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Correlative Study of Serum Th1/Th2 Cytokines Levels in Patients with Systemic Lupus Erythematosus with SLEDAI

Manal El- Sayed*, Eman Nofal*, Sahar Al Mokadem*, Inas Al Makhzangy*, Hala Gaballah* and Hossneia Akl****

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** Department of Dermatology & Venereology, Faculty of Medicine, Zagazig University*

*** Clinical pathology Department, Faculty of Medicine, Zagazig University*

****Rheumatology & Rehabilitation Department, Faculty of Medicine, Zagazig University*

Mail to: derma1.san@sghgroup.net

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Abstract

Objective

The aim of this study is to investigate the imbalance between Th-1 and Th-2 cytokines in systemic lupus erythematosus patients (SLE) and to assess if any of these cytokines could be related to disease activity.

Methods

Twenty three SLE patients and 11 healthy individuals were rolled in this study. Blood samples were collected to evaluate, using ELISA method serum levels of cytokines .Th-1 type: Interferon (IFNγ), tumor necrosis factor (TNF α) and Th-2 cytokines: Interleukin (IL-4, IL- 10). Disease activity was assessed using the SLE disease activity index (SLEDAI).

Results

Levels of IFN-γ were significantly higher in patients than in healthy controls ($P<0.01$), as well as IFN/ IL-10, IFN/ IL4, TNF/ IL-10, and TNF/ IL-4 ratios ($P<0.01$), suggesting a major participation of Th1 over Th2 cytokines. Nevertheless, a direct correlation between Th1 (IFN-γ and TNF-α) and Th2 (IL-4 and IL-10) cytokines was observed in patients ($P<0.001$), indicating a mutual Th1- Th2 participation. TNF-α levels and the TNF/IL-10 ratio were higher in patients with inactive disease compared with patients with active disease and controls ($P<0.05$). IL-10 levels were associated with anti-DNA response ($P<0.001$).

Conclusion

Our results show that Th1 as well Th2 cytokines can be elevated in SLE patients suggesting that lupus is a complex disease that may involve different cytokine patterns in different time points. Also, suggest that high level of TNF-α could be a protective factor in SLE patients, and IL-10 influences the autoimmune response (autoantibody production).

Introduction

Systemic lupus erythematosus (SLE) is a heterogenous, chronic autoimmune disease characterized by the deposit of immune complexes in different organs. The disease primarily affects women between the third and fourth decades of life. Even though the etiology of SLE is unknown, many predisposing factors have been found, including genetic, environmental, infections, and hormonal factors [1].

The immune dysregulation that lead to overt SLE is complex but has two main characteristics. One is the peculiar B lymphocyte hyperactivity accompanied by immunoglobulin repertoire changes leading to an increased production of autoantibodies [1]. The other is the impaired cell mediated immunity which results from both T lymphocyte and antigen-presenting cell abnormalities [2,3].

The abnormality in the T- cell response is manifested by an imbalance in the production of cytokines. Cytokines have been functionally divided into 2 subgroups: Th1, mainly interleukin (IL)-2, IL-12, interferon (IFN) γ, and tumor necrosis factor (TNF) α and β, which mainly activate the cellular machinery of the immune system, and Th2 (IL-4, IL-5, IL-10, and IL-13) cytokines, which activate the humoral machinery [4, 5, 6,7].

IL-10 is a potent inducer of B lymphocyte differentiation, as well as inhibitor of helper T cell and antigen presenting cell function [8,9]. Some have proposed that the immunologic imbalance in SLE may be related to an abnormally high production of IL-10. B lymphocyte hyperactivity may result from autocrine and paracrine effects of IL-10 signaling [10,11].

In patients with SLE, B- cell hyperactivity has been associated with a high production of Th2 cytokines, leading to excessive autoantibody production. However, the participation of Th1 cytokines has been equally demonstrated. Both Th1 and Th2 cytokines can participate in promoting or inhibiting autoimmune diseases; thus, a clear-cut distinction between Th1 and Th2 patterns is not without complexity [12].

In the present study, the relationships between the levels of Th1 (IFNγ, TNF α)- Th2 cytokines (IL-10, IL-4) with autoimmune response and disease activity of SLE in a group of patients was evaluated.

Patients and Methods

Patients:

Twenty three patients with SLE (21 female, 2 male, mean age was (38.2±15.8) who fulfilled 4 or more of the American college of Rheumatology criteria for the classification of SLE [13] were studied.

No patients fulfilling these criteria were excluded.Patients were on treatment with: prednisolone 10±5mg (80% of patients), hydroxychloroquine 250 mg (45%), azathioprine 50-150 mg (10%), either singularly or in combination.

Eleven healthy people unrelated to the patients, without inflammatory or autoimmune disease as normal control subjects (10 female, 1 male; mean age was 39.1±14.2) were studied.

SLEDAI:

Disease activity was assessed according to the systemic lupus erythematosus disease activity index (SLEDAI) [14, 15].

Table (1): SLEDAI (Systemic Lupus Erythematosus Disease Activity Index)[15]

weight	SLEDAI score	Descriptor	Definition
8	-	Seizure	Recent onset. Exclude metabolic, infectious and drug causes.
8	-	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes.
8	-	Organic brain syndrome	Altered mental function with impaired orientation, memory or other intellectual function, with rapid onset of fluctuating clinical feature. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes.
8	-	Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid or optic neuritis. Exclude hypertension, infection , or drug causes.
8	-	Clinical nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	-	Lupus headache	Severe, persistent headache; may be migrainous, but must be non responsive to narcotic analgesia.
8	-	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	-	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4	-	Arthritis	More than 2 joints with pain and signs of inflammation (i.e., tenderness, swelling, or effusion.
4	-	Myositis	Proximal muscle aching/weakness, associated with elevated creatinine phosphokinase/aldolase or electromyogram changes or biopsy showing myositis.
4	-	Urinary casts	Heme-granular or red blood cell casts.
4	-	Hematuria	>5 red blood cells/high power field. Exclude stone, infection, or other causes.
4	-	Proteinuria	>0.5 gm/24 hours. New onset or recent increase of more than 0.5 gm/24 hours.
4	-	Pyuria	> 5 white blood cells/high power field. Exclude infection.
4	-	New rash	New onset or recurrence of inflammatory type rash.
4	-	Alopecia	New onset or recurrence of abnormal, patchy or diffuse loss of hair.
4	-	Mucosal ulcers	New onset or recurrence of oral or nasal ulcerations.
4	-	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
4	-	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation
4	-	Low complement	Decrease in CH50, C3, or C4 below the normal limits of normal for testing laboratory.
4	-	Increased DNA binding	>25% binding by Farr assay or above normal range for testing laboratory.
4	-	Fever	>38°C. Exclude infectious causes.
4	-	Thrombocyto penia	<100,000 platelet/mm^3
4	-	Leukopenia	<3,000 white blood cell/mm^3. Exclude drug causes.

Two stages of SLE were considered as a function of the SLEDAI score; inactive SLE when SLEDAI ≤ 6 points and active SLE when SLEDAI >6 points.

Cytokines:

Blood samples from all patients and controls were taken during morning. Measurements of IL-4, IL-10, TNF-α and IFN-γ in serum samples collected from patients and controls were performed as follows:

- IL-4 levels were evaluated using enzyme linked immuno-sorbent assay (ELISA) with commercially available kits (Medgenix) according to the manufacture's instruction.
- IL-10 assay was performed by solid phase sandwich enzyme linked immunosorbent assay (ELISA) (Predicta, Genzyme. Diagnostics).
- IFN-γ was measured by a commercially available enzyme immunoassay kit (Endogen Boston, MA) with lower limit of sensitivity of 5 pg/ mL (0.1/ u / mL).
- Measurement of plasma tumor necrosis factor alpha was carried out using a commercially available enzyme immunoassay (EIA). Kit (Accucyte TNF-α EIA), Supplied by (Cytimmune sciences Inc. 8075 Green Mead Drive College Park, Maryland 20740).

Autoantibodies:

Antinuclear antibodies (ANA), anti- DNA were determined by indirect immuno fluorescence.

Statistical Analysis:

Data were entered, checked and analyzed by using EPI-INFO (2005). Data were expressed as mean ±standard deviation for quantitative variable, number and percentage for quantitative ones. T-test, ANOVA and chi-squared test and correlation(r) were used when appropriate. $P<0.05$ was considered significant [16].

Results

A total of 23 patients with SLE and 11 healthy controls were studied. Patients were on treatment with prednisone 10mg ±5 mg/d, (80% of patients), hydroxychloroquine 250mg/d (45%), as azathioprine 50-150 mg/d (10%). With regard to disease activity, 10 patients had inactive disease and 13 had active disease (Table 2).

SLE patients had higher levels of IFN- γ, and TNF-α than controls ($P<0.01$). They also had higher levels of IL-10 but not reach the significant levels than normal controls ($P=0.06$) (Table 3).

No significant differences were seen with respect to Th2 response. No correlation between disease duration and the cytokines levels or antibody titers was found.

IL-10 levels were significantly higher in patients with anti- DNA. In addition, a direct correlation between the IL-10/ TNF- α ratio and the levels of anti- DNA ($r= 0.57$, $P< 0.01$) antibodies was observed (Table 4)

The 2 groups of SLE patients had significantly higher titers of IFN-γ than controls (P<0.01); however, there were no differences in IFN-γ titers with respect to disease activity.

TNF -α levels were significantly higher in the inactive and active groups when compared with the controls (P<0.001). Patients with inactive disease had statistically higher levels than those in the active group (P< 0.05). Finally, no significant differences were found in Th2 (IL-4, IL-10) cytokines as a function of disease activity (Table 5).

Table (2): General characteristics of the studied groups:

	Cases N = 23		**Controls N = 11**
Gender, female, male	21:2		10:1
Age (years)	38.2±15.8		39.1±14.2
Duration of SLE (y)	4.8 ± 6.5		
Activity of disease	No	%	
≤ 6	10	43%	
> 6	13	57%	

Table (3): Levels of cytokines and autoantibodies in patients with SLE and in healthy individuals:

Cytokines	Control (n=11)	SLE (n=23)	P
IL-4 pg/ml			
$\overline{X}$ ± SD	122.11 ± 20.1	139.6 ± 36.6	0.14
Range	100-143	100-200	
IL – 10 pg/ml			
$\overline{X}$ ± SD	81.8 ± 11.5	112.4 ± 40.8	0.06
Range	65-103	65-224	
INF-γ pg/ml			
$\overline{X}$ ± SD	117.7 ± 22.8	144.2 ± 26.6	0.009
Range	95-148	95-200	
TNFα pg/ml			
$\overline{X}$ ± SD	23.9 ± 11	97.96 ± 38.44	0.001^{+}
Range	10-35	45.0-160.0	
Autoantibodies UI			
Anti-DNA +ve	0 (0.0%)	16 (69.6%)	0.001^{+}
ANA +ve	0 (0.0%)	22 (95.6%	0.001^{+}

Table (4): Pearson correlation between cytokines and autoantibodies in patients with SLE:

	Duration R	IL4 r	IL 10 r	INF r	TNF R
Anti DNA	-0.03	- 0.14	-0.07	-0.11	-0.01
ANA	-0.14	-0.09	-0.13	-0.08	-0.12
TNFα	-0.19	0.81^{+}	0.7^{+}	0.58^{+}	
INFγ	-0.09	0.65^{+}	0.62^{+}		
IL 10	0.08	0.72^{+}	0.55^{+}		
IL 4	0.12				

r = correlation coefficient.

Table (5): TH1/TH2 cytokines levels in patients with SLE in Function of Disease Activity

	SLEDAI≤6 (n=10)	SLEDAI > 6 (n=13)	Controls (n=11)	P
IL-4 pg/ml	129.9±41.4	143.1 ± 27.3	122.1 ± 20.1	0.12
TNFα pg/ml	98.6±42.2	84.2±44.3	23.9±11	0.001
IFN-γ pg/ml	130±13.4	147.7 ±20.6	117.7±22.8	0.0015
IL-10 pg/ml	107.8±40.7	118.3±42.4	81.8 ±11.5	0.15

Discussion

SLE is a disease characterized by variable autoimmune inflammatory tissue destructions. Defective T cell regulation has been postulated to play a crucial role in its pathogenesis and in the disease manifestations. However, the predominance of Th1 or Th2 cytokines in SLE has not been well defined, and the mechanisms that lead to the aberrant auto-inflammatory syndrome are not clearly understood [17]. In the present study we evaluated the relationship between Th1- Th2 cytokine levels with the production of auto antibodies and the activity of SLE. Our results showed a mutual participation of Th1 and Th2 cytokines in the disease.

TNF-α acutely up regulates the function of immune system, but following prolonged exposure, excessive TNF-α is immunosuppressive. We found that TNF-α levels were diminished as a function of disease activity, suggesting a possible protective role in SLE as has been previously reported [18, 19, 20]. TNF-α acts as a 2- face cytokine in SLE. First, it could be an immunosuppressive mediator, chronically produced as a defense mechanism or acting as a suppressor of autoantibody synthesis at

the T- lymphocyte level. Second, it might be a pro-inflammatory factor acutely released in the local tissues [21].

IL-10 is a B- cell stimulatory cytokine that also inhibits type 1 cytokine response [22, 23]. Several lines of evidence suggest that IL-10 plays a critical role in the immuno-pathogenesis of SLE. Although previous studies have suggested pathogenic roles for raised level of IL-10 in SLE, any involvement of this cytokine with specific clinical manifestation has remained unclear. The absence of a correlation between levels of IL-10 and active clinical subsets in SLE or overall disease activity scores is in agreement with previous studies [24, 25]. We observed a significant correlation between IL-10 levels and anti-DNA antibodies-which has been previously reported as supporting a role for IL-10 in active disease in SLE. Serum IL-10 levels have been shown to correlate with overall disease activity when serial measurements were taken in the same patients' overtime [26].

IFN-γ is produced principally by T cells, CD 4^+ as well as CD 8^+ and natural killer cells. Its main function is the activation of macrophages in innate and acquired response. Its activity increases in the presence of TNF-α and TNF-β [20]. In this study we observed significant increase of IFN-γ in patients than controls, although no association to disease activity was found.

Previous studies showed an increase in IFN-γ and IL-18 concentration, and there was a positive correlation between both [27] .IFN- γ showed a negative correlation with Th2 cytokines (IFN-γ with IL-4 and IL-10), $P<0.001$.

IL- 4 is produced by a subpopulation of activated T- lymphocytes (Th2). In our study, we did not find significant differences in IL-4 levels between SLE patients and controls, nor was there a statistical difference between the levels of this cytokine and disease activity. However, a direct correlation between IL-4 and the levels of Th1 cytokines was noticed. On contrary to other studies have shown similar results or diminished levels[28], suggesting that IL-4 does not have such a preponderant role in SLE.

Our results showed that Th1 as well Th2 cytokines can be elevated in SLE patients suggesting that lupus is a complex disease. That may be supported by different cytokine patterns in different time- points. In accord with the most recent literature we observed that a sharp distinction between Th1 and Th2 cytokine pattern is not feasible nor meaningful in SLE patients; either because the mechanism regulating this disease is too complex or because too many factors such as disease activity, treatment , organ involvement have to be taken into consideration [29] .Thus, to extend the findings of the present work, it would be necessary to design longitudinal studies to establish a cause- effect relationship, and to develop methods that not only evaluate cytokine levels, but also determine the function and regulation of their expression, including their gene polymorphism [20]. The results obtained should enhance our understanding of the immunology of SLE and other autoimmune diseases.

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