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MOLECULAR PHYSIOLOGY AND GENETICS

DISEASE-MODIFYING THERAPY IN RHEUMATOID ARTHRITIS AND CHANGES IN CYTOKINE LEVELS DEPENDING ON GENETIC CHARACTERISTICS

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Abstract

Among the factors that influence the occurrence of rheumatoid arthritis, a complex interaction of genetic, environmental, immunological, hormonal, socio-economic and psychological prerequisites is noted. Genetic factors in interaction with the environment and an individual's lifestyle play an important role in changing gene expression and the clinical realization of inherited or acquired pathology, including multifactorial. That is why the study of genetic mutations and their association in patients with rheumatoid arthritis is important today not only from a scientific point of view, but also for practical health care.

Keywords: rheumatoid arthritis, eNOS gene promoter *T-786C* polymorphism, disease-modifying therapy, cytokine profile.

Introduction. Despite a number of studies establishing the role of the above-mentioned genetic factors in the occurrence of certain vascular, infectious and autoimmune diseases, including rheumatoid arthritis (RA) [1, 2, 3, 4], individual immunological mechanisms of RA development remain unstudied and require detailed targeted research.

A characteristic feature of RA is the presence of a chronic inflammatory process in the joints, accompanied by pain, swelling and loss of function of the affected joint, as well as general symptoms (weakness, rapid fatigue, fever). However, the main manifestation of RA is the joint syndrome, manifested by pain as a result of irritation of the nerve endings of the joint capsule; swelling - an increase in the volume of synovial fluid, hypertrophy of the synovial membrane and thickening of the joint capsule and stiffness of the joint due to fibrous or cystic ankylosis and contractures, which lead to permanent deformation of the joint. A diagnostic sign of RA is morning stiffness that lasts more than 30 minutes and weakens during the day. This is related to the biorhythm of the production of corticosteroid hormones with a peak in the afternoon [5].

Currently, scientists are paying more and more attention to studying the role of tumor necrosis factor-α (TNF-α), interleukin (IL) IL-6, IL-12, IL-18 and IL-10 in RA. It has been established that in RA, an excessive amount of pro-inflammatory cytokines (IL-6, IL-1, IL-17, IL-18) is produced in the tissues of the joints, with minimal production of anti-inflammatory cytokines (IL-10, IL-2, IL-3, IL -4, gamma interferon) [6, 7, 8]. These cytokines ensure the development of the immune response within the framework of the so-called "cytokine network" [9].

Therefore, this indicates that the imbalance of the cytokine chain is a significant factor in the development of RA and mutually aggravates its course.

Material and methods. The general provisions on the procedure for conducting clinical research with human participation GCP (1996), the Helsinki Declaration of the World Medical Association on the ethical principles of conducting scientific medical research with human participation (1964-2013), the Council of Europe Convention on Human Rights and Biomedicine were observed (dated 04/04/1997), Order of the Ministry of Health of Ukraine No. 1169 dated 09/26/2017 and on the procedure for conducting clinical trials of medicinal products and examination of clinical trial materials in accordance with Articles 7 and 8 of the Law of Ukraine "On Medicinal Products".

To study the *T-786C* polymorphism of the *eNOS* gene promoter, 2 groups of patients were selected: a control group , n=20 and an experimental group (RA patients, n=60).

The clinical diagnosis of RA was verified according to the criteria of the American College of Rheumatology and the European Antirheumatic League (ACR/EULAR 2010) [10]. Medical care for a patient with RA was provided in accordance with the Unified clinical protocol of primary, secondary (specialized), tertiary (highly specialized) medical care and medical rehabilitation "Rheumatoid Arthritis", approved by the Order of the Ministry of Health of Ukraine No. 263 of April 11, 2014 [11].

Levels of IL-12, IL-18, and IL-10 cytokines in plasma were determined using a standard set of reagents from "Bender MedSystems GmbH" (Austria) by solid-phase ELISA, and IL-6 - using "Cytokin" reagent sets (Russian Federation). An increase in cytokine production was considered to exceed the upper quartile of the control group: for IL-12 >13.57 pg/ml, IL-18 >206.89 pg/ml, IL-6 >14.41 pg/ml, and a decrease for IL- 10 <1.37 pg/ml, respectively. The specific reagents of the set are monoclonal antibodies to cytokines, sorbed on the surface of the wells of a collapsible polystyrene tablet, which are incubated with the test and control samples at the first stage of the study.

Research results and discussion.

The distribution of patients with RA depending on the polymorphic variants of the *eNOS* gene (rs 2070744) taking into account the levels of cytokine production showed that the relative frequency of individuals with a level of IL-12, IL-6 in the blood above normal and with a lower content of IL-10 prevailed among carriers of *TC*- and *CC* genotypes: IL-12 – by 75.0% (p=0.005), IL-6 – by 39.14% (p=0.008) and 75.0% (p=0.005), IL-10 – by 47.82% (p=0.008) and 75.0% (p=0.005), respectively. On the other hand, the relative frequency of individuals with a high IL-18 content prevailed among *TT* carriers by 37.94% (χ2=8.34; p=0.004) (Table 1).

Table 1

The level of cytokines in the blood serum of patients with rheumatoid arthritis arthritis depending on polymorphic variants of the eNOS gene (rs 2070744)

Indicator	Production levels, n	Gene genotypes *eNOS*, n (%)		
		TT, n=29	*TC*, n=23	*CC*, n=8
IL-12	Within the norm, n=24	14 (48,28)	9 (39,13)	1 (12,50)
	Above the norm, n=36	15 (51,72)	14 (60,87)	7 (87,50)
χ^2; p		χ^2=1,60 p>0,05	χ^2=2,17 p>0,05	p=0,005
IL-18	Within the norm, n=20	9 (31,03)	9 (39,13)	2 (25,0)
	Above the norm, n=40	20 (68,97)	14 (60,87)	6 (75,0)
χ^2; p		χ^2=8,34 p=0,004	χ^2=2,17 p>0,05	χ^2<1,0 p>0,05
IL-6	Within the norm, n=22	14 (48,28)	7 (30,43)	1 (12,50)
	Above the norm, n=38	15 (51,72)	16 (69,57)	7 (87,50)
χ^2; p		χ^2=1,60 p>0,05	χ^2=7,04 p=0,008	p=0,005
IL-10	Within the norm, n=19	12 (41,38)	6 (26,09)	1 (12,50)
	Above the norm, n=41	17 (58,62)	17 (73,91)	7 (87,50)
χ^2; p		χ^2=1,72 p>0,05	χ^2=7,04 p=0,008	p=0,005

In addition, the content of IL-12 and IL-6 in carriers of the unfavorable *CC* genotype is higher than that in carriers of the *TT* genotype (by 2.25 times and 2.1 times) and *TC* genotypes (by 2.3 times and 1 .65 times) respectively (p<0.05) (Table 2).

Table 2

Dynamics of the cytokine profile after treatment in patients with rheumatoid arthritis depending on polymorphic variants of the *eNOS* gene (rs 2070744)

Genotypes of the eNOS gene		IL-12	IL -18	IL -10	IL -6
CONTROL		8,70±2,57	138,35±37,10	2,40±0,49	8,71±1,45
TT, n=21	Before treatment	17,96±6,1 p=0,049	321,66±42,17 p<0,001	1,26±0,27 p<0,001	20,52±4,61 p=0,007
	After treatment	15,01±4,97 p_1<0,05	318,17±42,59 p=0,002	1,90±0,19 p_1<0,001	16,48±2,81 p,p_1=0,05
TC, n=15	Before treatment	17,36±5,22 p=0,017	217,8±38,47 p=0,048	1,40±0,30 p=0,01	24,55±6,54 p=0,002
	After treatment	13,93±2,61 p_1<0,05	166,96±33,18 p_1<0,05 p_{TT}=0,01	1,85±0,26 p_1<0,001	21,39±4,24 p,p_1<0,05
CC, n=6	Before treatment	38,96±10,93 p<0,001	378,95±45,23 p=0,014	0,88±0,19 p=0,007	40,31±4,96 p<0,001
	After treatment	33,02±11,56 p,p_1<0,05 p_{TC}<0,05 p_{TT}<0,05	329,13±125,06 p,p_1<0,05	1,20±0,13 p,p_1<0,05 p_{TC}<0,05	34,50±6,78 p,p_1<0,05 p_{TT}<0,05

Note: p is the probability of differences in indicators with the control group; p1 – the probability of differences between indicators before and after treatment; p*TT* – the probability of differences in indicators after treatment with carriers of the *TT* genotype; p*TC* is the probability of differences in indicators after treatment with carriers of the *TC* genotype.

It was found that after treatment, the concentration of pro-inflammatory cytokines (IL-12, IL-18, IL-6) in patients with RA, depending on the polymorphic variants of the *eNOS* gene (rs 2070744), decreased, while the level of anti-inflammatory IL-10, on the contrary, increased (table. 2).

After the use of disease-modifying therapy, a decrease in the content of IL-12 and IL-6 was observed in

carriers of the TT genotype - by 19.65% ($p<0.05$) and 24.51% ($p<0.05$), in carriers of TC -genotype - by 24.62% ($p<0.05$) and 14.77% ($p<0.05$), and in CC genotype carriers - by 18% ($p<0.05$) and 16.8% ($p<0.05$) respectively. At the same time, the content of IL-12 and IL-6 in the blood plasma of carriers of the CC genotype remained higher than that of carriers of the TT and TC genotypes: the level of IL-12 was 2.20 times ($pTT<0.05$) and 2.37 times ($pTC<0.05$), IL-6 concentration – 2.09 times ($pTT<0.001$) and 1.61 times ($pTC<0.05$), respectively (Table 2). The content of proinflammatory IL-18 after treatment decreased mainly in carriers of the TC genotype - by 30.45% ($p<0.001$) with a slight decrease in carriers of the CC genotype - by 15.14% ($p<0.05$) with no significant difference between carriers of the TT genotype ($p>0.05$). The level of IL-10 after the proposed complex treatment probably increased in all genotypes of the analyzed gene: among carriers of the TT genotype - by 50.79% ($p<0.001$), TC genotype - by 32.14% ($p<0.05$), and CC genotype - by 36.36% ($p<0.05$). However, the content of IL-10 in the blood serum of carriers of the unfavorable CC genotype remained lower than that of carriers of the TT and TC genotypes by 1.58 times ($pTT<0.05$) and 1.54 times ($pTC<0.05$) respectively (Table 2).

Thus, according to the results of the researchers, IL-6, which is synthesized by T- and B-lymphocytes, fibroblasts, endothelial cells, macrophages, mesangial and glial cells, cells of the synovial membrane of joints, adipose and tumor tissues, is considered the main mediator of inflammation in the development of RA [12].

A prominent place in the pathogenesis of RA is occupied by IL-12, which is the main stimulator of IFN-γ production, promotes the differentiation of Th0 with their transformation into Th-1, has a stimulating effect on T-killers and NK cells, participates in the regulation of hematopoiesis and the initiation of apoptosis in cells An elevated level of IL-12 correlates with the degree of RA activity [13,14].

Conclusion. Therefore, the obtained data indicate that the imbalance of the cytokine chain is an important factor in the development of RA and the mutual burden of the course of the disease in the presence of *CC* genotype of the *T-786C* polymorphism of the *eNOS* gene promoter.

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