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The Molecular Basis of the Functioning of the Cytokine System and Anti-Cytokine Therapy in Rheumatoid Arthritis

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Abstract The cytokine system is a universal, polymorphic, regulatory network of mediators designed to control the processes of proliferation, differentiation and functional activity of cellular elements in the hematopoietic, immune and other homeostatic systems of the body. One of the important issues of the pathogenesis of rheumatoid arthritis (RA) is the role of innate immunity mechanisms in the development of autoimmune inflammation. The aim of the study is to highlight the role of cytokines in the pathogenesis of RA and to improve anti-cytokine therapy. DNA samples of RA patients and healthy individuals of the therapeutic department No. 1 of the multidisciplinary clinic No. 1 of SamSMU served as the material for the study. The group of patients consisted of 49 people aged 25-45 years and a control group of 71 practically healthy individuals. It has been established that the cytokine system is a polymorphic structure and such a mechanism as allelic polymorphism is important in the formation of its polymorphism. The presented results of clinical and laboratory studies show the development of torpidity to methotrexate therapy in A subgroup of patients of both groups. Thus, the use of anti-cytokine therapy is a great achievement in the treatment of rheumatoid arthritis.

Keywords Rheumatoid arthritis, Cytokines, Gene polymorphism, Interleukins, Tumor necrosis factor (TNFα), Monoclonal antibodies

1. Introduction

The cytokine system is a universal, polymorphic, regulatory network of mediators designed to control the processes of proliferation, differentiation and functional activity of cellular elements in the hematopoietic, immune and other homeostatic systems of the body. Numerous studies carried out over the past 10 years have demonstrated the existence of new mechanisms for the formation of the polymorphic structure of the cytokine system [1-2,4-5]. These are allelic polymorphism of cytokine genes and alternative splicing of cytokine genes. On the one hand, these mechanisms form an even more complex polymorphic cytokine network in the body, but on the other hand, they allow us to look at its organization from a new angle.

The effect of cytokines as participants in a complex network complicates the analysis of the functions of individual individual cytokines, the effect of polymorphism of their genes on the development of the immune response [8,11,19,20]. There are significant individual differences in cytokine production [6,7]. The differences between the maximum and minimum levels of production of some cytokines often reach tenfold values, and these indicators are constant at different time intervals. By studying the allelic polymorphism of genes, attempts are being made to determine the genetic basis of interindividual differences in the immune response by determining the relationship between individual polymorphic alleles, or haplotypes of cytokine genes and in vitro protein product production [12,15,23].

Having studied a sufficient number of candidate genes, it is possible to identify certain genetic profiles of polymorphic cytokine genes. For example, individuals with gene variants responsible for high IFNγ production, high TNFα content and low IL-10 content have an association with inflammatory processes. Such genotypes are of functional importance, because they make it possible to explain individual susceptibility to many autoimmune, infectious diseases.

Rheumatoid arthritis is a chronic systemic inflammatory disease of connective tissue with damage mainly to peripheral joints by the type of progressive symmetrical erosive-destructive polyarthritis [3], as well as characteristic extra-articular manifestations. Despite the great achievements in the study of the pathogenesis of rheumatoid arthritis (RA), which made it possible to create a fundamentally new class of fundamentally sound therapeutic agents, many immunological aspects remain not fully understood. One of

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the important issues of the pathogenesis of not only RA but also all rheumatic diseases is the role of innate immunity mechanisms in the development of autoimmune inflammation.

Rheumatoid arthritis (RA) is the most common inflammatory joint disease, the prevalence of which in the population is about 1%, and the economic losses from RA for society are comparable to coronary heart disease. Despite the ongoing research, RA is still a disease with an unknown etiology. Moreover, there are good reasons to assume that even if it is possible to prove the role of an infectious agent in the development of some forms of RA, its elimination with the help of antibacterial or antiviral drugs is unlikely to "cure" the disease. Chronic inflammatory process leads to excessive synovial hyperplasia with proliferation of synovial cells, generation of new vessels, and diffuse or nodular infiltration by mononuclear cells [1,4,5]. The hyperplastic synovial membrane in RA is infiltrated mainly by plasma cells, dendritic cells, macrophages, which, along with synoviocytes, turned out to be the main source of "pro-inflammatory" cytokines. In addition, these cells may play a role in the presentation of local antigen to T-lymphocytes in the synovial membrane. A large number of suspected autoantigens have been described using autoantibodies present in the serum of RA patients. Despite this, there is little evidence of their involvement in the pathogenesis of RA. Antigens associated with joint tissues, such as type 2 collagen, human chondrocyte glycoprotein 39, as well as those not associated with joint tissues, such as citrulinated peptides, glucose-6–phosphate isomerase, heat shock proteins, act as antigens in RA [13]. During the immune response in RA, two closely interrelated processes occur: 1). Activation of CD4+ T-lymphocytes by Th1 type, characterized by excessive synthesis of interleukin (IL)-2, interferon-γ and IL-17; 2). Imbalance between hyperproduction of proinflammatory cytokines of predominantly macrophage nature, such as tumor necrosis factor-α (TNF-α), IL-1, IL-6, IL-8 and anti-inflammatory cytokines (IL-4, IL-10, soluble antagonist IL-1, soluble TNF-α receptors), with predominance the products of the first over the second [17]. An important role in the induction and maintenance of inflammation in the joint in RA of the proinflammatory cytokine IL-17, which is produced by CD4+ activated memory cells (CD45RO+), has been proven [24]. IL-17 stimulates the production of MMP-1 and MMP-9 and the degradation of proteoglycans, increases the expression of IL-6 and leukemia-inhibiting factor in fibroblast-like synovial cells [9,10,14,20].

A more complete understanding of the mechanisms involved in the development and maintenance of rheumatoid inflammation has recently allowed the development of numerous new therapeutic approaches to its treatment. The main therapeutic task is to control the production and activity of factors involved in pathogenesis. A small part of this task was solved by drugs from the group of biological agents. For the treatment of RA, the following drugs are currently approved: Infliximab, Etanercept and Anakinra. Etanercept is a complex drug that contains two copies of the soluble recombinant TNF receptor (gp75) associated with the Fc fragment of immunoglobulin G1, etanercept blocks the biological activity of TNF by binding it, while competing with receptors on target cells.

The aim of this study is to highlight the role of cytokines in the pathogenesis of RA and to improve anti-cytokine therapy.

2. Research Material and Methods

DNA samples of RA patients and healthy individuals of the Samarkand region of the Republic of Uzbekistan served as the material for the study. The set of materials was carried out on the basis of the therapeutic department No. 1 of the multidisciplinary clinic No. 1 of SamSMU. Molecular genetic analysis was carried out in the laboratory of the RSSPMC of Hematology of the Ministry of Health of the Republic of Uzbekistan (Tashkent).

The group of patients consisted of 49 people aged 25-45 years. The comparison group consisted of 71 practically healthy individuals aged 25-46 years.

3. Results and Discussions

As mentioned above, the synovial membrane of the joints in RA is infiltrated by a wide range of cells that support the immune response in the affected joint. The severity and progression of synovitis largely depends on the local interaction, activation of these cells and their release of cytokines, which in turn regulate the growth, differentiation and activation of other cells involved in inflammation and immune response in the affected joint. Local and systemic production of these cytokines is the cause of many clinical and laboratory manifestations of RA. An important place among the mechanisms of joint damage in RA is given to the so-called "pro-inflammatory" cytokines: tumor necrosis factor-α (TNF-α), interleikin-6 (IL-6) and (IL-17A). Using a variety of methodological approaches, including the use of appropriate DNA probes to assess the expression of RNA cytokines, as well as biological and immunochemical methods, it was shown that all these cytokines are synthesized in excess by synovial cells and are contained in high concentrations in synovial fluid. The studies revealed that in the group of patients with RA, the level of TNF–α was significantly increased, although to a lesser extent, but also has the ability to stimulate chondrocytes, thereby causing degradation of cartilage tissue, and also participates in bone resorption. Of fundamental importance is the fact that TNF is synthesized by cells found in excess at the junction between the pannus and articular cartilage, that is, in the area from which the destruction of the joint begins. Hyperproduction of proinflammatory cytokine IL-17 was determined in RA patients in comparison with KG. TNF-α are powerful inducers of the synthesis of another pro-inflammatory cytokine - IL-6, the concentration of which closely correlates with the clinical and laboratory parameters of the activity of the inflammatory process in RA. IL-6 is actually the only

cytokine directly inducing the synthesis of acute-phase proteins by hepatocytes (Table 1).

Table 1. Cytokine levels in patients with RA and KG, pg/ml

Cytokines	Group with RA (n=49)	Control group (n=71)	The value of p
TNF-α	49,1% [7,8; 23,4]	17,2% [5,5; 12,6]	0,037
IL6	34,1% [7,7; 15,8]	51,2% [2,3; 9,2]	0,03
IL17A	16,6% [2,1; 3,2]	12,2% [1,7; 2,6]	0,039

The inflammatory process occurring in the joint cavity and the local release of proinflammatory cytokines are accompanied by extra-articular manifestations that are a consequence of systemic inflammation in RA. The most frequent extra-articular manifestation of RA is anemia, the cause of which is excessive production of proinflammatory cytokines. According to the study, it was revealed that the conditioned environment from peripheral blood mononuclear of RA patients suppressed the development of erythroid burst-forming and colony-forming units. When adding monoclonal AT to various cytokines to the media, it was shown that the main cytokine suppressing erythropoiesis in vitro is TNF-α (Table 2).

Table 2. Indicators of general blood analysis and iron metabolism in RA patients, (M±m)

Indicators	Control n=71	Patients with RA n=49
Hb, g/l	128,3±1,8	94,5±2,8
RBC (1x106/ml	4,51±0,31	4,08±0,24
MCV (fl)	88,4±3,56	80,91±5,73
MSN (pg)	29,01±0,89	24,96±2,01
MSNS (pg)	331,86±6,78	318,0±11,23
RDW (%)	11,9±0,51	15,6±0,43*
Morphology of erythrocytes	Norm	Moderate hypochromia
Serum iron (mm/L)	24,7±2,01	20,21±1,47
OHSS (mm/ml)	66,91±5,05	74,56±4,78
Ferritin (ng/ml)	87,6±4,78	106,4±1,56*
Soluble transferrin receptor (ng/ml)	1,89±0,12	4,63±0,21*

The clinical and laboratory activity of RA correlates with the level of proinflammatory cytokines in the blood serum. Thus, it was shown that patients with a high level of ESR had a higher concentration of TNF- α in the blood serum (Table 3).

Thus, proinflammatory cytokines play an important role in the pathogenesis of RA. The maintenance and carbonization of inflammation largely depends on the ability of cells to produce high levels of their production. This may be largely due to the presence of certain genetic profiles characterized by inheritance of combinations of allelic variants of cytokine genes.

Table 3. Indicators of the degree of activity of RA and TNF-α

Degrees of activity	ESR mm/hour	TNF-α pg/ml
I	Until 20 – 9,7%	5,8
II	20-40 – 52,4%	6,2
III	Higher 40 – 37,86%	8,4

Note: * – p<0.05 in comparison with the control.

It has been established that the cytokine system is a polymorphic structure and such a mechanism as allelic polymorphism is important in the formation of its polymorphism. The TNF-α gene polymorphism at such points as -238 and + 489 relative to the transcription site was studied in two different subgroups of RA patients. Subgroup A, patients with severe course, unresponsive to standard therapy, having more than six swollen joints and maintaining high activity despite treatment for 6 months, and subgroup B, patients with mild course, having less than 3 swollen joints and a good response to Methotrexate and other traditional therapy. Healthy donors were studied as a control group.

As a result, it was revealed that in the first group in 100% of cases there was – 238 G/G genotype, the same genotype was in 95.5% in the second group of patients and in 91.2% of healthy individuals. Thus, the genotype – 238 A/G- was absent in patients with severe RA. Genotype + 489 G/G – had some tendency to prevail in people with severe RA, but it was not statistically significant [16]. From other data, it was also noted that the genotype - 238 G/A- is associated with low RA progression and fewer erosions in patients [23]. Thus, the genotype –238G/G indicates a predisposition to a more severe course of RA. Also, many studies have been conducted in the study of the TNF-α gene polymorphism at the point -308(G→A), as a result, it was revealed that patients with the genotype –308G/A have a more severe course of RA than those carrying the G/G genotype, patients with G/A had an earlier onset of the disease, more high activity, a greater number of erosions.

It has been shown that the polymorphism allele C in the 5'-flanking region of the IL-6 gene (174G→C) in RA patients is associated with a reduced level of IL-6 in plasma, and the C/C genotype is significantly lower in the group of patients and can play a protective role against the development of this disease. IL-10 is known as an important endogenous regulator of the production of inflammatory cytokines by macrophages and T-lymphocytes in an inflamed joint in RA [21,24]. In addition, this cytokine is highly polymorphic, has single nucleotide substitutions in the gene promoter and two microsatellite loci of IL10.R and IL10.G. At the same time, it was found that the allele associated with high production was much more common in patients with RA in comparison with the control. Thus, when studying IL-10 polymorphism (-2849A→G), it was revealed that the genotype associated with high production of IL-10, namely the presence of the G allele, was more common in individuals with severe articular destruction and high titers of rheumatoid factor. In the study of IL17A polymorphisms (C-590T and 2 or 3 repeats of 70pn in the third intron), it was revealed that the RP1

allele (2 repeats of 70pn in the third intron) statistically significantly prevails in patients with RA. A hypothesis is put forward about the possible influence of the VNTR copy number on the transcriptional activity of the IL17A gene.

Thus, when studying the polymorphism of cytokine genes in RA patients, associations of certain allelic variants with susceptibility to the development of the disease, with the nature of the course and with sensitivity to therapy were revealed. The identification of alleles associated with a high level of proinflammatory cytokine production in patients explains the prospects of using anti-cytokine therapy and a more selective approach to it.

After the therapy was completed, we again evaluated clinical and laboratory changes in the examined groups of RA patients. Our research has provided convincing data proving the effectiveness of new biological agents in reducing the progression of RA. Etanercept showed a decrease in the inflammatory symptoms of RA and a slowdown in radiological progression, in addition, a comparative study proved a higher efficacy of etanercept monotherapy than taking Methotrexate, also as monotherapy for 2 years [1,2]. The higher efficiency of TNF-inhibiting agents is also confirmed by the fact that the neutralization of TNF–α suppresses the production of IL-17A, IL-6 in the culture of synovial cells of RA patients. Taking into account the peculiarities of the pathogenesis of RA, including the predominance of pro-inflammatory cytokines over anti-inflammatory ones, the use of the latter as therapeutic agents seems to be effective.

The use of IL-6 seems to be the most promising, in various studies it has been proven that it is a powerful anti-inflammatory agent that significantly suppresses the production of TNF-α and IL-17A by activated monocytes and synovial cells ex vivo in RA patients (Table 4).

Table 4. Analysis of the relationship of genetic polymorphisms IL-6 (rs202078), IL17A (rs2275913) and TNF-α (rs206983) with the effectiveness of methotrexate therapy in patients with RA

Gene polymorphisms	Alleles	Genotypes
IL-6 (rs202078)	A: χ^2 =0.979; p=0.331; OR=0.638; 95% CI: 0.262 - 1.554	G/A: χ^2=0.123; p=0.730; OR=0.833; 95% CI: 0.3 - 2.313
IL17A (rs2275913)	A: χ^2 =1.147; p=0.287; OR=1.554; 95% CI:0.694 - 3.482	G/A:χ^2 =1.203; p=0.277; OR=1.714; 95% CI: 0.654 - 4.489
TNF-α (rs206983)	G: χ^2 =0.78; p=0.40; OR=1.498; 95% CI: 0.611 - 3.675	G/A: χ^2=1.29; p=0.26; OR=1.865; 95% CI

The presented results of clinical and laboratory studies show the development of torpidity to methotrexate therapy in A subgroup of patients of both groups.

The development of torpidity to methotrexate is explained by the influence of genetic markers on the mechanism of action of the drug. In particular, when assessing the effect of proinflammatory cytokine genes on the effectiveness of methotrexate therapy, there was a tendency to decrease the protective role of the A allele by 1.6 times and the G/A genotype by 1.7 times in the IL17A gene polymorphism (rs2275913), the G/A genotype by 1.9 times in the TNF-α gene polymorphism variant (rs206983) in terms of effectiveness ongoing therapy with methotrexate.

Thus, the use of anti-cytokine therapy is a great achievement in the treatment of rheumatoid arthritis. Blocking cytokines, which play a crucial role in the pathogenesis of RA, allows to slow down the inflammatory process, while significantly reducing the progression of the disease and improving the quality of life of patients.

4. Conclusions

Pro-inflammatory cytokines play a leading role in the initiation and maintenance of the inflammatory process in the joint in RA. The increased production of their synoviocytes, mononuclear cells of peripheral blood of RA patients, has been proven by many researchers. In addition, high concentrations of the latter were found in synovial fluid and blood serum. Their main action is aimed at potentiating bone destruction, degradation of cartilage tissue by activating synovial cells, monocytes, macrophages, T- and B-lymphocytes, endothelial cells and granulocytes and their release of inflammatory mediators. Predisposition to high production of proinflammatory cytokines may be associated with inheritance of certain combinations of allelic variants of their genes. In addition, allelic polymorphism of cytokine genes affects the susceptibility to the development of RA, its severity and sensitivity to treatment. The use of anti-cytokine therapy is a great achievement in the treatment of rheumatoid arthritis.

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