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Decreased serum interleukin 27 in Brazilian systemic lupus erythematosus patients

Angela Luzia Branco Pinto Duarte · Andréa Tavares Dantas · Henrique de Ataíde Mariz · Flaviana Alves dos Santos · Juliana Cruz da Silva · Laurindo Ferreira da Rocha Jr. · Suely Lins Galdino · Maira Galdino da Rocha Pitta

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Abstract The immunological role of interleukin 27 has been reported in various inflammatory diseases, but its importance in systemic lupus erythematosus pathogenesis is not completely established. The aim of this study was to evaluate serum levels of IL-27 in SLE patients and its correlation with clinical manifestations and disease activity. IL-27 levels were assessed in 70 SLE patients and 30 healthy controls by ELISA. Clinical and laboratory parameters were recorded. Statistic analyzes were performed by Graph Prism 3.02 software. The IL-27 serum levels were significantly decreased in SLE patients compared with controls (mean 899.92 and 1,531.22 pg/ml, $P = 0.0005$). There was a correlation between IL-27 levels and C3 levels ($P = 0.004$). Nevertheless, there was no association of serum IL-27 levels with disease activity evaluated by SLEDAI score ($P = 0.9605$). No significant difference was found regarding IL-27 levels between SLE patients with and without nephritis, haematuria, proteinuria and positive anti-dsDNA. Correlation analysis between serum IL-27 levels and SLEDAI, SLICC, proteinuria levels, C4 and CH50 levels also showed no association. These data demonstrated decreased serum levels of IL-27 in SLE patients but further studies are needed to clarify the precise role of this cytokine and its potential use as therapeutic target.

Keywords Systemic lupus erythematosus · Cytokines · Interleukin 27 · Nephritis · SLEDAI

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown etiology that affects mainly young women. Pathologic hallmarks of lupus are an increased autoantibody production, complement activation and immune complex deposition, leading to tissue and organ damage. Recently, it has been demonstrated that cytokine-mediated immunity plays a crucial role in the pathogenesis of SLE [1].

Traditionally, SLE is characterized by an imbalance of Th1/Th2 cytokines. It has been reported a relative deficiency in Th1-cytokines and a relative excess in Th2-cytokines [2]. Recently, it has been described the participation of a novel TH effector cell subset, the TH17 cell subset, in the development of SLE, because of the ability of TH17 cells to produce cytokines like IL-17 and IL-21 that can drive both inflammatory and humoral responses [3, 4].

Interleukin 27 (IL-27) is a heterodimeric member of IL-12 cytokine family, which consists of two subunits: an IL12p40-related protein, encoded by the Epstein-Barr virus (EBV)-induced gene 3 (EBI3, also known as IL27), and a unique IL12p35-like protein, Il27p28 [5]. IL-27 receptor complex is constituted of IL-27R (also known as WSX-1)

Angela Luzia Branco Pinto Duarte, Andréa Tavares Dantas are contributed equally to this work.

A. L. B. P. Duarte · A. T. Dantas · H. de Ataíde Mariz · L. F. da Rocha Jr.
Hospital das Clínicas, Universidade Federal de Pernambuco, Recife, PE, Brazil

A. T. Dantas · H. de Ataíde Mariz · F. A. dos Santos · J. C. da Silva · L. F. da Rocha Jr. · S. L. Galdino · M. Galdino da Rocha Pitta (✉)
Laboratório de Imunomodulação e Novas Abordagens Terapêuticas (LINAT), Núcleo de Pesquisa em Inovação Terapêutica Suely Galdino (Nupit SG), Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, 1235, Cidade Universitaria, Recife, PE 50670-901, Brazil
e-mail: mgrpitta@gmail.com

and glycoprotein 130 (gp130) and both components were found to be co-expressed in a wide range of immune cells including monocytes, B cells and T cells [5]. The role of IL-27 in the immune response is not fully understood, since pro- and anti-inflammatory responses were described. Some studies reported that IL-27 could promote naive $CD4^+$ T cells to differentiate into Th1 cells trough an increase of IFNγ production [5]. Furthermore, there is some evidence that IL-27 has immunosuppressive functions: it suppresses IL-2 production by $CD4^+$ T cells [6], inhibits differentiation of Th17 cells [7] and promotes IL-10 production by $CD4^+$ T cells [8].

There are few studies that evaluated IL-27 serum levels in autoimmune rheumatic patients. Patients with rheumatoid arthritis have elevated IL-27 levels and this was associated with disease activity and interstitial lung disease [9]. In systemic sclerosis patients, serum IL-27 levels are elevated and correlate positively with the extent of skin involvement and pulmonary fibrosis [10]. In the same way, psoriatic patients have higher IL-27 levels and these levels have association with disease severity and IFN-γ levels, although in patients with established disease IL-27 suppress proinflammatory factors in the presence of TNF [11]. These findings emphasize the controversial role of IL-27 in autoimmune regulation. The role of IL-27 in SLE pathogenesis is not yet established. Thus, the aim of this study was to evaluate serum levels of IL-27 in SLE patients and its correlation with clinical manifestations and disease activity.

Materials and methods

Patients and controls

Seventy Brazilian patients with SLE (3 men and 67 women; mean age 37.8 ± 9.5 years) were recruited from Rheumatology Division at Hospital das Clínicas of Universidade Federal de Pernambuco, Brazil. All patients included in the study fulfilled four or more of the American College of Rheumatology (ACR) classification criteria for SLE [12] and answered a clinical questionnaire from which demographic, clinical and laboratorial data were collected (Table 1). Additional information was obtained from hospital records and reviewed by experienced physicians. Disease activity was evaluated by SLE disease activity index 2000 (SLEDAI-2K) and active disease was defined by a score of 6 or higher [13]. Patients were also classified as active lupus nephritis if they scored at least one of specific renal fields such as proteinuria, urinary casts, haematuria and pyuria. Thirty healthy individuals (20 men and 10 women, mean age 34.9 ± 9.9 years) were also included as a control group.

Table 1 Demographic, clinical and laboratory findings in SLE patients

Number of patients	70
Age (years)	
Mean (range)	37.8 ± 9.5 (20–61)
	N (%)
Sex	
Female	67 (95.7)
Male	03 (04.3)
Disease duration (mo)	
Mean (range)	95.8 (1–480)
Anti-dsDNA	
Positive	12 (17.1)
Negative	58 (82.9)
Complement	
Decreased	43 (61.4)
Normal	27 (38.6)
Treatment	
Steroids	54 (77.1)
Antimalarial agents	41 (58.6)
Azathioprine	22 (31.4)
Micophenolate mofetil	05 (7.1)
Thalidomide	02 (2.9)
Disease activity (SLEDAI)	
<6	53 (75.7)
≥6	17 (24.3)
Nephritis	
Active	15 (21.7)
Inactive	54 (78.3)

Peripheral blood samples were obtained from all subjects and serum was separated immediately and frozen at −20 °C until use. Tests for anti-dsDNA and serum complement (C3, C4 and CH50) were conducted by standard methods.

All subjects signed a consent form approved by Ethical Committee of Universidade Federal de Pernambuco—Brazil.

Measurement of serum IL-27 levels

Serum IL-27 concentrations were determined by specific ELISA kits according to the manufacturer′s recommendation (R&D Systems). Each sample was tested in duplicate. The results were expressed as pictograms per milliliter.

Statistical analysis

Associations of serum IL-27 levels with clinical and laboratory parameters of SLE patients were analyzed by

univariate comparisons using nonparametric tests (Mann–Whitney tests). $P < 0.01$ was considered as indicating a significant association and $P < 0.05$ as a suggestive association. The results are shown considering the mean value. All quantitative data were plotted with Graph Prism 3.02 software.

Results

Seventy SLE patients were included, 17 (24.3 %) of whom were patients with active disease (SLEDAI ≥6) and 15 (21.7 %) with lupus nephritis. SLE patients presented significantly lower levels of serum IL-27 than healthy controls (mean 899.92 and 1,531.22 pg/ml, $P = 0.0005$) (Fig. 1). There was no association between lupus treatment (corticosteroids, antimalarial agents, azathioprine or micophenolate mophetil) and IL-27 levels.

There was no association of serum IL-27 levels with disease activity evaluated by SLEDAI score using cut-off ≥6 (mean 895.02 ± 629.35 vs 890.64 ± 604.60 pg/ml, $P = 0.9605$). In addition, IL-27 presented lower levels in the anti-dsDNA positive patients (mean 725.53 ± 547.42 pg/ml) when compared with the anti-dsDNA negative group (mean 936.01 ± 623.24 pg/ml), not statistically significant ($P = 0.2585$). There was no difference regarding IL-27 levels between patients with and without nephritis, haematuria and proteinuria (Table 2).

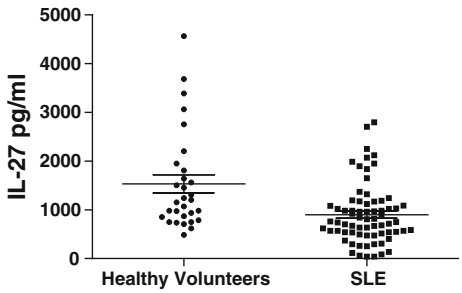

Fig. 1 Serum IL-27 levels in SLE patients and controls ($P = 0.0005$)

Table 2 Associations of serum IL-27 levels with clinical and laboratory parameters of SLE patients

Group	±	Number of patients	Serum IL-27 level (pg/ml)	*P* value
Active nephritis	+	15	916.22 ± 657.03	0.8268
	–	54	886.76 ± 608.15	
Proteinuria	+	15	916.22 ± 657.03	0.9016
	–	54	886.76 ± 608.15	
Haematuria	+	4	1,395.66 ± 862.23	0.2325
	–	65	862.23 ± 589.46	
Decreased complement	+	43	895.78 ± 612.07	0.9581
	–	26	904.19 ± 636.77	
Anti-dsDNA	+	12	725.53 ± 547.42	0.2585
	–	58	936.01 ± 623.24	

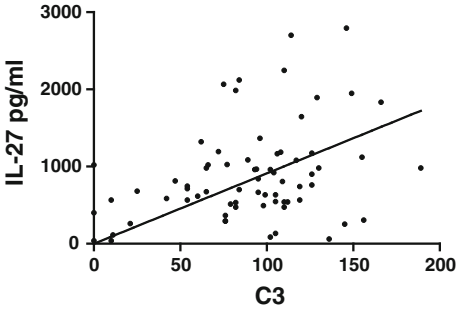

Fig. 2 Correlation between serum IL-27 levels C3 levels in SLE patients ($P = 0.004$)

We found a significant correlation between serum IL-27 levels and C3 levels ($r_s = 0.3419$, $P = 0.004$) (Fig. 2). However, correlation analysis between IL-27 levels and SLEDAI, SLICC, proteinuria levels, C4 and CH50 levels showed no association.

Discussion

The present study showed that IL-27 levels are significantly decreased in SLE patients compared to healthy controls. Furthermore, we find a significant correlation between serum IL-27 levels and C3 levels. Indeed, we find lower IL-27 levels in anti-dsDNA positive patients but it lacked statistical significance.

Experimental studies using MRL/lpr mice suggest the immunosuppressive effect of IL-27 in SLE pathophysiology [14]. This study demonstrated that transgenic overexpression of the WSX-1 gene in MRL/lpr mice resulted in suppressing the development of glomerulonephritis, with improvement in renal function, reduction of proteinuria, decreased anti-dsDNA production and increased survival rate. In line with these findings, a Chinese study with 56 new-onset SLE patients showed lower serum IL-27 levels in SLE patients, especially in patients with lupus nephritis compared to healthy controls [15]. Although it had been observed a correlation with serum C3 levels, one of parameters used to evaluate disease activity, our study could not demonstrate an association with nephritis or active disease.

On the other hand, WSX-1 or EBI-3 deficient MRL/lpr mice showed a change in the type of immune response developed, with the development of membranous glomerulonephritis (GN) with Th2-type Ig deposition rather than proliferative difuse GN, which presents Th1-type deposition [16, 17]. This confirms the role of IL-27 in promoting Th1

response, increasing its pro-inflammatory function. A recent study reported that SLE patients have a higher urinary IL-27 expression than healthy controls and IL-27 expression was inversely correlated with the SLEDAI score. Besides, after 6 months of treatment, urinary IL-27 expression was higher in patients with complete response but remained unchanged in those with partial or no response [18].

The participation of IL-27 in SLE pathogenesis has been suggested because of its implication in the regulation of Th17 responses. Elevated levels of IL-17 have been detected in the serum of SLE patients [4, 19] and it was showed higher frequencies of circulating Th17 cells in SLE patients with nephritis and elevated serum levels of Th17-related cytokines [3]. Since some studies have demonstrated that IL-27 promotes inhibition of IL-17 pathway [20–22], this cytokine may play a crucial role in SLE.

One of the limitations of our study is that some patients included were in regular use of steroids and/or immunosuppressive drugs and this represents a possible confounding effect. As IL-27 levels remain low in patients taking immunosupressive drugs, it suggests that neither corticosteroids or these immunosupressive drugs (antimalarial agents, azathioprine and micophenolate mophetil) are able to modulate IL-27 levels. Therefore, further studies with a higher sample of patients with active disease and nephritis are needed to comprehend the role of IL-27 in SLE pathogenesis.

In conclusion, our study showed a lower IL-27 serum level in SLE patients but it could not demonstrate any association with disease activity. Since few studies evaluated IL-27 in human and animal models of SLE, future research is needed to clarify the precise role of this novel cytokine and its potential use as therapeutic target.

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