Review paper

Pro-inflammatory cytokines gene polymorphisms in Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease, characterized by a number of immunological disturbances and synovium involvement leading to joint destruction. The etiology is unclear, since the causal factor is still unknown. RA affect all ethnic groups. The frequency of the disease, depending on population, amounted about 0,5-2% and women are affected three times more frequently then men.

Genetic factors like HLA DRB1 and cytokine genes, play an important role in susceptibility and severity of the disease. In the pathogenesis of RA exist a hierarchy of cytokines expression, which favor the proinflammatory cytokines. Cytokines production is stimulated by the Th-cells. They control all phases of immune response: autocrine, endocrine and paracrine, but also play a key role in the inflammation. In the RA a pivotal role play IL-1 and TNF-α. TNF-α is responsible for the inflammatory and proliferative aspects, and IL-1 is responsible for the destructive aspects of RA. Other proinflammatory cytokines also play very important role in RA, because they are responsible for activation of enzymes in synovial fluid, which induce degradation of bone.

The determination of single nucleotide polymorphisms (SNP) of proinflammatory cytokines seems to be of great importance. Since we are dramatically lacking any prognostic factors for the development of RA, there is a possibility that SNPs may serve as the markers of susceptibility and severity of the disease.

Key words: *Rheumatoid Arthritis, interleukin-1, interleukin-6, tumor necrosis factor, gene polymorphism.*

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Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease characterized by the joints involvement leading to deformity, disability and even to death [1]. The etiology is still unclear. It seems, that RA, as a multifactorial disease, is caused by an interaction between environmental and genetic factors [2]. The joints abnormalities are associated by a number of immunological disturbances. Inflammation is responsible for stimulating destructive process in the joint. The inflamed synovial membrane contains dendritic cells, synovial fibroblasts, macrophages, and also T lymphocytes. Synovial macrophages play a key role in joint destruction and inflammation [3].

RA is one of the autoimmune disease, where the activation of antigen-specyfic T cell, particularly CD4+ helper cells, initiate autoimmune response. A naive CD4+cells differentiates into a Th1 (T helper type 1) and Th2 cells, which are the dominant cell type in the synovial filtrate of patients with RA. Th1 cell induces production of IFN-γ and play a role in elimination of intercellular pathogenes and tissue damage by activating macrophages or by cytotoxic T-cell response [4, 5]. However Th2 cell stimulates secretion of IL-4 and activation of self-reactive B cells to produce autoantibody [4].

Recent studies have shown that not only T-cells, but also B-cells play a central role in the pathophysiology of RA. B-cells stumulate secretion of proinflammatory cytokines, production of rheumatoid factors (RF) and activation of T cells. In RA, the RF are usually IgM anti-IgG, which react with the Fc fragment IgG and create IgM-IgG immune complexes leading to some tissue damage [6].

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RA affects all ethnic groups at any age, including children, with a peak between 35 and 55. Women are affected three times more frequently than men. Sometimes disease goes into remission in pregnant women, however symptoms tend to increase their intensity after delivery. The frequency of RA depending on population varies between 0,5-2%, [7]. Morbidity carries out about 50 cases on 100,000 persons. The progression of the disease is the highest during the first 5 years. During the first 10 years up to 50% patients come to disability. The spontaneous remissions are sometimes observed in early period of the disease.

The family RA appears in about 10-30% patients and 20-40% monozygotic twins [8]. Genetics factors like HLA DRB1 and IgG gens can be associated with familial aggregation. It is possible that many different gens are involved in RA pathogenesis and each of them makes only a small contribution to disease susceptibility. Genetic factors play a role not only in susceptibility and severity of the disease, but also may serve as a useful prognostic toole in the management of RA.

The most definite genetic association with RA is with HLA (human leukocyte antigen) region. The HLA is a very large group of genes, which encods HLA class I and class II molecules [7]. It has been described, that special sequences (called shared epitope – SE) within HLA-DRB1 gene are associated with the development of rheumatoid diseases [7, 9]. This sequence encodes amino acid at position 70-74 and 86, which is located in the third hypervariable region of the DR beta chain (table 1).

Immunological factors play an important role in the pathogenesis of RA. Cytokines are produced in huge amount during activation of Th1 cells and immune complexes, start processes leading to synovial membrane inflammation and consequently to joint damage [10]. The number of cytokines taking part in pathogenesis of RA is really immense. Since, we are unable to describe all proinflammatory cytokine inducing RA inflammation, we have chosen a few which seemes to us to be of the greatest importance.

Table 1. The sequence of the third HV region connected with RA

Allel:	**Aminoacid position**					
	70	**71**	**72**	**73**	**74**	**86**
shared epitope	**Q(R)**	**R(K)**	**R**	**A**	**A**	**G(V)**
0101	Q	R	R	A	A	G
0401	Q	K	R	A	A	G
0404	Q	R	R	A	A	V
0408	Q	R	R	A	A	G
1402	Q	R	R	A	A	G

A – alanine, G – glycine, K – lysine, Q – glutamine, R – arginine, V – valine.

The role of cytokines in pathogenesis of rheumatoid arthritis

It has been already demonstrated, that cytokines take part in destructive processes accompanying RA and they are also responsible for some systemic features [11]. Cytokines are soluble proteins that serve as chemical messengers between cells and the immune system. They play a role in many important biological activites, including cell growth, proliferation, differentiation, inflammation, tissue repair and regulation of the immune response [7]. Cytokines are called immmune factors, which production is stimulated by the Th-cells, because they not only control all the phases of immune response: autocrine, endocrine and paracrine, but also they play a key role in the inflammation process. Each cytokine may have multiple, pleiotropic actions, and that's way many functions of the cytokines can be redundant. They can influence the production, function or expression of other cytokines. [3]. Additionally, cytokines activate synovial cells to produce hydrolitic enzymes, such as collagenase and cathepsin L causing further destruction within the joints.

Cytokines are usually divided into anti-inflammatory and pro-inflammatory ones (table 2). In the pathogenesis of RA exists a hierarchy of cytokines expression, favoring proinflammatory cytokines (figure 1), which play a very important role in the pathogenesis of RA and contribute to bone and cartilage destruction [12].

Still unknown inducing factor stimulates production of TNF-α, next TNF-α induces production of IL-1 and other proinflammatory cytokins and mediators. IL-1 induces productions of TNF-α, other proinflammatory cytokines and also itself. Both cytokines also induce expression of anti-inflammatory cytokines. That's way TNF-α and IL-1 are called "master regulators" of inflammatory response [13].

Table 2. More important anti- and pro-inflammatory cytokines in RA

Pro-inflammatory cytokines	**Anti-inflammatory cytokines**
IL-1	IL-1Ra
IL-2	IL-4
IL-6	IL-10
IL-8	IL-13
IL-15	IL-15R-α
IL-17	IL-18BP
IL-18	TGF-β
TNF-α	
IFN-γ	
GM-CSF	

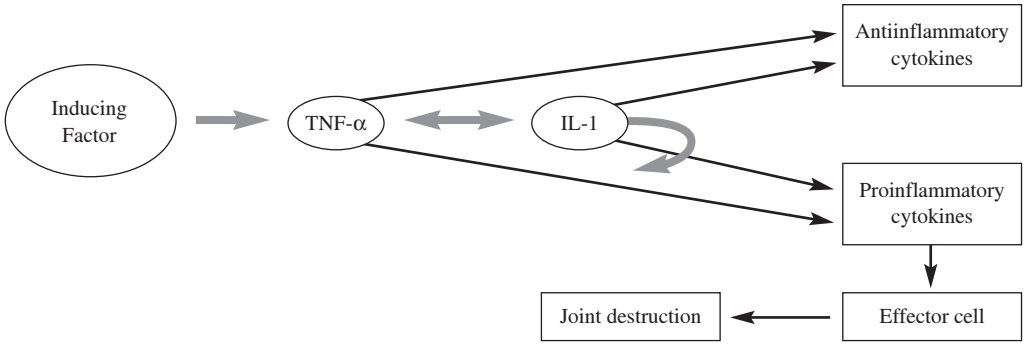

Fig. 1. Cascade of the cytokines in the rheumatoid arthritis

Therefore, it seems that IL-1 and TNF-α play a pivotal role in RA. They have numerous functions throughout the body and they are present in synovial tissue and synovial fluid. Both cytokines increase the production of COX-2, nitric oxide, prostaglandin-E2, upregulate expression of cell adhesion molecules of endothelial cells and also stimulate the development of proinflammatory phenotype on responding cells. TNF-α and IL-1 stimulate activation of the T and B- cells, stimulate hepatocytes to release acute phase reactants, and also they have actions in the central nervous system. TNF-α is responsible for the inflammatory and proliferative aspects, and IL-1 is responsible for the destructive aspects of the RA [3, 7].

Another proinflammatory cytokines also play an important role in the RA, because they are responsible for activation of enzymes in synovial fluid, which induces bone degradation. TNF-α, IFN-γ, IL-2 and particularly IL-6 play an essential role in the pathogenesis of mineralization disturbance.

Proinflammatory cytokines in the Rheumatoid Arthritis

Interleukin-1

The IL-1 family consist of IL-1α, IL-1, IL-1RI, IL-1RII, IL-1RA and IL-1 converting enzyme-ICE, but most important is IL-1α and IL-1. IL-1 is a 15-18 kD protein that is derived from a 30 to 35 kD precursor under the influence of cysteine protease, and IL-1α is a 17 kD protein. They are encode by two diffrent genes, which are located on chromosome 2. The gene encoding IL-1α is located on 2q21, and the IL-1 gene is located on 2q14. IL-1α and IL-1 have just only 26% homology in the sequences [14] and they were cloned in 1984.

IL-1 was previously known as endogenous pyrogen, lymphocyte activating factor and catabolin. IL-1 is produced by macrophages, monocytes, keratinocytes, chondrocytes, Langerhans' cells, glial cells, mesangial cells, endothelial cells and T cells and B cells during the inflammation process, but this cytokine is not produced by the cells described above in healthy patients. IL-1 demonstrates constant expression in healthy tissue, such as hypothalmus and male gonad [14]. Experession of IL-1 is induced by IL-2, IL-3, IL-12, IL-1, TNF-α, PHA, ConA, MDP, viruses, yeast and bacteria.

IL-1 is one of the most potent proinflammatory cytokines because it possesses several biological properties and it plays a central role in joint destructions and inflammation [15, 16]. IL-1 induces production of IL-2 and TNF-α by T lymphocytes, IL-6 by macrophages and endothelial cells, and IL-6 nad IFN-gamma by fibroblasts. Another important property of IL-1 is stimulation of T lymphocytes proliferation and ability to increase the expression of adhesion molecules on endothelial cells and other cell surfaces [16]. The cytokine stimulates proliferation and differentiation of B lymphocytes, fibroblasts and miocytes. Morover it stimulates inflammatory and immune response.

Polymorphisms of IL-1 and RA

IL-1 is a pivotal cytokine involved in RA. It is a potent stimulator of osteoblasts, synviocytes and chondrocytes, and amplifies the disease proccess in RA. Its plasma levels correlated with disease activity and it is a key mediator of synovial inflammation, pannus formation and destruction of bone and cartilage. In RA patients IL-1 upregulates the production of pro- and anti- inflammatory mediators, and prostaglandins. The levels of interleukin-1 is higher in RA patients with erosive disease [17].

A proinflammatory effects of IL-1 can be inhibited by IL-1Ra, which is produced and secreted by all cells. The balance between IL-1Ra and IL-1 may contribute to the pathogenesis of RA [18].

IL-1 manifests two single nucleotide polymorphisms (SNPs) at position -511C/T and +3953C/T exon 5, which may be important in determining the pathogenesis of RA. Allel +3953T is associated with incereased production of

IL-1, higher disease activity. It can be useful in predicting the severity of RA. Allel +3953T is associated with high bone turnover, lower bone mineral density (BMD) [19]. IL-1 +3953 gene polymorphism may be an important genetic marker for the susceptibility or severity of joint destruction in RA [18, 20]. Additionally, IL-1 +3953 gene polymorphism may cause cardiac-vascular complications in RA patients.

Increased production of interleukin-1 by SNPs at position +3953 play an important role of some neurologic and psychiatric diseases, and also in the Alzheimer's disease. Allel -511C is associated with light disease course, gastric cancer and *Helicobacter pylori* infection [21].

Interleukin-6

IL-6 is a 22-29 kD glycoproteine [22], which gene is located on chromosome 7p21. It was cloned in 1986. IL-6 is produces by monocytes, macrophages, fibroblasts, endothelial cells, T and B lympyocytes, keratinocytes and chondrocytes. Production of interleukin-6 is induces by IL-1, TNF, interferon, LPS and viruses. IL-6 is a cytokine with pro-inflammatory and anti-inflammatory proporties.This cytokine stimulates differentiation and proliferation of stem cells and B lymphocytes. IL-6 play a role in regulation of metabolism and stimulation of bone resorption. Also it is involved in induction of synthesis CRP and activation of T lypmhocytes, stimulation of growth and differentiation of haematopoietic precursor cells and proliferation of synovial fibroblasts [23, 24]. Upregulation of IL-6 production has been observed not only in RA, but also in other autoimmune diseases, such as type I diabetes, multiple myeloma, systemic sclerosis or leukemia. This cytokine is not produced in healthy patients, but IL-6 appears as results of inflammation process. The level of its production and releasing is dependent on stimuli intensity [22]. IL-6 plays a very important role in the immune respone, inflammatory reaction and hemopoesis.

Polymorphisms of IL-6 and RA

IL-6 is present in a high concentration in synovial fluid and tissue. The levels of IL-6 permit monitoring the changes in the inflammation process. Expression of interleukin-6 in RA synovial tissue is regulated by the transcription factors, such as NF-κB and C/EBP([25]. This cytokine plays a role in degradation process of articular cartilage, because it blocks proliferation of chondrocytes and creation of proteoglikans. In the patients with RA IL-6 is responsible for appearance of fever and catabolism causing cachexy [26].

IL-6 manifests some single nucleotide polymorphisms (SNPs) at position -174G/C, -373A9T11, -572G/C, -597G/A and -634C/G. The polymorphisms at position -174G/C of the IL-6 gene is most important in the pathogenesis of RA. This polymorphism involves a DNA-biding site for NF-IL-6. It causes interaction of transcription factor with estradiol/estrogen receptor complex to regulate IL-6 gene expression [27]. IL-6 -174G/C gene polymorphism may be an important genetic markers for the susceptibility and activity of disease in RA. A G/C polymorphism at position -174 of the IL-6 gene in the patients with juvenile rheumatoid arthritis displays a typical daily spiking fever, lymphadenopathy, serositis, myalgia and arteritis. Allel -174C may contribute with the pathogenesis of RA.

The SNP described above are associated with some different diseases or conditions. IL-6 -174G/C and -572G/C promotor polymorphisms may serve as a marker of bone resorption in postmenopausal women [27]. The SNPs at position -174G/C may modulate the effects of alcohol on carotid atherosclerosis [28]. IL-6 -373A9T11 gene polymorphisms is associated with reduced susceptibility to chronic periodontitis and decrease serum IL-6 level [29]. The polymorphism at position -634C/G of the IL-6 gene is associated with osteoporosis and causes BMD elevation. IL-6 -597G/A promotor polymorphism is associated with hyperandrogenesis.

Tumor Necrosis Factor (TNF)

TNF-α is a 17kD trimeric molecule, that is derived from 26kD precursor and has 157 amino acids [30]. Its gene is located on chromosome 6p21.1-p21.3. It was cloned in 1984. Tumor necrosis factor has a two different receptors, 55kD receptor called a proteine p55 or TNF-R1 and 75kD receptor called a proteine p75 or TNF-R2. These receptors are trans-membrane proteins and activate different intra-cellular signal-transduction pathways. This cytokine is produced by monocytes, macrophages, limphocytes, fibroblasts, mast cells and NK cells. Tumor necrosis factor has a catabolic, proinflammatory and immunostimulatory activities [2]. TNF-α play a key role in the immune and inflammatory response. It is very important mediator in the inflammatory response of RA [31]. TNF-α induces proliferation and differentiation of B lymphocytes, T lymphocytes and NK cells. It can stimulate the production of PGE2 and collagenase by synovial cells, IFN-γ by lymphocytes and IL-1, IL-6, GM-CSF, M-CSF, NGF and EGF by macrophages. TNF-α induces angiogenesis and proliferation of melanocytes.

TNF-α and RA

Interestingly, high levels of TNF do not correspond to the activity of RA. However it is present in about 50% of RA patients [30]. Strong local expression of TNF-α results in a chronic inflammation with tissue destruction. TNF induces production of PGE2 and COX-2 in RA. It may also stimulate expression of cell adhesion molecules on endothelial cells. TNF-α being a powerful immune modulator of joint destruction serves as a marker of RA genetic susceptibility [32].

Polymorphisms of TNF-α

The production of TNF-α is probably associated with TNF promoter polymorphisms, which are playing an

independent role in RA susceptibility. TNF manifests some SNPs at position -1031, -863, -857, -575, -376, -308, -244, -238, +70, +489. The -238GG and +489GG homozygots are associated with a more severe radiological course and higer number of erosions within hand joints [2, 33]. TNF-α -238 gene polymorphism can be also associated with RA susceptibility. Allel -308A strongly activate transcriptional signal in the human B cell line and is over-represent in patients with severe RA. Allel -308A can be use as a prognostic marker for the development of severe RA [31, 32]. In the patients with RA, SNP at position -308 is associated also with anaemia and kidney pathology. Finally, TNF-α +489 gene polymorphisms seems to be associated with the development of RA, and may indicate the worse outcome of the disease [34].

Conclusions

The management of RA is still a challenge, since ethiology is unclear. In spite of a huge progress in our understending of pathogenetic mechanisms we are unable to start efficient and causal treatment in RA patients.

A number of new drugs, called biological agents, were introduced in the treatment of RA recently, however none of them fulfils a causal therapy criteria. Since IL-1 and TNF-α, according to our present knowledge, are the most important in the inflammatory process in RA, the most demanded are drugs, which would neutralize both cytokines concurrently.

The difficulty in the management of RA are also related with our insufficient knowledge connecting progression of the disease mechanisms. Based on the clinical data we are unable to choose a group of cases with rapidly progressed RA. Therefore, we dramatically need a markers of early progression of the disease and it is possible that some genetical factors may by usefull. The determination of gene polymorphisms of proinflammatory cytokines seems to be of some importance, because they may serve as the markers of susceptibility and severity of RA. It seems that estimation of IL-1 polymorphisms may be very promising and useful in predicting the RA outcome.

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