

“High” and “Low” Gene Expression Signatures in Rheumatoid Arthritis: an Emerging Approach for Patient Stratification and Therapy Choice

Elena V Tchetina

Elena V Tchetina, Principal Investigator, Clinical Immunology Laboratory, Nasonova Research Institute of Rheumatology, 34A Kashirskoe Shosse, Moscow, 115522, Russia

Correspondence to: Elena V Tchetina, PhD, DSc, Principal Investigator, Clinical Immunology Laboratory, Nasonova Research Institute of Rheumatology, 34A Kashirskoe Shosse, Moscow, 115522, Russia

Email: etchetina@mail.ru

Telephone: +7-909-647-6991

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease with unknown etiology that affects various pathways within the immune system, involves many other tissues and is associated with joint destruction. Current treatments fail to address pathophysiological and biochemical mechanisms involved in joint degeneration and the induction of pain. Moreover, RA patients are extremely heterogeneous and require specific treatments, the choice of which is complicated by the fact that not all patients respond to treatment. At present, patient assessment involving clinical, immunological, and radiological traits does not permit the prediction of the anti-rheumatic treatment response in approximately half of RA subjects. Recent data suggest that patient stratification, in accordance with the level of pro-inflammatory gene expression, may help to predict response to RA therapy.

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Key words: Rheumatoid arthritis; Gene expression; Chinese traditional medicine; TNF α ; Type I IFN-response genes; Patient stratification; Response to therapy

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial hyperplasia, mononuclear cell infiltration, articular cartilage and bone erosion, and joint destruction. The synovium is a major site of the pathologic process, which involves synovial hyperplasia and pannus formation. Synovial tissue dysfunction in RA affects the interchange of fluid between blood vessels and intraarticular space because synovial fluid serves as a lubricant and, along with subchondral bone, provides nutrition for articular cartilage^[1]. Synovial tissue is invaded by macrophages, fibroblasts, and activated lymphocytes. T-lymphocytes are involved in the production of a wide range of pro-inflammatory cytokines, predominantly of tumor necrosis factor and interleukin superfamilies, as well as growth factors^[2-4]. The role of B-lymphocytes is associated with the production of autoantibodies, such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (ACPA)^[5]. Later, the invasion of the articular cartilage and bone occurs by the secretion of degrading enzymes, primarily matrix metalloproteinases (MMPs), presumably through the small region at the insertion of the joint capsule where bone is covered by synovium only (“bare area”)^[1].

Rheumatoid arthritis is a heterogeneous condition. Heterogeneity could be associated with various factors, including ACPA and/or RF positivity^[6-8], the pace of the disease course^[9-11], and variability in response to treatment^[10-13]. This might suggest the involvement of different pathophysiological mechanisms. Therefore, adequate treatment of RA requires specific diagnostic tests and optimal biomarkers to distinguish between different manifestations of the disease.

Gene expression profiling, the analysis of the expression of numerous genes, could provide a comprehensive description of changes in biological functions associated with the disease^[14]. Current accomplishments in RA gene expression studies were described in detail in a profound recent review^[15]. The latter demonstrates that gene expression profiling is valuable in the investigation of RA pathogenesis, the recognition of a preclinical phase of the disease, and as a research tool for the identification of prognostic factors associated with disease progression and its severity. Moreover, gene expression signatures permit the distinction of specific predictors of responses to major RA therapeutic agents, such as methotrexate, and biological drugs including anti-tumor necrosis factor (TNF) agents, rituximab, anakinra, and tocilizumab. However, signatures acquired by gene expression profiling do not always overlap and have not always been re-validated; they often lack reproducibility and might reflect drug specificity rather than pathophysiological mechanisms of the disease^[15,16].

Another approach to RA gene expression studies is the examination of several functionally associated genes. As inflammation represents a key trait of RA, its intensity was suggested to be related to patient heterogeneity^[17]. RA patient categorization into “low” and “high” pro-inflammatory gene expression subsets could help to overcome some of the abovementioned troubles.

GENE EXPRESSION ASSOCIATED WITH LEVELS OF INFLAMMATION AS A BASIS FOR RA PATIENT STRATIFICATION

In Chinese traditional medicine (TCM), RA patients are categorized according to articular manifestations in cold and hot patterns. A cold feeling in joints and pain relieved with warming defines the “TCM cold” pattern, while a hot feeling and pain relieved with cooling defines the “TCM hot” pattern. In addition, a hot feeling in joints is always related to red joint color, indicating inflammation^[18,19].

Gene expression profiling of CD4+ T cells has shown that TCM cold and hot patterns involve the activation of unique signaling pathways^[20,21]. T cell proliferation associated with the up-regulation of transforming growth factor (TGF) β -, vascular endothelial growth factor (VEGF), Wnt-signaling pathway, and insulin signaling pathways were related to the TCM hot pattern. These signaling pathways have been also associated with the inflammatory response and tumor formation in RA^[22-26]. In addition, RA patients with the TCM hot pattern demonstrated the increased expression of genes related to glycolysis and the citric acid cycle as well as ATP-synthase and NADH-dehydrogenase activity, indicating the up-regulation of energy generation pathways^[20]. In contrast, the TCM cold pattern was associated with the up-regulation of alanine, aspartate, and tyrosine amino acid metabolism^[20]. The enzymes involved in the metabolism of these amino acids are also essential for RA pathogenesis^[27-29]. Purine and fatty acid metabolism, small G-protein signaling pathways, and T cell proliferation-associated gene expression, although observed in patients of both subsets, was higher in TCM hot pattern patients^[20,21]. Therefore, RA patients stratified according to the intensity of inflammation in TCM exhibited different gene expression signatures, indicating dissimilar metabolic disturbances associated with the disease.

The up-regulation of proinflammatory cytokine gene expression signatures in RA is expected, as RA is traditionally viewed as a TNF α -driven disease^[30]. This concept is based on the effectiveness of TNF α -blocking agents in RA and also results from genetic studies,

in which an association was found between T-cell activation and ACPA-positive RA, while ACPA-negative RA was associated with type I interferon (IFN)-related gene up-regulation^[31-33]. According to this model, ACPA-positive RA is predominantly driven by TNF α based on in vitro observations that TNF α downregulates the effects of type I IFNs and vice versa. However, gene expression levels of proinflammatory cytokines could vary among RA patients. For example, gene expression profiling in the peripheral blood revealed the increased expression of type I IFN-response genes in about a half of RA patients (IFN “high” patients). The IFN “high” group exhibited significantly upregulated pathways involved in coagulation and complement cascades and fatty acid metabolism compared with healthy controls, while in IFN “low” patients the expression of these genes was equal to that in control subjects despite no clinical differentiation^[34]. Moreover, plasma levels of TNF α were equal between IFN “high” and IFN “low” subsets. Therefore, the presence of TNF α and IFNs are not mutually exclusive but might indicate the simultaneous operation of multiple immune mechanisms in RA^[13].

In RA patients with an activated type I IFN signature, gene expression levels were highly correlative and linked to the intensity of global IFN signature activation^[35]. A “high” type I IFN signature was associated with a lower level of disease activity; it also predicted a decrease in DAS28 when the swollen joints were controlled. An IFN signature was observed equally often in seropositive and seronegative RA patients and was associated with the persistence of ACPA after TNF blockade^[36]. Therefore, the type I IFN signature might be involved in RA pathogenesis^[13].

Deleterious effects of type I IFNs have been associated with the enhancement of B-cell survival through the direct stimulation of B-cells or through the production of B-lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL)^[13] and also by the stimulation of T-cells and dendritic cells^[31,37]. At the same time, IFN β can reduce the secretion of proinflammatory cytokines, such as interleukin (IL)-6, MMPs, and prostaglandin E (PGE)₂ by fibroblast-like synoviocytes. It possesses antiangiogenic properties and can inhibit osteoclastogenesis^[38,39]. Hence, it was suggested that IFN signature activation in RA synovium could be a reactive attack to limit inflammation^[38].

The difference in the gene expression of major metabolic regulators, such as mammalian (or mechanistic) target of rapamycin (mTOR) could also provide a molecular basis for RA patient stratification related to pro-inflammatory gene expression. mTOR is a key regulator of cell growth and proliferation^[40]. mTOR inhibition resulted in the downregulation of mitogen-induced T- and B-lymphocyte proliferation and IL-1 and TNF α production *in vitro*^[41,42]. In addition, animal studies have shown that mTOR inhibition alleviated paw swelling in antigen-induced arthritis^[43]. At the same time, mTOR up-regulation has been associated with interleukin (IL)-1, TNF α production, synovial fibroblast proliferation, and osteoclast formation^[41,42,44,45].

Mammalian target of rapamycin was differentially expressed in the peripheral blood cells of naïve early RA patients. Expression was either downregulated or upregulated in naive RA patients with clinically similar phenotypes at diagnosis compared to healthy controls. A “high” mTOR gene expression signature was associated with a significant up-regulation of cell cycle progression inhibitor (p21) and apoptosis- and autophagy-related genes. In patients with “low” mTOR gene expression, the abovementioned genes were downregulated. Pro-inflammatory cytokine TNF α and IL-6 expression was upregulated in both patient subsets compared to control. However, the expression of these genes was significantly

higher in “high” mTOR patients versus “low” mTOR patients^[46].

Therefore, the differential expression of type I IFN-response genes and mTOR in the peripheral blood cells might be used to stratify RA patients into “low” or “high” subsets, each requiring unique approaches for treatment.

BASELINE PREDICTION OF THE RESPONSE TO THERAPY BASED ON THE LEVEL OF PRO-INFLAMMATORY GENE EXPRESSION

Treatment strategies in RA primarily involve disease-modifying anti-rheumatic drugs (DMARDs) and biological agents^[47]. As these drugs are capable of alleviating symptoms and slowing the disease progression, a good response to treatment in RA is suggestive of remission, which is associated with a decrease in inflammation and pain^[48]. However, modern anti-rheumatic drugs are not efficient in all patients, as only approximately 30% of patients attain remission in response to methotrexate (MTX) treatment^[49,50]. TNF α -blocking agents in combination with MTX were effective in 60-80% RA patients^[51-53] while rituximab, a chimeric human monoclonal antibody against the B cell marker CD20, which depletes CD20-positive B cells, is effective only in 40-50% of RA subjects^[54,55]. Hypothetically, a good response to RA treatment should restore normal cellular metabolism, which might be associated with the recovery of gene expression to levels comparable to healthy controls^[56]. Therefore, the identification of anti-rheumatic drug responders at baseline is important for the selection of adequate treatment.

Methotrexate

Methotrexate (MTX) is considered the most conventional disease-modifying anti-rheumatic drug for RA, with the best efficacy and fewest adverse effects^[57,58]. Although the mode of action of MTX in RA remains unclear, it is established that MTX primarily interferes with folate homeostasis^[59,60]. In view of this observation, it has been shown that inflammatory conditions in MTX-naïve RA patients that were associated with the up-regulation of folate metabolism gene expression in the peripheral blood were restored to the level of healthy controls after MTX treatment^[61]. In addition, the subset of “high” mTOR RA patients, which exhibited high pro-inflammatory cytokine TNF α and IL6 gene expression at baseline, has demonstrated a better response to MTX therapy. Patients in the group demonstrated lower concentrations of RF, less number of swollen and tender joints, and a lower DAS 28 value compared with those in the “low” mTOR gene expression subset after 24 months of follow-up. This was accompanied by the downregulation of mTOR and TNF α gene expression^[46,62]. Moreover, a negative correlation between baseline mTOR and TNF α gene expression in the peripheral blood of these RA patients and joint inflammation markers measured after 24 months of follow-up further indicates the relationship between high level of expression of these genes and better MTX treatment results^[63].

Therefore, a better response to MTX treatment could be expected when pro-inflammatory TNF α , mTOR, and folate metabolism-related genes are upregulated at baseline in the peripheral blood of early RA patients.

Anti-TNF α blockade

Several studies reported that patients with higher levels of synovial inflammation and synovial TNF α expression respond better to TNF blockade^[64-63]. The up-regulation of inflammation-related gene

expression, such as IL2 receptor beta, SH2 domain 2A, and GOS2, in the peripheral blood of RA patients has also been shown to be predictive of good response to anti-TNF therapy^[67]. Moreover, high baseline gene expression of TNF α in patients whose serum C-reactive protein (CRP) decreased to the levels observed in normal subjects after treatment has been shown to be a useful marker of infliximab treatment efficacy^[68]. In addition, the increased expression of pro-inflammatory genes in responders normalized faster than in non-responders^[69]. This might be associated with the downregulation of various immune-related pathways, including inflammation in the course of anti-TNF treatment^[70,71]. Therefore, a high baseline level of TNF α gene expression might help identify anti-TNF α therapy responders.

The level of type I IFN bioactivity has also been shown to be associated with the clinical response to TNF α blockade in RA patients although, these results were not always consistent^[36,72,73]. Some studies demonstrated that relatively high plasma levels of type I IFN activity prior to the initiation of therapy were associated with a better clinical response to TNF-antagonists^[35,73]. This may be related to the fact that increased TNF α expression is associated with an overall higher level of inflammatory activity in patients with “high” IFN signature compared with “low” IFN signature patients. In addition, the better response to anti-TNF α treatment in patients with “high” IFN activity may involve anti-inflammatory effects of high levels of IFN β .

At the same time patients with a “low” baseline IFN signature, which did not respond to anti-TNF blockade, showed an increase in type I interferon response gene expression in the course of treatment^[35,72], indicating that the neutralization of TNF α in these patients favors the up-regulation of genes that were previously silenced by TNF α ^[74,75]. However, the up-regulation of IFN bioactivity was also suggested to be deleterious in RA or represented a failed attempt to counter-regulate inflammation^[35].

Therefore, the baseline up-regulation of TNF α and type I IFN-related gene expression in the peripheral blood and/or synovium might suggest a better response to anti-TNF α treatment in RA patients.

Anti-IL6 treatment

Anti-IL6 treatment is capable of decreasing the expression of numerous chemokine and T cell activation genes in the synovium^[76]. IL6-blocking therapy in RA was efficient when type I IFN response gene expression was increased in the peripheral blood mononuclear cells^[77], although type I IFNs have been reported to enhance IL-6 signaling by providing docking sites for STAT1 and STAT3 on the phosphorylated IFN α receptor 1 (IFNAR1) in close proximity of the gp130 chain of the IL-6 receptor^[37,78]. As both anti-IL6 and anti-TNF α blocking therapies appeared to be more efficient when IFN activity is increased, it was suggested that molecular and cellular mechanisms underlying the therapeutic effects of IL6 and TNF α antagonists share a similar pathway in the pathophysiology of RA^[79,80].

Therefore, the baseline up-regulation of type I IFN-response genes in the peripheral blood suggests a better response to anti-IL6 treatment in RA patients.

Anti-B cell treatment

A good response to anti-B cell treatment by rituximab (RTX) was observed if genes involved in inflammation, primarily nuclear factor kappa B (NF κ B) - and TGF β -signaling were upregulated and IFN-response genes were downregulated at treatment onset^[13,81-84]. In contrast, activated baseline type I IFN-response gene expression is a

predictive biomarker for a lack of response to RTX. The response to RTX was also associated with the downregulation of gene expression related to cell proliferation and growth, the regulation of cell cycle progression, apoptosis, autophagy, inflammation, genes associated with bone and articular cartilage turnover, and urea cycle as well as with the up-regulation of innate immune response-related genes^[85,86].

Therefore, the baseline downregulation of type I IFN-response genes and the up-regulation of pro-inflammatory mediator gene expression in the peripheral blood suggests a better response to anti-B cell therapy in RA patients.

GENE EXPRESSION SIGNATURES AND JOINT DESTRUCTION

Joint destruction is a major problem in RA, as RA patients exhibit radiographic progression despite the fact that clinically, they are in a state of low disease activity^[87,88]. Moreover, dissimilar gene clusters and distinct molecular signatures specifically expressed during early or long-standing RA suggest the involvement of different pathophysiological mechanisms in the disease course as a function of disease progression^[89]. In view of this, studies of early RA patient synovial tissue demonstrated higher levels of TNF α -related gene expression, while in long-standing RA patients, higher levels of expression of IFN-response genes correlated with the downregulation of protein biosynthesis and metalloproteinase inhibitor gene expression^[89,90].

Blood-based gene expression examination may also be useful for joint destruction assessment, as a correlation of gene expression at the diseased sites and in the peripheral blood for matched subjects was demonstrated^[30,91]. Moreover, the increase in erosion numbers after 24 months of MTX treatment in seropositive early RA patients was accompanied by the up-regulation of MMP-9 and cathepsin K gene expression in their peripheral blood^[56]. These gene counterparts in the joint are involved in bone and articular cartilage degradation in RA^[92-94]. In contrast, the treatment of RA patients with RTX, which did not augment erosion numbers or joint space narrowing indices, was associated with a decrease in MMP-9 and cathepsin K gene expression in the peripheral blood^[85].

In addition, gene expression examination in the peripheral blood indicated that radiographic severity monitored by erosion assessment in RA patients was associated with the up-regulation of IFN- and TGF β -signaling and apoptosis activity and the downregulation of oxidative phosphorylation and mitochondrial function both at baseline and after three years of disease^[95]. Another study identified a set of 14 genes involving pro-inflammatory and growth arrest-related genes, which were up-regulated in the peripheral blood, that predict severity of the disease^[96]. Moreover, the majority of long-standing RA patients undergoing joint replacement exhibited “high” mTOR and proinflammatory cytokine gene expression signatures compared to healthy controls^[46].

Therefore, the monitoring of gene expression associated with inflammation and bone and articular cartilage degradation in the peripheral blood could identify RA patients more predisposed to joint destruction.

CONCLUSIONS

The findings presented here indicate that high TNF α -related gene expression in the peripheral blood is a prerequisite of a good response of RA patients to MTX and biological therapy. In this regard, the combined up-regulation of TNF α - and type I IFN-response gene

expression would indicate good responders to anti-TNF α blockade. In contrast, RA patients with high TNF α and low type I IFN-response gene expression would better respond to anti-B cell therapy. The up-regulation of type I IFN-response genes denotes a positive response to tocilizumab, while high TNF α , mTOR, and folate metabolism gene expression would indicate good responders to methotrexate therapy. RA patients with lower levels of TNF α expression, who also have the expression of other genes at the level of healthy subjects, should be stratified into a different subset requiring specific therapy, which might involve targets other than pro-inflammatory cytokine signaling pathways. Further detailed studies on gene expression signature alterations in the course of the disease might provide opportunities for the identification of new targets for therapeutic intervention in RA, which could slow the disease progression and reduce symptoms.

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CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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Peer reviewer: Huma Jawed, Ph.D, Pharmacology Unit, 1H.E.J Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.