Reviews

Nitric oxide as the main multifunctional regulator of immunocompetent and endothelial cells

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Nitric oxide (NO) plays an important role in metabolism. Consequently, every tissue of our body is affected by changes in concentrations of nitric oxide and its metabolites. Here we analyze phenotypic effects of NO and its metabolites in macrophages, NK-cells and lymphocytes that perform protection of the host organism against malignant tumors and intracellular pathogens. Immune system imbalance may result from misregulation of NO synthesis. Nitric oxide also plays an important role in endothelial dysfunction including inhibition of platelet adhesion and aggregation, vasoconstriction and vasodilation disorders of blood vessels, blood rheology changes and the development of atherosclerotic plaques. Thus an imbalance in the synthesis of nitric oxide in the vascular endothelium may be an early marker of lesions of various origins.

K e y w o r d s: NO-synthases, lymphocytes, immune system, inflammation, endothelial dysfunction, endotheliocytes.

Introduction

Nitric oxide [(NO)], easily penetrating through biological membranes, is a natural unique secondary messenger, since it not only takes part in implementation of physiological processes, but causes many pathological states in a living organism. The NO effect on cells depends on its amount, which in turn depends on different NO-synthases isoforms (NOS), but, as a rule, this effect is primarily caused by inducible NO-synthases (iNOS) [1–3]. The key function of NO produced by iNOS is involving in the immune processes, including antipathogenic reactions, non-specific cytotoxicity, antitumor protection, transplant rejection reactions *etc.*

Nitric oxide and immune system. Nitric oxide has been recently determined as one of the most diverse factors that exhibit their regulative effect on the immune system affecting inter- and intracellular molecules and mediating the immune response [4–6]. NO is involved in pathogenesis and control of infectious, tumor, autoimmune processes and chro-

nic degenerative diseases [7–9]. However, nowadays there is no simple and clear picture of the NO effect on the immune system. The protective and toxic NO effects can often be observed simultaneously [10, 11]. Being produced by the range of cells involved in immune and inflammatory reactions (macrophages, dendritic cells, lymphocytes, neutrophils, eosinophils, monocytes, Kupffer cells, hepatocytes, microglia, endothelial, epithelial cells, fibroblasts *etc.*), NO plays an important role affecting the processes of their maturation, differentiation, proliferation and apoptosis [12–15]. According to the literature data [5, 16–20], NO produces an active effect on:

– selection and maturation of T-lymphocytes in the thymus;
– lymphocyte migration and recirculation and balance of the population-clonal composition;
– maintaince of the T-helper-suppressor balance;
– thymus age involution;
– IFNγ production by NK-cells and support of their cytolytic properties;
– decrease/increase of cytokines synthesis resulting in stimulation/inhibition of cytotoxic function of immunocompetent cells] [21, 22].

Nowadays the question of synthesis of NOS isoforms by primary T- and B-lymphocytes is still open [23–25]. At the early stage of immune response, NO induces the series of important effector functions of immune cells [26]. In the first four hours after thepathogenic agent entrusion into the organism, the nonspecific mechanisms of innate immunity are activated; neutrophils, macrophages, NK-cells and the complement system take part in the implementation mechanisms. The phagocytes, which are activated by the pathogens, produce proinflammatory cytokines (TNFα, IL-12 *etc.*), which induce thesynthesis of IFNγ by stimulated NK-cells. TNFα and IFNγ are promoters of the iNOS in immunocompetent cells – the NO, which is produced by them, mediates killing microbial pathogens, but excessive concentration of the radicals in NK-cells impairs their function [27–32]. A capacity of T-cells of switching the IFNγ program from proliferation to apoptosis under the influence of NO has been proved. Additionally, NO is able to induce interferon-independent apoptosis of T-cells by affecting their proliferation directly [11, 33–36].

The lymphocyte migration and recirculation processes, antibody production, proliferation of T- and B-lymphocytes, production of cytokines and a number of proteins and enzymes, formation of different cell adhesion molecules, such as VCAM-1, ICAM-1, E-selectin (CD62E) and P-selectin (CD62R), are inhibited as a consequence of significant increase of NO level [37–45].

The progression of an allergic type of inflammation is related to the effect of elevated concentrations of NO and its derivatives on eosinophils due to suppression of Fas-dependent apoptosis. At the same time, the induction of NO-dependent neutrophilic apoptosis contributes to the inhibition of allergic inflammation. Cytostatic-cytotoxic effects of nitric oxide and peroxynitrite, caused by NO action in the process of «respiratory explosion» in macrophages, contribute to more active elimination of infectious agents [43–45].

Some authors [17, 29] describe the NO impact on the change in the helper T-lymphocytes subpopulations pattern that causes modification of cytokines set produced by Th1 and Th2 types of cells, and as a consequence – the qualitative and quantitative imbalance in the immune sys-

tem [34, 46]. Under these circumstances, the regulatory function of T-lymphocytes is impaired resulting in "slipping out" the transformed cells immunologic control and possible tumor development [47–49]. The NK-lymphocytes use NO or its derivatives for elimination of bacteria and transformed cells. However, an excess of NOS inducible isoform and NO metabolism products, causing the immunity disbalance, reduces the NK cytotoxic capacity by stimulating premature apoptosis and even necrosis [50–53].

In cases of immune and inflammatory processes, the iNOS expression clearly correlates with the disease progression that can point to its ototoxic and / or immunosuppressive effect [54–57].

The NO role in the autoimmune diseases development is being actively studied. The nitration of proteins caused by NO increases their antigenicity resulting in a severe course of autoimmune processes and manifestations of autoimmune disease [58, 59]. Thus, experimental autoimmune disease [60] was accompanied by a significant increase in the NO production with an increased level of NO_2^- / NO_3^- in blood. Difficult course of sclerodermia was accompanied by a change in the NO synthesis [61]. The NO effect is also important in the rheumatoid arthritis pathogenesis [62]. The iNOS activation, mediated by cytokines and TNFα, causes a cytotoxic effect in the cells of synovial fluid of joints [63, 64]. According to [65], the level of nitrites / nitrates in patients with rheumatoid arthritis increased regardless of gender and correlates directly with the stage of disease. The iNOS activity increased upon multiple sclerosis causing not only a cytotoxic effect but also a damage of blood-brain barrier and its higher permeability [8]. High concentration of NO and products of its me-

tabolism is also determined in blood and cerebrospinal fluid of patients with systemic lupus erythematosus (SLE) – synthesis of NO is being increased with the disease progression [58, 66]. Additionally, inhibition of proliferation and increased apoptosis of lymphocytes and macrophages cause the development of secondary immunodeficiency [67].

Along with a cytotoxic effect of NO, there are many examples of its cytoprotective effect, particularly, in the B-lymphocytes and human cell cultures *in vitro*, such as splenocytes, eosinophils, hepatocytes, endothelial cells [68]. The NO protective effect in different cell types is mediated by cAMP [6]. Probably, cAMP synthesized under the NO effect can activate cAMP-dependent protein kinases, which, in turn, affect apoptotic proteins (e.g., caspases, Vs1-2). Antiapoptotic effect of NO associated with inhibition of caspases is of interest as well. Active forms of NO influence cysteine, a residue in the caspase active center, by nitration of SH groups, causing inhibition of caspase cascade. Some authors [53, 69] emphasize the enhanced expression of heat shock proteins (Hsp) that protect cells against various stresses; the NO antiapoptotic effect correlates with an increase of Hsp synthesis.

Currently there is almost no data on the role of NO metabolism arginase pathway in immunocompetent cells [25]. The fact is that arginase starts to work in activated macrophages and lymphocytes in parallel with NO-synthase. Under such conditions, arginase can compete with NO-synthase for the common substrate arginine and even inhibit the formation of NO. Excessive arginase synthesis [5, 25, 70] in the organism correlates with pathology, moreover, with the tendency to develop chronic processes

[71]. Enhanced arginase production results in the imbalance in biosyntesis of polyamines and plays an important role in the proliferation of all mammalian cells. Polyamines noncovalently interact with various biomacromolecules, being a part of chromatin involved in replication of DNA, and their concentration significantly increases in the periods of active cell division and tissue growth [40, 72]. Disturbed metabolism of polyamines may be a serious factor of carcinogenesis. The arginase activity is increased in tumor cells, and, therefore, can be used as a marker in early diagnosis of tumors. A reduction of polyamines synthesis causes inhibition of the tumor cells metastasis *in vitro* and *in vivo* [25].

Consequently, it can be assumed that imbalance of two pathways – oxidative (NO-synthase) and not oxidative (arginase) – leads to serious changes in immunocompetent cells and immune reactions.

L-arginine nitric oxide system and endothelium. A number of recent studies have obviously changed the opinion concerning the vascular endothelium and its role in the overall homeostasis [73–76]. It was revealed that endothelium synthesizes a large amount of biologically active substances that play an important role in various processes of living organism, in norm and pathology. These substances are mostly paracrine (in neighboring cells) or autocrine-paracrine (in endothelium) [2, 77]. However, a vascular wall is a dynamic structure, its endothelium is constantly being renewed, dead fragments containing biologically active substances enter blood, spread throughout the organism and may affect systemic circulation [78–80].

Endothelium regulates hemodynamic reactions, hemostasis, immune reactions, regenera-

tion *etc.*; it is considered to be one of the diffuse endocrine systems [81]. Endothelial cells synthesize several regulatory hormonal substances called histohormons. The location on the border between tissues and blood underlies a special structure of endothelium. The intimal layer of the vessel wall is extremely smooth and covered with a mucous membrane – glycocalyx. Glycocalyx is composed of mucopolysaccharides possessing antiadhesive properties: they protect blood cells against adhesion to the blood vessel walls. There are a large number of specific receptors on the glycocalyx surface. It supports the binding of thrombin and prevents the adhesion of platelets to the endothelium [82].

The L-arginine-nitric-oxide system is a main biochemical unit to implement all physiological functions of endothelium in the organism [25]. NO is contained in all endothelial cells regardless of the size or function of the vessel. Small-caliber blood vessels synthesize more NO than larger ones, thus regulating the peripheral resistance, blood pressure and distribution of vascular blood flow. The basal level of NO production is higher in arteries than in veins [56]. Therefore, an indicator of normal endothelium function is its capacity for continuous synthesis of NO, required to maintain normal vascular tone. The NO synthesis is stimulated by the activation of multiple receptors located on the membrane of endothelial cells with biologically active substances: acetylcholine, kinins, serotonin, thromboxane, thrombin [43]. Being formed in the endothelium, NO diffuses into smooth muscle cells and causes dilatation of blood vessels [58]. The vasodilation is regulated by calcium and potassium ions within the vascular smooth muscle cells through the cGMP-dependent mechanism. Thus, cGMP, as a secondary mes-

senger, mediates a range of biological effects of nitric oxide, including control of vascular tone [83, 84]. It is very important, because in the absence of NO the vasodilators do not react to acetylcholine appropriately, whereas the nature of the blood vessel reaction to acetylcholine is an indicator of the vessel physiological state. Such type of reaction is called endothelium-dependent [85, 86].

The vascular wall containing nitric oxide synthesized in platelets and leukocytes with the help of two synthases (macrophage and endothelial), prevents adhesion and aggregation of platelets, as well as the secretion of substances, like thromboxane or serotonin, in other words, implements the atrombogene effect and does not allow an excessive vasoconstriction [87–90].

A prolonged exposure to various damaging factors (hypoxia, intoxication, inflammation, hemodynamic overload) causes a gradual depletion and distortion of compensatory dilation property of endothelium. In these circumstances, vasoconstriction is a function of smooth muscle cells [91]. Accumulation of vasoconstrictive factors in the endothelium may lead to the proliferative effect relative to the inner and middle membranes of vessels, disruption of baroreceptors structure, an increase of blood pressure, and the development of atherosclerosis [92, 93]. The cardiovascular system is also getting disrupted, as the endothelium is damaged, its dysfunction supports the synthesis and release of vasoconstrictors, and as a result, small arteries are prone to cramps that may cause tissue hypoxia, the development of arterial hypertension and myocardial damage [92–94].

It is well-known that the inflammatory process is a basis of various pathological states [37]. The endothelium, activated under inflammatory conditions, secretes inflammatory mediators, stimulating the expression of cell adhesion molecules [95], like receptors for selectins and integrins, which are present in all immune cells. This facilitates the migration of immune cells through the endothelial barrier into the tissue, where the inflammatory process is developing [25, 37]. Each phase of the inflammatory process is associated with the appropriate NOS isoforms.

At the early stage of inflammatory reaction, under the influence of mediators (histamine, bradykinin, prostaglandins and leukotrienes) [87] a stimulation of NO production through iNOS is observed. Within the cells of vascular endothelium, NO activates soluble guanylate cyclase that leads to the intensive synthesis of cGMP, resulting in relaxation of smooth muscle cells of the vascular wall and increment of its permeability [85].

In the course of chronic inflammation, NO stimulates the release of proinflammatory cytokines – IL-1, IL-2, IL-3, IL-6, leukotrienes, chemokines, which stimulate the migration of leukocytes to the site of inflammation [61, 96–100].

Endotheliocytes lose anti-aggregatory and anti-inflammatory properties during prolonged and intensive activation, providing protrombogene vascular surface and stimulating further inflammation, which leads to the development of degenerative and necrotic changes [1]. Deficiency of endothelial NOS in the course of inflammation impairs the ability of inflammatory mediators to inhibit the expression of proinflammatory genes that prevents resolution of inflammation. Chronic inhibition of the NO synthesis (in experimental condition) provokes early manifestations of inflammatory changes within the vascular wall with varying extent

of its damage [101, 102]. The activity of iNOS is also stimulated resulting in the excessive production and accumulation of peroxynitrite, nitrogen dioxide and hydroxyl groups, the potential targets of which are DNA and cellular proteins. High concentrations of aggressive oxygen forms activate LDL oxidation, and launch another mechanism of endothelial damage [103–105]. Under such conditions [54, 106–108] endothelium plays a key role in the pathogenesis of a number of systemic pathologies (atherosclerosis, hypertension, stroke, pulmonary hypertension, heart attack, diabetes, *etc.*). On the other hand, oxidative and nitro active types of stress lead not only to the cellular damage by free radicals and increased synthesis of proinflammatory mediators but also to the modification of endothelial NO-receptors [109–114].

In the course of NO metabolic biotransformation, nitric oxide is intensively synthesized, therefore, significant changes in its concentration cause the microcirculation disturbance in the area of pathological process [115]. The content of nitrite anion was found to be significantly reduced with the age. Obviously, its decrease is associated with a reduction of the cNOS activity. Instead, the iNOS activity is increased resulting in the excessive production and accumulation of peroxynitrite in tissues with age. A reduction in both oxidative (NO-synthases) and non-oxidative (arginase) ways of the arginine conversion is observed, whereas the arginase activity and urea increased with age [78, 116]. The reason for these changes may vary from the age-related disorders in endothelial cells, reduction in the genes expression, accumulation of calcium and oxidative stress, to the lipid membranes restructuring. These

disorders are considered to be a primary factor in the age-dependent pathologies of the cardiovascular system [83, 117–120].

Thus, NO, participating in the regulation of vascular tone stabilization of the permeability of the vascular wall membrane, improvement of rheological properties of the blood and suppression of proliferation of vascular smooth muscle cells and monocytes, prevents pathological reconstruction of the vascular wall. The violation of these processes is the basis for pathological changes, namely, the development of atherosclerosis, hypertension and other manifestations of endothelial dysfunction. However, a wide range of NO bioregulatory activity is not limited to these properties. Participating in the maintenance of all homeostatic parameters, it is directly or indirectly associated with each pathological process or extreme state of the organism. Therefore, the systems of NO synthesis and degradation, including the L-arginine-nitric oxide system, play an important role in the development of pathological processes within the organism. Consequently, the laboratory determination of NO metabolites or substances regulating their production is a perspective way for prognoses of many diseases.

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Оксид азоту як основний поліфункціональний регулятор імунокомпетентних та ендотеліальних клітин

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Оксид азоту (NO) відіграє важливу роль в метаболізмі. Отже, кожна тканина нашого тіла піддається ушкодженню при зміні концентрації оксиду азоту і його метаболітів. У статті проаналізовано фенотиповий ефект оксиду азоту та його метаболітів у макрофагах, NK- клітинах, лімфоцитах, який забезпечує захист організму від злоякісних новоутворів та внутрішньоклітинних збудників. Дисбаланс в імунній відповіді може бути спричинений дисрегуляцією синтезу NO. Оксид азоту також відіграє важливу роль в розвитку ендотеліальної дисфункції, яка полягає в інгібуванні адгезії та агрегації тробмоцитів, порушенні регуляції вазоконстрикції та вазодилатації, змінах реологічних властивостей крові та формуванні атеросклеротичних бляшок. Отже, дисбаланс в синтезі оксиду азоту ендотелієм судин може бути раннім маркером розвитку різноманітних патологій .

Оксид азота как основной полифункциональный регулятор иммунокомпетентных и эндотелиальных клеток

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Оксид азота (NO) играет важную роль в метаболизме. Поэтому, каждая ткань нашего тела поддается повреждению при смене концентрации оксида и его метаболитов. В статье мы анализируем фенотипический ефект оксида азота и его метаболитов в макрофагах NK-клетках, лимфоцитах, который обеспечивает защиту организма от злокачественных новообразований и внутриклеточных возбудителей. Дисбаланс в иммунном ответе может бить обусловлен дисрегуляцией синтеза NO. Оксид азота играет важную роль в разитии эндотелиальной дисфункции, суть которой в ингибировании адгезии и агрегации тромбоцитов, нарушении регуляции вазоконстрикции и вазодилатации, изменении реологических свойств крови и формировании атеросклеротических бляшек.Поэтому дисбаланс в синтезе оксида азота эндотелием сосудов может бить ранним маркером развития разнообразных патологий.