MOLECULAR BASIS OF THE FUNCTIONING OF THE CYTOKINE SYSTEM AND ANTICYTOKINE THERAPY FOR RHEUMATOID ARTHRITIS

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Annotation : *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 in the pathogenesis of rheumatoid arthritis (RA) is the role of innate immune mechanisms in the development of autoimmune inflammation. The purpose of the study is to highlight the role of cytokines in the pathogenesis and improve anti-cytokine therapy. The material for the study was DNA samples from RA patients and healthy individuals of the therapeutic department No. 1 of the multidisciplinary clinic No. 1 of Samara State Medical University. The patient group consisted of 49 people aged 25-45 years and the control group, 71 practically healthy individuals. It has been established that the cytokine system is a polymorphic structure and in the formation of its polymorphism such a mechanism as allelic polymorphism is important. The presented results of clinical and laboratory studies show the development of torpidity to methotrexate therapy in subgroups of patients in both groups. Thus, the use of anti-cytokine therapy is a major advance in the treatment of rheumatoid arthritis.*

Key words: *rheumatoid arthritis, cytokines, gene polymorphism, interleukins, tumor necrosis factor (TNFα), monoclonal antibodies.*

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 perspective.

the action of cytokines as participants in a complex network complicates the analysis of the functions of individual cytokines and the influence of their gene polymorphism on the

development of the immune response [8, 11, 19, 20]. There are significant individual differences in cytokine production [6, 7]. Differences between the maximum and minimum levels of production of some cytokines are often tenfold, and these values are constant over different periods of time. Through the study of allelic gene polymorphism, attempts are made to determine the genetic basis of interindividual differences in immune response by determining the relationship between individual polymorphic alleles, or haplotypes, of cytokine genes and protein product production in vitro [12, 15, 23].

By examining a sufficient number of candidate genes, specific genetic profiles of polymorphic cytokine genes can be identified. For example, individuals with gene variants responsible for high production of IFNγ, high TNFα and low IL-10 are associated with inflammatory processes. Such genotypes are of functional importance because make it possible to explain individual susceptibility to many autoimmune diseases

Rheumatoid arthritis is a chronic systemic inflammatory disease of connective tissue affecting predominantly peripheral joints in the form of progressive symmetrical erosive-destructive polyarthritis [3], as well as characteristic extra-articular manifestations. Despite 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 incompletely studied. One of the important issues in the pathogenesis of not only RA but also all rheumatic diseases is the role of innate immune mechanisms in the development of autoimmune inflammation.

Rheumatoid arthritis (RA) is the most common inflammatory disease of the joints, 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 ongoing research, RA still remains a disease of unknown etiology. Moreover, there are good reasons to assume that even if it is possible to prove the role of any infectious agent in the development of some forms of RA, its elimination with the help of antibacterial or Antiviral drugs are unlikely to "cure" the disease. the chronic inflammatory process leads to excessive synovial hyperplasia with proliferation of synovial lineage cells, generation of new vessels, and diffuse or nodular infiltration of mononuclear cells [1,4,5]. The hyperplastic synovial membrane in cancer is infiltrated mainly by plasma cells, dendritic cells, and 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 presenting local antigen to T cells in the synovium. A large number of putative autoantigens have been described using autoantibodies present in the serum of patients with cancer. Despite this, there is little evidence of their involvement in the pathogenesis of cancer. Antigens in RA include antigens associated with joint tissues, such as collagen type 2, human chondrocyte glycoprotein 39, as well as those not associated with joint tissues, for example, citrullinated peptides, glucose-6-phosphate isomerase, heat shock proteins [13]. During the immune response in RA, two closely interrelated processes occur: Activation of CD4+ T lymphocytes by Th1 type, characterized by excessive synthesis of interleukin (IL)-2, interferon-γ and IL-17; 2). Imbalance between the hyperproduction of pro-inflammatory cytokines predominantly of 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 IL-1 antagonist , soluble TNF-α receptors), with a predominance of production of the former over the latter [17]. An important role in the induction and maintenance of inflammation in the joint in RA has been proven for the pro-inflammatory cytokine IL-17, which is produced by CD4+ activated memory cells (CD45RO+) [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 inhibitory 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 goal is to control the production and activity of factors involved in pathogenesis. A small part of this problem has been solved by drugs from the group of biological agents. For the treatment of RA, the currently approved drugs are 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 through its binding, while competing with receptors on target cells.

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

MATERIAL AND RESEARCH METHODS

The material for the study was DNA samples from patients with RA and healthy individuals from the Samarkand region of the Republic of Uzbekistan. The collection of material 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 Republican Scientific Research and Medical Center 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.

RESULTS AND DISCUSSIONS

As mentioned above, the synovium of joints in cancer is infiltrated by a wide range of cells that support the immune response in the affected joint. the severity and progression of synovitis depends largely 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 the immune response in the affected joint. Local and systemic production of these cytokines is responsible for many of the 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 RNA expression of cytokines, as well as biological and immunochemical methods, it has been shown that all of these cytokines are synthesized in excess by synovial cells and are contained in high concentrations in synovial fluid. Studies have revealed that in the group of RA patients the level of TNF-α was significantly increased, although to a lesser extent, it also has the ability to stimulate

chondrocytes, thereby causing degradation of cartilage tissue, and also takes part in bone resorption. Of fundamental importance is the fact that TNF-α is synthesized by cells found in excess at the junction between the pannus and articular cartilage, that is, in the zone from which joint destruction begins. Hyper production of the pro-inflammatory cytokine IL-17 was determined in patients with RA in comparison with CG. TNF-α are powerful inducers of the synthesis of another pro-inflammatory cytokine - IL-6, the concentration of which closely correlates with clinical and laboratory parameters of the activity of the inflammatory process in RA. IL-6 is actually the only cytokine that directly induces the synthesis of acute-phase proteins by hepatocytes (Table 1).

Table 1

Levels of cytokines in patients with RA and CH, pg/ml

Cytokines	Group with RA (n = 49)	Control group (n = 71)	p valve
TNF – a	49,1 % [7,8 ; 23,4]	17,2 % [5,5 , 12.6]	0,037
IL	33,4 % 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, which are a consequence of systemic inflammation in RA. The most common extra-articular manifestation of RA is anemia, which is caused by excessive production of proinflammatory cytokines. According to the study, it was found that the conditioned medium from peripheral blood mononuclear cells of RA patients suppressed the development of erythroid burst-forming and colony-forming units. When monoclonal antibodies to various cytokines were added 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 count and iron metabolism in RA patients, (M±m)

Indicators	Control (n = 71)	Patient with RA (n = 49)
Nv , g/l	128,3±1,8	94.5±2.8
RBC (1 * 106/ ml)	4,51±0,31	4.08±0.24
MSV (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
Morphology Red blood cells	11,9±0,51	15.6±0.43*

Serum iron	Normal	Moderate hypochromia
OZHSS	24,7±2,01	20.21±1.47
Ferritin	66,91±5,05	74.56±4.78
Soluble transferrin Receptor	87,6±4,78	

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

Table 3

Indicators of the degree of activity of RA and TNF-α

Activity levels	ESR mm/hour	TNF-a pg/ml
I	Up to 20 – 9.7%	5,8
II	20-40 – 52.4%	6,2
III	Above 40 – 37.86%	8,4

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

It has been established that the cytokine system is a polymorphic structure and in the formation of its polymorphism such a mechanism as allelic polymorphism is important. Polymorphism of the TNF-α gene at points such as -238 and + 489 relative to the transcription site was studied in two different subgroups of RA patients. Subgroup A, patients with a 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 a 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, the 238 G/G genotype was present in 100% of cases, the same genotype was present in 95.5% of 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. The + 489 G/G – genotype had a slight tendency to prevail in individuals with severe RA, but this was not statistically significant [16]. From other data, it was also noted that the 238 G/A genotype is associated with low progression of RA and fewer erosions in patients [23]. Thus, the –238G/G genotype indicates a predisposition to more severe RA. Also, many studies have been conducted to study the polymorphism of the TNF-α gene at the –308(G→A) point, as a result, it was revealed that patients with the –308G/A genotype have a more severe course of RA than those carrying the G/G genotype, in patients with G/A- there was an earlier onset of the disease, higher activity, and a greater number of erosions.

It has been shown that the C allele of the polymorphism in the 5'-flanking region of the IL-6 gene (174G→C) in patients with RA 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 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 the 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 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 compared to controls. Thus, when studying the polymorphism of IL-10 (-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 joint destruction and high titers of rheumatoid factor. When studying IL17A polymorphisms (C-590T and 2 or 3 repeats of 70 bp in the third intron), it was revealed that the RP1 allele (2 repeats of 70 bp in the third intron) statistically significantly prevails in patients with RA. A hypothesis has been 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 patients with RA, 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 identified. The identification of alleles associated with high levels of production of pro-inflammatory cytokines in patients explains the prospects of using anti-cytokine therapy and a more selective approach to it.

After completion of therapy, we again assessed clinical and laboratory changes in the examined groups of RA patients. Our studies provide compelling data demonstrating 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 the higher effectiveness of etanercept monotherapy than taking Methotrexate, also as monotherapy for 2 years [1,2]. The higher effectiveness of TNF-inhibiting agents is also confirmed by the fact that neutralization of TNF-α suppresses the production of IL-17A and IL-6 in the culture of synovial cells of RA patients. Considering the features of the pathogenesis of RA, including the predominance of pro-inflammatory cytokines over anti-inflammatory ones,

the use of the latter as therapeutic agents appears to be effective.

The most promising is the use of IL-6; various studies have 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 patients with RA (Table 4).

Table 4

Gene polymorphisms	Allels	Genotypes
IL-6 (rs202078)	A: $\chi 2$ =0.979; p=0.331; OR=0.638; 95%	G/A: $\chi 2$=0.123; p=0.730; OR=0.833; 95%

	CI: 0.262 - 1.554	CI: 0.3 - 2.313
IL17A (rs2275913)	A: $\chi 2$ =1.147; p=0.287; OR=1.554; 95% CI:0.694 - 3.482	G/A:$\chi 2$ =1.203; p=0.277; OR=1.714; 95% CI: 0.654 - 4.489
TNF-α (rs206983)	G: $\chi 2$ =0.78; p=0.40; OR=1.498; 95% CI: 0.611 - 3.675	G/A: $\chi 2$=1.29; p=0.26; OR=1.865; 95% CI

Analysis of the relationship between genetic polymorphisms IL-6 (rs202078), IL17A (rs2275913) and TNF-α (rs206983) with the effectiveness of methotrexate therapy in patients with RA The presented results of clinical and laboratory studies show the development of torpidity to methotrexate therapy in A subgroups of patients in 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 influence of pro-inflammatory cytokine genes on the effectiveness of methotrexate therapy, a tendency was established to reduce the protective role of the A allele by 1.6 times and the G/A genotype by 1.7 times for the polymorphism of the IL17A gene (rs2275913), the G/A genotype by 1 .9 times variant in the polymorphism of the TNF-α gene (rs206983) in relation to the effectiveness of methotrexate therapy.

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

CONCLUSIONS

Proinflammatory cytokines play a leading role in the initiation and maintenance of the inflammatory process in the joint in RA. Their increased production by synoviocytes, mononuclear cells of the peripheral blood of patients with RA, 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 mediators of the inflammatory process. A predisposition to high production of proinflammatory cytokines may be associated with the inheritance of certain combinations of allelic variants of their genes. In addition, allelic polymorphism of cytokine genes influences susceptibility to the development of RA, its severity and sensitivity to treatment. The use of anticytokine therapy is a major advance in the treatment of rheumatoid arthritis.

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