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THE INVESTIGATION OF CYTOKINES AND OXIDATIVE STRESS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

PROUČAVANJE CITOKINA I OKSIDACIONOG STRESA KOD BOLESNIKA SA SISTEMSKIM LUPUSOM ERITEMATODESOM

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Summary: Numerous factors can influence the onset of SLE and development of some clinical disease manifestations with various organ involvements and occurrence of characteristic symptoms and disease signs. This paper studies the balance between proinflammatory and antiinflammatory cytokines, investigates the presence of oxidative stress measuring certain prooxidative factors and determines the activation of antioxidative protection pathways aiming to establish possible correlations between the studied parameters. ELISA, enzymatic spectrophotometry and colorimetric methods were used to determine the above-mentioned parameters. The results obtained indicate that disturbed pro/antioxidative status is associated with the change of antioxidative factors, with the fall od SOD activity and increase of GPx and CAT activity in the erythrocytes of all studied groups of patients. At the same time, the cytokine production was altered, not only compared to the healthy control samples, but also in various clinical disease manifestations. Altered relationships of pro and antiinflammatory cytokines and the consequential disorders of other studied systems provide us with useful strategic targets for diagnostic monitoring and possible therapeutic interventions in SLE patients.

Keywords: cytokines, antioxidative enzymes, systemic lupus erythematosus, autoimmune diseases

Kratak sadržaj: Mnogobrojni faktori mogu uticati na nastanak sistemskog lupusa eritematodesa (SLE) i razvoj pojedinih kliničkih manifestacija bolesti sa zahvatanjem različitih organa i pojavom karakterističnih simptoma i znakova bolesti. U ovom radu je ispitivana ravnoteža između proinflamatornih i antiinflamatornih citokina, proučavano je postojanje oksidacionog stresa merenjem određenih prooksidacionih činilaca i određivano aktiviranje mehanizama antioksidacione zaštite sa ciljem proučavanja eventualnih korelacija između ispitivanih parametara. Za određivanje pomenutih parametara korišćene su ELISA, enzimske spektrofotometrijske i kolorimetrijske metode. Dobijeni rezultati pokazuju da poremećaj pro/antioksidacionog statusa prati promena antioksidacionih činilaca sa padom aktivnosti SOD i porastom aktivnosti GPx i CAT u eritrocitima svih ispitivanih grupa bolesnika. Istovremeno je izmenjena i produkcija citokina, ne samo u odnosu na zdrave kontrolne uzorke već i u različitim kliničkim manifestacijama oboljenja. Promene odnosa pro i antiinflamatornih citokina i njihovi posledični poremećaji ostalih ispitivanih sistema omogućavaju dobijanje vrednih strateških ciljeva za dijagnostičko praćenje i eventualno terapijsko intervenisanje kod bolesnika sa SLE.

Ključne reči: citokini, antioksidacioni enzimi, sistemski lupus eritematodes, autoimunske bolesti

Introduction

Autoimmune diseases, the prototype of which is systemic lupus erythematosus (SLE), are characterized by autoimmune reactions directed towards one's own widely present determinants. Though numerous self-antigens may be involved in an autoimmune disease, these diseases can occur as the result of an abnormal immune reaction against only one

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antigen present in various organs. Numerous studies, primarily on experimental animals, confirm that autoimmune diseases can be the result of a wide range of genetic and immunologic disorders, which may occur earlier or later in life, depending on the action of promoting endogenous or exogenous factors. Gene studies done so far have indicated that a »well« combined mixture of genes of susceptibility and protection can have an impact on the development of autoimmune diseases (1). Studies on twins have demonstrated that in many high risk individuals there is no evident disease development (2, 3). When a deleterious event initiating disorders has happened, later development of autoimmune diseases is characterized by chronic and indolent inflammation, which is markedly different from the tempo of most host immune responses to infectious agents. Incomplete penetration of genetic risk can be explained by different epigenetic events. In particular, the presence of autoreactive T cells and antibodies is not sufficient to induce an autoimmune disease, but additional immune disorders are required as well.

Cytokines play an important role in the induction and regulation of autoimmune diseases. They mediate the expansion and differentiation of T helper (Th) cells in order to generate autoantigen-reactive pathogenic and protective effectors which support the production of autoantibodies by autoreactive B cells, but they are also involved in the mediation of tissue damage in the target organ. Cytokines form a central co-ordinating network of soluble effector molecules, which has a key role in each phase of the development of autoimmune diseases: generation of pathogenic or protective effectors, transmission, ie. recruitment of pathogenic cells in the target organ, mediation of tissue damage or tissue tolerance in the target organ (4). It is believed that, out of all immune system cells, autoreactive CD4+ T cells have the most important role in the induction and regulation of autoimmune diseases. Mossman et al. have suggested that naïve Th cells are differentiated after activation into separate functional groups, characterized by a pattern of cytokine secretion (5). Nowadays we know that this differentiation is not only into Th1 and Th2 cells, but some other subgroups can be identified as well, such as Th3 and T regulatory 1 (Tr1) cells (5). However, some effector T cells have combined cytokine secretion patterns and cannot be classified into any of the described Th categories. Studies on transgenic T cells have demonstrated that cells with identical T cellular receptors (TCR) possess the potential to differentiate into various phenotypes and that these cells can have different effects on autoimmunity dependent on their state of differentiation.

Four essential characteristics contributing to the pathogenicity of autoreactive T cells are: 1. the nature of the target antigen, 2. epitope specificity, 3. cytokine profile, and 4. expression of particular surface molecules (adhesion molecules, chemokine receptors, death receptors). In cases of antibody-mediated diseases, such as SLE, myastenia gravis (MG) or some forms of rheumatoid arthritis (RA), for the disease to be induced the expansion of CD4+ T cells and autoreactive B cells is required, producing pathogenic antibodies of the appropriate isotype (6, 7).

The difference between cytokines as phenotype markers and cytokines as inflammation and tissue damage mediators should be stressed. Disease-inducing or protecting T cell phenotypes have been extensively studied in experimental autoimmunity models, while the data on human autoimmune diseases are scarce since the pathogenic effects of T cells cannot be tested (6–8). In certain autoimmune diseases, Th1 cells producing some particular cytokines have been mentioned as pathogenic effectors, but in others there are indications that an autoimmune disease is mediated by Th2 cells producing IL-4, IL-10 and IL-13. In order to properly understand autoimmunity, the central question is: how a Th precursor cell (Thp) acquires its cytokine profile during expansion and differentiation? Comprehension of the fundamental mechanisms of T cell differentiation control is the road to the strategy of modulation of the cytokine profile and prevention of tissue damage and autoimmune diseases, promoting therefore protection from these (9).

Cytokines in systemic lupus erythematosus

SLE is a prototype of systemic autoimmune diseases. It is a chronic, inflammatory, multisystem disorder of the connective tissue with a general population incidence of 1/2000, the activation of which is the result of a combination of environmental »triggers« in the context of genetic susceptibility. During the reaction to a foreign antigen, an immune system undergoes dramatic proliferation and expansion. Prolonged periods of immune response to external/ environmental factors such as UV radiation or viral infection can also cause immune system dysregulation. It is thought that SLE pathogenesis is a three-phase process with a loss of tolerance and antibody production, generalized increase and dysregulation of the immune system and destruction of the target tissue or organs mediated by direct antibody binding and deposition of immune complexes (10).

Many cytokines participate in SLE activity and involvement of various organs. It is known that cytokine production in SLE patients significantly differs from the production in healthy controls and patients with other diseases, such as RA. It should be noted that cytokine production is not just altered in SLE patients compared to healthy controls, but it is different in different disease phenotypes. IL-6 is thus most commonly increased in the cerebrospinal fluid of patients with changes of the central nervous system

(CNS) in SLE, but not in those without neurologic symptoms (11). Similar to other systemic diseases, cytokine balance is of more significance in phenotype and disease severity determination than in susceptibility determination.

Free radicals and antioxidative enzymes in systemic lupus erythematosus

In addition to cytokines which have an important role in the induction and regulation of autoimmune diseases, free radicals have an important role in the production of autoantibodies out of self-reactive B cells and in the mediation of tissue damage in the target organ. Considering the underlying pathology and up-to-date knowledge of the pathophysiologic mechanisms of SLE development, it is supposed that in these patients in the acute disease phase there is an increase of oxidation processes. The results of numerous studies have demonstrated that free radicals play a major role in cell damage and that their production is increased in various pathologic conditions such as inflammatory diseases, ischemic-reperfusion tissue damage, immune-mediated injury and cancerogenesis. Free radicals, via their oxidative action, are able to damage all biomolecules. Oxidative injury to nucleic acids may induce mutations and cancerogenesis (12), while the oxidation of the protein thiol groups alters their structure and function (13). The connective tissue function can also be altered via the alteration of hyaluronic acid structure under the action of free radicals. Deleterious effects are most pronounced in cellular biomembranes and cell organelles, where a chain, autocatalytic process is taking place, altering the structure and function of the membrane – lipid peroxidation.

In the peripheral blood of SLE patients there are signs of increased superoxide anion production and intense lipid peroxidation on one hand, and reduced activity of tissue antioxidative enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) on the other. The measured values of the intensity of oxidative process indicate the presence of »oxidative stress« in SLE patients. The term has been introduced to account for the disbalance in physiologic conditions between the oxidative processes and antioxidative system.

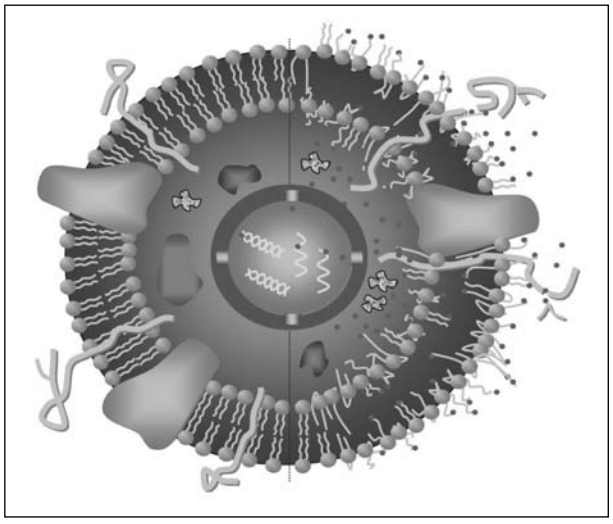

Figure 1 Free radical-induced cell damage.

Blood cells, in addition to immunocompetent cells and macrophages, are involved in the process of inflammation associated with SLE as an autoimmune disease. Through the exchange of variously directed messages aided by cytokines, the activation is created of various metabolic pathways within them, providing thus the propagation or limitation of inflammatory processes. Cytokines and thrombocyte activation factor via the activation of particular enzyme systems – phospholipase A_2, myeloperoxidase, NO synthase and receptor systems on blood cells, endothelial cells and macrophages, lead to the production of inflammation mediators, such as leukotrienes and prostaglandins, microcirculation disorders and immune effects with the production of immunoglobulins and various reactive oxidation metabolites (14). In addition to other oxidases, one of the powerful free radical generators is the enzyme xanthine oxidase (XO). Systemic lupus erythematosus may lead to microvasculature disorders and, consequently, to partial or total ischemia of the portions of tissue or organs. Restoration of circulation after antiinflammatory treatment results in tissue reperfusion and release of free oxygen radicals, associated with intense activity of xanthine oxydase. Consequently released neutrophils may lead to the occlusion of local vasculature inducing further ischemia and release of new quantities of free oxygen radicals in the reaction with endothelial cells (15).

In view of the vulnerability of many cellular structures in the organs which may be affected by SLE, in the conditions of accumulation of inflammatory cells in the region affected by inflammation, lipid peroxidation process intensity is increased. This autocatalytic process is the consequence of the action of reactive oxygen species or cytokines, regardless of whether it is spontaneous or controlled by the enzymes of arachidonic acid cyclooxygenase pathway. As the result of lipid peroxidation in chronic inflammation associated with SLE, the concentration of malondialdehyde (most commonly studied lipid peroxidation product) is markedly increased (16–18).

Material and Methods

We used the biologic material of the SLE patients treated at the Institute for Prevention, Treatment and Rehabilitation of Rheumatic and Cardiovascular Diseases »Niška Banja« and the Clinic of

Neurology, Clinical Centre Niš. Diagnosis and classification of the patients were done according to the list of 11 clinical and laboratory criteria suggested by the American Rheumatology Association (ARA) (19). Some additional SLE-diagnostic tests were utilized too: pathohistologic study of biopsy material, videocapillaroscopy, computerized tomography, electroencephalography. Complete hematologic, biochemical and immunologic laboratory processing of the biomaterial after the previously mentioned methods, enabled classification of SLE patients (n=55), 47 men and 8 women, aged 42±20 years, into the following groups: patients with predominant skin disease manifestation, S-SLE, n=17; patients with neurolupus, N-SLE, n=5; patients with predominant joint changes, J-SLE, n=11; patients with blood vessel changes – vasculitis, V-SLE, n=22. Twenty healthy volunteers, blood donors, comprised the control group.

Concentration of proinflammatory cytokines (TNF-α, IFN-γ, IL-1β), antiinflammatory cytokines (IL-4, IL-10, IL-13) and adhesion molecules, intercellulary adhesion molecule (ICAM) and vascular cellular adhesion molecule (VCAM) was determined by commercial ELISA tests (Quantikine Immunoassay Kit, R&D, Minneapolis, USA; BioSource International Camarillo, USA).

SOD activity was determined indirectly based on the competition reaction of SOD and indicators of superoxide anion radical produced in the xanthin-xanthin oxidase system. Ransod (Randox) test was applied on a multi-channel analyzer Olympus AU400. Commercial Ransel (Randox) test was used to determine the activity of glutathione peroxidase (GPx) on the Olympus AU400 analyzer. The test is based on the Paglia and Valentine method (20). To determine catalase activity in the erythrocytes we used the traditional Beutler (21) method, while the same enzyme in the serum was measured according to the kinetic-spectrophotometric method by Goth (22).

Results

Numerous factors take part in the determination of disease activity and involvement of various organs in SLE patients. This investigation is the review of the role of particular pro- and antiinflammatory cytokines and possible pathways of their action in particular organs; at the same time, it tries to assess the degree of produced oxidative stress and consequential activation of antioxidative protection mechanisms.

Cytokines and adhesion molecules are important products of cell activation with the key role in the process of intercellular signaling. In the process of development of autoimmune diseases, the network of pro- and antiinflammatory cytokines has special significance. To the group Th1 belong the proinflammatory cytokines IL-1β, IFN-γ and TNF-α, while to the Th2 group belong the antiinflammatory cytokines IL-4, IL-10 and IL-13.

Table I Values of IL-1β and IFN-α in the blood of SLE patients.

Group	n	IL-1β (pg/mL)	IFN-γ (pg/mL)
Control	20	16.65 ± 1.03	10.32 ± 5.52
S-SLE	17	18.13 ± 3.24	10.97 ± 4.75
N-SLE	5	18.62 ± 2.20	10.98 ± 3.12
J-SLE	11	17.86 ± 1.70	8.50 ± 2.94
V-SLE	22	17.68 ± 6.42	10.93 ± 1.77

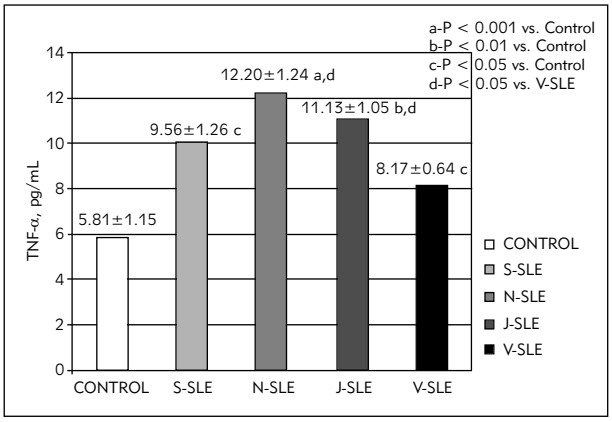

Figure 2 TNF-α concentration in the plasma of SLE patients.

Table I presents the values of proinflammatory cytokines IL-1β and IFN-γ. These two parameters do not demonstrate any significant differences compared to controls.

TNF-α, a cytokine primarily created by activated macrophages, possesses the ability to influence numerous target cells. In this study, we have recorded a statistically significant increase of this cytokine related to controls (*Figure 2*). The increase was at its highest in patients with neurolupus ($p<0.001$) and joint disease ($p<0.01$), while skin and vascular forms were of lesser significance ($p<0.05$). Comparing the groups, we noticed significant TNF-α increase in joint and neurolupus related to vascular SLE ($p<0.05$). It is believed that the increase of TNF-α is spontaneous and in accordance with disease eruptions, which means that it is a parameter characteristic for the acute disease phase.

IL-4, secreted by Th2 type lymphocytes, mast cells and basophilic leukocytes, demonstrated a statistically significant increase in neurolupus patients, 11.96 ± 2.91 pg/mL, and vascular lupus, 10.93 ± 1.77 pg/mL, compared to control values 8.97 ± 1.90 pg/mL for $p<0.05$ (*Figure 3*). IL-4 induces an inflammatory reaction rich in monocytes and eosinophilic leukocytes.

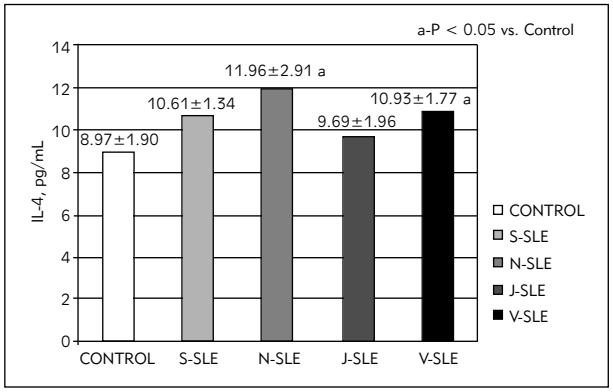

Figure 3 IL-4 concentration in the plasma of SLE patients.

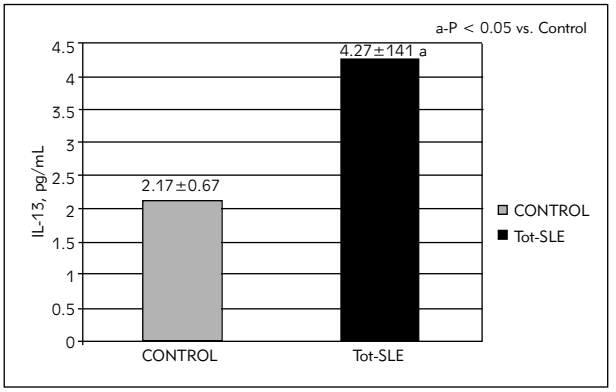

Figure 5 IL-13 concentration in the plasma of SLE patients.

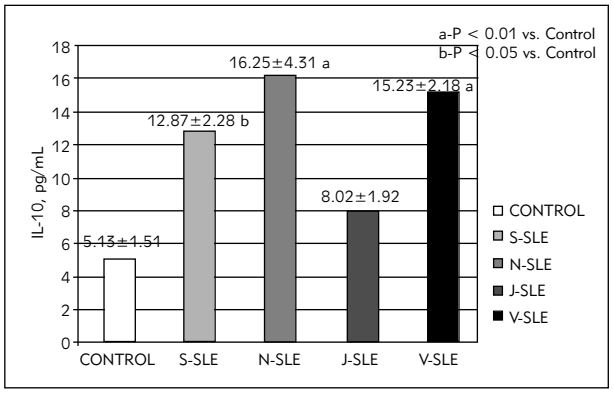

Figure 4 IL-10 concentration in the plasma of SLE patients.

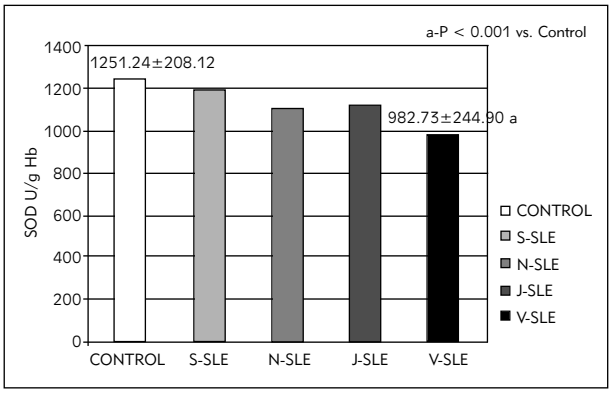

Figure 6 SOD activity in the erythrocytes of SLE patients.

The results of IL-10 concentration measurements are shown in *Figure 4*. The increase of the concentration is of statistical significance in neurolupus patients (16.25 ± 4.31 pg/mL) and in vascular disease (15.23 ± 2.18 pg/mL) compared to controls (5.13 ± 1.51, for $p<0.01$) and skin disease (12.87 ± 2.28 pg/mL), with somewhat lower significance of $p<0.05$.

Interleukin-13, as the product of Th2 cells, has a role in the regulation of monocyte, macrophage and B cell function. It demonstrates homology with IL-4 and inhibits the production of proinflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α. *Figure 5* presents the increase of IL-13 concentration in the whole SLE group (4.27 ± 1.41 pg/mL) related to controls (2.17 ± 0.67 pg/mL) at the level of significance of $p<0.05$.

Intercellular adhesion molecules (ICAM), as one of the integral parts of cell-cell and cell-matrix receptors and initial markers of inflammatory response, demonstrate only a slight increase without statistical significance compared to control values. As for the vascular cellular adhesion molecule (VCAM) produced by endothelial cells, there is a marked increase in all SLE patient-groups related to controls ($p<0.05$).

We assessed the antioxidative response by measuring the activity of the following enzymes: SOD, GPx and CAT in the plasma and erythrocytes. The activity of SOD, the enzyme which affects dismutation of two superoxide anion radicals into less reactive hydrogen peroxide, is markedly reduced in patients with vascular disease form, 982.73 ± 244.90 U/gHb, ($p<0.001$) compared to healthy controls, 1251.24 ± 208.12 U/gHb, while in other groups there were no significant changes (*Figure 6*). The graph presents the average values of this parameter.

Glutathione peroxidase is an especially important enzyme of the glutathione redox cycle in the protection system from reactive metabolites. The enzyme is significantly elevated in skin, M=30,11 U/gHb, joint M=30,31 U/gHb and vascular disease form M=31,43 U/gHb ($p<0.01$), as well as in neurolupus M=29,77 U/gHb, ($p<0.05$), related to control values (*Figure 7*). The graph shows median values.

Catalase is an antioxidative enzyme with significant effects in the reduction of free radicals production. Determination of the activity of the same enzyme in erythrocytes demonstrated elevated values related to controls, 8.89 ± 1.38, with the same degree of significance ($p<0.001$). *Figure 8* shows the average values of catalase in erythrocytes: S-SLE=11.57 ± 1.84 U/gHb × 10^4; N-SLE=12.19 ± 1.60 U/gHb × 10^4; J-SLE=11.49 ± 1.52 U/gHb × 10^4, V-SLE=11.67 ± 1.28 U/g Hb × 10^4. We obtained

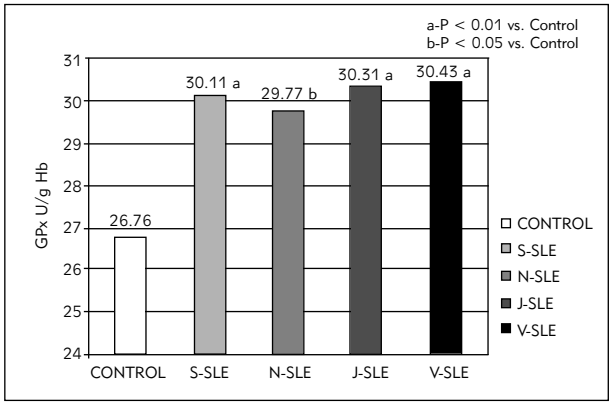

Figure 7 GPx activity in the erythrocytes of SLE patients.

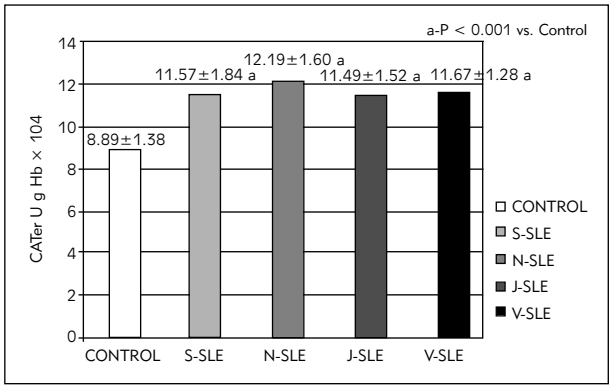

Figure 8 CAT activity in the erythrocytes of SLE patients.

statistically significant elevated values of catalase in the plasma compared to control values in healthy examinees ($p<0.001$). S-SLE has the value of M=96.14 kU/L; neurolupus M=96.32 kU/L; joint disease form M=95.60 kU/L and vascular disease M=102.90 kU/L. The control value was M=39.70 kU/L.

Discussion

The balance between proinflammatory (Th1) and antiinflammatory or immunosuppressive cytokines is of special significance in the onset of SLE. These signaling molecules have a central role in the induction and regulation of autoimmune diseases. It is believed that they are principal mediators in the expansion and differentiation of Th cells, acting towards the generation of autoantigen-reactive pathogenic or protective effectors. Moreover, cytokines control tissue tolerance and prevent the advancement of inflammation within organs. Together with the selectin and integrin molecules, cytokines control the transport and storage of auto-reactive cells in target organs. They thus form a kind of a coordination network which has a central role in each phase of SLE development. They participate in the generation of pathogenic or protective effectors, transport and recruitment of pathogenic cells and tissue damage or tolerance mediation in the target organs.

SLE patients are characterized by the reactivity of B cells, which produce a whole set of antibodies. Namely, these are the autoantibodies and antibodies against other exogenous antigens. The help of T cells, required for antibody production, is mediated by cytokines. When there is a tolerance and autoantibody production drop-off, it is quite probable that cytokines play their role in the process. There is a question whether cytokines are involved in tolerance drop-off via the production of α-ds DNA antibodies. It is thought that the acute phase response components, C reactive protein (CRP) and serum amyloid protein (SAP), participate in the tolerance to apoptotic fragments. Particularly CRP and SAP are bound to circulating DNA and RNA, making them non-immunogenic. Acute phase protein release from the liver is induced by proinflammatory cytokines, IL-6, IL-1 and TNF-α, promoting thus the removal of circulating autoantigens. This assumption is further corroborated by the fact that in SLE patients producing low levels of TNF-α nephritis onset is more probable as a complication related to the presence of anti-ds DNA antibody (23).

It was established that some SLE patients have relatively low levels of TNF-α. The ratio of TNF and soluble TNF receptor is of key significance since the receptor acts as a TNF inhibitor, reducing its biologic activity. TNF-α can be protective in SLE patients (24). In numerous SLE models it has been demonstrated that the pathologic activity of TNF-α and IL-1 is determined by the dose and stage of disease activity. We determined the TNF-α value in 55 patients with different clinical manifestations of SLE in the acute relapse phase. A statistically significant increase of this cytokine in the plasma was observed compared to controls in all studied patient groups, which correlates with the results of Borodin et al. (25) and Aringer and Smolen (26). The highest increase was observed in those with neurolupus (N-SLE) and joint disease manifestations (J-SLE), and a lesser significance was noted in patients with skin (S-SLE) and vascular (V-SLE) forms of the disease.

It is thought that particular haplotypes of TNF-α gene are associated with lower production of this cytokine. They are present in SLE families in Great Britain, Sweden, and in some patients in Greece (27). These haplotypes are associated with lower production of TNF-α by monocytes. Our investigated patients most probably do not belong to this haplotype – they have a strong TNF-α response. The protective role of this cytokine is also described. The confirmation of its protective role came from the studies of TNF-α and its soluble receptor TNF-sR2 in the plasma of 9 patients with active lupus nephritis – the values obtained were higher compared to control samples. The method of immunofluorescence on the

biopsy material helped proving that TNF-α protein can be deposited along the damaged vascular endothelium, and in patients with lupus nephritis along the glomerules and tubules. By *in situ* hybridization and RT-PCR amplification, local expression of this cytokine was demonstrated, meaning that it can be locally synthesized as well (28).

Neurolupus patients have elevated values of TNF-α produced by the lymphocytes in peripheral circulation. The results of this paper indicate that TNF-α can be of special importance in the N-SLE pathology. Very important is the question whether the process of inflammation in N-SLE is intrathecal or systemic. The number of cells in the cerebrospinal fluid (CSF) can be very low, so that mRNA for the production of TNF-α, IFN-γ, IL-4 and IL-10 cannot be detected in the lymphocytes of SLE patients (29). These results differ from the previous studies, where in the patients with other systemic diseases, such as MS, elevated IFN-α and IL-10 were observed. It indicates the possibility that passive diffusion or passage through the damaged blood-brain barrier can induce high cytokine concentration in the intrathecal space. Therefore, a valid possibility is that peripheral inflammatory mediators such as TNF-γ induce intrathecal inflammation. Interleukin-1 and TNF-γ released from inflammatory cells act synergistically in the circulation, inducing peripheral vasodilatation, increase of vascular permeability and alteration of endothelial function favoring thrombosis.

Interferon γ has a significant immunomodulatory role, with a marked antiviral effect and a role in the cell growth regulation. It is an integral part of the cytokine network, with synergistic action with some cytokines, while in relation to IL-4 it has a clear antagonistic action. In our results we can observe that there is no significant elevation of IFN-γ in view of the above-mentioned antagonism with the Th2 response cytokines, which demonstrate significant elevation. According to the literature data, IFN-γ production by peripheral mononuclear cells significantly correlates with the global disease activity score (SLEDA I). In its proliferative response to IL-4, B cells can be inhibited by IFN-γ, confirming antagonistic actions of these two cytokines. Though IFN-γ can induce increased production of antibodies by all or by some B cells, its principal effect is inhibitory and T cell-dependent (30).

Numerous studies indicate that genetic regulation of IL-10 is of significance in most SLE patients and that production of this interleukin is controlled on the transcription level. Serum levels of IL-10 in SLE are elevated, which can mainly be attributed to increased production by monocytes, part of B cells and CD4+ CD45 RO+ memory T cells. Studies of twins and families demonstrate that around 75% of variations in IL-10 production are genetically conditioned. In the families with more than 2 members with SLE, it was established that IL-10 production by mononuclear cells is increased in healthy relatives as well. However, if only 1 family member has SLE, there is no such increase of IL-10 production. Genetic regulation of IL-10 is essential in most SLE patients. Serum levels of this cytokine are increased in SLE patients (31) compared to healthy controls, which was proven by our results as well. IL-10 was increased in all studied groups, especially in neurolupus, skin and vascular manifestations of the disease, while the changes were most unconspicuous in SLE with joint manifestations.

Increased IL-10 can be attributed to its increased production in monocytes, part of B cells and $CD4^+CD45RO^+$ memory T cells. Literature data indicate positive correlation of IL-10 with the titre of antids DNA antibodies and SLEDA I score, while the correlation with the level of C_3 complement fraction is negative (32). In healthy subjects IL-10 stimulates B cell proliferation and IgG synthesis. It also influences the release of soluble TNF receptors and inhibits ICAM 1 expression.

Increase of IL-10 is associated with neuropsychiatric manifestations of the disease, which correlates with our results. Neurolupus (N-SLE) was present in our study with 9.1% of the patients. In the literature, three IL-10 haplotypes were described: GCC, ACC, ATA. In patients with neuropsychiatric manifestations, APA haplotype was present in 30% of the cases, which is markedly higher compared to control samples (10%) and non-psychiatric SLE forms (17%). GCC haplotype was present in a significantly lower percent (33). Some experimental models have demonstrated that immune complex generation can affect nerve structures and induce neurolupus. Pathogenetic mechanism in human pathology can be similar to this experimental model of the disease. IL-10 can be to a significant extent responsible for increased production of immune complexes in patients with neuropsychiatric SLE manifestations.

Interleukin-10 controls inflammatory processes suppressing the expression of proinflammatory cytokines, chemokines, adhesion molecules, as well as antigen-presenting and co-stimulatory molecules in monocytes, macrophages, neutrophils and T cells. A large number of inflammatory proteins suppressed by IL-10 is controlled by NF-κB, which indicates that antiinflammatory features of this cytokine are the result of inhibition of this transcription factor. NF-κB activity can be inhibited through two mechanisms. High doses of IL-10 lead to a significant increase of p105 and p50 proteins and selective nuclear transcription of p50. IL-10 treatment inhibits high constitutive secretion of IL-6 and MIP-2α in p105/p50. NF-κB is an exclusive transcription factor in IL-6 induction, produced in response to TNF-α action (34). Investigations in this direction can help in the better understanding of the mechanisms through which IL-

10 controls inflammatory processes and can contribute to the development of new therapeutic approaches in the surveillance of chronic inflammatory and autoimmune processes such as SLE.

Interleukin-4 is a pleiotrophic cytokine primarily produced in activated B lymphocytes, mast cells and basophils. It exerts a multiple immune response since it modulates the function of various cell types. It is an important modulator of T precursor cell differentiation into Th2 response, and thus participates in the humoral immunity and production of antibodies. Its antitumoral activity has been proven *in vivo* and *in vitro*. The gene for IL-4 on the chromosome 5q is close to the genes for IL-3, IL-5, IL-13 and GM-CSF, which may explain the similarity in around 30% of amino acid sequences.

Approximately 1/3 of SLE patients have cutaneous (skin) disease form as a photosensitive erythematous »butterfly« rash with malar distribution. Many of the cases may have alopecia (35). In this study, 30.9% of the patients had skin disease form. Cutaneous manifestations have been commonly initiated by the exposure to UV radiation (36). Experimental studies with UV components of solar irradiation demonstrate that the 320–400 nm wave length light induces accumulation of reactive oxygen species, which suggests that free radical action is involved in the photosensitivity in SLE (37). Patients with this form of lupus more commonly have m-RNA for IL-2, IL-4 and IL-5 in the specimens of skin biopsy, that cannot be detected in healthy controls. The findings suggest the role of Th2 type cells in the skin pathology of SLE. The results of this paper demonstrate a significant increase of this cytokine in SLE. The increase is most striking in N-SLE and V-SLE, while in S-SLE it is not so evident. In joint disease form there were no statistically significant changes.

Interleukin-13 is a T cell derivative regulating the function of monocytes, macrophages and B cells. It demonstrates 25–30% of structural homology with IL-4. During the Th2 response, it influences the recruitment of eosinophils in the tissues, acts upon endothelial cells and induces VCAM-1. Our results demonstrate a significant increase of IL-13 in SLE patients related to control values, which agrees with the results of Xu and Chen (38). It demonstrates the most striking increase in N-SLE and Z-SLE, while it is less marked in skin and vascular disease forms. It seems probable that the most severe forms of SLE induce intense production of antiinflammatory cytokines.

Transcription of all cytokine genes, the gene for IL-13 as well, requires cooperative and synergistic action of specific transactivating elements. Some factors, such as NFκB, induce transcription of multiple cytokine genes, but lately factors have been identified which are limited in their expression to Th1 and Th2 cells. A transcription factor identified as GATA-3 is Th2 specific and increases transcription of most Th2 cytokines, including IL-4, IL-10 and IL-13.

Adhesion molecules, vascular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), are essential for cellular interactions with a significant role in cellular activation and adhesion. Soluble adhesion molecules can be detected in the plasma as very useful and usable markers of leukocyte and endothelial cells activation in various diseases such as SLE, vasculitis and rheumatoid arthritis.

Inhibitors of cytokine production are being extensively studied as potential therapeutic agents in various immunologic and inflammatory diseases. Cytokine studies have two important applications in the treatment of autoimmune diseases. The first would be the creation of important disease markers, disease progression markers and markers of treatment efficacy. It is of utmost importance to assess the impact of possible therapies on cytokine regulation using the model of autoimmune disease and to assess the elements of possible efficacy in patients. The second application refers to the identification of targets for specific interventions. The application may refer to the targeting of one or more effector cytokines in order to inhibit target organ damage or to enhance normal immunoregulation mechanisms. The second approach is more advantageous since this is the way to inhibit recruitment and expression of new pathogenic T cell clones from the natural pool of naive Thp cells (39).

Free radical induced damage has a very important role in the pathogenesis of autoimmune diseases. Phagocytes, as an important part of the immune system, are the first line of defence against various pathogens. They ingest and destroy opsonized extracellular pathogens, secrete proinflammatory cytokines and generate various reactive oxygen species (ROS) in the process known as respiratory explosion. In ideal circumstances, the ingestion of pathogens, their opsonization and secretion of ROS, enable removal or prevent their further spreading. Secreted proinflammatory cytokines and chemokines recruit immune system cells in the protection against a pathogen. Low concentrations of intracellular ROS can be useful if they activate transcription factors such as NFκB, which is involved in the regulation of production of inflammatory mediators, such as TNF-α, IL-1β and a number of metalloproteinases. There is the question what is the role of reactive oxygen species and how they influence the setting-up of immune response in SLE.

Extreme production of free radicals can have a significant role in SLE pathogenesis, where it can influence reduced SOD activity. The imbalance between proinflammatory factors and antioxidative enzymes such as SOD, CAT, GPx, is associated with active

disease phase and has a key role in SLE pathophysiology. The results of this study suggest reduced SOD activity, intensively spent in the reaction of dismutation of superoxide anion radical or binding to SOD-antibodies. SOD can be inactivated with hydrogen peroxide which acts upon the creation of Cu(II)-OH or its ionized form (40). At the same time, due to creation of high concentrations of hydrogen peroxide as the substrate for GPx and CAT, we may observe an increase in the activity of these enzymes and their synthesis may be compensatory or absent as the result of induction by some cytokines (41). Reduced SOD activity is most evident in vascular lupus, while GPx and CAT increase of activity is present in all disease forms. The elevation of values of conjugated diens and MDA (6.97 vs. 4.17 nmol/μL) as well as the reduction of SOD activity and presence of antibodies binding SOD have all been described in the paper by Kurien and Scofield (42). The presence of antibodies 1 against SOD is most probably the cause of damage in systemic lupus erythematosus.

There is an ample body of primary data regarding the role of various cytokines in the induction and regulation of autoimmunity; however, a clear consensus has not been reached. This investigation aimed at the synthesis of available and new information on the role of cytokines in autoimmunity and it should be of help in learning and understanding the ways cytokines and oxidative stress are involved in various clinical SLE manifestations. The comprehension of complex interactions between the studied parameters is aimed towards the development of specific cytokine immunomodulatory therapy.

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