-17 to control the migration of B cells, leading to their prolonged retention within the germinal center where they presumably could receive enhanced help from T cells.[8] In addition, down-regulation of IL-17 production by T cells has been shown to correlate with the amelioration of murine lupus after treatment with either low-dose peptide tolerance therapy or nasal anti-CD3 antibodies.[9,10] These findings suggest that T_H17-related cytokines might be implicated in the pathomechanism of SLE.

Recently, IL-27 was identified, which belongs to the IL-12 cytokine family. These family members play important roles in the regulation of T_H cell differentiation. It has been revealed that IL-27 is also involved in the regulation of T_H17 responses, suppressing T_H17 differentiation and IL-17 production. In this context, IL-27 has an immunosuppressive feature.[11] In addition, it has been demonstrated that IL-27 has 2 conflicting properties: proinflammatory and anti-inflammatory.[12] However, whether IL-27 plays a role in the pathogenesis of SLE and which property it works with are still unclear. Therefore, in the present study, we investigated the serum IL-27 level in SLE and its relations to disease activity.

MATERIALS AND METHODS

Patients and Controls

Fifty-six patients with SLE (55 females and 1 male; mean age, 35.9 [13.1] years; age range, 17–60 years) were recruited from the departments of rheumatology of Anhui Provincial Hospital and of the First Affiliated Hospital to Anhui Medical University. All patients had new-onset SLE, who were included in the study according to the following criteria: (1) first-time diagnosis of SLE and (2) no history of corticosteroids or immunosuppressive drugs use before registration. The diagnosis of SLE was established by the presence of 4 or more American College of Rheumatology diagnostic criteria.

Thirty healthy volunteers (29 females and 1 male; mean age, 36.5 [13.5] years; age range, 16–60 years) were included as healthy controls; all of them were without evidence of rheumatologic conditions. Sera obtained from cases and controls were stored at −80°C until tested. All subjects gave their written consent to participate in the study.

Collection of Clinical and Laboratory Data

Demographic, clinical, and laboratory data were collected from hospital records or by questionnaire and were reviewed by experienced physicians.

Renal involvement was defined by persistence of proteinuria (≥0.5 g/24 h), the presence of cellular casts, persistent hematuria, or renal biopsy showing mesangial, focal proliferative, diffuse proliferative, or membranous glomerulonephritis.

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Disease activity was quantified using the SLE disease activity index (SLEDAI) score. The SLEDAI scores were evaluated by 2 physicians and reviewed by a senior rheumatologist. More active SLE was defined by a SLEDAI score of 6 or higher, whereas less active SLE was defined by a SLEDAI score[13,14] of less than 6.

Detection of IL-27

Serum IL-27 concentrations were determined by enzyme-linked immunosorbent assay kits (R&D Systems, Inc, Minneapolis, MN) according to the manufacturer's recommendation. Briefly, 96-well plates coated with biotinylated IL-27 were incubated with 100-μL sera for 120 minutes at 37°C. Then, the plates were washed 6 times with the wash solutions of the kits. Biotinylated abs of 100 μL was pipetted into each of the microplate wells and incubated for 60 minutes at room temperature. After 6 washes, 100 μL of enzyme conjugate was added and incubated for 30 minutes at 37°C and then washed as before. A 100-μL tetramethyl benzidine solution was added and incubated for 15 minutes in dark. Color release was stopped with the addition of 100 μL of stop solution. The microplate wells were read at 450-nm reference filter within 30 minutes, and the optical density value of each well was recorded. Then, the concentrations were calculated according to the optical density values, and the results were expressed as picograms per milliliter (intraassay: coefficient of variation, <10%; interassay: coefficient of variation, <15%).

Statistical Analysis

The data were analyzed by the SPSS 10.01 software (SPSS, Inc, Chicago, IL). For comparing the level between different groups, the nonparametric Mann-Whitney 2-sample test was used. For the correlation analysis between serum IL-27 level and SLEDAI, the Spearman rank correlation test was used. $P < 0.05$ in 2-tailed tests was considered as a criterion of significance.

RESULTS

Among the 56 patients with SLE who were included in the study, 25 had lupus nephritis (LN) and 47 had more active SLE. Results showed that the serum IL-27 level was significantly decreased in the SLE patients, compared with that in the healthy controls ($P < 0.001$). The serum IL-27 level in SLE patients with LN was significantly lower than that in SLE patients without LN ($P < 0.05$). No significant difference was found between less active and more active SLE (Table 1).

There was no association of serum IL-27 level with clinical and laboratory parameters of SLE patients ($P > 0.05$).

TABLE 1. Comparison of Serum IL-27 Levels Between Different Groups

Group	No.	Serum IL-27 Levels: Median (Interquartile Range), pg/mL	*P*
Healthy controls	30	56.91 (30.94–68.92)	
SLE	56	17.15 (9.54–36.61)	<0.001*
SLE without nephritis	31	27.47 (10.89–57.25)	
SLE with nephritis	25	13.26 (7.00–24.77)	<0.05†
Less active SLE	6	19.35 (11.07–31.96)	
More active SLE	50	17.15 (9.29–42.61)	0.949‡

*Versus healthy controls.

†Versus SLE without nephritis.

‡Versus less active SLE.

DISCUSSION

Recent discoveries and characterization of T_H17, which is involved in various inflammatory diseases by production of IL-17 and other proinflammatory cytokines, revealed that IL-27 is also involved in the regulation of T_H17 responses.[11] T helper 17 with its cytokines has been implicated in a variety of autoimmune diseases, such as experimental autoimmune encephalitis (EAE), collagen-induced arthritis, and SLE.[1]

Furthermore, it has been shown that IL-27 signaling could ameliorate T_H17-driven inflammation in models of EAE in vivo.[15] Another study also found that overexpression of IL-27 receptor (WSX-1) ameliorates the development of autoimmune disorders in the MRL/lpr mice, which are considered as an experimental model of SLE in humans.[16] Consistent with the results of these 2 studies, our data indicated that serum IL-27 level was significantly decreased in patients with SLE as compared with healthy controls. Yu and Gaffen[17] considered that IL-17 can promote the recruitment of inflammatory cells to target organs such as kidney. Whereas T_H17 differentiation and IL-17 production could be suppressed[11] by IL-27, this could explain partially that serum IL-27 level in patients with LN is lower than that in patients without LN in our study.

Because of its ability to suppress or antagonize proinflammatory cytokines, such as IL-2, IL-6, and IL-17, and to suppress proliferation of immune cells, IL-27 might prove to be useful as a therapeutic immune modulator.[18] Recently, 2 models showed that IL-27R−/− mice were highly susceptible to EAE induced by adoptive transfer of encephalitogenic T_H17 cells and EAE induced by active immunization,[19,20] which suggested that IL-27 might have a therapeutic potential in autoimmune diseases such as SLE.

Another recent study reported that SLE patients have a higher urinary IL-27 expression than healthy controls and that IL-27 expression is found to be inversely correlated with the SLEDAI score.[21] Microarray analysis of glomerular gene expression in murine LN also showed a high level of Epstein-Barr virus–induced gene 3 (Ebi3), a subunit[22] of IL-27. Our findings seem to contradict with the results. The cause of this paradoxical pattern of change in IL-27 expression remains elusive. However, there are several explanations. Firstly, it might be that IL-27 is produced within the kidney where it exerts local effects. Secondly, it is also possible that urine and serum levels of IL-27 are not correlated. Thirdly, RNA was measured rather than protein in some studies. Lastly, some studies looked at cells deriving from renal inflammation/capillary leak, whereas we looked at circulating levels. Therefore, further studies are needed to clarify the role of IL-27 in human SLE.

However, several limitations of this study should be noted. First, considering the limited subjects included in the study, our results should be interpreted with caution. Because we aimed to recruit patients with new-onset SLE, many patients who have a history of corticosteroids or immunosuppressive drugs use before registration were excluded, and only 56 patients were eligible for this study. Of course, further studies with a large sample size are needed to confirm this preliminary result. Second, this is only a descriptive study, and which property of IL-27 works in SLE is still unclear; thus, future pathologic

mechanism studies are urgently needed. Despite these limitations, our study has 2 advantages. First, there have been no studies evaluating circulating IL-27 level in SLE patients, so a study such as this is novel and could add to the existing literature. Second, we have enrolled patients before initiation of immunosuppressive therapy, which is unique and removes the confounding effects of treatment.

In summary, a decrease in serum IL-27 level in patients with SLE suggests that it may be implicated in the pathomechanism of this disease. In addition, IL-27 is negatively associated with the occurrence of nephritis in patients with SLE, suggesting that IL-27 may play a role in the development of nephritis in SLE patients.

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