

SERUM LEVELS AND IN VITRO PRODUCTION OF Th1- AND Th2-TYPE CYTOKINES BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS SUFFERING FROM SYSTEMIC LUPUS ERYTHEMATOSUS

SERUMSKI NIVOI I IN VITRO PRODUKCIJA Th1 I Th2 CITOKINA U MONONUKLEARNIM ČELIJAMA PERIFERNE KRVI KOD BOLESNIKA SA SISTEMSKIM LUPUSOM ERITEMATODESOM

Vidosava B. Đorđević¹, Lilika Zvezdanović², Vladan Ćosić², Predrag Vlahović³, Slavica Kundalić², Tatjana Jevtović-Stoimenov¹, Bojana Stamenković⁴, Dragoslav R. Mitrović⁵

¹Institute of Biochemistry, Faculty of Medicine, Niš, Serbia

²Center of Medical Biochemistry, Clinical Center, Niš, Serbia

³Institute of Nephrology and Hemodialysis, Clinical Center, Niš, Serbia

⁴Institute for Rheumatic and Cardiovascular Diseases, Niška Banja, Serbia

⁵INSERM-U-606, Lariboisière Hospital, Paris, France

Summary: Th1-type and Th2-type cytokine profiles and adhesion molecules in the serum of patients suffering from systemic lupus erythematosus and the cytokine production by peripheral blood mononuclear cells (PBMC) were studied. Tumor necrosis factor-alpha (TNF- α), interferon-gamma (IFN- γ), interleukin-1 β (IL-1 β), IL-4, IL-10, IL-13, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were measured using ELISA technique in the sera of 16 systemic lupus erythematosus patients without vasculitis (SLE), 30 SLE patients with vasculitis (LV), and in 18 healthy controls. The cytokines were also measured in the culture media of unstimulated, concanavalin-A (Con-A) and phorbol-12-myristate-13-acetate (PMA) stimulated PBMC. TNF- α serum levels were significantly elevated in both SLE and LV patients and those of IL-1 β in SLE patients. TNF- α was also significantly increased in SLE compared to LV patients. Serum levels of all three Th-2 cytokines were significantly elevated in both SLE and LV patients compared to healthy controls. Serum IFN- γ and Th2 cytokine levels were significantly increased in patients with more active disease. Both ICAM-1 and VCAM-1 were significantly increased in SLE patients and only VCAM-1 in LV patients. ICAM-1 showed a significant correlation with IL-1 β , IFN- γ , IL-4 and IL-10 in both

Kratak sadržaj: U serumu bolesnika sa sistemskim lupusom eritematodesom određivani su Th1 i Th2 citokini i adhezioni molekuli a u kulturi mononuklearnih ćelija periferne krvi (PBMC) produkcija citokina. Faktor tumorske nekroze-alfa (TNF- α), interferon-gama (IFN- γ), interleukin-1b (IL-1 β), IL-4, IL-10, IL-13, interćelijski adhezioni molekul-1 (ICAM-1) i vaskularni ćelijski adhezioni molekul-1 (VCAM-1) mereni su ELISA tehnikom u serumu 16 bolesnika sa sistemskim lupusom eritematodesom bez vaskulita (SLE), 30 bolesnika sa SLE koji su imali vaskulitis i 18 zdravih kontrola. Citokini su, takođe, određivani u kulturi nestimuliranih kao i konkanavalinom-A (Con-A) i forbol-12-miristat-13-acetatom (PMA) stimuliranih PBMC. Serumski nivoi TNF- α su značajno povišeni kod obe grupe bolesnika, dok je IL-1 β povišen samo u grupi sa SLE. Serumski TNF- α je, takođe, značajno viši kod bolesnika sa SLE u poređenju sa LV grupom. Sva tri Th2 citokina su značajno viša kod obe grupe bolesnika u poređenju sa zdravim ispitanicima. Nivoi IFN- γ i Th2 citokina značajno su viši kod bolesnika sa težim oblikom bolesti. Kod bolesnika sa SLE značajno su povišene koncentracije oba adhezijska molekula, dok je kod bolesnika sa LV povišen samo VCAM-1. Kod obe grupe bolesnika postoji značajna korelacija ICAM-1 i IL-1 β , IFN- γ , IL-4 i IL-10. U grupi sa SLE VCAM-1 značajno kore-

Address for correspondence:

Prof. Vidosava B. Đorđević, PhD
Institute of Biochemistry, Faculty of Medicine
Bul. dr Zorana Đinđića 81, 18000 Niš, Serbia
Tel: ++381 18 232 210, Fax: ++381 18 238 770
e-mail: vidadjordjevic@yahoo.com

List of abbreviations: SLE – systemic lupus erythematosus; VEC – vascular endothelial cells; IL-1 β – interleukin-1 β ; TNF- α – tumor necrosis factor-alpha; IFN- γ – interferon-gamma; IL-4 – interleukin-4; IL-10 – interleukin-10; IL-13 – interleukin-13; ICAM-1 – intercellular adhesion molecule-1; VCAM-1 – vascular cell adhesion molecule-1; Con-A – concanavalin A; PMA – phorbol-12-myristate-13-acetate; PBMC – peripheral blood mononuclear cells; LV – lupus vasculitis; ACR – American College of Rheumatology; SLEDAI – SLE Disease Activity Index.

patient groups. In the SLE group VCAM-1 correlated significantly only with ICAM-1, but in the LV group only with IL-1 β and IFN- γ . Compared to healthy controls, basal TNF- α and IL-4 production by unstimulated PBMC derived from SLE patients were significantly increased. Con-A-stimulated PBMC of both SLE groups produced significantly more IFN- γ , IL-4 and IL-13 than Con-A-stimulated control cells. Con-A-stimulated cells derived from LV patients produced much more IFN- γ than cells from SLE patients. PMA strongly stimulated IFN γ , TNF α and IL-13 production by cells derived from both SLE groups but had no effect on IL-4 production. In addition, it had little if any effect on the production of IFN γ and IL-13 by PBMC derived from healthy donors. These findings suggest that the altered pattern of cytokine production by PBMC may play an important role in the SLE pathophysiology, accounting for differences in the clinical expression of the disease. The differences in adhesion molecules production and their correlation with cytokines suggest ICAM-1 and VCAM-1 as useful markers in SLE patients stratification.

Keywords: Th1/Th2 cytokines, adhesion molecules, serum concentrations, *in vitro* production, Con-A, PMA, systemic lupus erythematosus, lupus vasculitis

Introduction

SLE is a chronic inflammatory disease of unknown etiology which may involve multiple tissues and organs, leading to/or resulting from a disturbance in immune regulation characterized by excessive production of autoantibodies directed against various cell and tissue antigens (1). This immune disturbance seems to result from the interplay of genetic, environmental and hormonal factors (1, 2). Cytokines are locally produced factors acting as autocrine or paracrine agents involved in the regulation of immune and inflammatory reactions, as well as of other pathological processes associated with tissue destruction (3, 4).

Vasculitis is characterized by perivascular infiltration with proinflammatory cells and by damage to blood vessel walls. It may be the primary manifestation of a disease or secondary component of another primary disease, including SLE (5). Vasculitis, associated with SLE, seems to be mediated by the deposition of immune complexes resulting from the interaction of various autoantibodies, including antiphospholipid antibodies (6) and their cell- and tissue-based antigens (3, 4). Recently, it has been suggested that the interaction between vascular endothelial cells (VEC) and circulating DNA fragments may be a crucial event in lupus vasculitis. A DNA fragment is suspected to interact with adhesion molecules, ICAM-1 and VCAM-1 expressed on VEC and thus activate the production of proinflammatory cytokines (IL-6, IL-8, TNF- α , and IFN- γ) (7). ICAM-1 is an initial marker of inflammatory response induced by IL-1, TNF- α , or IFN- γ , whilst VCAM-1 production is regulated by TNF- α , IL-1, and IL-4.

lira samo sa ICAM-1, a u grupi sa LV sa IL-1 β i IFN- γ . U poređenju sa kontrolama, bazalna produkcija TNF- α i IL-4 od strane nestimuliranih PBMC bolesnika sa SLE je značajno povišena. Konkanavalinom-A stimulirane PBMC obe grupe bolesnika proizvodile su značajno više IFN- γ , IL-4 i IL-13 nego Con-A stimulirane ćelije kontrolne grupe. Konkanavalinom-A stimulirane ćelije bolesnika sa LV produkovale su mnogo više IFN- γ nego ćelije bolesnika sa SLE. U ćelijama obe grupe bolesnika PMA je izazvao jaku stimulaciju produkcije IFN- γ , TNF- α i IL-13, ali nije pokazao uticaj na produkciju IL-4. Isti stimulator pokazao je mali ili nikakav efekat na produkciju IFN- γ i IL-13 u PBMC zdravih osoba. Ovi nalazi sugerišu da poremećeni profil produkcije citokina u PBMC može imati značajnu ulogu u patofiziologiji SLE, čime se mogu objasniti razlike u kliničkom ispoljavanju bolesti. Razlike u produkciji adhezionih molekula i njihovoj korelaciji sa citokinima sugerišu da ICAM-1 i VCAM-1 mogu biti korisni markeri u stratifikaciji bolesnika sa SLE.

Ključne reči: Th1/Th2 citokini, adhezioni molekuli, serumske koncentracije, *in vitro* produkcija, Con-A, PMA, sistemski lupus eritematosus, lupus vasculitis

Proinflammatory cytokines TNF- α and IL-1 are known to induce the synthesis and expression of VEC adhesion molecules ICAM-1 and VCAM-1, ELAM-1, E selectin (8, 9), and the production of other proinflammatory cytokines, including chemotactic IL-8, and neutrophil degranulation factor (10, 11). They also activate proinflammatory cells and vascular endothelial cells to produce free radicals and NO, other cell-tissue damaging species (11). TNF- α and IL-1, secreted by VEC, may also attract and activate T lymphocytes, directing their aggressiveness toward the blood vessel wall.

The disturbed immune regulation, and upregulated production and expression of cytokines and adhesion molecules by immune and VEC are common to the pathogenesis of lupus vasculitis. This study was designed to evaluate the levels of Th-1 and Th-2 cytokines and adhesion molecules in the serum of SLE patients with particular interest in lupus vasculitis. Since cytokine levels in serum may not adequately reflect the cytokine-producing potential of immune cells, we also measured *in vitro* cytokine production by peripheral blood mononuclear cells (PBMC).

Patients and Methods

Patient selection

A total of 46 patients were diagnosed with SLE at the Institute for Rheumatic and Cardiovascular Diseases, Niška Banja, Serbia, between October 2002 and April 2004, and included in the study. Based on clinical manifestations of the disease, the

patients were subdivided into two groups: group I (SLE) – 16 patients without vasculitis (12 women and 4 men; mean age at admission 43 years, range from 20 to 72 years), and group II (LV), SLE with vasculitis – 30 patients (17 women and 13 men; mean age at admission 47 years, range 31–72 years). SLE patients fulfilled four or more of the revised American College of Rheumatology (ACR) classification criteria (12, 13). All patients were in an active phase of the disease with clinical manifestations, including fever, arthritis, malar or discoid rash, photosensitivity, hair loss, persistent discoloration of the fingers, glomerulonephritis, psychoneurological disorders and others. These were accompanied by laboratory abnormalities (proteinuria, leucopenia, anemia, thrombocytopenia, increase in auto-antibodies and complexes, significant decrease in complement components). In addition, group II vasculitis patients presented with ulcers and gangrenes. In six LV and in two lupus nephritis patients neuropsychiatric manifestations were detected. SLE disease activity was measured using the SLE Disease Activity Index (SLEDAI) (14, 15). SLEDAI between 6 and 13 was scored as mild or moderate disease, and above 13 the disease was scored as severe.

All patients were examined and disease evaluated by qualified physicians (internists and rheumatologists) and possess documented medical files. Blood was obtained by vein puncture from all patients and 18 healthy control individuals (11 women and 7 men, aged 19 to 65 years). The control group included volunteers randomly selected at the Institute of Blood Transfusion, Niš. None of the control participants reported any history of acute or chronic disease. Informed consent was obtained from all subjects. Serum was prepared in a routine way. It was stored at -20°C for a period of two weeks before it was used for assays. In addition, heparinized blood samples were also taken from 20 patients with SLE (10 patients without and 10 with vasculitis) and 6 healthy controls. The blood was immediately processed for PBMC isolation and culture.

Isolation and culture of human PBMC

In order to assess cytokine production, PBMC were isolated from 10 mL of freshly drawn heparinized (50 IU/mL) whole blood by density gradient centrifugation on a Histopaque-1077 (Sigma, Germany), according to manufacturer's recommendations. The cells were washed twice by centrifugation with RPMI 1640 culture medium (Sigma, Germany) and resuspended in the same medium at a density of 10^6 cells/mL. The culture medium consisted of RPMI 1640 supplemented with a 10% heat-inactivated fetal calf serum, 25 mmol/L HEPES buffer, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. The 0.5 mL of cell suspension was distributed to the wells of a 24-well tissue culture plate. The cells were incubated at 37°C in humidified 5%

CO_2 and 95% air atmosphere. They were stimulated with 10 $\mu\text{g}/\text{mL}$ concanavalin A (Con-A; Serva, Germany), known as a stimulator of T lymphocyte proliferation (15), or with 10 ng/mL phorbol-12-myristate-13-acetate (PMA; Sigma, Germany), known as a potent activator of phosphokinase C (PKC) (16). Control cultures received an equivalent amount of the vehicle used to dissolve Con-A or PMA. The incubation was allowed to proceed for the next 72 h. Finally, the cells were drawn by centrifugation. The clear supernatants were aspirated and stored at -20°C until assayed.

Cytokine and adhesion molecules assays

Cytokines and adhesion molecules were determined by ELISA technique with commercially available kits. IL-1 β , TNF- α , IL-10, ICAM-1 and VCAM-1 kits were obtained from R and D Systems (Abingdon, Oxon, UK). IFN- γ kits were from Bender Med System Diagnostics (Vienna, Austria), and IL-4 and IL-13, were from Beckman Coulter, Immunotech (Marseille, France). Cytokines were determined in the sera and culture media according to manufacturers' instructions. The sensitivities for individual assays are as follows: TNF- α , < 4.4 pg/mL; IL-1 β , 1 pg/mL; IFN- γ , 1.5 pg/mL; IL-4, < 2 pg/mL; IL-10, < 0.5 pg/mL; IL-13, 1.5 pg/mL.

Data analysis

Data were analyzed using both the Mann-Whitney Rank Sum Test (for medians and range determination) and the standard computer SPSS program including ANOVA test with Post Hoc analysis, Tukey HSD and Dunnett »t« test to evaluate significant differences between groups. Differences were considered significant at $p < 0.05$.

Results

Patient characteristics

Table 1 reports the clinical and therapeutic characteristics of 16 patients classified as SLE without vasculitis and 30 patients suffering from LV. In 26 LV patients vasculitis was confirmed by skin or kidney biopsy. In 6 LV patients neurological (headache, epileptic seizures, polyneuropathy) or psychiatric disorders (confusio-oid syndrome) were diagnosed. According to SLEDAI, all patients were classified as having mild or moderate SLE (SLEDAI scores ranged from 5 to 13), or severe SLE (SLEDAI scores ranged from 13 to 30). At the time of inclusion 89% of all patients were being treated with corticosteroids with doses ranging from 10 to 70 mg per day, 39% were being treated with cytostatics (methotrexate, immuran, cyclophosphamide), and 39% with antimalarial drugs. Certain patients also received some other symptomatic therapy including antihypertensive, analgesic or cardioprotective drugs.

Table I Baseline, clinical and therapeutic characteristics of patients with SLE and LV.

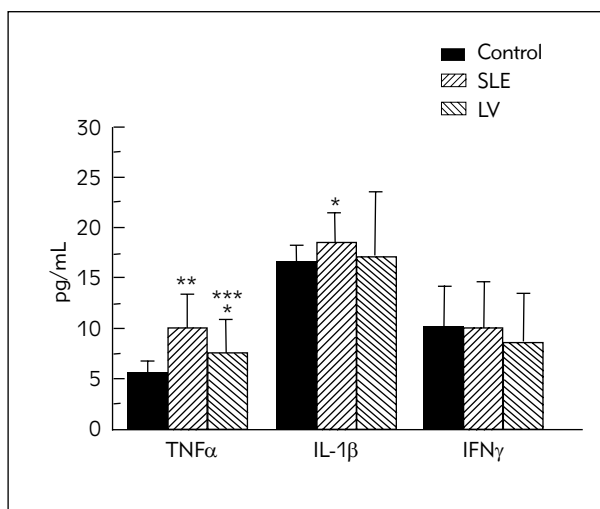
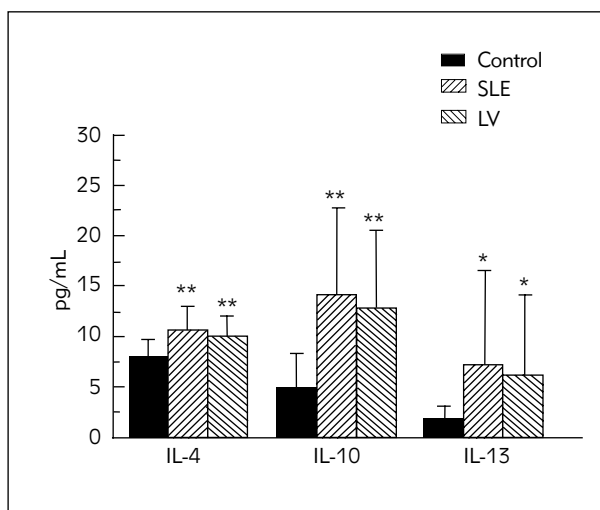
	SLE	LV
Age, years	20–72	31–72
Sex	12 f 4 m	17 f 13 m
Disease duration, years	14–33	1–13
Malar and/or discoid rash, %	44	63
Photosensitivity, %	26	35
Arthritis, %	87	60
Serositis, %	0	10
Renal disorder, %	12	20
Hematological disorders, %	50	40
Fever, %	50	26
Raynaud, %	12	23
Ulcerations, %	0	33
Gangrene, %	0	17
Neurological disorders, %	13	24
Psychosis, %	0	11
ANA test, %	31	40
C ₃ ↓, %	25	17
Corticosteroids, %	94	86
Cytostatics, %	38	40
Antimalarial drugs, %	50	33

Cytokine serum concentrations

Proinflammatory Th-1 and antiinflammatory Th-2 cytokine profiles in the sera of patients with SLE and with LV are shown in *Figure 1* and *2*, respectively. IFN- γ serum levels were similar in all three groups. TNF- α concentrations were significantly increased in both SLE (9.6 ± 3.3 pg/mL; $p < 0.001$) and LV patients (7.3 ± 4.7 pg/mL; $p < 0.05$) compared to healthy controls (5.8 ± 1.2 pg/mL). Also, the values in SLE group were significantly increased compared to LV group ($p < 0.05$). IL-1 β was slightly but significantly increased (18.8 ± 2.8 pg/mL; $p < 0.05$) in SLE patients, only compared to healthy controls (16.8 ± 1.7 pg/mL).

As shown in the *Figure 2*, serum concentrations of all three investigated antiinflammatory cytokines (IL-4, IL-10 and IL-13) were significantly increased in both patient groups ($p < 0.001$ for both IL-4 and IL-10, $p < 0.05$ for IL-13) compared to healthy controls. Comparing cytokine serum concentrations as related to therapy, TNF- α was significantly increased in patients receiving cytostatics and corticosteroids as compared to patients treated with corticosteroids and antimalarial drugs ($p < 0.05$). Serum IFN- γ , IL-4, IL-10 and IL-13 concentrations were also significantly increased in patients suffering from more severe disease (*Figure 3*).

Serum ICAM-1 levels were significantly elevated in SLE patients in comparison with both the controls

**Figure 1** Serum levels of Th1 cytokines in patients with SLE and LV. Mean values \pm SD are presented. * – $p < 0.05$ vs. control; ** – $p < 0.001$ vs. control; *** – $p < 0.05$ vs. SLE.**Figure 2** Serum levels of Th2 cytokines in patients with SLE and LV. Mean values \pm SD are presented. * – $p < 0.05$ vs. control; ** – $p < 0.001$ vs. control.**Table II** Serum adhesion molecules levels in patients with SLE and LV.

Group	n	ICAM-1 (ng/mL)	VCAM-1 (ng/mL)
Control	18	239.5 \pm 21.7	428.8 \pm 76.2
LV	30	240.3 \pm 18.0	495.3 \pm 77.2**
SLE	16	254.3 \pm 13.2*,***	514.2 \pm 69.9**

The results are presented as mean \pm SD.

* – $p < 0.05$ vs. controls

** – $p < 0.01$ vs. controls

*** – $p < 0.05$ vs. LV

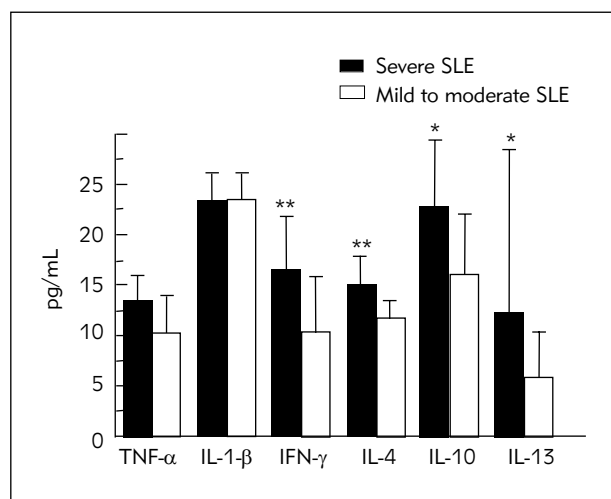


Figure 3 Serum levels of Th1 and Th2 cytokines in patients with different clinical severity of SLE. Mean values \pm SD are presented. * – $p < 0.05$ vs. mild to moderate SLE, ** – $p < 0.001$ vs. mild to moderate SLE.

Table III Correlation between adhesion molecules and cytokines.

Group		IL-1 β	IFN- γ	IL-4	IL-10	ICAM-1
SLE	ICAM-1	$r=0.502$ $p < 0.01$	$r=0.390$ $p < 0.05$	$r=0.392$ $p < 0.05$	$r=0.642$ $p < 0.01$	
	VCAM-1					$r=0.393$ $p < 0.05$
LV	ICAM-1	$r=0.420$ $p < 0.01$	$r=0.285$ $p < 0.05$	$r=0.517$ $p < 0.01$	$r=0.534$ $p < 0.01$	
	VCAM-1	$r=0.382$ $p < 0.01$	$r=0.314$ $p < 0.05$			

($p < 0.05$), and LV patients ($p < 0.05$). VCAM-1 was significantly increased in both patient groups compared to controls ($p < 0.01$) (Table II). A significant correlation was found between ICAM-1 and IL-1 β , IFN-g, IL-4 and IL-10 in both patient groups (Table III). In the LV group VCAM-1 significantly correlated with IL-1 β and IFN-g. In the SLE group there was no significant correlation between VCAM-1 and cytokines, but this adhesion molecule significantly correlated with ICAM-1 ($p < 0.05$).

Cytokine production by PBMC

The INF- γ and TNF- α production by cultured PBMC are presented in Figure 4 and those for IL-4 and IL-13 in Figure 5. From Figure 4 it is apparent that INF-gamma basal production by unstimulated PBMC shows small if any significant variations in all groups, whereas that of TNF- α is significantly increased in patients suffering from SLE ($p < 0.01$). Con-A-activated cells produced significantly greater amounts of INF- γ , IL-4 and IL-13 compared to Con-

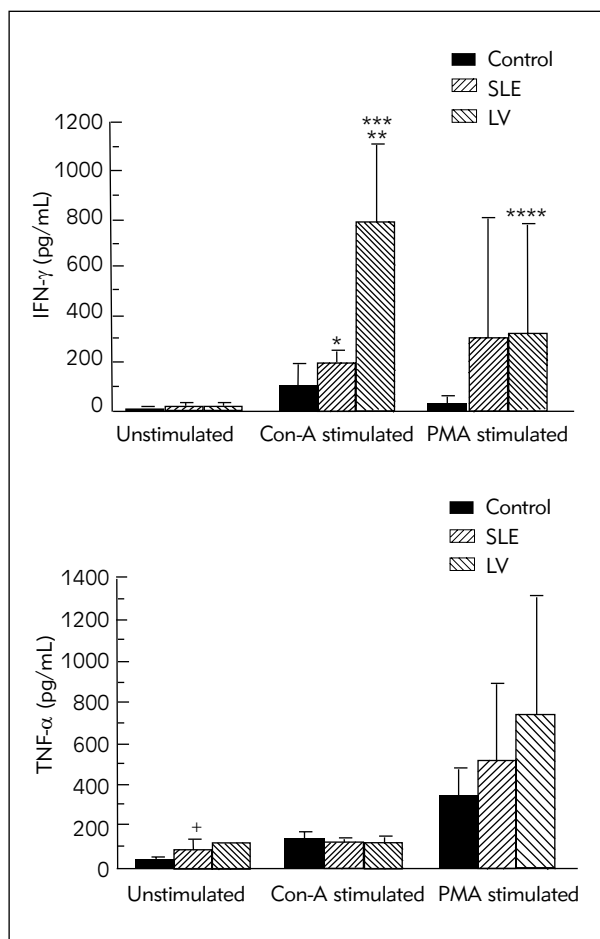


Figure 4 Spontaneous and stimulated production of inflammatory cytokines by PBMC from SLE and LV patients and healthy controls. Unstimulated and Con-A (10 μ g/mL) and PMA (10 ng/mL) stimulated PBMC were cultured for 72 hours, and the concentrations of TNF- α and INF- γ in the supernatant were measured by specific ELISA. Mean values \pm SD are presented. * – $p < 0.05$ vs. Con-A stimulated control, ** – $p < 0.001$ vs. Con-A stimulated control, *** – $p < 0.0001$ vs. Con A stimulated SLE, **** – $p < 0.05$ vs. PMA stimulated control, + – $p < 0.05$ vs. unstimulated control group.

A-treated controls. It also increased the TNF- α production only in healthy controls. With the exception of TNF- α the cells derived from patients suffering from SLE responded much better to Con-A than cells from healthy controls ($p < 0.01$ for both SLE groups). Also, Con-A-treated cells derived from LV patients produced significantly more INF γ than their counterparts derived from patients suffering from SLE without vasculitis ($p < 0.05$).

PMA stimulated the production of INF- γ , TNF- α and IL-13, but failed to stimulate IL-4 production. The PMA-activated cells derived from SLE-affected patients produced significantly more INF- γ and IL-13 than the activated cells derived from healthy donors. It failed to stimulate significantly the IL-4 production by any type of cells.

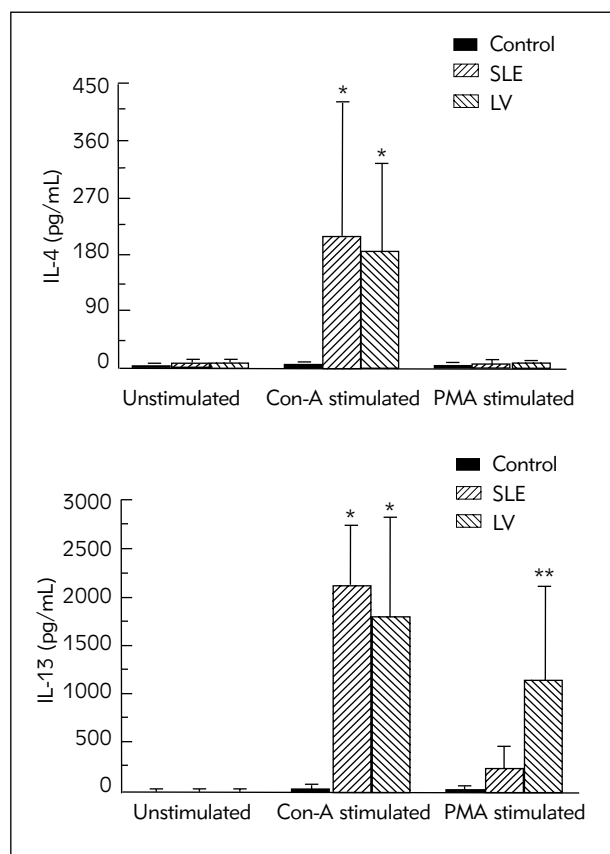


Figure 5 Spontaneous and stimulated production of anti-inflammatory cytokines by PBMC from SLE and LV patients and healthy controls. Unstimulated and Con-A (10 $\mu\text{g}/\text{mL}$) and PMA (10 ng/mL) stimulated PBMC were cultured for 72 hours, and the concentrations of IL-4 and IL-13 in the supernatant were measured by specific ELISA. Mean values \pm SD are presented. * $-p < 0.05$ vs. Con-A stimulated control, ** $-p < 0.05$ vs. PMA stimulated control.

Discussion

To our knowledge, this is the first study reporting the pro- and anti-inflammatory cytokine pattern in the sera and culture media of PBMC derived from both SLE patients without and with vasculitis, and from healthy donors. Although the cytokine profiles in patients suffering from SLE have been studied extensively, the results were mostly related to TNF-alpha, IL-1 beta and IL-10. To date the cytokine profile in LV has not been investigated in spite of the opinion that the cytokine pattern in SLE is associated, to a certain extent, to clinical manifestations of this disease (2, 17).

The opinions about TNF- α crucial role in the pathogenesis of SLE are conflicting. Based on findings in both animal models and humans, previous studies have suggested that this cytokine could be involved in SLE pathophysiology as an inductive or even protective mediator (18–20). Moreover, certain studies reported that serum TNF- α levels in patients

suffering from SLE do not differ from those of healthy controls (21–24). This was explained by the fact that TNF- α molecules are bound to circulating soluble TNF- α receptors, that occur in abundance in patients with SLE (19, 20, 24–28). In contrast to these findings, we found a significant increase in the serum levels of TNF- α in SLE patients compared to healthy controls and, also, in SLE patients without vasculitis as compared with those suffering from vasculitis. Furthermore, cultured PBMC derived from SLE patients produced, under basal conditions, ten times more TNF- α than did the cells from healthy controls. This finding is in agreement with the results of another study (22), demonstrating an increased number of circulating lymphocytes expressing mRNA specific for TNF- α and IFN- γ in patients suffering from SLE and presenting with neurological and psychiatric symptoms. Interestingly, Con-A failed to stimulate TNF-alpha production by PBMC derived from SLE patients. Although PMA stimulated PBMC to produce TNF-alpha, no significant differences were observed between the three groups.

With respect to IFN-gamma conflicting findings have been reported in SLE patients. The IFN- γ serum concentrations and its production by PBMC were found in some studies to be increased (28, 29), unchanged (23, 30) and decreased (31, 32). In this study, serum IFN- γ levels in SLE patients were not significantly different neither from those of healthy controls nor LV patients. However, IFN- γ seems to be increased in the sera of patients suffering from more severe SLE. Also, stimulated-PBMC from SLE patients produced significantly more IFN- γ compared to cells from healthy controls, as well as Con-A-stimulated cells derived from LV patients compared to SLE without vasculitis. Since IFN- γ was not elevated in patients' sera this finding suggests that *in vivo* patient PBMC were not activated to produce this cytokine.

The results published for IL-1 β have also been controversial. This proinflammatory cytokine was detected in increased amounts in serum (33, 34) and in lipopolisaccharide-stimulated PBMC (34) from patients with SLE, but also its expression and production by PBMC were found to be decreased or unchanged compared to control healthy subjects (36, 37). In the present study, IL-1 β was found to be slightly but significantly increased only in the sera of SLE patients. Failure to demonstrate IL-1 β increase in an active SLE vasculitis may be due to the increased production and release of the soluble IL-1 β receptors (34, 36–39) that have not been investigated here.

The proinflammatory cytokines, including TNF- α and IL-1 β , have been detected in increased amounts in the sera and blood vessel walls of patients suffering from various types of vasculitides such as Wegener's granulomatosis, Kawasaki disease and Behcet disease, conditions characterized by perivascular granulomatous infiltration with macrophages and CD4+ and CD8+ T lymphocytes (4).

Also, we measured the three Th2-antiinflammatory cytokines, IL-4, IL-10, and IL-13. In our patients all three cytokines were found to be significantly increased in serum and their concentrations positively correlated with disease activity. Although the IL-10 occurrence in SLE is well documented, scarce information, reporting conflicting results, is available in the literature related to IL-4. The serum levels of this cytokine, to our knowledge, have not been reported. However, its production by PBMC was reported to be increased in CD8(\pm) cells (31, 34), unchanged (30) or decreased (29) in SLE, compared to control PBMC derived from healthy subjects. In this report, IL-4 basal production by PBMC derived from SLE patients was slightly, but significantly, increased in comparison with that of control cells. Con-A-stimulated PBMC from SLE patients also produced significantly more IL-4 compared to the stimulated control cells. Surprisingly, PMA which is known to activate protein kinase C (16) with a subsequent rise in intracellular Ca and activation of other important biochemical pathways, such as increased production of prostaglandins and free radicals, failed to increase significantly the production of IL-4 in all three types of culture. This finding suggests that the PKC activation is not implicated in IL-4 production by human PBMC.

Previous studies have reported increased IL-10 serum concentrations (23, 32, 41–43) and its spontaneous production by mononuclear cells derived from patients suffering from SLE (29–32, 44). These increased IL-10 levels were often (41, 43) but not always (23, 33, 42) found to correlate with disease activity or certain serological findings. Based on IL-10 suppressive effects on Th-1 type cytokines its therapeutic administration, or its upregulated expression may be beneficial in patients suffering from lupus vasculitis (45).

IL-13 is a pleiotropic cytokine with a wide spectrum of actions including the inhibition of production of a series of Th-1 cytokines. Serum IL-13 levels were significantly higher in both active and inactive SLE patients as compared with normal controls, and were also correlated with SLEDAI (46). Significantly higher serum levels of IL-13 correlated with those of rheumatoid factor, but not with any other antinuclear autoantibody, total immunoglobulin levels or the main clinical features of the disease (47). In SLE patients with high levels of IL-12 serum levels of IL-13 were significantly lower than in patients with normal IL-12 levels (48). The determination of the IL-12/IL-13 ratio showed that IL-12 levels may be above, equal to or below IL-13 levels and the latter suggests a predominance of Th2 that could drive the autoantibody production in SLE (49). In this study, the serum levels and production of this cytokine by stimulated PBMC were found to be significantly increased in SLE. In addition, significantly increased production by PMA-stimulated PBMC derived from LV patients compared to SLE patients without vasculitis has been found.

However, in our patients, elevated serum IL-13 and IL-10 levels and their increased production by PBMC were not associated with decreased serum levels or production of TNF-alpha, as one would expect based on down-regulatory and suppressive effects of these two cytokines on Th-1 counterparts.

Our findings regarding the failure of Con-A and PMA to stimulate TNF-alpha and IL-4 production, respectively, could not be attributed to an altered cell response in SLE, since control PBMC reacted in the same way. However, several reports have described altered reactivity of PBMC or T lymphocytes from patients suffering from SLE when stimulated with T-cell mitogens or PMA (42, 45, 50). This was not investigated in this study, but deserves further consideration.

Adhesion molecules, ICAM-1 and VCAM-1, are essential for cell-cell interactions, being also important in cell activation and adhesion (51). Soluble adhesion molecules may be detected in patient sera as useful markers of leucocytes and endothelial cells activation in different human diseases, including SLE and LV. Serum levels of ICAM-1 found were significantly elevated (more than 2 SD above the mean in normal controls) only in 7 of 35 patients with SLE (52). In this study, ICAM-1 was found significantly increased in SLE patients, and VCAM-1 in both patient groups. Only in SLE patients there was a significant correlation between ICAM-1 and VCAM-1. These findings suggest that ICAM-1 and VCAM-1 may be useful markers in SLE patients stratification into SLE patients without vasculitis and SLE patients suffering from vasculitis.

A significant correlation between VCAM-1 and IL-1 β and IFN-g observed in LV patients was another difference between the patient groups. This correlation indicates a stronger activation of p65 subunits of NF-kB in LV patients, which is involved in a specific regulation of VCAM-1 gene expression (53).

In conclusion, the serum cytokine profile and *in vitro* response of PBMC of both patient groups significantly differ from controls. TNF- α is the only serum cytokine differing between the SLE and LV group. The PBMC derived from LV patients respond strongly to mitogen stimulation by increasing INF- γ and IL-13 production. These different cytokine SLE and LV patient profiles may contribute, at least in part, to different clinical features of SLE, pointing to more specific cytokine-based modulation therapy in the control of the disease. The results related to adhesion molecules suggest that ICAM-1 and VCAM-1 may be useful markers in SLE patient stratification.

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