ISSN 2079-0570, Advances in Gerontology, 2013, Vol. 3, No. 4, pp. 243–254. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © B.I. Kuznik, N.S. Linkova, S.I. Tarnovskaya, V.Kh. Khavinson, 2013, published in Uspekhi Gerontologii, 2013, Vol. 26, No. 1, pp. 38–51.

Cytokinis and Regulatory Peptides: Age-Related Changes, Atherosclerosis, and Thrombotic Diseases

B. I. Kuznik[a, b], N. S. Linkova[b], S. I. Tarnovskaya[b], and V. Kh. Khavinson[b, c]

[a] *Chita State Medical Academy, ul. Gor'kogo 39a, Chita, 672000 Russia*
e-mail: macadem@mail.chita.ru

[b] *St. Petersburg Institute of Bioregulation and Gerontology, pr. Dinamo 3, St. Petersburg, 197110 Russia*

[c] *Pavlov Institute of Physiology, Russian Academy of Sciences, Nab. Makarova 6, St. Petersburg, 199034 Russia*

Abstract—The review describes different aspects concerning the participation of cytokines in age-related diseases. The authors observe their own and literature data and reveal the role of short peptides in molecular mechanisms of homeostatic regulation of the immune, cardiovascular, and hemostatic systems. Short regulatory peptides were found to implement their geroprotective effect by changing the gene expression of immune and anti-inflammatory cytokines and γ-interferon.

Keywords: short peptides, cytokines, immune cells, aging

DOI: 10.1134/S2079057013040048

GENERAL CONCEPTS OF CYTOKINES

Over the past three decades, numerous data have been accumulated that evidence to the immediate relationship between the innate and adaptive immu-
1 2 nity systems and hemostasis performed through mac-
rophages, leukocytes, platelets, vascular wall, comple-
3 ment, prostaglandins, leukotrienes, and fibronectin [2, 3, 13, 14, 16, 20, 44, 80]. A special role in this list belongs to cytokines, one of whose main functions is the relationship between immunocompetent cells. In the figurative expression of Paltsev et al. [27], cytokines constitute a microendocrine system. They are not only involved in the regulation of the immune response and hematopoiesis but also have a pronounced effect on a variety of bodily functions [7, 14, 19, 27, 33, 35]. Today, approximately 200 cytokines are identified. All of them have the following common features: they

(1) are synthesized during either the unspecific protection or the immune response;

(2) like hormones, exhibit their activity at very low concentrations (10^{-10}–10^{-11} M);

(3) are mediators of the immune response and the inflammation reaction and have paracrine, autocrine, and endocrine activities;

(4) act as growth and differentiation factors of various cells;

(5) form an extensive regulatory network, where individual compounds exhibit synergistic or antagonistic effects;

(6) have a polyfunctional activity and overlapping functions, which provides a high reliability of their action;

(7) do not have antigen specificity [7, 34].

Conventionally, all cytokines are divided into four groups: (1) interleukins (IL), (2) interferons (INF [α, β, γ, and ω]), (3) hematopoietic colony growth factors, and (4) factors preventing the tumor growth (tumor necrosis factor (TNF) [α and β], oncostatin M, leukemia inhibitory factor, etc.) [7, 33]. The main function of interleukins is to provide the relationship between individual cells during the immune response. Specifically this property led to their name. It was further found, however, that interleukins have a wider spectrum of action. Currently the name "interleukin" is assigned to a newly discovered mediator only if it meets the following criteria: (1) the molecular cloning and the gene expression of a new cytokine, (2) the presence of the unique nucleotide and the corresponding peptide sequence, (3) the formation of neutralizing monoclonal antibodies, (4) the mandatory formation in cells involved in the immune response, (5) the presence of the important biological function in the regulation of the immune response, and the additional properties, which do not allow one to assign a different name to a found compound [7, 35].

Cytokines are synthesized by macrophages and all 2
types of leukocytes (mostly monocytes, lymphocytes, thrombocytes, endotheliocytes, fibroblasts, epithelial and mast cells, and different cells (glia, astrocytes) of CNS). Most cytokines, except for *IL*-1 and *IL*-4, act mainly locally almost in all organs: bone marrow, liver, blood vessels, central nervous system, endocrine system, etc. In the case of the failure of local protective reactions, cytokines fall in circulation and act at the systemic level.

By affecting the CNS, cytokines change all complex of behavioral responses. The synthesis of many hormones and acute-phase proteins increases, the ionic composition of the plasma and cells shifts, and genes of growth and differentiation factors begin to be expressed.

Cytokines are divided into four groups according to their characteristics: (1) lymphocyte cytokines (*IL*-2, *IL*-4, *TGFβ*), (2) prior-to-immune cytokines (*TNF*, *IL-1*, *IL-6*, chemokines), (3) cytokines-regulators of the immune inflammation (*Ifγ*, *IL*-5, *IL*-10, *IL*-12, etc.), and (4) cytokines-growth factors (*c-kit*-ligand, *GM-CSF*, *G-CSF*, *IL*-3, *IL*-7, *IL*-9, *IL*-11, etc.).

On the basic mechanism of their action, cytokines are divided into five groups: (1) growth factors, which control hematopoiesis, including the production of immune competent cells, (2) proinflammatory cytokines, which ensure the mobilization and activation of cells involved in inflammation, (3) antiinflammatory cytokines, which restrict the development of infection and inflammation, (4) immune cytokines, which regulate the course of the cell and humoral immunity, and (5) effector cytokines with antiviral, cytotoxic, and other effects [7, 34]. Cytokines of a separate group are lymphopoietins (most of the interleukins), eosinophilopoietic cytokines (*IL*-3, *IL*-5, *GM-CSF*), and chemokines or chemoattractants (*IL*-8, *IL*-16, etc.) [7].

CYTOKINES AND LIFESPAN

Cytokines are known to be synthesized as a response to the stimulation of producing cells. The expression level of cytokines is regulated by both the negative feedback mechanism mediated by prostaglandins and corticosteroids and the mechanism of self-regulation. The expression of cytokines is controlled by *cis*-regulating elements localized in a promoter. It is found that the transcription complex of the majority of interleukins contain factors *AP*-1, *GATA*-3, *Oct*1/2, *YY*1, *Ets*1, and *NF-AT* [84].

Today, it is conclusively proven that the content of many cytokines that play a key role in the mechanism of the innate and adaptive immunity varies greatly with age [38]. In particular, it was found that the production of *IL*-2, which is secreted mainly by the mitogen- or antigen-activated *T*-helper cells of the first clone (Th_1), significantly decreases with human aging. The decrease in the *IL*-2 level in response to the stimulation leads to the fact that the functional activity of *T*-cells, macrophages, and natural killer cells (NK) is reduced in older people and, as a result, the antiviral, antibacterial and antitumor protection of the body is deteriorated [84].

It should be noted that *IL*-2 is a stimulator of the synthesis of *IFNγ* by the cytotoxic and *NK* cells. Moreover, *NK* cells begin to produce *IFNγ* most intensively only after the interaction with cancer or virus-infected cells, and this process is enhanced by *IL*-12. Meanwhile, as some authors showed [38, 67, 68], the concentration of *IL*-2 and *IL*-12 in older people significantly decreases.

In adult patients with bronchial asthma, the content of *IL*-5 decreases compared with children, which is an extremely unfavorable factor for the prevention and suppression of allergic reactions and local inflammatory processes [12].

The level of cytokines in blood is known to depend on the intensity of the immune response induced by various foreign and self-antigens of the body. With age, the number of antigens in the body steadily increases, which should affect the content of cytokines [43]. Therefore, the reduced reaction of proinflammatory cytokines in old age in response to antigens is unfavorable and can lead to the development of pathological states and even death.

According to data of Sukmanova et al. [38], the concentration of proinflammatory cytokines (*TNFα* and *IL*-1β) in elderly people is significantly increased. In 1996 when examining healthy people, Hager et al. [66] revealed the linear correlation between the level of *IL*-6 and human lifespan: the higher the concentration of *IL*-6 was in blood, the lower appeared to be the lifespan. The content of *IL*-6 during a year increased on average by 0.016 pg/mL of blood. At the same time, the level of *IL*-6 appeared to be linearly associated only with fibrinogen, α_2-macroglobulin, and *D* dimer, which indicated the significant enhancement of the intravascular coagulation and the inhibition of fibrinolysis. The level of *TNFα* in elderly people is significantly increased in the absence of a pathological process [12]. In this case, the inadequate response of proinflammatory cytokines (the insignificant increase of their concentration) on pathogenic stimuli is observed in patients older than 60 years. Moreover, the production of *TNFα*, *IL*-1β, *IL*-6, and *IL*-17 stimulated with *LPS* mononuclears decreases in donors with age [31, 73]. Similar data were obtained by Nyugen et al. (2010) on 17 young (21–32 years) and 17 elderly (66–89 years) people [86]. It was found that the production of *IL*-6 and *TNFα* decreased and expression of *TLR*-1 by monocytes is reduced in people over 60 years as compared with young people. At last, Hartford et al. [69] indicate that there is a close relationship between the level of *IL*-18, total mortality, and mortality from cardiovascular diseases.

The presented data suggest that the initial level of proinflammatory cytokines increased in elderly people and people in old age, whereas their stimulated production significantly reduced.

There is no doubt that the increase in the concentration of proinflammatory cytokines in elderly and old age people is the adaptive response aimed at the activation of the immune system and the fight with diseases pursuing elderly and senile people. However, there is a flip side of the coin of this reaction. Without exception, all proinflammatory cytokines significantly enhance the process of blood clotting and inhibit

fibrinolysis [2, 3, 13, 14, 20, 22, 60, 65], which inevitably gives rise to thromboembolic diseases, including myocardial infarction and ischemic stroke in the presence of predisposing factors (atherosclerosis, pathology of the cardiovascular system, thrombophilia).

The concentration of *IFNγ*, which is synthesized mainly by cytotoxic $CD8^+$ lymphocytes stimulated by antigens or mitogens and *NK* cells ($CD3^+CD16^+$ and $CD3^-$ $CD16^+$), significantly changes during aging. At the early phase of infection, *IFNγ* is practically absent or contained at a minor concentration. The formation of *IFNγ* and its secretion occurs only after remeeting presensitized lymphocytes with antigen. This cytokine is unable to directly influence the infectious agent. It acts mainly via stimulation of monocytes, macrophages, and *NK* lymphocytes. In addition, *IFNγ* increases the production of antibodies and leads to the formation and secretion of proinflammatory cytokines. *IFNγ* can dramatically increase the permeability of vessels for macromolecules and induce the formation and secretion of chemokines providing leukocyte chemotaxis in the complex with *TNFα* [8, 52, 53, 71].

The formation of *IFNγ* stimulated with mitogen and mononuclears decreases in blood of elderly people [31, 38]. Moreover, Cytanot et al. [56] showed that the level of *IFNγ* in nocturnal primates (*Microcebus murinus*) correlates with their lifespan. On the basis of the experiments, the authors come to a sensational conclusion that the concentration of *IFNγ* in plasma can "predict" the lifespan of primates.

CYTOKINES AND CARDIOVASCULAR DISEASE

In recent years, great importance has been attached to cytokines in the development of atherosclerosis and cardiovascular disasters. It is found in particular that the increase in the level of *IL*-1, *IL*-12, *IL*-18, and *IFNγ* in experimental animals ensures the progressive development of atherosclerosis, while the blockage of these cytokines decreases the manifestation of atherosclerotic changes by 15–69% [47]. Moreover, proinflammation cytokines *IL*-1 and *TNFα* increase the production of the *MCP*-1 protein (*monocyte chemoattractant protein*-1), which induces migration of monocytes into the intima and, thereby, is a potent activator of atherosclerosis [82]. In addition, it should be noted that all of these cytokines without exception promote the expression of the tissue factor (*TF*) and the von Willebrand factor (*vWF*) and inhibit fibrinolysis [2, 3, 13, 14, 49, 60, 61, 80], which, thus, stimulate the occurrence of thrombotic complications.

IL-1β and *TNFα*, which are major stimulants of physiological and, particularly, pathological angiogenesis, play an especially important role in the development of atherosclerosis. The mRNA level of *IL*-1β in atherosclerotic areas significantly increases. Oxidized (modified) low-density lipoproteins (LDL) cause the increase in the *IL*-1β level in human macrophages by factors of 6–10. It is assumed that the same reaction occurs also in endothelial cells. Furthermore, Th_1, which infiltrates the plaque, can secrete *IL*-2, *IL*-12, *IL*-18, *TNFα* and *β*, *IFNγ*, and other proinflammatory cytokines [51]. In addition, *IFNγ* and *IL*-18 can synthesize and secrete *NK* and *TNK* and stimulated smooth muscle cells. A direct correlation is found between the level of *C*-reactive protein (CRP), *IL*-1α, *IL*-8, and *TNFα* and the thickness of the intima-media complex, which indicate the early involvement of the inflammation process (and consequently proinflammatory cytokines) in the formation of atherosclerotic plaques [45]. All of the listed cytokines lead to the activation of other damaging cells (neutrophils, $CD8^+$), and thus, increase vascular inflammation and progression of atherosclerosis [67].

IL-6 plays an important role in the development of atherosclerosis, atheromatosis, and infarction. The increase in its concentration (higher than 3.2 pk/mL) increases the risk of death from cardiovascular events by a factor of more than two [70].

Apparently, the proinflammatory cytokines, which increase vascular-platelet hemostasis and the process of blood coagulation and inhibit fibrinolysis, are independent predictors of cardiovascular events. In particular, Hartford et al. [69] evaluated the relationship between the level of proinflammation cytokine *IL*-18 on the first day of hospitalization of patients with acute coronary syndrome with the risk of myocardial infarction and death in the near (3 months) and long (7.6 years) observation periods [69]. It is found that, in the long term, there is a close relationship between the initial level of *IL*-18 and both the total mortality and mortality from cardiovascular diseases. It should be added that, under the influence of *IL*-18, the production of proinflammatory cytokines *TNFα*, *IL*-1β, *IL*-6, and *IL*-8 and immune cytokine *IL*-2 [67] dramatically increases, which ensure the enhancement of vascular-platelet and coagulation hemostasis and lead to the development of thrombotic complications.

The blockage of receptors or the knockout of the *IFNγ* and *TNFα* genes significantly decreases the manifestations of atherosclerosis in experimental mice [67, 91]. At the same time, the blockage or the removal of cytokines *IL*-10 and *TGFβ* leads to the enhancement of atherosclerosis and atherothrombosis. These experimental data allow for the conclusion that anti-inflammation cytokines prevent the formation of atherosclerotic plaques [63].

Of course, the increase in the level of proinflammatory cytokines indicates the presence of latent inflammation directly in the blood vessels. Oxidized lipoproteins enhance the induction of adhesive molecules and the release of proinflammatory cytokines from endothelial and smooth muscle cells. Since plaques contain macrophages and monocytes, *TNFα* are intensively concentrated there, which facilitates the activation of

T lymphocytes and induces apoptosis of surrounding cells. The introduction of direct anticoagulants during a long period of time inhibits the infiltration of monocytes into the vessel wall and prevents the development of plaques [85].

T helpers of type 1 (Th_1) are accumulated in the atherosclerotic plaque from the earliest stages, and their number significantly exceeds Th_2, *Tc* ($CD8^+$), and *NK* cells. Each of the populations and sub-populations of lymphocytes contribute to the mechanism of development of atherosclerosis. Th_1 and dendrite cells produce *IFNγ* and other proinflammation cytokines as well as *IL*-2, which leads to proatherogenic immune response. In this case, the number of Th_2 cells, which synthesize proinflammation cytokines, decreases [28]. Monocytes were found throughout the fibrous cap, whereas *T* lymphocytes are located in marginal areas [5, 46]. Macrophages, including dendrite cells, produce *HLA*-II and interact with *T* lymphocytes, which results in the activation of the immune response [83, 89]. The found changes are, apparently, the result of infection and deposition of LDL [28].

The population of macrophages in the center of atherosclerotic lesions is heterogeneous and consists of three sub-populations: (1) foam cells, where, as known, the cytokine expression is suppressed, (2) macrophages, which closely contact with lymphocytes and are involved in the production of *IL*-1 and *TNFα*, and (3) macrophages, which synthesize only *TNFα*. $CD4^+$ lymphocytes contact with LDL and VLDL and become proatherogenic [88] and activate $CD8^+$ providing, thus, the inflammatory process in the vascular wall. The atherosclerotic plaque contain also *B* lymphocytes, which are activated by *T* cells through the interaction with *CD40L*. *B* cells in the atheromatose plaque may move into plasma cells and produce *IgG*.

IL-6, *IL*-8, and *IL*-18 of monocyte chemotactic protein (MCP-1) and endothelial monocyte-activating polypeptide (EMAP-II) are present at the increased concentration in men without acute coronary syndrome in unstable atherosclerotic plaques of coronary arteries. The MMP-7 and MMP-9 content is increased and, at the same time, the activity of tissue inhibitor of metalloproteinases TIMP-1 is decreased in the unstable plaques. At earlier stages of the formation of stable atherosclerotic plaques, they contain a high concentration of *IL*-1, *TNFα*, and MMP-3 and a low concentration of TIMP-1. The increased level of *IL*-6, *IL*-8, MCP-1, and MMP-7 is found in unstable atherosclerotic plaques of the lipid type, while the enhanced destructive activity is revealed in unstable plaques of the necrotic type caused by both the presence of *TNFα* and the reduced content of TIMP-1 [4, 29, 30, 69, 91].

Macrophages and monocytes produce more than 100 biologically active compounds in mature atherosclerotic plaques, including *IL*-1, *TNFα*, erythropoietin, insulin-like growth factor, platelet mitogenic factor, and ACTH [64]. *MCP*4/*CCL*13, *MIP*1a/*CCL*3, *MIP*1b/*CCL*4, *RANTES*/*CCL*5, *PARC*/*CCL*18, *IL*-8/*CXCL*8, *IP*10/*CXCL*10, *MIG*/*CXCL*9, and other chemokines are found in atherosclerotic plaques.

Chemokines, such as *CXCL*16 and *GROα*, are of great importance in the development of cardiovascular pathology, including atherosclerosis. Their sources are macrophages and dendrite, endothelial, and smooth muscle cells. These chemokines are expressed at sites of smooth muscle lesions. One of them, *CXCL*16, involves *T* lymphocytes in the inflammation areas and promotes the integration in the intima of modified oxidized LDL, increases the chemotaxis and adhesion of endothelial cells, significantly induces angiogenesis, leads to the formation of foam cells, and becomes actively involved in the inflammation process and the accumulation of cholesterol [93]. The other chemokine, *GROα*, produced by endothelial cells and macrophages, blocks the ability of monocytes to adhesion and chemotaxis, which leads to the decreased involvement of these cells in inflammation. At the same time, *GROα* is the atherosclerosis mediator because it promotes the destabilization of plaques and the accumulation of fat in them, increases the secretion of MMP from smooth muscle cells and, thus, is involved in the degradation of intracellular matrix [55]. *MIG*, *I*-309, *SDF*-1, *MCP*-3, and *IL*-13 are found in the atherosclerotically damaged and undamaged sections of the aortic intima. In most cases, there is the enhanced expression of MCP-1, *MIP*-1β, *I-TAC*, MCP-2, *RANTES*, and *TGF*β and, in a smaller number of cases, *SDF*-1 and *MCP*-3 [23]. All this shows that the concentration of proinflammatory cytokines, chemokines, and adhesion molecules undergoes phase changes in the developing atherosclerotic plaques.

The binding of *Mac*-1 to *Gp*Iβ or fibrinogen, which interacts with *Gp*IIβ/IIIα, may exacerbate the proinflammatory damaging effect of platelets on blood vessels and, thus, contribute to atherothrombosis [90]. This reaction is likely enhanced by the action of chemokines MCP-1 and *IL*-8 on monocytes and neutrophils. Migration of monocytes significantly increases during the cleavage of fibrinogen with plasmin [24].

The most important factor in fatty degeneration of the arterial wall is cytokine from the alarmin family, HMGB1 (*High-mobility group box chromosomal protein* 1), which is intensively produced by monocytes and macrophages and supports chronic inflammation in atherosclerotic plaques. This cytokine is contained in almost all nuclear cells. Being inside the nucleus, HMGB1 is involved in the stabilization of nucleosomes and regulates the gene expression [25]. One of the properties of HMGB1 is its ability to bind heparin, which indicates its participation in hemostasis. The role of HMGB1 in the atherosclerotic process may be in stimulation of endothelial cells, which produce proinflammation cytokines, which, in turn, increase the expression of adhesion molecules. At the same

time, the level of the inhibitor of HMGB1, thrombomodulin, decreases. This promotes the development of hypercoagulation and thrombotic complications along with the increased expression of *TF* [49].

It is found that free (extracellular) HMGB1 is involved in all phases of inflammation from the tissue damage to the tissue repair. It activates endothelial cells and their precursors. In this case, the expression of adhesive molecules *ICAM*-1 and *VCAM*-1 along with proinflammatory cytokines increase, which is accompanied by adhesion of monocytes/macrophages, neutrophils, and, probably, platelets on inflamed endothelium [92].

The potent activator of neutrophils is proinflammatory cytokine, chemokine *IL*-8, capable of binding to proteoglycans on the apical surface of endothelial cells. Furthermore, E-selectin provides adhesion of neutrophils to stimulated endothelium and, thus, prepares ploynuclears for the subsequent activation of *IL*-8, which binds to its receptor on the surface of blood vessels [7, 38].

The role of secretory phospholipase A_2 (sFA_2) in the mechanism of the progression of atherosclerosis should be especially mentioned. It is known that sFA_2 hydrolyzes not only membrane phospholipids but also lipoproteins promoting the release of free fatty acids and lysophospholipids, which are precursors of proinflammatory mediators, such as prostaglandins, leukotrienes, thromboxanes, and *PAF*. Proinflammatory cytokines (*IL*-1α, *IL*-6, *IL*-8, *TNFα*) dramatically increases the expression of sFA_2 in stherosclerotic plaques. sFA_2 increases the expression of chemokines and adhesive molecules in endothelial cells and, thereby, enhances dysfunction of endothelium. In addition, the effect of *sFA*2 promoting the atherosclerosis development is aimed to inhibition of the paraoxonase activity.

Currently, a cytokine theory of atherosclerosis is put forward, which consists in the idea that lipoproteins penetrated into the intima are subjected to a slight modification under the influence of endothelial cells. At the same time, the intima is infiltrated with monocytes/macrophages, which induce free radicals. This leads to the further modification of lipoproteins with the formation of modified LDL (mLPNP). Under the influence of the latter, macrophages intensively synthesize and secrete *IL*-1β, which implements the autocrine regulation of the expression of chemoattractants, and this results in the accumulation of monocytes and lymphocytes in these zones. Moreover, *IL*-1β stimulates proliferation of endothelial cells followed by the deterioration of the dense contacts between them. *IL*-18 promotes the enhancement of the synthesis and secretion of *IL*-6, *IL*-8, the adhesive *ICAM*-1 molecule, metalloproteinases, which destroy the extracellular matrix, and provide the inhibition of age involution of the thymus [6, 37, 69].

Titov [39] expresses an original point of view on the mechanism of atherosclerosis and the role of the inflammatory reaction in this process. He believes that it is necessary to face with sober eyes the modern theory of atherosclerosis and acknowledge its failure. According to the author, atherosclerosis is a violation of the functions of each of the cells in vivo in the synthesis of eicosanoids (prostacyclin, thromboxanes, and leukotrienes) not from arachidonic essential polyene fatty acid but from aphyziological endogenous dihomo-γ-linolenic fatty acid. Moreover, the change in the composition of phospholipids in plasma membranes also influences the development of atherosclerosis. In this case, the excess of saturated fatty acids and triglycerides in LDL does not allow the apo-*B*-100 protein to take a physiological conformation and to express the apo-*B*-100 ligand onto membrane, and lipoproteins in the blood become biological garbage and disrupt the function of purity of the intercellular environment. All garbage is moved in the intima of the arteries after physiological denaturation due to peroxide oxidation by endothelial transcytosis. The author believes that the intima is a pool of the collection and the utilization of the biological garbage from the intravascular cellular environment. However, intimal macrophages (scavenger cells) do not contain acid esterases in lysosomes; when utilizing lipoproteins, all essential polyene fatty acids are deposited in the intima and cause its destruction and inflammation. Therefore, the basis of atherosclerosis is the impaired function of the external supply (exotrophy), and atheromatosis is a violation of the function of maintaining cleanliness of the intercellular medium. The pathogenetic mechanisms of these processes are completely different. Atherosclerosis is the disease of the conformation and pathological compensation, and atheromatosis is the pathological process of the utilization of biological garbage in the intima. Of course, Titov's hypothesis of the pathogenesis of atherosclerosis requires a careful examination and new facts to prove its correctness.

REGULATORY PEPTIDES AND CYTOKINES

It was found by numerous studies at the St. Petersburg Institute of Bioregulation and Gerontology that short peptides (SP), which were designed due to examining the amino acid composition of the complex of polypeptides from the thymus and epiphysis, can modulate immune responses and significantly increase the average and maximal lifespan in experimental animals [1, 50, 74, 75, 76]. SP molecules were shown to normalize the gene expression of heat shock proteins in humans and animals under pathological states and stress [18, 21, 78, 81]. The influence of SP on the expression of heat shock proteins is in the basis of antiinflammatory effect. Moreover, the geroprotective action of SP is caused by their ability to tissue-specific stimulate proliferation and differentiation and

Possible binding sites of epitalon in promoter regions of genes of different cytokines

mRNA	Sequence (5' → 3')
IL-2 (*NM_*000586.3)	1 *agttccctat cactctcttt aatcactact cacagtaacc tcaactcctg ccacaatgta* 61 *caggatgcaa ctcctgtctt gcattgcact aagtcttgca cttgtcacaa acag*
IL-5 (*NM_*000879.2)	1 *atgcactttc tttgccaaag gcaaacgcag aacgtttcag agccatgagg atgcttctgc* 61 *atttgagttt gctagctctt ggagctgcct acgtgtatgc c*
IL-1α (*NM_*000575.3)	1 *agctgccagc cagagaggga gtcatttcat tggcgtttga gtcagcaaag*
IL-6 (*NM_*000600.3)	1 *aatattagag tctcaacccc caataaatat aggactggag atgtctgagg ctcattctgc* 61 *cctcgagccc accgggaacg aaagagaagc tctatctccc ctccaggagc ccagctatga* 121 *actccttctc cacaagcgcc ttcggtccag ttgccttctc cctggggctg ctcctggtgt* 181 *tgcctgctgc cttccctgcc cca*
IL-17α (*NM_*002190.2)	1 *gcaggcacaa actcatccat ccccagttga ttggaagaaa caacg*
*TNF*α (*NM_*000594.2)	1 *ctccctcagc aaggacagca gaggaccagc taagagggag agaagcaact acagaccccc* 61 *cctgaaaaca accctcagac gccacatccc ctgacaagct gccaggcagg ttctcttcct* 121 *ctcacatact gacccacggc tccaccctct ctcccctgga aaggacacc*
*IFN*γ (*NM_*000619.2)	1 *cacattgttc tgatcatctg aagatcagct attagaagag aaagatcagt taagtccttt* 61 *ggacctgatc agcttgatac aagaactact gatttcaact tctttggctt aattctctcg* 121 *gaaacg*

suppress cells apoptosis [40–42]. However, it cannot be the only explanation of the geroprotective action of SP. Short peptides were shown to have a normalizing effect on the expression of the genes of oxidative stress. It has been found that dipeptide Vilon (*Lys-Glu*) stimulates the expression of the *c-fos* gene in rat hypothalamic neurons [1, 50]. This result shows the ability of Vilon to induce the adaptive reactions under stress because the *c-fos* gene and the corresponding protein are activated in response to stress. In addition, tetrapeptide epitalon (*Ala-Glu-Asp-Glu*) has been demonstrated to increase the expression of the *c-fos* protein in rat epiphysis [1, 50], which indicates the common regulatory mechanisms of SP.In recent years, it has been shown that regulatory peptides can also influence the genome state, which causes the synthesis of different cytokines. One of these compounds is epitalon. Immunomodulating and geroprotective effect of epitalon may be partly due to the influence on a complex of genes, which regulate the synthesis of immune, proinflammatory, and anti-inflammatory cytokines. As a result, the functional activity of cells is recovered. It was suggested that the di-, tri-, and tetrapeptides bind to DNA in the promoter region of the genes [77]. It is possible that epitalon, after penetration into the nucleus and nucleolus of the cell [62], binds to the *ATTTS, GTTTC, ATTTG* sequences, and Vilon binds to the *GCAG* sequence. Vilon and epitalon were shown to increase the expression of the *IL*-2 gene [76].

We have withdrawn from GenBank the nucleotide sequences of mRNA of the mentioned cytokines (table) and determined the localization of the complementary to epitalon binding sites in the promoter regions of the genes of cytokines *IL*-2 and *IL*-5. It has been shown that epitalon binds to the interleukin genes in the regulatory region and activates the gene expression or acts as a cofactor in the gene transcription process. As a result, the expression of interleukins and the restoration of the immunity increase.

The domains of the transcription factors interacting with DNA have an α-helical structure. When forming the secondary structure (α-helix) of the protein molecule, one turn of the helix contains 3.61 residues; i.e., the SP molecule is a minimal fragment for the formation of the α-helical structure. The distance between the first and the last carbon atoms in epitalon is 5.43 Å in physiological conditions, which corresponds exactly to the turn of α-helix in the protein molecule [77]. Therefore, tetrapeptide epitalon may interact with the major groove of DNA similar to transcription factors.

It is known that *IL*-2 is expressed mainly by antigen-activated cells Th_1, Th_0, and *NK* cells. *IL*-2 is produced by 90% and 10% of cells-producers for $CD4^+$ and $CD8^+$, respectively. *IL*-2 stimulates growth of all types of *T*-lymphocytes and especially cytotoxic *T* cells. *IL*-2 not only facilitates the proliferation of *T* lymphocytes but also leads to the secretion of *IFNγ* by Th_1 and macrophages and induces the expression of proto-oncogenes [80]. Receptors for *IL*-2 are found in *Th*, macrophages, *B* lymphocytes, *NK* cells, and platelets. *IL*-2 can stimulate the differentiation of cytotoxic *T* cells only in the presence of additional factors, such as *IL*-4, *IL*-6, *IL*-7, and *IL*-12 [52].

One of the most important functions of *IL*-2 is the stimulation of *NK* cells, which have the unspecific cytotoxic effect, including the action on tumor cells. *IL*-2 enhances the proliferation and differentiation of *B* lymphocytes and the synthesis and secretion of *IgM*,

IgG, and *IgA* [7, 58]. *IL*-2 acts on monocytes and strengthens the generation of main oxygen forms and its peroxides. Furthermore, it participates in hematopoiesis and stimulates the formation of eosinophils and platelets but, at the same time, inhibits erythroid and myeloid islands of hematopoiesis. The prolonged use of *IL*-2 in humans leads to eosinophilia and short-term lymphopenia followed by acute lymphocytosis. The latter is due to the increase in activated *NK* lymphocytes (*LAC*) in blood capable of spontaneously killing cancer and other foreign cells. In this case, normal cells remain intact [33].

The mechanism of the action of *IL*-2 on the malignant growth is still poorly understood. Undoubtedly, it is not limited by the stimulation of macrophages and cytotoxic *T* lymphocytes. *IL*-2 increases proliferation and antitumor activity of *NK* cells by factors of 8–10 and 5–10, respectively. Furthermore, Th_1 and Th_2 are also involved in this reaction. The antitumor effect of *IL*-2 is associated with other cytokines, which are formed as a result of increasing concentration of *IL*-2. It cannot be excluded that, in a number of tumors, *IL*-2 directly affects nonhematopoietic tissues and decreases the expression of adhesion molecules, which prevents metastasis of cancer cells. Due to the wide spectrum of the activity, *IL*-2 increases the content of many cytokines, including *IL*-3, *IL*-4, *IL*-5, *IL-6,* *IL*-8, *IL*-10, *IL*-12, *IL*-18, *TNF*α, and *IFN*γ [52, 55].

The presented data show the extremely diverse functions of *IL*-2. It is not surprising that short peptides, which enhance the expression of the *IL*-2 gene, inhibit the development of many pathological processes; this is one of the mechanisms of the geteroprotective action of SP.

The function of *IL*-5 deserves special attention. It enhances the growth and proliferation of not only eosinophils and basophils but also thymocytes. It directly affects the transition of *B* lymphocytes to plasma cells intensively producing *IgM*. The main role of *IL*-5 is to participate in allergic inflammation. *IL*-5 stimulates the synthesis of serum *IgA* and secretory *IgA*, which provides the local protection of the mucosa. To a lesser extent, *IL*-5 influences the synthesis and secretion of *IgE*. The ability of *IL*-5 to maintain proliferation of *B* lymphocytes occurs in advanced stages of the activation along with the action of *IL*-2, but later than the action of *IL*-1 and *IL*-4. If *IL*-5 is introduced along with *IL*-4, the effect of the former increases by factors of 2–3 [52]. *IL*-5 increases the production of leukotrienes and reactive oxygen radicals in eosinophils. Through the mentioned mechanisms, *IL*-5 promotes the involvement of eosinophils in inflammation process and antiparasitic and antitumor protective reactions.

Thus, epitalon may act through the molecular mechanism similar to transcription factors of cytokines and largely duplicate their biological effects. Epitalon stimulates the cytokine synthesis and, thus, activates the function of Th_1 and Th_2 in persons above 60 and influences the cellular and humoral immunity. The enhancement of the expression of genes encoding the *IL*-2 synthesis should lead to the increase in the activity of *NK* lymphocytes and *cytotoxic T lymphocytes*, which ensure the antitumor defense.

But this is not the only problem. It is known that hypercoagulability is developed and fibrinolysis is inhibited in elderly and senile patients [9–11, 14, 17]. These effects are especially strong with cardiovascular diseases, which are the major cause of death worldwide. According to the Bulletin of the WHO, cardiovascular diseases cause the death in 49% of cases and the premature death before 65 in 30% of cases in Europe. In Russia, the mortality because of cardiovascular diseases and stroke is 48.6% and 41.1%, respectively [26, 36].

At the same time, it is found that there is a close link between the immune system and hemostasis. Moreover, the innate and adaptive immunity, along with the system of hemostasis, form a single humoral defense system [3, 15, 80]. In this case, the molecules that combine immunity and hemostasis are cytokines. The normalization of the immune system should eliminate hypercoagulation shifts and lead to the stimulation of fibrinolysis, which should lead to the decrease in the mortality because of cardiovascular diseases and to the increase in lifespan.

Thus, epitalon penetrating into the cellular nucleus and nucleolus can regulate the expression of the *IL*-2 and *IL*-5 genes by the mechanism typical for the transcription factors. As a result, a cascade of molecular/cellular reactions starts, which lead to differentiation and proliferation of lymphocytes, which, in turn, is accompanied by the enhanced antitumor and immunomodulatory activity.

However, the geroprotective action of SP is not limited by these functions. We determined the localization of the binding sites complementary to Vilon and epitalon in the promoter regions of the genes of cytokines *TNF*, *IL*-1α, *IL*-6, and *IL*-17 and showed that Vilon and epitalon being bound to the promoter regions of these genes activate their expression or act as cofactors in the DNA transcription process.

And what is the connection between our results and geroprotective properties of SP? This question can be answered only after the more detailed consideration of the function of the studied proinflammatory cytokines.

IL-1 is produced during antigen stimulation by monocytes, macrophages, white dendritic epidermocytes, dendritic cells, keratinocytes, brain astrocytes and microglia, endothelial, epithelial, meso telialnymi cells, fibroblasts, *NK* lymphocytes, neutrophils, *B* lymphocytes, smooth muscle cells, interstitial cells, sustentocytes, etc. The formation and secretion of this cytokine in all cells is the response to the infectious and inflammatory agents, microbial toxins, vari-

ous cytokines, active complement fragments, certain activated clotting factors, etc. [7, 32, 33]. According to the figurative expression of Bellau, *IL*-1 is a family of molecules for all occasions. *IL*-1 is divided into two fractions, α and β, which are products of different genes but have similar biological properties. Almost all *IL*-1α remains inside cells or binds to membranes. It has been found that this cytokine has not less than 50 various functions, and cells of almost all organs and tissues are its targets. *IL*-1 mostly affects Th_1, although it may also stimulate Th_2 and *B* lymphocytes [59]. It induces the formation of acute-phase proteins in liver cells [79, 80]. Furthermore, it accelerates vasculogenesis after vascular injury. At last, *IL*-1 increases the amount of circulating nitric oxide. It is known that the latter participates in the regulation of blood pressure, promotes platelet disaggregation, and enhances fibrinolysis. All this indicates that *IL*-1 stimulates the development of a complex of protective reactions of the body, which aim at the limitation of the spread of infection, elimination of invading microorganisms, and recovery of damaged tissues.

The continuous stimulation of endothelial cells by *IL*-1α leads to their accelerated aging, which is associated with the increased activity of β-galactosidase. In this case, most of cells are at the G_0-G_1 phase of cell division. The following factors are revealed in stimulated endothelial cells: defects in DNA, increase in the concentration of reactive oxygen species and MDA, and decreased levels of antioxidant enzymes, which control the regeneration and aging of cells. Obviously, the mentioned mechanism is of great importance in the development of atherosclerosis and aging [82, 83].

Many proinflammatory effects of *IL*-1 occur in the complex with *TNFα* and *IL*-6: induction of fever, anorexia, effects on hematopoiesis, involvement in nonspecific anti-infectious protection, secretion of acute-phase proteins, etc. [34, 35, 72]. *IL*-6 is produced by stimulated monocytes, macrophages, endothelial cells, Th_2, fibroblasts, hepatocytes, sustentocytes, cells of the nervous system, thyrocytes, white dendritic epidermocytes, etc. Along with *IL*-4 *IL*-10, it promotes the growth and differentiation of *B* lymphocytes providing their transition to antigen producers. In addition, *IL*-6, like *IL*-1, stimulates hepatocytes, which results in the formation of acute-phase proteins. *IL*-6 affects hematopoietic cells and, in particular, stimulates megakaryocytopoiesis. This compound has also the antiviral activity.

It should be noted that in mice with a knockout of the gene encoding a shared component of receptors for cytokines of the *IL*-6 family, numerous abnormalities incompatible with life are developed in different systems of the body. In addition to the violation of cardiogenesis, one can observe in the embryos of these mice a sharp decline in the number of hematopoietic precursor cells of different hematopoietic series, as well as a dramatic reduction in the size of the thymus. These facts suggest the critical importance of *IL*-6 in the regulation of physiological functions [48].

There are complicated mutually regulating relations among proinflammatory cytokines, which act as synergists [61]. For example, *IL*-6 inhibits the production of *IL*-1 and *TNFα*, although both these cytokines induce the synthesis of *IL*-6. This feature of *IL*-6 determines its dual role in the progression of inflammation. Being a typical proinflammatory cytokine, it also has an anti-inflammatory effect and restricts the production of other proinflammatory cytokines. The biological significance of this phenomenon is obvious: *IL*-6 is the agent that terminates the formation of the inflammatory process [66].

IL-6 does not play the final role in the cardiovascular events [70] because it stimulates the vascular-platelet and coagulation homeostasis and inhibits fibrinolysis [13, 20, 66].

Finally, *IL*-6 affects the hypothalamic pituitary system and enhances the production of cortisol, which inhibits the expression of the *IL*-6 gene as well as the genes of other proinflammatory cytokines [55].

IL-17 is formed by macrophages and special *T*-helpers named *Th*-17. It affects stromal cells of many tissues. It enhances the expression of intercellular adhesive molecules and, in particular, *ICAM*-1 in fibroblasts and stimulates endothelial and epithelial cells. It is found that it plays a significant role in the autoimmune and cancer diseases and in the transplant rejection [34, 35].

Under the influence of *IL*-17, human macrophages intensively produce proinflammatory cytokines (IL-1β and *TNFα*) in the direct dependence on the dose of the studied cytokine. Moreover, *IL*-17 stimulates the formation and secretion of *IL*-6, *IL*-10, *IL*-12, PgE_2, antagonist *RIL*-1, and stromalysine. Anti-inflammatory cytokines *IL*-6 and *IL*-10 completely abolish the formation of *IL*-1β induced by *IL*-17, and $CTF\beta_2$ and *IL*-13 only partially block this effect. The presented facts strongly suggest that *IL*-17 should play an important role in triggering and maintaining the inflammatory process [52].

IL-17 plays an important role in the destruction of collagen and cartilage. *IL*-17 in suboptimal doses has a powerful synergistic effect with *TNFα*, *IL*-1, *IL*-6, and oncostatin *M* on the destruction of collagen. This process is inhibited by *IL*-4 and *IL*-13.

TNF is divided into two fractions, α and β. Both fractions have similar biological properties and affect the same cellular receptors. *TNFα* is secreted in monocytes, macrophages, *Th*1, endothelial and smooth muscle cells, keratinocytes, *NK* lymphocytes, neutrophils, astrocytes, osteoblasts, etc. *TNFα* is formed to a lesser extent in some cancer cells. The main inductor of the synthesis of *TNFα* is bacterial lipopolysaccharide and other components of bacterial origin. In addition, the synthesis and secretion of *TNFα* is stimulated by cytokines *IL*-1, *IL*-2, *IFNα*

and β, *GM–CSF*, etc. In contrast to this, the synthesis of *TNF* is inhibited by Epstein-Barr virus, *IFNα*, β, *IL*-4, *IL*-6, *IL*-10, *G-CSF*, *TGFβ*, etc. [8].

This cytokine plays a key role in the development of the inflammatory response. It affects the synthesis of *IL*-1 and *IL*-6. Moreover, it acts as chemoattractant for neutrophils, activates macrophages, and stimulates proliferation of *T* and *B* lymphocytes.

TNF is involved in the pathogenesis of most infectious and immunopathological diseases, where it acts as a mediator of the development of the inherited immunity.

It was assumed for a long time that the main manifestation of the biological activity of *TNFα* is its effect on some tumor cells. It was noted that *TNFα* led to hemorrhagic necrosis and thrombosis of bringing blood vessels [87]. Synchronously, *TNFα* enhances the natural cytotoxicity of monocytes, macrophages, and *NK* cells. The regression of the tumor cells was especially intensive under the simultaneous action of *TNFα* and *IFNγ*.

Recently, however, these data are subject to revision. Back in the mid-1990s, it was shown that *TNFα* has a tumorigenic effect in vitro and in vivo. It has been found that, besides a direct impact on the formation of tumor tissue, *TNFα* also promotes the angiogenesis and the expression of adhesion molecules, which are involved in the metastasis of transformed cells. These data largely explain why the adverse outcomes are observed at a very high level of *TNFα* in cancer.

TNFα inhibits the synthesis of lipoprotein kinase, one of the main enzymes that regulates lipogenesis. *TNFα* being the mediator of cytotoxicity may inhibit the cellular proliferation, differentiation, and functional activity of many cells [57].

TNFα is directly involved in the immune response. It plays an extremely important role in the early stages of inflammatory reactions because of the activation of the endothelium and the expression of adhesion molecules, which leads to the granulocyte adhesion to the inner vessel surface. This cytokine activates granulocytes, monocytes, and lymphocytes and induces the formation of other proinflammatory cytokines: *IL*-1, *IL*-6, *IFNγ*, and *GM–CSF*, which are synergists of *TNFα* [52].

Being locally formed, *TNFα* dramatically increases the phagocytic activity of monocytes and neutrophils in the site of inflammation or infection and promotes complete phagocytosis by enhancing the peroxide oxidation processes. When acting along with *IL*-2, *TNFα* significantly increases the formation of *IFNγ* by *T* lymphocytes. *TNFα* is also involved in the destruction and reparation processes because affects the growth of fibroblasts and stimulates angiogenesis [57].

TNF is the important regulator of hematopoiesis. Alone or in combination with other cytokines, TNF influences all types of hematopoietic cells. It enhances the function of the hypothalamic-pituitary-adrenal axis system and certain endocrine glands, i.e., thyroid, testis, ovary, pancreas, etc. [7]. Even this short list of properties of the *TNF* molecule indicates the important role of the studied cytokines in the regulation of not only the immunity but also the different physiological functions of the body.

SP influence the gene expression of proinflammatory cytokines and, therefore, should not only contribute to the elimination of the pathological process but also prevent thrombotic complications.

The results allow for the explanation of one of the possible mechanisms of geroprotective action of epitalon. Tetrapeptid epitalon influences the expression of the *IL*-2 and *IFNγ* genes and, thereby, ensures the normalization of the functions of not only the cellular (through cytotoxic and *NK* lymphocytes) but also humoral (through Th_2) immunity and reduces morbidity and mortality among the elderly. Certainly, this does not deny other effects of epitalon, i.e., the normalization of the functions of heat shock proteins, antioxidant protection, the hemostasis system, etc. [18, 19, 21, 41, 42, 44, 76, 77].

The data presented in this review, no doubt, suggest that short peptides, while acting on the gene expression of immune and inflammatory cytokines and *IFNγ*, play the essential role in maintaining the activity of the immune, nervous, endocrine, cardiovascular, hemostatic, and other systems of a body. It means that short peptides trigger the hidden reserves of the biological reliability of the body and, thus, act as geroprotective agents.

REFERENCES

1. Anisimov, V.N., *Molekulyarnye i fiziologicheskie mekhanizmy stareniya* (Molecular and Physiological Mechanisms of Senescence), St. Petersburg: Nauka, 2008, pp. 364–370.
2. Vitkovskii, Yu.A. and Kuznik, B.I., Influence of interleukin-1 on ability of lymphocytes synthesize factors affecting adhesion and aggregation of thrombocytes, blood fibrillation, and fibrinogenesis, *Ross. Fiziol. Zh. im. I. M. Sechenova*, 2002, vol. 88, no. 4, pp. 468–475.
3. Vitkovskii, Yu.A., Solpov, A.V., Shenkman, B.Z., and Kuznik, B.I., IL-1B, IL-2, IL-10, and IL-16 effect upon lymphocytic-thrombocytic aggregate interaction with extracellular matrix, *Immunologiya*, 2006, vol. 27, no. 3, pp. 141–143.
4. Egorova, E.N., Kalinkin, M.N., and Mazur, E.S., Immune mechanisms in pathogenesis of acute cardiac insufficiency, *Pat. Fiziol. Eksper. Med.*, 2012, no. 2, pp. 56–61.
5. Zhdanov, V.S., Chumachenko, P.V., and Drobkova, I.P., Inflammatory and immunological cell reaction in intima of aorta and pulmonary artery, and development of atherosclerosis, *Kardiologiya*, 2004, no. 2, pp. 40–44.
6. Zenkov, N.K., Men'shikova, E.B., and Shkurupii, V.A., Senescence and inflammation, *Usp. Sovrem. Biol.*, 2010, vol. 130, no. 1, pp. 20–37.

7. Ketlinskii, S.A., Simbirtsev, A.S., *Tsitokiny* (Cytokines), St. Petersburg: Foliant, 2008.
8. Kiselev, O.I. and Ershov, F.I., Interferon gamma: new cytokine used in clinical practices, in *Mater. nauchn. konf. "Interferonu 50 let"* (Proc. Sci. Conf. "50 Anniversary of Interferon"), Ershov, F.I., Ed., Moscow, 2007, pp. 15–46.
9. Korkushko, O.V., Age-related changes of hemostasis system as one of the important factors for thrombosis in elderly and senile people, *Krovoobrashch. Gemostaz.*, 2010, no. 4, pp. 5–16.
10. Korkushko, O.V. and Kovalenko, A.I., *Sistema svertyvaniya krovi pri starenii* (The System of Blood Coagulation during Aging), Kiev: Zdorov'ya, 1988.
11. Korkushko, O.V. and Lishnevskaya, V.Yu., Endothelial function during aging, in *Mater. I Vseros. nauchn. konf. "Klinicheskaya gemostaziologiya i gemoreologiya v serdechno-sosudistoi khirurgii"* (Proc. I All-Russ. Sci. Conf. "Clinical Hemostasiology and Hemorheology in Cardiovascular Surgery"), Moscow, 2003, p. 32.
12. Kudryashova, I.A. and Polunina, O.S., Comparative study of production of proinflammatory cytokines in community-acquired pneumonia in different periods of the disease depending on the age of the patients, *Fundam. Issled.*, 2007, no. 12, pp. 113–114.
13. Kuznik, B.I. The relationship immunity and hemostasis in experiment and clinical practice, in *Mater. IV Vseros. nauchn. konf. "Klinicheskaya gemostaziologiya i gemoreologiya v serdechno-sosudistoi khirurgii"* (Proc. IV All-Russ. Sci. Conf. "Clinical Hemostasiology and Hemorheology in Cardiovascular Surgery"), Moscow, 2009, pp. 267–269.
14. Kuznik, B.I., *Kletochnye i molekulyarnye mekhanizmy regulyatsii sistemy gemostaza v norme i patologii* (Cellular and Molecular Regulatory Mechanisms of Hemostasis in Norm and Pathology), Chita: Ekspress Izd., 2010.
15. Kuznik, B.I. and Lozhkina, A.N., The relationship of the complement system, kallikrein-kinin system and system of hemostasis, in *Fiziologicheskie sistemy gemostaza* (Physiological Systems of Hemostasis), Baluda, V.P., Ed., Moscow, 1995, pp. 150–160.
16. Kuznik, B.I., Vasil'ev, V.N., and Tsybikov, N.N., *Immunogenez, gemostaz i nespetsifichnaya rezistentnost' organizma* (Immunogenesis, Hemostasis, and Nonspecific Resistance of Organism), Moscow: Meditsina, 1989.
17. Kuznik, B.I., Vitkovsky Yu.A., Lulkina?E.V. Age features of haemostasis in human, *Usp. Gerontol.*, 2005, no. 16, pp. 38–48.
18. Kuznik, B.I., Linkova, N.S., and Khavinson, V.Kh., Heat shock proteins: aging changes, development thrombotic diseases and peptidergic regulation of genes, *Usp. Gerontol.*, 2011, vol.?24, no. 4, pp. 539–552.
19. Kuznik, B.I., Morozov, V.G., and Khavinson, V.Kh., *Tsitomediny* (Cytomedines), St. Petersburg: Nauka, 1998.
20. Kuznik, B.I., Tsybikov, N.N., and Vitkovskii, Yu.A., United cellular-humoral protection system of organism, *Tromboz, Gemostaz Reologiya*, 2005, no. 2, pp. 3–16.
21. Kuznik, B.I., Lin'kova, N.S., Khavinson, V.Kh., and Tsybikov, N.N., Heat shock proteins, atherosclerosis, thrombosis, disseminated intravascular coagulation syndrome, and life expectancy. Peptide mechanisms of regulation, *Gemostazilogiya*, 2011, no. 2, pp. 28–52.
22. Kuznik, B.I., Likhanov, I.D., Tsepelev, V.L., and Sizonenko, V.A., *Teoreticheskie i klinicheskie aspekty bioregulyatsii terapii v khirurgii i travmatologii* (Theoretical and Clinical Aspects of Bioregulatory Therapy in Surgery and Traumatology), Novosibirsk: Nauka, 2008.
23. Kukhtina, N.B., Aref'eva, T.I., Aref'eva, A.M., et al., Expression of chemokines and cytokines in the atherosclerotic plaques and internal shell of arteries in patients with coronary heart disease, *Ter. Arkh.*, 2008, no. 4, pp. 63–69.
24. Kukhtina, N.B., Rutkevich, P.N., Shevelev, A.Ya., et al., Involvement of b2-intergrins CD11b/CD18 and CD11c/CD18 in adhesion and migration of the cells on fibrinogen, *Ross. Fiziol. Zh.*, 2011, vol. 97, no. 6, pp. 601–608.
25. Mayanskii, A.N. and Mayanskii, N.A., Late cytokine HMGB1: mediator functions and prospects, *Immunologiya*, 2009, no. 4, pp. 232–237.
26. Oganov, R.G., Pozdnyakov, Yu.M., and Volkov, V.S., *Ishemicheskaya bolezn' serdtsa* (Coronary Heart Disease), Moscow: Sinergiya, 2002, pp. 7–20.
27. Pal'tsev, M.A., Ivanov, A.A., and Severin, S.E., *Mezhkletochnye vzaimodeistviya* (Intercellular Interactions), Moscow: Meditsina, 2003.
28. Pigarevsky, P.V., Maltseva, S.V., Voskanyants, A.N., Seliverstova, V.G., and Snegova, V.A., Morphometric study of Th1 and Th2 cells in the vessel wall in human atherogenesis, *Tsitokiny Vospalenie*, 2010, vol. 9, no. 1, pp. 13–16.
29. Ragino, Yu.I., Chernyavski, A.M., Polonskaya, Ya.V., Volkov, A.M., Semaeva, E.V., Tsimbal,?S.Yu., and Voevoda, M.I., Changes of proinflammatory cytokines and destructive metalloproteinases contents during formation of unstable atherosclerotic plaque, *Kardiologiya*, 2009, vol. 49, no. 6, pp. 43–49.
30. Ragino, Yu.I. Chernyavski, A.M. Polonskaya, Ya.V., Volkov, A.M., and Kashtanova, E.V., Activity of the inflammatory process in different types of unstable atherosclerotic plaques *Bull. Exp. Biol. Med.*, 2012, vol. 153, no. 2, pp. 186–189.
31. Ryzhikova, Yu.V., Druzhinina, Yu.G., Ryabicheva, T.G., et al., A new kit of reagents for the cultivation and mitogenic cell activation of the whole blood, *Novosti Vektor-Best*, 2009, no. 4, pp. 4–12.
32. Simbirtsev, A.S., Interleukin-8 and other chemokines, *Immunologiya*, 1998, no. 4, pp. 9–13.
33. Simbirtsev, A.S., Cytokines as a new system, regulating body defense reactions, *Tsitokiny Vospalenie*, 2002, vol. 1, no. 1, pp. 9–16.
34. Simbirtsev, A.S., Clinical use of cytokines-based drugs, *Immunologiya*, 2004, vol. 25, no. 4, pp. 247–251.
35. Simbirtsev, A.S., The role of cytokines in the regulation of physiological functions of the immune system, *Fiziol. Patol. Immun. Sist.*, 2004, no. 10, pp. 3–9.
36. Smirnova, A.V. and Naumcheva, N.N., Solar activity and cardiovascular system diseases, *Klin. Med.*, 2008, no. 1, pp. 10–17.
37. Stoika, R.S., Filchenkov, A.A., and Zalessky,?V.N., Cytokines and target cells in regulatory system of

atherogenesis, *Usp. Sovrem. Biol.*, 2003, vol. 123, no. 1, pp. 82–97.

38. Sukmanova, I.A., Yakhontov, D.A., Pospelova, T.I., Kuzinskaya,?O.S., and Kosoukhov, A.P., The clinical picture of morphofunctional parameters, and endothelial function in patients with systolic chronic heart failure in different age groups, *Tsitokiny Vospalenie*, 2010, vol. 9, no. 3, pp. 30– 34.
39. Titov, V.N., Atherosclerosis – the problem of general biology: biological dysfunction of nutrition and ecology, *Usp. Sovrem. Biol.*, 2009, vol. 129, no. 2, pp. 124–143.
40. Khavinson, V.Kh., *Peptidnaya regulyatsiya stareniya* (Peptide Regulation of Senescence), St. Petersburg: Nauka, 2009.
41. Khavinson, V.Kh., Kuznik, B.I., and Lin'kova, N.S., Influence of peptide bioregulators and heat shock proteins on formation of age-related changes in hemostatic system, *Tromboz, Gemostaz Reol.*, 2011, no. 4, pp. 15–32.
42. Khavinson, V.K., Lin'kova, N.S., Dudkov,?A.V., Polyakova, V.O., and Kvetnoi, I.M., Peptidergic regulation of expression of genes encoding antioxidant and anti-inflammatory proteins, *Bull. Exp. Biol. Med.*, 2012, vol. 152, no. 5, pp. 615–618.
43. Khaitov, R.M., *Fiziologiya immunoi sistemy* (Physiology of Immune System), Moscow: Vseross. Inst. Nauchnoi Tekh. Inf., Ross. Akad. Nauk, 2005.
44. Tsybikov, N.N. and Kuznik, B.I., Immune regulatory mechanism of hemostasis, *Probl. Gematol. Perelivaniya Krovi*, 1986, no. 2, pp. 23–28.
45. Shavrin, A.P., Khovaeva, Ya.B., Chereshnev, V.A., and Golovskii, B.V., Markers of inflammation in the development of an atherosclerosis, *Kardiovask. Ter. Prof.*, 2009, no. 3, pp. 13–15.
46. Shlychkova, T.P., Cherpachenko, N.M., Chumachenko, P.V., et al., Pathomorphological features of atherosclerotic plaques at acute coronary syndrome, *Kardiologiya*, 2003, no. 12, pp. 42–46.
47. Yakushenko, E.V., Lopatnikov, Yu.A., and Sennikov, S.V., A role of interleukin-18 in immune response, *Med. Immunol.*, 2005, no. 4, pp. 355–364.
48. Yarilin, A.A., Cytokines in the thymus: functions and biological action, *Tsitokiny Vospalenie*, 2003, vol. 2, no. 2, pp. 1–11.
49. Abeyama, K., Stern, D.M., and Ito, Y., The N-terminal domain of thrombomodulin sequesters high mobility group B1 protein, a novel anti-inflammatory mechanism, *J. Clin. Invest.*, 2005, vol. 113, pp. 1267–1274.
50. Anisimov, V.N. and Khavinson, V.Kh., Peptide bioregulation of aging: results and prospects, *Biogerontology*, 2010, vol. 11, pp. 139–149.
51. Baars, J.W., de Boer, J.P., Wagstaff, J., et al., Interleukin-2 induces activation of coagulation and fibrinolysis: resemblance to the changes seen during experimental endotoxaemia, *Br. J. Haematol.*, 1992, vol. 82, no. 2, pp. 295–301.
52. Balkwill, F.R., *Cytokine Cell Biology*, Oxford Univ. Press, 2001.
53. Baron, S., Tyring, S., Fleschmann, W., et. al., The interferons mechanisms of action and clinical applications, *JAMA, J. Am. Med. Assoc.*, 1991, vol. 266, pp. 1375–1384.
54. Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., et al., Database issue, *GenBank, Nucleic Acids Res.*, 2009, vol. 37, no. 10, pp. 26–31.
55. Berezhnaya, N. and Sepiashvili, R., Microenvironment and immuno-rehabilitation in various pathologies, *Int. J. Immunorehab.*, 2009, vol. 11, no. 2, pp. 173–176.
56. Caytanot, F., Nygard, M., Perret, M., et al., Plasma levels of interferon g correlates with age-related disturbances of circadian rhythmus and survival in a no-human primate, *Chronobiol. Int.*, 2009, vol. 26, no. 8. P. 1587–1601.
57. Chandler, S., Miller, K.M., Clements, J.M., et al., Matrix tumor necrosis factor and multiple sclerosis, *J. Neuroimmunol.*, 1997, vol. 272, pp. 155–161.
58. Clark, I.A., The advent of the cytokine storm, *Immunol. Cell. Biol.*, 2007, no. 4, pp. 271–273.
59. Dinarello, C., Biologic basis for IL-1 in disease, *Blood*, 1996, no. 6. Ð. 2095–2147.
60. Esmon, C.T., The interactions between inflammation and coagulation, *Br. J. Haematol.*, 1999, vol. 84, pp. 254–259.
61. Esmon, C.T., Fukudome, K., Mather, T., et al., Inflammation, sepsis and coagulation, *Haematologica.*, 2005, vol. 131, pp. 417–430.
62. Fedoreyeva, L.I., Kireev, I.I., Khavinson, V.Kh., and Vanyushin, B.F., Penetration of short fluorescence-labeled peptides into the nucleus in HeLa cells and *in vitro* specific interaction of the peptides with deoxyribooligonucleotides and DNA, *Biochemistry*, 2011, vol. 76, no. 11, pp. 1505–1516.
63. Galkina, E. and Ley, K., Immune and inflammatory mechanisms of atherosclerosis, *Ann. Rev. Immunol.*, 2009, vol. 27, pp. 165–197.
64. Habenicht, A.J., Hawroth, P., Schweigerer, L., et al., The role of endothelial cells in the maintenance of arterial wall integrity and their potential role in the pathogenesis of arteriosclerosis, *Beitr. Infusionsther.*, 1988, no. 23, pp. 18–25.
65. Hack, C.E., Derangements of coagulation end fibrinolysis in infections diseases, *Contrib. Microbiol.*, 2003, vol. 10, pp. 19–37.
66. Hager, K., Machein, U., Krieger, S., et al., Interleukin 6 and parameters haemostasis, *Fibrinolysis.*, 1996, suppl. 1, p. 54.
67. Hansson, G.K., Inflammation, atherosclerosis and coronary artery disease, *N. Engl. J. Med.*, 2005, vol. 352, no. 16, pp. 1685–1695.
68. Hansson, G.K., Inflammatory mechanisms in atherosclerosis, *J. Thromb. Haemostasis.*, 2009, vol. 7, suppl. 1, pp. 328–331.
69. Hartford, M., Wiclung, O., Hilten, L., et al., Interleukin-18 as a predictor of further events in patients with acute coronary syndromes, *Arterioscler., Thromb., Vasc. Biol.*, 2010, vol. 30, no. 10, pp. 2039–2046.
70. Hedman, A., Larson, P.T., Alam, M., et al., CRP, IL-6 and endothelin-1 levels in patients undergoing coronary artery bypass grafting, *Int. J. Cardiol.*, 2007, vol. 120, pp. 108–114.
71. Heitmeier, M.R., Scarim, A.I., and Corbet, J.A., Interferon g increases the sensitivity of islets of Langerhans for inducible nitric-oxide synthase expression induced

by interleukin-1, *J. biol. Chem.*, 1997, vol. 272, no. 21, pp. 13697–13704.

72. Ikejima, T., Okusawa, S., and Dinarelio, C.A., Syndrome is mediated by interleukin 1 and tumor necrosis factor, *Rev. Infect. Dis.*, 1989, no. 11, pp. 320–323.
73. Jovanovic, D.V., Di Battista, J., Martel-Pelletier, J., et al., IL-17 stimulates the production and expression of prainflammatory cytokines, *J. Immunol.*, 1998, no. 7, pp. 3513–3521.
74. Khavinson, V.Kh., *Peptides and Ageing* Ohio, USA: Neuroendocrinol. Lett., 2002, special issue.
75. Khavinson, V.Kh. and Linkova, N.S., Morphofunctional and molecular bases of pineal gland aging, *Hum. Physiol.*, 2012, vol. 38, no. 1, pp. 119–127.
76. Khavinson, V.Kh. and Malinin, V.V., *Gerontological Aspects of Genome Peptide Regulation*, Basel, Switzerland: Karger AG, 2005.
77. Khavinson, V.Kh., Shataeva, L., and Chernova, A., DNA double-helix binds regulatory peptides similarly to transcription factors, *Neuroendocrinol. Lett.*, 2005, vol. 26, no. 3, pp. 237–241.
78. Khavinson, V.Kh., Linkova, N.S., Trofimov, A.V., et al., Morphofunctional Fundamentals for peptide regulation of aging, *Biol. Bull. Rev.*, 2011, vol. 1, no. 4, pp. 390–394.
79. Klark, I.A., The advent of the cytokine storm, *Immunol. Cell. Biol.*, 2007, no. 4, pp. 271–273.
80. Kuznik, B.I. and Tsibikov, N.N., Cytokines, immunoglobulins and hemostasis, *Hematol. Rev.*, 1996, vol. 7, part 2, pp. 43–70.
81. Kuznik, B.I., Lin'kova, N.S., and Khavinson, V.Kh., Heat shock proteins: changes related to aging, development of thrombotic complications, and peptide regulation of the genome1, *Adv. Gerontol.*, 2012, vol. 2, no, pp. 175–186.
82. Libbi, P., Suchova, G., Lee, R.T., and Galis, S.Z., Cytokines regulate vascular functions related to stability of the atherosclerotic plaque, *J. Cardiovasc. Pharmacol.*, 1995, vol. 25, suppl. 2, pp. 9–12.
83. Lusis, A.J., Atherosclerosis, *Nature*, 2000, vol. 407, pp. 233–241.
84. Ngoc, P.L., Gold, D.R., and Tzianabos, A.O., Cytokines, allergy, and asthma, *Curr. Opin. Allergy Clin. Immunol.*, 2005, vol. 5, pp. 161–166.
85. Nilsson, J., Lipoproteins and inflammation in cardiovascular disease, *Fibrinolysis*, 1996, vol. 10, no. 1, suppl., p. 43.
86. Nyugen, J., Sudhanshu, A., Sastry, G., et al., Impaired functions of peripheral blood monocyte subpopulations in aged humans, *J. Clin. Immunol.*, 2010, vol. 30, no. 6, pp. 806–813.
87. Osterud, B., Tissue factor and its role in hemostasis and thrombosis, *Trombozy, Krovotochivost Bolezni Sosudov*, 2008, suppl. 6, pp. 3–6.
88. Robertson, A.K. and Hansson, G.K., T-cells in atherogenesis: for better or for worse? *Arterioscler., Thromb., Vasc. Biol.*, 2006, vol. 26, no. 11, pp. 2421–2432.
89. Ross, R., Atherosclerosis – an inflammatory disease, *N. Engl. J. Med.*, 1999, vol. 340, pp. 115–126.
90. Simon, D.L., Hen, Z., Hu, H., et al., Platelet glycoprotein Iba is a counter receptor for the leukocyte integrin Mac-1, *J. Exp. Med.*, 2000, vol. 192, pp. 193–204.
91. Tenger, C., Sundborger, A., Jawien, J., and Zhou, X., IL-18 accelerates atherosclerosis accompanied by elevation of IFNg and CXCL16 expression independently of T-cells, *Arterioscler., Thromb., Vasc. Biol.*, 2005, vol. 25, pp. 655–657.
92. Yang, H., Wang, H., and Tracey, K.G., The cytokine activity of HMGB1, *J. Leukocyte Biol.*, 2005, vol. 78, pp. 1–8.
93. Yang, H.L., Hu, Y.Y., Du, L.F., et al., Chemokine CHCL16 expression in peripheral blood of patients with acute coronary syndrome, *Clin. Med. J.*, 2008, vol. 121, no. 2, pp. 112–117.

Translated by A. Levina

SPELL: 1. hemostasis, 2. macrophages, 3. leukotrienes