Rheumatoid Arthritis and Cytokine Gene Polymorphism

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Abstract

Rheumatoid Arthritis is a chronic, painful, systemic, inflammatory, autoimmune disease with unknown etiology and it is characterized by the proliferation of synovial cells, dysfunction of joints. According to the common view accepted today, the disease of RA begins with the antigenic stimulation of the environmental factors that we do not yet know exactly in genetically predisposed individuals and is considered to be chronic with complex interactions in the immune system. Cytokine polymorphisms have been linked to many inflammatory, infectious and autoimmune diseases and it is now known that polymorphisms in regulatory regions of the cytokine genes affect their secretion. Inheritance of the polymorphisms is influenced by the ethnicity and ethnic differences in patterns of cytokine gene polymorphisms have been associated with predisposition to various diseases in different populations. Here we discus some cytokine gene polymorphism and Rheumatoid arthritis.

Keywords: Rheumatoid Arthritis, Cytokine gene, polymorphism

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Introduction

Rheumatoid Arthritis is a chronic, painful, systemic, inflammatory, autoimmune disease with unknown etiology and it is characterized by the proliferation of synovial cells, dysfunction of joints. According to the common view accepted today, the disease of RA begins with the antigenic stimulation of the environmental factors that we do not yet know exactly in genetically predisposed individuals and is considered to be chronic with complex interactions in the immune system. The imbalance between pro-inflammatory and anti-inflammatory cytokines released from macrophages, monocytes and synovial fibroblasts is speculated to play a role in the inflammation of joints. In addition to Class II MHC genes belonging to human leukocyte antigen (HLA) loci, multiple cytokine polymorphisms have been implicated as genetic risk factors predisposing to RA **(1).**

Cytokine polymorphisms have been linked to many inflammatory, infectious and autoimmune diseases and it is now known that polymorphisms in regulatory regions of the cytokine genes affect their secretion. Inheritance of the polymorphisms is influenced by the ethnicity and ethnic differences in patterns of cytokine gene polymorphisms have been associated with predisposition to various diseases in different populations **(2)**

Here we discus some cytokine gene polymorphism and Rheumatoid arthritis

TNF and Rheumatoid arthritis:

A-Biological regulation of TNF-α production and function:

The biological functions of the TNF-α are varied and complex, where on one hand it confers disease resistance and on the other causes pathological complications. Indeed, TNF-α plays contradictory role which may be related to genetic polymorphisms in the genes regulating its production and effect. In the acute situation, local production of TNF-α is clearly beneficial. It increases the expression of adhesion molecules on the vascular endothelium to allow immune cells, in particular neutrophils and macrophages, to translocate to sites of tissue damage and infection **(3).**

Initially, TNF-α potentializes T and B leukocytes activation, which also affects *via* feedback chemotaxis, macrophages and Natural Killer (NK) cells, both Antigen Presenting Cells (APC) belonging to the innate immunity. Such cell-to-cell interaction leads to a cascade of events, also stimulating the adaptive immunity to interact, which is represented mainly by T and B lymphocytes, responsible for antibody production. TNF-α also triggers Prostaglandins (PG) production, increasing fever induction, and the release of the acute inflammation phase proteins, such as C-Reactive Protein (CRP), gene expression of cytokines and chemokines, and endothelial cell activation. TNF-α had initially been referred to as Cachectin or Differentiation Inducing Factor (DIF), with two bioactive forms: transmembrane TNF-α (tmTNF-α) and soluble TNF-α (sTNF-α) **(4).**

Cells from mice deficient of TNFR1 or TNFR2 produce substantially more TNF-α upon stimulation. Although TNF-α may be produced by many cell types, macrophages are the main source of this cytokine. TNF-α is produced as a membrane-bound 26 kDa molecule from which is released the soluble 17 kDa active TNF-α molecule by enzymatic cleavage. The enzyme involved is a metalloproteinase disintegrin called TNF-α converting enzyme (TACE). Remarkably, TACE also acts on membrane anchored TNFR2 protein thus controlling the amount of soluble circulating TNFR2. This adds another layer to the regulation of TNF-α function because soluble TNF receptor affects the activity of TNF-α. TNF-α itself can suppress the production of more TNF-α, an effect mediated through the TNFR1 and TNFR2 cell surface receptors for TNF-α **(5).**

Physiologically, TNF-α is a crucial component for a normal immune response. TNF-α can activate the immune system to regulate; however, the inappropriate or excessive production of TNF-α can be

harmful and may lead to disease. Rheumatoid arthritis is induced by the abnormal secretion of TNF-α; thus, TNF-α can be classified as a key factor in the pathological development **(6).**

Due to the involvement of TNF-α in the pathogenesis of autoimmune diseases, TNF-α inhibitors have been successfully developed and applied in the clinical treatment of autoimmune diseases such as Crohn's disease (CD) and RA. Therapeutic drugs act as antagonists by blocking the interaction of TNF-α with TNFR1/2 or, in some cases, as agonists by stimulating reverse signaling, causing the apoptosis of TNF-α producing immune cells. Several TNF-α inhibitors have been approved for clinical use: etanercept, infliximab, adalimumab, golimumab, and certolizumab. The impact of TNF-α signaling on each type of autoimmune disease will be introduced in this review, as well as the evaluation of current TNF-α inhibitors utilized as therapeutic drugs against autoimmune diseases **(7).**

B-Tumor Necrosis Factor Alpha SNPS and Rheumatoid arthritis:

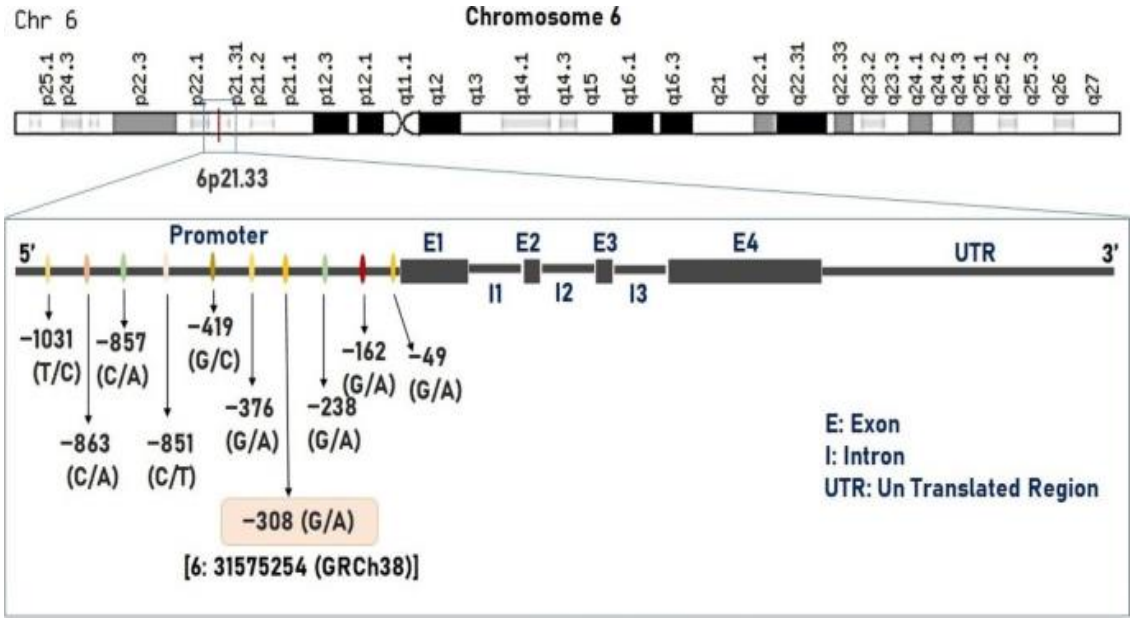
TNF gene is located in close proximity to the HLA-B locus in both humans and mouse. The 5′ flanking region of the TNF gene contains multiple potential regulatory sites, including consensus sequences for the AP-1 and AP-2 sites, the cAMP-responsive element, and sequences similar to the kappa B sequences found in immunoglobulin and cytokine regulatory elements **(3).**

The TNF-α gene is located on the short arm of chromosome 6 within the major histocompatibility complex, where genetic altera- tions in the TNF-α locus are now known to be involved directly in high TNF-α production. The gene that encodes TNF-α is located in the short arm of chromosome 6, in the 6p21.3 region **(8).**

Single Nucleotide Polymorphisms (SNPs) are the most common variation in the human genome that differentiate from the rare mutations because they present frequency of at least 1% of the less common allele in the population. One SNP takes place in virtually 1 out of a thousand base pairs and their most common replacement (2/3 of the overall) is of one cytosine by a tymine (C/T) **(9).**

One SNP may influence the genic product by different manners: (**i**) variations in un-translated region 5' (5'UTR - 5' Un-translated Region) may modify the mRNA translation; (**ii**) variation in un-translated region 3' (3'UTR - 3) may affect the clivage, stability and the transport of mRNA, thus altering protein productions which clinically may imply in differentiated cell functions, for better or for worse **(10).**

SNPs may occur in the coding region, or in the regulatory region of the gene, leading to changes in the amino acid sequence of the encoded protein, or its production rate. For being bi-allelic, SNPs can be detected by using techniques that discriminate any different combinations in the nucleotides Adenine (A), Thymine (T), Cytokine (C) and Guanine (G). Among the SNPs, the -308 G/A (rs1800629) in the promoter region is described as being able to increase the gene expression and therefore the level of TNF-α production due to the transition from wild-type G allele to the variant A allele **(11).**



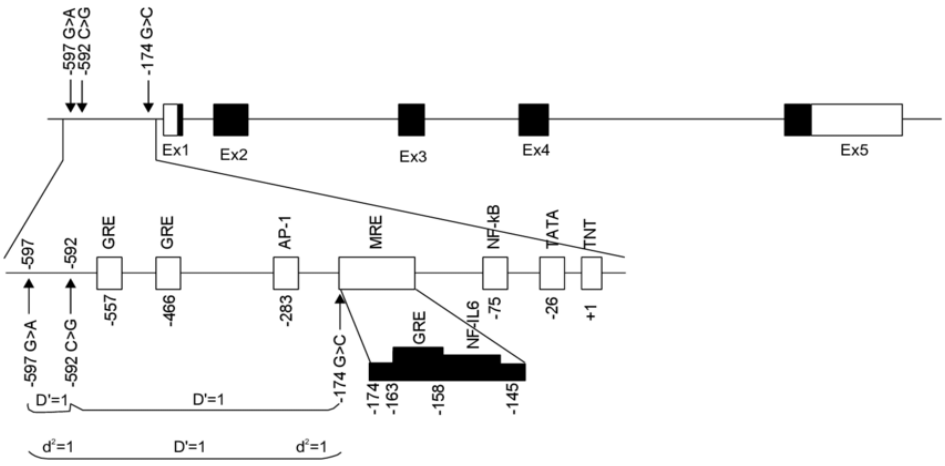
Figure(1): Location and structure of TNF-alpha gene and list of upstream variants (12).

TNF-α is encoded in the promoter region -308 G/A (rs1800629) and admits three different kinds of genetic profiles: The homozygous form G/G, and the mutant variant forms G/A and A/A. The wild type genetic profile G/G is most commonly found in a specific population studied, usually associated with low level TNF-α-producing individuals. The mutant form, G/A, on the other hand, is related to intermediate production, while the rarer form, A/A, is associated with high level TNF-α- producing individuals. Expectable as it seems, it is possible to speculate that the presence of such genetic polymorphisms could exacerbate the inflammatory responses depending of the genetic individual profile, modulating the course of the disease, and therefore the immune response of the organism **(10).**

TNF-α was initially identified as a factor that can induce tumor cell necrosis, but was latterly found to be involved in the pathogenic process of autoimmune diseases as an important pro-inflammatory factor**.** The accumulation of TNF-α can ultimately contribute to the development of chronic inflammation and tissue destruction. It has been reported that *TNF-α* promoter -308A allele in Crohn's disease increased TNF production promoting inflammatory activity and were associated with worse responsiveness to anti-TNF-α therapy **(**13**).** On this basis, we speculated that *TNF-α* -308A may also have a similar role in autoimmune diseases such as AS, RA, and PsA.

There is an association between HLA-DR4 and RA but there is a lack of linkage disequilibrium between the TNF-308 polymorphism and this HLA antigen. More recently, other TNF-SNP polymorphisms have been shown to correlate with the severity of RA but not with the initial susceptibility to the disease **(**14**).**

2-IL-6 and Rheumatoid arthritis:



Figure(2): Map of human IL6 on chromosome 7p21. Exons, UTR, and SNPs are shown with the putative transcriptional factor-binding site. Coding exons are marked by shaded blocks and 5' and 3' UTR by white blocks (15)

Interleukin 6 (IL-6) is a glycosylated polypeptide with a molecular weight of about 28 kDa, composed of 184 amino acids. The gene encoding IL-6 is located on chromosome 7p15.3 and consists of 4 introns and 5 exons. The production of IL-6 is mainly stimulated by IL-1, TNF-α, interferons, lipopolysaccharides, and viruses. IL-6 is produced by cells of the immune system—neutrophils, T and B lymphocytes, monocytes, and NK cells—but also other cells—fibroblasts, osteoblasts, keratinocytes, vascular endothelial cells, and even some neoplastic cells (**16).**

Circulating IL-6 is found in the blood of healthy humans at low concentration (#1 pg/mL), and significantly increases during inflammatory conditions, reaching concentrations in the range of μg/mL during sepsis. In fact, this cytokine critically contributes to host defense against infections and tissue injuries by stimulating acute-phase immune response and hematopoiesis, but it also regulates metabolic, regenerative, and neural processes under physiological conditions. Once released, IL-6 exerts its pleiotropic biological effects by activating a unique IL-6R signaling system, including the IL-6R and downstream signaling molecules **(17).**

The receptor for IL-6 consists of two subunits: gp130, the main function of which is to transmit a signal to the interior of cells, and α-gp80, which recognizes and binds IL-6. The gp130 subunit is present on the surface of most cells, while α-gp80 only appears on neutrophils, lymphocytes, monocytes, and hepatocytes. IL-6 has both pro-inflammatory and anti-inflammatory properties. It participates in acute phase reactions, and depending on the type of cells it affects, it can lead to their activation, proliferation, differentiation, and apoptosis **(18).**

Currently, IL-6 is considered an important marker of many disease states. IL-6 has been found to play an important role in the pathogenesis of RA by initiating neutrophil migration and osteoclast maturation, resulting in synovitis, joint destruction, and pannus formation. Elevated levels of IL-6 are

observed in both the synovial fluid and serum of RA patients, and the concentrations in the fluid are much higher than in the serum, which may indicate its local production **(19).**

IL-6 signal transduction:

After IL-6 binding, receptor homodimerization promotes the interaction between the gp130 chain with the tyrosine kinase JAK (Janus kinase), resulting in their mutual transactivation. In turn, JAK activation triggers three main intracellular signaling pathways, via phosphorylation of two key proteins, ie, 1) the Src Homology domain-containing protein thyrosin Phospatase-2 (SHP-2), and 2) the signal transducer and activator of transcription proteins (STAT1–STAT3). Once phosphorylated, SHP-2 can, on one hand, interact with Grb2 (growth factor receptor bound protein 2), leading to the activation of the Ras/ERK/MAPK (rat sarcoma protein/ extracellular signal-regulated kinase/mitogen-activated protein kinase) cascade, and/or, on the other hand, can activate the PI3K/Akt (phosphoinositol-3 kinase/protein kinase B) pathway. On the contrary, phosphorylation of STATs proteins induces the formation of heterodimers (STAT1/STAT3) or homodimers (STAT1/STAT1 and/or STAT3/STAT3), which subsequently translocate into the nucleus. In all cases, the final result of the activation of these intracellular pathways is the induction of the transcription of multiple target genes accounting for the pleiotropic biological activities of IL-6 **(20).**

Abbreviations: IL-6, interleukin-6; mIL-6R, transmembrane interleukin-6 receptor; sIL-6R, soluble interleukin-6 receptor; gp130, 130 kD signal-transducing chain; JAK, Janus kinase; SHP-2, Src Homology domain-containing protein thyrosin Phospatase-2; STATs, signal transducer and activator of transcription proteins; Grb2, growth factor receptor bound protein 2; Ras/ERK/MAPK, rat sarcoma protein/extracellular signal-regulated kinase/mitogen- activated protein kinase; PI3K, phosphoinositol-3 kinase; Akt, protein kinase B.

Pleiotropic biological effects of IL-6:

Effects on the immuno-inflammatory response

IL-6 is a pleiotropic cytokine that has both pro- and anti-inflammatory properties and plays multiple roles in adaptive immunity. In RA patients, IL-6 showed increased levels in serum and synovial fluid, and such increase was positively correlated with disease severity and joint destruction. Synovitis is also promoted by IL-6 via inducing neovascularization, in which vascular endothelial growth factors mediate the proliferation and outcomes in inflammatory cell infiltration and hyperplasia of synovium **(21).**

Further evidence suggests that IL-6 is a mediator of joint erosion and causes bone resorption by inducing the formation of osteoclasts. Cartilage degeneration is also caused by IL-6 via matrix metalloproteinases produced by synovial cells and chondrocytes. IL-6 is also responsible for mediating many of the inflammatory manifestations in RA patients; for instance, it induces hepatocytes to produce C-reactive protein (CRP) **(22).**

Extra-immunoinflammatory effects:

Besides its key involvement in the immune-inflammatory response, IL-6 also plays an important role under physiological conditions by modulating a number of multisystemic functions such as embryogenesis,25 glucose and lipid metabolism,26 bone remodeling,27,28 liver regeneration,29 neural tissue homeostasis, cognitive function, sleep, memory, pain, and emotional behavior. The knowledge of these extra-immunoinflammatory effects may help explain the pathogenesis of some systemic manifestations observed in RA and other chronic inflammatory diseases characterized by persistently elevated IL-6 levels **(23).**

Polymorphisms in the IL-6 promoter include rs1800795, rs1800796 and rs1800797. rs1800796 has been reported to be associated with inflammatory disorders such as RA, osteoarthritis (OA), diabetic nephropathy and HCV etc **(23).**

The most often studied SNPs of the IL-6 gene in RA are IL-6 rs1800795 (-174 G > C) and rs1800796 (-572 G > C). Conducted studies showed that both allelic and genotypic frequencies of rs1800795 SNP did not differ between RA patients and healthy controls in Polish, Turkish, Mexican, and Indian populations Opposite results were obtained in the Chinese Han and Egyptian populations **(23).**

IL-6 is involved in a wide range of physiological processes, such as the immune response, inflammation, and bone metabolism, and has also been implicated in the pathogenesis of RA. Anti-IL-6 receptor antibody was first approved for RA in 2008, and high efficacy in the treatment of RA has been demonstrated. Due to the wide range of concentration of IL-6 in RA patients, clinical application of anti-IL-6 antibody seems to be difficult. In fact, clinical trials of the anti-IL-6 antibody sirukumab for RA were not successful **(24).**

The Food and Drug Administration (FDA) have declined the approval of this agent because of a trend for increased overall mortality with sirukumab vs. placebo. The mortality was mainly associated with cardiovascular events, infection, and malignancy. IL-6 activates the JAK-STAT system, and JAK inhibitors have a remarkable effect on RA. Various cytokines regulate a number of downstream signaling molecules in RA, and these are potential therapeutic targets However, inhibition of pathways other than the JAK-STAT system is not considered to be easy due to the problem of crosstalk in which signals enter from other pathways **(2)**

3- TGF-β and Rheumatoid arthritis:

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology, characterized by chronic inflammation that affects diarthrodial joints, has a progressive course and causes joint functional disability, deformity and, ultimately, a decreased quality of life and reduced life expectancy. Its pathogenesis is characterized by an alteration of cellular and humoral immunity, and impaired resident cell components of the connective tissue of the synovial membrane (SM), which behave in a pseudo-tumoral fashion, invading and destroying adjacent tissue. The first manifestations of RA joint

inflammatory response appear to be due to microvascular changes and an increase in the number of cells or synovial lining hyperplasia. These changes are accompanied by an altered regulation of cytokines, an increase in the number of fibroblasts and excess proliferation of inflammatory cells, mainly macrophages and lymphocytes, tissue destruction and angiogenesis **(25).**

Transforming growth factor TGF-β1 is a multifunctional cytokine that regulates cell growth, adhesion, and differentiation in a wide variety of cell types. Among the activities of TGF-β1 are those associated with growth modulation, immunosuppression, and pro-inflammation. TGF-β1 is produced in the synovial fluid of patients with RA and acts as an important regulator in the course of the disease. TGF-β1 was reported to increase the expression of pro-inflammatory cytokines and metalloproteinase-1 by synovial fibroblasts. These results suggest that TGF-β1 contributes to the progression of inflammation and joint destruction in RA **(26).**

Angiogenesis is a remarkably active process in RA, especially in early stages and is regulated by various pro-angiogenic mediators such as TGF-β1, angiopoietin, placenta growth factor, FGF, and vas- cular growth factor (VEGF). These factors activate endothelial cells and induce the production of proteolytic enzymes that degrade the basement membrane and perivascular extracellular matrix **(25).**

TGF-β1 SNPS and RA:

TGF-β1 gene is located in the 19q13 chromosome region. TGF-β1 displays many functions which are dependent on the cell type, and the state of differentiation. There are five described polymorphisms in this gene: two in the promoter region at positions 800 G/A and 509 C/T and three located in the coding sequence at positions 869 T/C, 915 G/C, and 1628 C/A. The mutation point in position 869 at codon 10 involves alleles T (leucine) and C (proline) and the second one in position 915 at codon 25 G (arginine) and C (proline) **(27).**

It has been proved that the ability of a human being to produce a higher or lower level of TGF-β1 may be genetically affected and polymorphisms in its gene regulate TGF-β1 expression. These polymorphisms may play in predisposing a person to various disease states, including Rheumatoid arthritis, colorectal carcinoma, diabetes mellitus, osteoporosis, asthma, Crohn's disease, and fibrotic diseases of the skin and kidney **(27).**

It was found that some locus gene polymorphisms of TGF-β1 were related to the expression level of TGF-β, and TGF-β 1 genotypes may determine the severity of bone damage and predict the progression of rheumatoid arthritis [15]. Currently, TGF-β1 +869T/C (rs1982073), -509T/C (rs1800469) and +915C/G (rs1800471) have been reported in variety of diseases **(28).**

4- IFNγ and Rheumatoid arthritis:

Pleiotropic effect of IFN-γ and RA:

Interferon-gamma (IFN-γ) is involved in the regulation of both the innate and adaptive immune responses. The major biological activities of IFN-γ include activation of cellular immunity. IFN-γ activates innate immune cells and induces major histocompatibility complex (MHC) class II antigen expression, possibly contributing to the development of rheumatoid arthritis (RA). In RA synovium, CD8+ T cells are thought to be a source of IFN-γ, which leads to down- stream cytokine cascades through by activating rheumatoid synovial infiltrating cells **(29).**

IFN-c in autoimmunity:

Autoimmunity against citrullinated proteins is another hallmark of RA. Anti-citrullinated protein/ peptide antibodies (ACPA) recognize and bind to citrullinated epitopes present on numerous proteins, such as vimentin, a-enolase, and fibrinogen. Among ACPA, anti-citrullinated vimentin antibodies are particularly associated with joint destruction, indicating citrullinated vimentin as a major autoantigen in RA **(30)**.

However, given that vimentin is expressed exclusively in mesenchymal cells including fibroblasts, it needs to be considered how such non- immune cells (non-professional antigen presenting cells) play a role in RA autoimmunity. Whereas professional antigen presenting cells, such as dendritic cells, monocytes/macrophages, and B cells, constantly express MHC class II, non-immune cells including synovial fibroblasts need IFN-c stimulation to express MHC class II. Although synovial fibroblasts lack costimulatory molecules CD80 and CD86, CD276 (B7-H3) on those cells has been shown to act as a CD28 ligand. Upon stimulation with IFN-c, synovial fibroblasts expressed MHC class II, CD274 (PD-L1), and CD273 (PDCD1LG2), while CD276 (B7-H3) was expressed regardless of the presence of IFN-c **(30)**.

Signaling pathway of IFN-c:

An important role of IFN-γ is the regulation of immune cell growth and differentiation by activating the transcription of target genes. IFN-γ signaling pathway is mediated by activating Janus kinase (JAK) and signal transducer and activator of transcription (STAT) 1. After binding to its receptor, IFN-γ activates the receptor-associated JAK1 and JAK2 by phosphorylation. The activated JAKs allow for subsequent phosphorylation of STAT1, which then dissociates from the IFN-γ receptor, translocated to the nucleus and binds to the promoter regions of IFN-γ-inducible genes. JAK inhibitors (JAKi) target multiple cytokines, including interleukin-6 (IL-6) or granulocyte–macrophage colony-stimulating factor (GM-CSF), and exhibit clinical efficacy in the treatment of RA. However, the molecular mechanisms for the negative regulation of IFN-γ-induced signal transduction pathways by JAKi have not been completely elucidated **(31).**

Interferon-c (IFN- γ), a T-helper 1 (Th1) cytokine has an important role in the pathogenesis of RA as it serves as a marker of activation of Th1 cells which promote and amplify autoimmune diseases. This cytokine has the potential to direct the inflammatory response by upregulating a variety of

proinflammatory mediators including TNF-a and IL-6. Moreover, data suggest that IFN-c may also be able to directly enhance activation of the pro-inflammatory nuclear transcription factor-jB (NF-jB) under certain conditions Adenine (7) to thymine (T) transition at position +874 (rs2430561) has been associated with increased IFN-c expression **(32).**

IFN-γ SNPS and RA:

IFN-γ gene is located in chromosome 12 (q14) and consists of four exons and three introns. Although several single nucleotide polymorphisms (SNPs) have been reported in the IFNG gene, an association of a common variant in the intron 1 (+874 T>A) has been widely investigated in various autoimmune diseases as this region is crucial for binding of a nuclear factor kappa B (NFKB). However, the functional relevance of the intronic polymorphism (+874 T>A) remained contradictory **(33).**

New insights into IFN-γ in rheumatoid arthritis: role in the era of JAK inhibitors:

The cytokine interferon-gamma (IFN-c), which is the sole member of type II interferons, plays an important role in the innate and adaptive immune responses. Based on the mutually antagonistic effect of IFN-c and tumor necrosis factor (TNF)-a, recombinant IFN-c was once attempted to treat RA. Anti-IFN-c and anti- TNF-a antibodies showed similar efficacy in patients with active RA **(34).**

Janus kinase (JAK) inhibitors target multiple cytokines including IFN-c and interleukin (IL)-6 and exhibit a beneficial treatment effect in patients with RA and inadequate response to conventional synthetic or biologic disease-modifying anti-rheumatic drugs (DMARDs). Even for patients refractory to anti-IL-6 therapy, the treatment effect of JAK inhibitors is promising, putatively indicating roles of multiple cytokines in the pathogenesis of RA **(34).**

5- IL-10 and Rheumatoid arthritis:

IL-10 cellular sources:

IL-10 was classified as a cytokine specifically secreted by T helper 2 (Th2) cells, however, it was subsequently widely recognized that it can be produced by many myeloid and lymphoid cells. Among these, CD4+ Th1, Th2 and Th17 cells, and Treg cells, DCs, monocytes and macrophages are main producers of IL-10. Recently, microglia and cardiac macrophages have been also identified as producers of IL-10 **(35).**

IL-10 systemic effects:

IL-10 was initially defined as "cytokine synthesis inhibitory factor" due to its inhibitory activity on IL-2 and interferon-γ (IFN-γ) release by Th1 cells, however it is now commonly considered as a key immunoregulatory cytokine with pleiotropic activities, exerting multiple and sometimes even opposite effects on immune cells **(36).**

IL-10 is a master regulator of immunity during infection, role in limiting or terminating inflammation and in the consequent host protection. IL-10 production by innate immune cells generally occurs later compared to that of pro-inflammatory cytokines released in the early phase of the inflammatory process. IL-10 secreted at the site of ongoing inflammation is responsible for maintaining the right balance between effective pathogen elimination and prevention of detrimental immune-mediated response against infections, resulting in the restoration of normal tissue homeostasis. At the same time, numerous pathogens induce IL-10 up-regulation during the infection and exploit the immunosuppressive activity of this cytokine to escape host immune system and promote a microenvironment that favors their tolerance and long-term survival (37).

IL-10 exerts strong immunosuppressive effects on monocytes, macrophages, which are the cells with the higher expression of IL-10R, and dendritic cells. It inhibits the ability of these cells to produce pro-inflammatory cytokines (including IL-1α and β, IL-6, IL-12, IL-18, and TNF-α) and chemokines (CCL2, CCL12, CCL5, IL-8, CXCL10, and CXCL2) and prevents their differentiation, maturation and migration to lymphoid organs (7).

It also suppresses the antigen-presenting capabilities to Th1 and Th2 of monocytes and APCs by down-regulating their expression of the class II major histocompatibility complex (MHC II). and the co-stimulatory molecules CD54 (intercellular adhesion molecule-1, ICAM-1), CD80 and CD56. Moreover, it can act on CD4+ T cells by inhibiting their antigen-specific activation and proliferation in lymph nodes, limiting their secretion of cytokines, such as IL-2, IFN-γ, IL-4, IL-5 and TNF-α, and their cytotoxic activity, and inducing their long-term anergy through the block of CD28 co-stimulatory signaling. Therefore, through these coordinated actions, IL-10 leads to the shutdown of the inflammatory immune response, both directly, by the suppression of macrophages and dendritic cells activity, and indirectly, by limiting T cells activation, differentiation and effector function and promoting peripheral tolerance (38).

On the other hand, IL-10 exhibits several immunostimulatory activities. This cytokine is a potent stimulator of B lymphocytes: it prevents apoptosis in germinal cells, enhances cell growth, proliferation and activation and drives differentiation into immunoglobulin-secreting plasma cells. IL-10 plays also an important role in differentiation and functioning of the Tregs. and promotes the survival of T cells otherwise destined to apoptotic cell death. Regulatory B cells (Bregs), representing B cells immune-suppressive fractions, regulate inflammation primarily through an interleukin 10 mediated inhibitory mechanism (39).

In addition, IL-10 induces thymocytes proliferation, by upregulating the expression of CD3 and CD8 molecule. It also enhances the production of IFN-γ and granzyme, improves MHC expression and facilitates antigen recognition, promoting in this way the survival, expansion and cytotoxic activity of antigen activated CD8+ T cells. IL-10 is critically involved in the generation and/or sustaining of effector CD8+ memory T cells too (40).

IL-10 promotes NK cell proliferation and migration and enhances their cytolytic activity and effector functions. Furthermore, IL-10 directly stimulates mast cells, enhancing their expansion, survival, and activation, upregulating their expression of high-affinity IgE receptors (FcϵRI) and increasing their production of pro-inflammatory cytokines. Furthermore, IL-10 is an important regulator of epithelial wound repair and plays a key function in gut homeostasis, promoting wound closure and stimulating intestinal epithelial cell proliferation **(38).**

Regulation of IL-10 production and its double role in immunological homeostasis:

IL-10 plays a fundamental role in maintaining host homeostasis at both local and global level, ensuring the fine equilibrium between pro- and anti-inflammatory immune response required to achieve an effective clearance of infecting pathogens and preventing, at the same time, tissue damage occurrence **(7).**

Therefore, in physiological conditions IL-10 production is under a highly dynamic and finely balanced modulation to orchestrate the different immunological activities in a cell-specific manner and to control the inflammatory response force and duration **(36).**

On the other hand, downregulation of IL-10 expression or defective signaling can also have both a beneficial and a detrimental impact to the host. An IL-10 deficiency occurring in the early phase of microbe infection triggers a rapid amplification of the innate and adaptive immune response and facilitates effective clearance of invading pathogens. If the deficiency persists, it leads to systemic, exaggerated inflammation and immune-mediated tissue damage and participates to the onset or aggravation of chronic inflammatory diseases and several autoimmune pathologies. IL-10 expression was found lower in psoriatic and asthmatic patients and IL-10 and IL-10R mutations, causing a loss of IL-10 function, were found to be associated with severe inflammatory bowel disease, including Crohn's disease and ulcerative colitis **(41).**

Signaling pathway of IL-10:

IL-10 is a member of the class II cytokine family and its biologically active form is a soluble 36 kDa homodimer, comprising two monomers with six α-helices structure and stabilized by two intrachain disulfide bonds. The cellular response to IL-10 starts with the binding of an IL-10 homodimer to a heterotetrametric IL-10 receptor (IL-10R) complex, belonging to the interferon receptor family and comprised of two ligand-binding IL-10R-alpha (IL-10RA) subunits and two accessory signal-transducing IL-10R-beta (IL-10RB) subunits. IL-10RA is the main responsible for directing ligand and target specificity: it recognizes IL-10 with high-affinity **(7).**and it is mainly expressed by lymphocytes, macrophages and dendritic cells at basal level, but can be upregulated by various cells upon their activation **(42).**

IL10 in Rheumatoid arthritis:

IL-10 is a potent immune-modulatory cytokine, concentrations of which are increased in both the serum and the synovial fluid of patients with RA. IL-10 inhibits neutrophil infiltration and activation in the synovial tissue, skews macrophage polarization towards an M2 phenotype and inhibits the expression of key pro-inflammatory cytokines such as TNF. In vitro, neutralization of IL-10 in RA synovial membrane cultures lead to increased expression of TNF and IL-1β **(41).**

Despite the unquestionable regulatory function of IL-10 in experimental arthritis, therapeutic administration of IL-10 in established RA initially delivered positive results but has since been disappointing. The reason for the poor performance of IL-10 in RA is unclear; however, IL-10 is thought to induce Fcγ receptor expression on monocytes and to increase their responsiveness to immune complexes, which could potentially outweigh the immune-regulatory function of IL-10 **(41).**

Almost all leukocyte subsets produce IL-10. Among them, Breg cells are increasingly being recognized as exerting an anti-inflammatory function through the secretion of IL-10. Impaired IL-10 production by CD1dhiCD5+B cells was responsible for the exacerbated arthritis seen in mice with B cell-specific deletion of hypoxia-inducing factor-1α (HIF1α) . In a previous study, overexpression of IL-10 restored the protective function of HIF1α-deficient Breg cells, confirming the crucial role of HIF1α in the production of IL-10 by Breg cells. Similarly, IL-10 produced by myeloid-derived suppressor cells also contributes to the attenuation of experimental arthritis, supposedly by influencing the balance between TH17 cells and Treg cells **(43).**

IL-10 SNPS and RA:

IL-10 appears to have a conflicting function in RA, decreasing pro-inflammatory cytokines while simultaneously fostering a mixed immunological response, as indicated by contradicting findings regarding IL-10's therapeutic effectiveness in RA. In recent years, genetic susceptibility to RA had been examined. Numerous genes associated with RA have been discovered as risk factors for the disease, and the IL-10 gene is one of the most extensively examined. Numerous studies suggest that various IL1082 A/G polymorphisms are related to a higher risk for RA disease **(44).**

IL-10 is an immunomodulatory cytokine encoded by the IL10 gene on chromosome 1q31–32, containing five exons separated by four introns. Polymorphisms located in the 5′-flanking region of the IL10 gene, at positions −1082 A>G, −819 T>C, and −592 A>C, are known to be involved in regulating the production of IL-10, with the first being the best characterised of them **(44).**

The best distinct SNP for the IL10 gene is IL-10-1082 A / G. This polymorphism is characterized by the substitution of guanine (G) to adenine (A) nucleotides. The resulting IL-10–1082 AA genotype is marked by lower IL-10 production. Numerous cells of adaptive and innate immune, including Th2 lymphocyte, B - lymphocyte, and macrophage, secrete IL-10, a very strong antiinflammatory cytokine with the capacity to downregulate antigen performance and activate macrophages **(44).**

Inhibition of B cell activation and antibody production has also been demonstrated with IL-10. IL-10 had been shown to suppress proinflammatory cytokine in rheumatoid joints and also to induce joint swelling and deformity reduction, while also cartilage necrotic decrease. Given the importance of IL-10 in the development of RA, various research had looked at IL-10 genetic diversity that might influence human association with RA, but the data have been mixed and inconclusive. Autoantibody status, such as rheumatoid factor (RF) and citrulline (anti-CCP) antibodies, has been used to separate RA patients into various hereditary groupings **(45).**

No Conflict of interest.

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