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THE SIGNIFICANCE OF CYTOKINES IN DIAGNOSIS OF AUTOIMMUNE DISEASES

Lilika Zvezdanović[1], Vidosava Đorđević[1], Vladan Ćosić[1], Tatjana Cvetković[3], Slavica Kundalić[1], Aleksandra Stanković[2]

[1]Centre of Medical Biochemistry, Clinical Centre – Niš, Serbia
[2]Institute for Prevention, Treatment and Rehabilitation of Cardiac and Rheumatic Patients »Zelengora«, Niška Banja, Serbia
[3]Institute of Biochemistry, Faculty of Medicine, Niš, Serbia

Summary: Autoimmune diseases are characterized by autoimmune reactions against one's own widespread determinants. Many cytokines are involved in activity regulation and organ involvement in various autoimmune diseases. It is well known that some tissues maintain a very high »entry barrier« concerning the development of immune-mediated inflammation, which leads to the state of »immune privilege« through the generation of specialized microenvironment. There are different patterns of cytokine synthesis in particular autoimmune diseases such as rheumatoid arthritis, type I diabetes, systemic lupus erythematosus and multiple sclerosis, and worth stressing is the difference between cytokines as phenotype markers and cytokines as inflammation and tissue damage mediators. In most autoimmune diseases the balance between proinflammatory and antiinflammatory cytokines determines the extent and spread of inflammation and can lead to conspicuous clinical effects. In SLE patients, for instance, we observed a significant elevation of TNF-α and IL-10 in all, but especially in neurologic disease form. Understanding of the fundamental mechanisms of T cell differentiation control is the road to the strategy of cytokine phenotype modulation and prevention of tissue damage and autoimmune diseases, promoting naturally the protection from them.

Key words: autoimmune diseases, cytokines, systemic lupus erythematosus, TNF-α, IL-10

Introduction

Autoimmune diseases are disorders with complex etiology, involving initiating external factors, the so-called »triggers«, which interact with the background of clearly present polygenic susceptibility. Gene studies done so far have indicated that »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

Address for correspondence:

Dr sc. med. Lilika Zvezdanović
Centre of Medical Biochemistry, Clinical Centre – Niš
Bul. Dr Zorana Đinđića 48
18000 Niš

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 (5) have suggested that naive Th cells are differentiated after activation into separate functional groups, characterized by a pattern of cytokine secretion. 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 in 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. After activation by a certain ligand, naive Th cells differentiate into Th1 cells secreting interleukin-2 (IL-2) and interferon-γ (IFN-γ), which in turn activate macrophages and incite delayed-type hypersensitivity reactions, or Th2 cells which produce IL-4, IL-10 and IL--13, significant for IgE production and suppression of cell-mediated immunity.

Four essential characteristics contributing to the pathogenicity of autoreactive T cells are: 1. the nature of 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 systemic lupus erythematosus (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 antibody of the appropriate isotype (6, 7). Cytokine profile acquired during expansion and differentiation by an autoreactive T cell is the key one for the outcome of autoimmune response. If a T cell acquires directly a pathogenic profile or one which supports autoreactive B cells in their production of pathogenic antibodies, tissue damage and induction of autoimmune disease will result. On the other hand, if during their expansion autoreactive T cells develop a non-pathogenic cytokine profile, pathogenic T cell response will not be generated and the ultimate outcome could perhaps be the organ protection from tissue damage and prevention of autoimmune disease (4).

The difference between the 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 autoimmune disease is mediated by Th2 cells producing IL-4, IL-10 i 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, therefore promoting protection from these (9).

T cell activation is a complex process dependent on the activation of the T cell receptor (TCR) which binds peptide complexes on the surface of antigen-presenting cells, but also on the co-stimulatory signals, such as CD28 signals. Blocking of the CD28 stimulation ameliorates the pathogenic response in many murine models of autoimmunity and in other immune system-related diseases (10). T cells produce a long list of cytokines in response to activation, which can be divided into several groups depending on the time of production and the basis of their ultimate function.

Cytokine receptor binding on the surface of T cells by IL-4 and IL-12 acts in concord with the signals from T cell receptors (TCR), which leads to the induction of specific transcription factors required for T cell differentiation. IL-12 induces STAT-4 (signal transducer and transcription activator) after binding IL-12 receptors in Thp cells, which is essential for IFN-γ production. IL-4 induces STAT-6 after binding IL-4 receptors on Thp, which helps IL-4 gene transcription. These STAT transcription factors do not act alone but in combination with the signals originating from TCR and through the chromatin remodeling process which induces opening of specific cytokine loci.

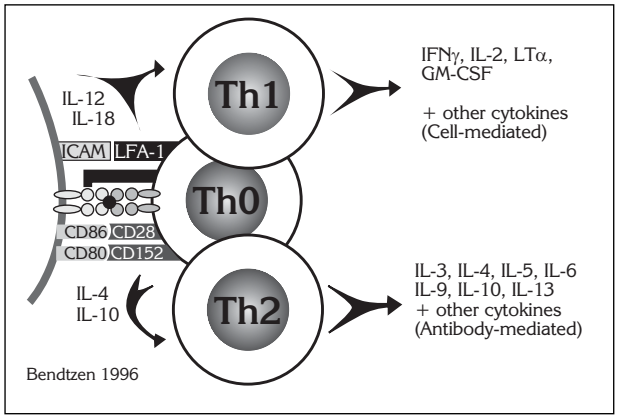

Figure 1 Th differentiation

Transcription of cytokine genes depends on the synergistic action of multiple and cytokine-promoter specific trans-activating elements involved in gene transcription. While some transcription factors, such as NF-κβ, induce transcription of multiple cytokine genes, recent investigations have identified specific transcription factors limited in their expression to Th1 and Th2 cells. GATA-3 is a Th2-specific transcription factor increasing transcription of most Th2 cytokines, including IL-4, IL-5, IL-10 i IL-13. One of the members of the T-box family of transcription factors, the so-called T-bet, has a powerful effect on IFN-γ production and suppresses IL-4 and IL-5 production. Also, T-bet can suppress GATA-3 and *vice versa* during Th2 development (11). Transcription factor regulation and chromatin remodelling represent molecular mechanisms which trigger and maintain T cell differentiation by cytokines.

As proven in experimental models, interventions aiming at the blockade of various cell surface molecules influence T cell phenotype and disease development. These effects can be the result of the changes in general signal potency or the qualitative signal effects from co-stimulators on T cell differentiation. One of the most significant influences on the cytokine balance, influencing T cell differentiation, is the CD28/CTLA-4-B7-1/B7-2 co-stimulation mechanism (12).

Th1 cells and their cytokines have an important role in the induction of a number of autoimmune diseases. Above all, these are SLE and MS, as antibody-mediated autoimmune diseases with strong Th1 autoimmune response component (8, 13). However, this does not mean that Th2 cells cannot induce autoimmune diseases and that Th2-dependent antibodies cannot induce tissue damage. Investigations of the cytokine expression in various inflammatory target tissues have demonstrated an increased expression of Th1 cytokines in experimentally induced autoimmune diseases and in biopsy specimens of different tissues in rheumatoid arthritis (RA) patients, Cron's disease, type I diabetes and multiple sclerosis (MS) (7, 8, 14). In order to establish whether a cytokine is directly pathogenic, its *in vitro* effect on different cell types has been studied, its action has been blocked by antibodies *in vitro,* cytokine expression has been increased by transgenesis *in vivo* in a general or tissue-specific manner, or the cytokine gene has been knocked out. The results have demonstrated that cytokines, though they can be very toxic, very often are not definitive mediators of tissue destruction by cytokine-activated macrophages. Naturally, disease development depends on the T cells differentiated under Th1 phenotype conditions, but the role of effector cytokines is essential as well, since they are key factors for tissue destruction and can be different in different diseases. TNF blockade can be very useful in RA treatment, while in MS patients it can aggravate the disease. These differences are the result of the different response of target tissues during the influx of inflammatory lymphocytes. Target tissues have different degrees of sensitivity to cytokine action. Bone and cartilage are often more damaged by cytokines which activate bone resorption than oligodendrocytes which are damaged by direct cytotoxicity.

Cytokines in type 1 diabetes

It is believed that several factors, including genetic associations and autoantibody presence, are involved in type 1 diabetes. The role of human leukocyte antigen (HLA) has been most extensively studied for susceptibility of HLA-DR3 and HLA-DR4 or resistence of HLA-DQ8 to this disease (15–18). In the evaluation of the risk of the disease, antibody titre determination to islet T cell antigens, such as glutamin decarboxylase 65, insulin and other antigens (2), has special significance.

The function of autoreactive T cells and their reactivity in type I diabetes has not been fully elucidated. Cytokines largely influence the immunologic function, initiation and progression of the disease. Abundant data indicate the key role of lymphocytes and macrophages in β cell damage and development of insulin-dependent type 1 diabetes (IDDM). Therefore, β cellular destructive insulitis is associated with increased expression of proinflammatory cytokines IL-1, tumor necrosis factor (TNF-α) and IFN-α, as well as Th1-type cytokines (IFN-γ, TNF-β, IL-2 and IL-12). On the other hand, non-destructive, benign insulitis is associated with increased expression of Th2 cytokines (IL-4, IL-10) and Th3 cytokine TGF-β (19, 20). Changes in the Langerhans' islets are characteristic mononuclear cell infiltrations. In animal models of diabetes, macrophages are the first to appear in a significant number as a sign of insulitis, and then T lymphocytes as well – together they induce cytokine release which influences the type and intensity of the immune response.

After β cell protein are released, they are absorbed by antigen-presenting cells (APC) in Langerhans' islets and transformed into antigenic peptides. This induces IL-1 and TNF secretion by APC and augmentation of co-stimulatory signals, which induces the expression of lymphokine genes in Th lymphocytes and synthesis of IFN-γ (21). IFN-γ stimulates IL-1 and TNF secretion. Increased IL-1 production is cytotoxic to β cells since it induces free radical production within the islets (22). The results of the human studies on the role of cytokines in diabetes type 1 have so far been very scarce. Functional experiments have been performed in order to evaluate the effects of IL-1 and TNF on pancreatic islets in animal models and in human subjects. It has been demonstrated that IL-1 selectively inhibits insulin secretion in α cells, but not glucagon secretion in β cells. These effects of IL-1 are supported by the action of addi-

tional cytokines, such as TNF-α, and other inflammatory mediators, such as free radicals. From numerous studies it can be deduced that cytokines, such as Il-1, TNF-α, TNF-β and IFN-γ, disturb insulin secretion, especially when added in experimental models in combinations of two or three.

The latest studies demonstrate that newly diagnosed IDDM patients have significantly higher levels of IL-2, IFN-γ, TNF-α and IL-1 in their blood compared to healthy controls, patients with insulin-independent type 1 diabetes and long-term DM patients. Various results related with TNF-α serum levels have been described (23–26). Several reports have demonstrated that Th2-type response in type 1 diabetes is reduced and that generalized IL-4 secretion defect may occur (27, 28).

Cytokines in rheumatoid arthritis

Rheumatoid arthritis (RA) is a disease which affects mainly diarthrodial joints, with synovial tissue inflammation associated with destruction of adjacent cartilage and bone and typical erosions as an ultimate effect and one of the diagnostic criteria for RA. The disease is fairly common and very serious, and analysis of the process of synovial inflammation is a matter of intense investigation in view of the fact that synovial tissue and fluid are easily accessible for detailed analysis. What is the reason for the selectivity of inflammation in RA and what are the mechanisms responsible for inflammation regulation? It is believed that selective T and B cellular immunity is responsible for tissue specificity, while cytokines and other regulatory molecules determine the intensity and character of inflammation. Latest findings suggest that the expression and regulation of cytokines and other regulatory molecules should be studied separately in inflammatory diseases affecting different organs. Also, the so-called »targeting« of regulatory molecules can be of great value in selective immunotherapy.

Especially significant in the study of RA pathogenesis is a detailed examination and description of the expression of the most important cytokines. Recent reports demonstrate wide variations of TNF-α expression in the inflamed synovium in different patients and differences in dependence on TNF-α in disease pathogenesis (29). Also, the relative role of various cytokines varies in different disease stages, which has been demonstrated in animal models of RA. Disease chronicity is regulated by the genes which do not affect disease susceptibility and intensity of inflammation in early phases. In order to understand the process dynamics, it is essential to analyze synovial biopsy specimens in different disease stages. One of the hypotheses is that T cells and T cell cytokines are less significant in RA due to a relatively small amount of T cell cytokines in rheumatoid synovitis. The evidence of certain pathogenetic RA principles obtained *in vivo* are related to human pathology as well, which has special significance in the process of introduction of targeted therapies against particular cells or cytokines.

TNF-α has a central role in the cytokine network, regulating the synthesis of other proinflammatory cytokines, IL-1, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (30, 31). It stimulates collagenase and production of prostaglandine E, inducing cartilage and bone destruction (33, 34) in cell culture or by osteoclast activation. TNF-α is one of the basic mediators of pathology in arthritis, expressed in synovia during the acute phases of antigen-induced arthritis (34). Investigations of collagen-induced arthritis have demonstrated that systemically applied TNF-α increases incidence and severity of the disease, while anti-TNF-α antibody offers protection from this type of arthritis (35).

In contrast to TNF-α, IL-1β expression occurs later in the course of the disease (36). An injection of recombinant IL-1β can increase disease incidence, while high doses of an IL-1β receptor antagonist prevent its development and delay the disease onset. Proinflammatory effect of IL-1 in RA reflects in the production of prostaglandin E and collagenase in the synovial fluid (33). Stimulation of proteoglycans, synthesis of glucosaminoglycans and metalloproteinase-1 are also the described effects of IL-1. It leads to increased expression of collagen type 1 and 3 in chondrocytes, induces fever and synthesis of acute phase proteins. In manifest disease, most efficient is the therapeutic combination of anti-IL-1α and anti-IL-1β, reducing inflammation and destruction of the cartilage, and the treatment ameliorates both early and developed disease. According to the latest data, IL-1 has a significant role in cartilage destruction, while TNF-α influences joint swelling. Antibodies given against IL-2 have demonstrated their prophylactic action in RA suppression, disease incidence has been reduced and the disease has been less severe (37). Together with TNF-α and IFN-γ, IL-6 is markedly expressed in the synovia in antigen-induced arthritis in experimental animals and may have an important role in the suppression of arthritic insult on the cartilage (38). It is produced by T cells, fibroblasts and macrophages. In RA patients, high concentrations of IL-6 have been detected in the synovial fluid, and serum levels of this cytokine highly correlate with markers of the disease activity, such as acute phase proteins. IL-6 can stimulate B cell proliferation and immunoglobulin production. It can also have an impact on the hepatic production of acute phase proteins.

In RA, IL-4, IL-10 and IL-13 have special significance as antiinflammatory cytokines. IL-10 is produced locally by the synovial monocytes. It can be detected in the synovial fluid of RA patients, with higher

levels than in the serum (39). IL-10 inhibits local IFN-γ production, while the expression of IL-4 in synovial fluid is very low in RA. Regarding the serum levels of IL-4, they are markedly elevated, compared to healthy control samples (40). IL-4 inhibits the production of proinflammatory cytokines and favors the synthesis of IL-1 receptor antagonists by synoviocytes. The main source of IL-13 in the synovial fluid are activated T cells, while exogenously administered IL-13 reduces the production of TNF-α and IL-1β in the synovial fluid. Also, together with IL-4, it inhibits bone resorption *in vitro* (41).

Cytokines in multiple sclerosis

Many investigators consider the etiology and pathogenesis of multiple sclerosis (MS) still unknown, though very modern is the hypothesis that MS is cellulary mediated autoimmune disease against the central nervous system (CNS) myelin, which may be associated with some viral infection. The disease can be best defined as a »recurrent inflammation of the CNS white matter leading to myelin destruction and progressive neurologic deterioration«.

Inflammation is associated in MS with increased IFN-γ expression, activation of endothelial cells with adhesion molecule expression and myelin destruction through receptor-mediated endocytosis. A »trigger« has to be present for the disease to be initiated, leading to sensitization – infectious agents possess the components which cross-react with myelin antigens, or via a limited brain infection occurs the release of myelin antigens. Th1 response in MS patients is characterized by IFN-γ secretion, and administration of this cytokine in MS patients causes disease exacerbation. Th2 responses are characterized by protective IL-4 secretion, which has been demonstrated in animal models (42) and, lately, CD4+ regulatory T cells have been described which suppress Th1 response and primarily secrete IL-10. Th3 response is characterized by TGF-β secretion, induced after mucous antigen presentation, and recovery in experimentally induced MS is associated with the appearance of cells which secrete TGF-β. The response of an unsusceptible person exposed to some myelin antigen is the absence of reaction, or the generation of Th2 or Th3 response, non-pathologic and protective, while in MS patients pathogenic Th1-type response is generated.

Immune disorders associated with MS can be identified and monitored in the peripheral blood of the patients, since activated T cells are present in the peripheral blood and cerebrospinal fluid in MS patients. One of the characteristics of relapsing remittent MS is the recovery after disease attack, accompanied by an increase of IL-10 secreting reactive cells. After the disease transformation from the acute into chronic progressive form, T cells enter the state of chronic activation, associated with immune system skewing towards Th1 response. In progressive MS, elevation of IL-12 secreting monocytes has been found (43). Progressive and relapsing-remittent MS react differently to immunomodulatory therapy. Many immunomodulatory treatments can influence the disease process, though not ideally and not in every situation. An effective MS treatment has to act on the antigen-specific myelin reactive cells, through the reduction of IFN-β secretion or the increase of Th2 and Th3 myelin reactive cells. Similar to other autoimmune diseases, MS is easier to stop in earlier phases since more advanced stages respond to therapy to a lesser extent.

Cytokines in systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases. It is a chronic, inflammatory, multisystem disorder of the connective tissue with general population incidence of 1/2000, the activation of which is the result of the 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 loss of tolerance and antibody production, generalized increase and dysregulation of the immune system and destruction of target tissue or organs mediated by direct antibody binding and deposition of immune complexes (44).

In SLE activity and involvement of various organs, many cytokines are involved. It is known that cytokine production in SLE patients significantly differs from the production in healthy controls and patients with other diseases, such as rheumatoid arthritis. 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 the patients with changes of the central nervous system in SLE, but not in those without neurologic symptoms (45). Similar to other systemic diseases, cytokine balance is of more significance in phenotype and disease severity determination than in susceptibility determination.

Based on the cytokines generated, the cytokine production pattern in various pathologic stages is often described as Th1, Th2 and Th3 response. Though helpful, Th1/Th2/Th3 paradigm has its limitations regarding cytokine classification. Classification of cytokines on the basis of their augmentation or weakening of the inflammatory cascade or response can be of much more use. In many inflammatory

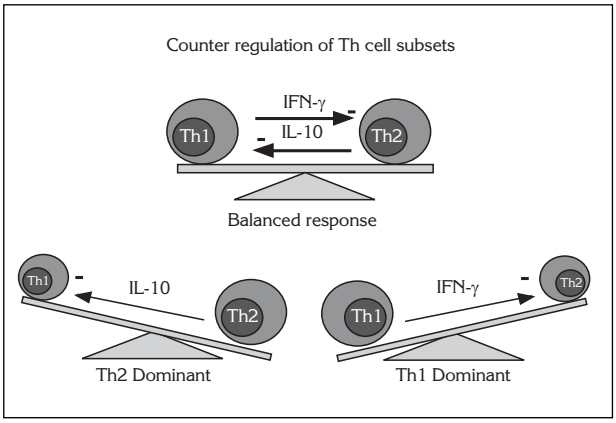

Figure 2 Th balancing, predominance of Th1, ie Th2 response

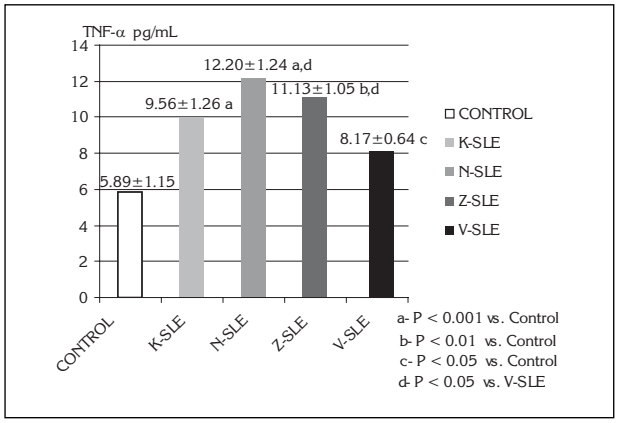

Figure 3 TNF-α concentration in the plasma of SLE patients

diseases such as SLE and RA, the balance between proinflammatory and antiinflammatory cytokines defines the degree and extent of inflammation and can lead to manifest clinical effects. Cytokine action is determined by the relative cytokine concentration related to the concentration of appropriate inhibitor or antagonist.

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 are playing 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 for 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, thus promoting 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 (46).

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 TNF inhibitor, reducing its biologic activity. TNF-α can be protective in SLE patients (47). In numerous SLE models, it has been demonstrated that pathologic activity of TNF-α and IL-1 is determined by the dose and stage of disease activity. We determined TNF-α value in 55 patients with different clinical manifestations of SLE in 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 (48) and Aringer and Smolen (49). The highest increase was observed in those with neurolupus (N-SLE) and joint disease manifestations (J-SLE), and less 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 (50). 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. 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 in comparison 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 (51).

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 (52). These results differ from the previous studies, where in the patients with other systemic diseases, such as MS, elevated IFN-γ and IL-10 were observed. It indicates the possibility that passive diffusion or passage

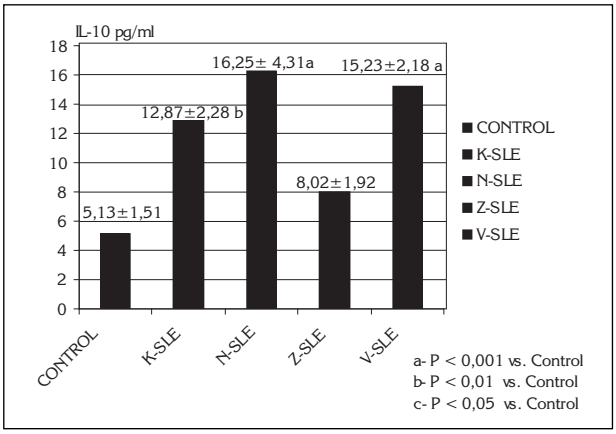

Figure 4 IL-10 concentration in the plasma of SLE patients

through the damaged blood-brain barrier can induce a high cytokine concentration in the intrathecal space. Therefore, a valid possibility is that peripheral inflammatory mediators such as TNF-α induce intrathecal inflammation. Interleukin-1 and TNF-α released from inflammatory cells act synergistically in the circulation, inducing peripheral vasodilatation, increase in vascular permeability and alteration of endothelial function favoring thrombosis.

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 is 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 in IL-10 production. Genetic regulation of IL-10 is essential in most SLE patients. Serum levels of this cytokine are increased in SLE patients (53) 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 manifestation 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 anti-ds DNA antibodies and SLEDA I score, while the correlation with the level of C3 complement fraction is negative (54). 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 in 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 (55). 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 are 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 significant increase in 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 (56). Investigations in this direction can help to better understand 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.

Inhibitors of cytokine production are being extensively studied as potential therapeutics 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 (57).

ZNAČAJ CITOKINA U DIJAGNOSTICI AUTOIMUNSKIH OBOLJENJA

Lilika Zvezdanović[1], Vidosava Đorđević[1], Vladan Ćosić[1], Tatjana Cvetković[3], Slavica Kundalić[1], Aleksandra Stanković[2]

[1]Centar za medicinsku biohemiju, Kliničkі centar – Niš, Srbija
[2]Institut za prevenciju, lečenje i rehabilitaciju srčanih i reumatskih bolesnika »Zelengora«, Niška Banja, Srbija
[3]Institut za biohemiju, Medicinski fakultet, Niš, Srbija

Kratak sadržaj: Autoimunske bolesti odlikuju se autoimunoreakcijama usmerenim protiv široko rasprostranjenih sopstvenih determinanti. Mnogi citokini učestvuju u regulisanju aktivnosti i zahvatanju organa kod različitih autoimunskih oboljenja. Poznato je da neka tkiva održavaju vrlo visoku »ulaznu barijeru« u pogledu razvoja imunoposredovane inflamacije, što vodi imunoj privilegovanosti putem generisanja specijalizovane mikrosredine. Postoje različiti obrasci sinteze citokina u pojedinim autoimunskim oboljenjima kao što su reumatoidni artritis, dijabetes tipa 1, sistemski lupus eritematodes i multipla skleroza, pri čemu treba istaći razliku između citokina kao markera fenotipa i citokina kao medijatora inflamacije i oštećenja tkiva. U većini autoimunskih oboljenja, ravnoteža između proinflamatornih i antiinflamatornih citokina određuje stepen i proširenost inflamacije i može voditi evidentnim kliničkim efektima. Tako je kod bolesnika sa SLE dobijen značajan porast TNF-a i IL-10 u svim, a posebno u neurološkoj manifestaciji bolesti. Razumevanje osnovnih mehanizama kontrole diferencijacije T ćelija predstavlja put ka strategiji modulacije fenotipa citokina i sprečavanja tkivnog oštećenja i autoimunskih bolesti i, naravno, promoviše i zaštitu od istih.

Ključne reči: autoimunske bolesti, citokini, sistemski lupus eritematodes, TNF-a, IL-10

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