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The Activity of JAK/STAT and NF-κB in Patients with Rheumatoid Arthritis*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Research is still being conducted in order to determine the mechanisms responsible for the initiation of rheumatoid arthritis (RA) as well as for its persistence and progression.
Objectives. The aim of this work was to establish the expression of the signal transducer and activator of transcription (STAT) transcription factors and the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) transcription factor in peripheral blood leukocytes and synovial fluid cells. The correlations between the activation level of the transcription factors and the activity of the disease were also analyzed.
Material and Methods. In total, the study included 34 RA patients and 19 healthy individuals as controls. The expression of NFκB, STAT1, STAT3, STAT4, STAT5 and STAT6 in peripheral blood leukocytes and synovial fluid cells was established. The immunocytochemistry method was used to determine the degree of activation of STAT and NF-κB transcription factors. For the location of the factors, primary polyclonal anti-STATs and monoclonal anti-NF-κB antibodies were used.
Results. The expression of STAT1, STAT3, STAT4, STAT5, STAT6 and NFκB was significantly higher in the group of RA patients than in the controls. No statistically significant differences were found between the expression of STATs in peripheral blood leukocytes and synovial fluid cells.
Conclusions. In comparison with the control group, the expression of the STAT and NFκB transcription factors in RA patients was higher, which may be helpful in better understanding the etiopathogenesis of the disease in the future, and may potentially have important therapeutic implications (**Adv Clin Exp Med 2016, 25, 4, 709–717**).

Key words: rheumatoid arthritis, STAT expression, NFκB expression.

Rheumatoid arthritis (RA) is a chronic autoimmune-based inflammatory disease that leads to progressive joint degeneration, disability, and increased risk of cardiovascular complications, which constitutes the main cause of mortality among RA patients [1]. The etiopathogenesis of RA is multifactorial and not fully known, which is characteristic of most autoimmune diseases. The autoimmune process underlying the disease leads to the dysregulation of cytokine synthesis, disturbances in the migration of immunocompetent cells and abnormal apoptosis. To date, the mechanisms through which inflammatory mediators, especially cytokines, influence effector cells have not been fully researched. One of the transduction pathways that researchers have found enabling this

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influence is the Janus tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) pathway. So far, seven different homologous proteins of the STAT family have been identified in mammalian cells: STAT1, -2, -3, -4, -5A, -5B and -6 [2]. A single STAT type can be activated by several different ligands, but cytokines usually preferentially utilize a particular transduction pathway, e.g., INFγ usually selects STAT1 [2]. When a cytokine is bound to a cell membrane receptor, the associated JAK tyrosine kinase is activated and the process of phosphorylation takes place; the molecules bind with subunits of cytoplasmic STAT proteins, which undergo dimerization. This complex is then translocated into the nucleus *via* importin. Once inside the nucleus, STAT is released and binds with a DNA fragment responsible for the expression of a particular arrangement of genes [3]. Previous studies have investigated the deregulation of the JAK/STAT pathway in RA. An increase in the expression of the STAT1, -3, -4 and -6 proteins was found during active RA synovitis [2]. Up-regulated levels of STAT3 mRNA in mononuclear cells from peripheral blood and synovial fluid, and elevated STAT1 expression in synovial fluid have been observed in active RA [4]. STAT3 has been found to be responsible for joint degradation in RA [5]. The effectiveness of treatment with tocilizumab (which influences STAT3 activation through IL-6) and tofacitinib (a JAK1 and JAK3 inhibitor) constitutes indirect evidence of the influence of JAK/STAT signaling on RA activity [6]. Decreased synovial STAT activity as a result of tofacitinib treatment correlates with disease activity and results in significant DAS28 change [6].

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is one of many transcription factors that can lead to numerous autoimmune diseases, including arthritis, when their activity is abnormally regulated [7]. NF-κB regulates the activity of many genes coding cytokines (including IL-1, IL-2, IL-6, IL-8, IL-12 and TNFa), monocyte chemotactic protein factor (MCP)-1, adhesion molecules, acute-phase proteins and lymphotoxin α and β.

Taking into consideration previous research of the JAK/STAT pathway and the NFκB transcription factor, as well as their role in the pathogenesis of RA, the present study aimed at assessing the activity of individual proteins in the STAT and NFκB systems in the peripheral blood leukocytes and mononuclear synovial membrane cells of RA patients. The correlation between the expression of the studied parameters and clinical disease activity scores was assessed as well. The study also focused on the extent to which disease modifying antirheumatic drugs and the presence of unfavorable prognostic factors influence the activity of the STAT proteins.

Material and Methods

The study included 34 Caucasian patients aged 51 ± 16 years (range: 19–76 years) meeting the 2010 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria for rheumatoid arthritis, and 19 healthy volunteers aged 40 ± 10 years (range: 28–62 years) as the control group. There were no statistically significant differences between the analyzed populations in terms of sex and age. Four patients were lost from follow-up and were excluded from the final analysis.

Exclusion criteria were the following: pregnancy or breastfeeding; coexistence of other systemic connective tissue diseases besides RA; clinically significant impairment of hepatic or renal function; alcohol abuse; infection with hepatotropic viruses; infections resistant to therapy; ongoing history of cancer if no cure was achieved; uncontrolled diabetes; unwillingness or inability to cooperate.

All the participants provided their written informed consent. The study was approved by the Wroclaw Medical University Ethics Committee.

Clinical Evaluation of RA Patients

The clinical evaluation was based on medical history, the number of painful and swollen joints, pain intensity as assessed by the patients on a 100-mm visual analogue scale (VAS), and laboratory tests, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) concentration, blood cell count, aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, serum creatinine and urea levels, and urinalysis.

The RA patients were classified into two groups based on Disease Activity Score 28 (DAS28) values: Patients with high disease activity (DAS28 > 5.1) and patients with low to moderate disease activity (DAS28 ≤ 5.1).

NF-κB, STAT1, STAT3, STAT4, STAT5 and STAT6 Expression

The expression of NF-κB, STAT1, STAT3, STAT4, STAT5 and STAT6 in peripheral blood leukocytes and synovial fluid cells was determined. The degree of STAT and NF-κB transcription factor activation was determined using immunocyto-

chemistry. In order to determine the expression of the JAK/STAT system proteins, the patients' peripheral blood samples and synovial fluid samples were collected into sodium heparin coagulant and then centrifuged on a density gradient with LSM 1077 Lymphocyte Separation Medium (Cytogen, Wetzlar, Germany) to isolate leukocytes. The obtained cells were placed on 1 mm ground-edge frosted Superfrost Plus slides (Thermo Fisher Scientific Gerhard Menzel B.V. & Co., Braunschweig, Germany) with the use of a cytospin cytocentrifuge (Thermo Shandon, USA. The cells were fixed at room temperature using 4% paraformaldehyde solution (POCh, Gliwice, Poland). Incubating the slides in 3% hydrogen peroxide solution in methanol (POCh, Gliwice, Poland) blocked the endogenous peroxidase activity. The preparations were treated with a blocking serum (from the Novostain Super ABC Universal Kit, Novocastra Laboratories Ltd., Newcastle, UK). The examined cells were initially incubated with primary antibodies specific to STAT and NF-κB transcription factors: Polyclonal rabbit anti-STATs IgG antibody and monoclonal mouse anti-NF-κB p65 subunit antibodies (Chemicon International Inc., Temecula, CA, USA) at a dilution of 1:100 of a stock solution. A negative control was applied during staining by using phosphate buffered saline (PBS) instead of the primary antibody. After washing, the cells were treated with a biotinylated secondary antibody that recognized rabbit or mouse IgG (also from the Novostain Super ABC Universal Kit). The preparations were subsequently treated with peroxidase-conjugated avidin (again from the Novostain Super ABC Universal Kit). Chromogen fast diaminobenzidine (Liquid DAB Substrate Kit for Peroxidase, Novocastra Laboratories Ltd., Newcastle, UK) was used for STAT and NF-kB staining. For improved contrast and cell structure staining, the samples were additionally stained with hematoxylin (Vector Laboratories Inc., Burlingame, CA, USA). A negative control was applied during staining by using PBS instead of the primary antibody. STAT and NF-kB factor activation were evaluated using a Nikon type 120 microscope (Nikon, Japan) with a video channel and appropriate computer software. The percentage of cells with stained nuclei reflected the degree of activation of the factors being studied (100 cells were counted on each slide in three independent observations).

The expression of NF-κB, STAT1, STAT3, STAT4, STAT5 and STAT6 was analyzed in RA patients, taking into account the presence of rheumatoid factor (RF), anti-CCP antibodies, and bone erosions. The influence of disease-modifying antirheumatic drugs (DMARDs) and glucocorticosteroids (GCs) on NF-κB and STAT expression was also assessed. The correlation between disease activity and NF-κB and STAT expression was analyzed as well.

Statistical Analysis

The normality of distribution was tested using the Kolmogorov–Smirnov test. Independent quantitative variables meeting the requirements for normal distribution were compared using Student's *t*-test. To assess correlations between the analyzed parameters, Spearman's correlation coefficient (*r*) was calculated. Results were considered statistically significant when $p < 0.05$. All tests were performed using STATISTICA v. 10 software (StatSoft, Tulsa, USA).

Results

The demographic and clinical characteristics of the RA group are presented in Table 1.

Most of the RA patients were being treated with methotrexate (67%), while 10% were taking leflunomide, 7% were being treated with sulphasalazin and 16% were not being treated with any DMARDs. Additionally, 62% of the RA patients were being treated with a stable dose of glucocorticoids (GCs) (5–15 mg prednisone daily).

Table 1. Demographic and clinical characteristics of RA patients

	RA patients (n = 30)
Age [years][a]	51 ± 16 (19–76)
Women[b]	23 (77)
RA duration [months][a]	9.8 ± 24.4 (1–134)
RF (+)[b]	22 (73%)
anti CCP (+)[b]	20 (66%)
ESR [mm/h][a]	36 ± 27 (4–107)
CRP [mg/dL][a]	34 ± 47 (0.3–226)
DAS28[a]	4.7 ± 1.6 (1.8–7.1)
Disease activity	
low (DAS28 < 3.2)[b]	5 (17%)
moderate (DAS28 > 3.2 < 5.1)[b]	10 (33%)
high (DAS28 > 5.1)[b]	15 (50%)

[a] Data are presented as mean ± standard deviation (range); [b] Data are presented as number of patients (%); anti-CCP – anti-cyclic citrullinated peptide antibodies; CRP – C reactive protein; DAS28 – disease activity score; ESR – erythrocyte sedimentation rate; n – number of patients; RA – rheumatoid arthritis; RF – rheumatoid factor.

Table 2. Mean NFκB and STAT expression in peripheral leukocytes in RA patients and the control group. The percentage of cells with stained nuclei reflected the degree of activation of the studied factors

	Control group (n = 19)	RA group (n = 30)	p-value
NFκB	5.0 ± 3.4	15.4 ± 9.9	0.0001
STAT1	3.9 ± 3.3	14.5 ± 9.7	0.0001
STAT3	4.5 ± 2.8	14.5 ± 8.4	0.0001
STAT4	3.3 ± 2.0	11.5 ± 10.3	0.001
STAT5	4.6 ± 3.5	11.1 ± 6.9	0.0004
STAT6	4.4 ± 3.1	10.8 ± 9.2	0.004

Data are presented as mean ± standard deviation; n – number of participants; NFκB – nuclear factor κB; STAT – signal transducer and activator of transcription.

The expression of NFκB and the analyzed STATs in peripheral leukocytes was significantly higher in the RA group than in the control group (Table 2).

The expression of STATs and NFκB in peripheral leukocytes and synovial fluid cells was determined in 19 RA patients. There was no significant difference in the expression of NFκB and STATs in peripheral leukocytes and synovial fluid cells in RA patients compared to the control group (Table 3).

The correlations between individual STATs and NF-κB transcription factors in patients with RA were analyzed. Statistically significant positive correlations were found between most of the analyzed transcription factors (Table 4). In the control group, significant positive correlations were detected only between NFκB and STAT5, and between STAT1 and STAT3.

When analyzing the correlation between STAT expression and age, RA duration, body mass and BMI, only a negative correlation between STAT3 and the age of the RA patients was found.

The group of RA patients with erosions visible in radiological imaging (11 patients) was also compared with the group of RA patients without erosions (19 patients). No statistically significant differences were found in the expression of the studied parameters. Moreover, no statistically significant differences were found in the expression of STATs and NFκB between RA patients with increased and normal rheumatoid factor titers and between patients with increased and normal anticyclic citrullinated peptide antibody titers.

The correlations between inflammatory parameters (CRP and ESR) and the expression of transcription factors were also analyzed. No correlation between CRP and NFκB or STATs was found. Negative correlations in RA patients were found between ESR and STAT1 as well as ESR and STAT4 ($r = -0.56$, $p = 0.002$ and $r = -0.47$, $p = 0.011$, respectively; Fig. 1).

The influence of disease activity on the expression of NFκB and STATs in peripheral leukocytes was also analyzed. Increased expression of STAT1 and STAT4 in RA patients with low to moderate disease activity was found (Table 5). Treatment with methotrexate (MTX) or other DMARDs was not found to have any influence on NFκB and STAT expression.

When comparing RA patients receiving GCs

Table 3. Mean NFκB and STAT expression in peripheral leukocytes and synovial fluid cells in RA patients. The percentage of cells with stained nuclei reflected the degree of activation of the studied factors

	Synovial fluid cells (n = 18)	Peripheral leucocytes (n = 30)	p-value
NFκB	12.1 ± 9.8	15.4 ± 9.9	ns.
STAT1	12.3 ± 3.2	14.5 ± 9.7	ns.
STAT3	14.8 ± 8.4	14.5 ± 8.4	ns.
STAT4	9.1 ± 8.3	11.5 ± 10.3	ns.
STAT5	8.8 ± 5.3	11.1 ± 6.9	ns.
STAT6	7.5 ± 5.5	10.9 ± 9.2	ns.

Data are presented as mean ± standard deviation; n – number of participants; NFκB – nuclear factor κB; ns. – not significant; STAT – signal transducer and activator of transcription.

Table 4. Spearman's correlations between NFkB and STAT expression in peripheral leukocytes in RA patients

	NFkB	STAT1	STAT 3	STAT4	STAT 5	STAT 6
NFkB		0.5226	0.6636	0.4042	0.1271	0.4297
		p = 0.002	p = 0.000	p = 0.018	p = 0.474	p = 0.011
STAT1	0.5226		0.6389	0.5475	0.3781	0.4777
	p = 0.002		p = 0.000	p = 0.001	p = 0.027	p = 0.004
STAT 3	0.6636	0.6389		0.6790	0.3481	0.4263
	p = 0.000	p = 0.000		p = 0.000	p = 0.044	p = 0.012
STAT4	0.4042	0.5475	0.6790		0.2299	0.7178
	p = 0.018	p = 0.001	p = 0.000		p = 0.191	p = 0.000
STAT 5	0.1271	0.3781	0.3481	0.2299		0.2748
	p = 0.474	p = 0.027	p = 0.044	p = 0.191		p = 0.116
STAT 6	0.4297	0.4777	0.4263	0.7178	0.2748	
	p = 0.011	p = 0.004	p = 0.012	p = 0.000	p = 0.116	

NFκB – nuclear factor κB; STAT – signal transducer and activator of transcription.

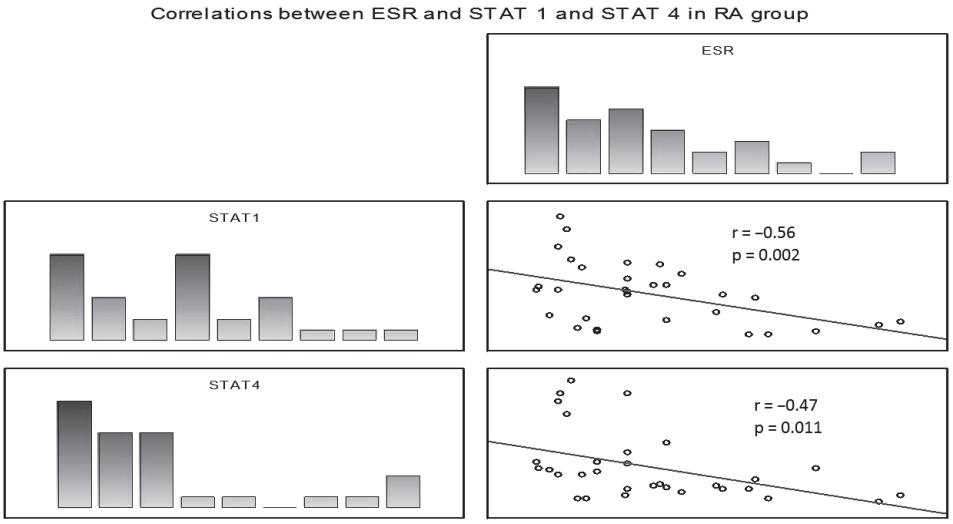

Fig. 1. Correlations between ESR and STAT1 and between ESR and STAT4 in the RA group

Table 5. Influence of disease activity on the expression of NFκB and STATs in peripheral leukocytes. The percentage of cells with stained nuclei reflected the degree of activation of the studied factors

	RA patients with high disease activity (n = 15)	RA patients with low to moderate disease activity (n = 15)	p-value
NFκB	15.2 ± 10.1	17.1 ± 10.3	ns.
STAT1	9.9 ± 7.4	17.7 ± 10.1	0.02
STAT3	13.7 ± 5.3	17.2 ± 10.6	ns.
STAT4	7.6 ± 5.2	15.9 ± 13.2	0.03
STAT5	10.9 ± 5.8	12.8 ± 7.5	ns.
STAT6	8.5 ± 4.0	13.9 ±12.7	ns.

Data are presented as mean ± standard deviation; n – number of participants; NFκB – nuclear factor κB; ns. – not significant; RA – rheumatoid arthritis; STAT – signal transducer and activator of transcription.

with patients who were not treated with GCs, lower STAT1 and STAT5 expression was found in synovial fluid cells obtained from the RA patients treated with GCs (8.37 ± 7.84 vs. 20.17 ± 11.67, p = 0.0208, and 6.77 ± 4.30 vs. 12.93 ± 4.85, p = = 0.0141, respectively). In the RA patients treated with GCs, STAT1 expression was also lower in peripheral leukocytes (10.32 ± 7.56 vs. 20.01 ± 10.09, p = 0.0032). There was no significant difference in disease activity between these subgroups.

Discussion

Cytokines and growth factors regulate numerous intracellular processes, including gene expression, activation processes, proliferation and cell differentiation. At the cellular level they utilize, among other things, the Janus-activated kinase/ /signal transducers and activators of transcription transduction pathway. The JAK-STAT pathway is the signaling target of a multitude of cytokines, including IFN, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17A, IL-19, IL-20, IL-21, IL-23 and IL-27, which are thought to have biologically important roles in rheumatoid synovial inflammation [8, 9]. The JAK tyrosine kinases are activated by cytokines though membrane receptors and take part in the phosphorylation of cytoplasmic receptor domains, through which latent cytoplasmic STAT proteins are activated. These proteins bind to DNA fragments which are the promoters of genes controlled by a given JAK/STAT system and activate transcription processes [2]. For instance, one of the main cytokines participating in RA etiopathogenesis, IL-6, activates the JAK/STAT pathway through JAK-1, JAK-2 and TYK-2 kinases. Janus kinases are constitutively associated with gp130, and their activation leads to the tyrosine phosphorylation of specific motifs within the intracellular domain of gp130. IL-6 activates gene transcription mainly *via* the STAT1 and STAT3 proteins [10]. The research conducted to date confirms the vital role of the JAK/STAT pathway in RA pathogenesis [9]. An increase in the expression of the STAT1, -3, -4 and -6 proteins was revealed during active synovial membrane inflammation in the course of RA [2]. It is, however, necessary to emphasize the heterogeneous image of RA. Its various subtypes may exhibit different STAT protein expression. Based on the analysis of the RNA separated from the synovial cells acquired from rheumatoid arthritis patients, two distinct subgroups of patients were found by Kraan et al. [11]. The first subgroup was characterized by high expression of inflammatory genes and STAT1 activation, while the second group exhibited insignificant STAT1 activation and tissue remodeling instead of inflammation [11].

The role of individual proteins in the pathogenesis of the disease varies. STAT1 seems to have a dual nature. Knowledge based on different models of arthritis indicates its predominant anti-inflammatory activity, resulting from its anti-proliferative and proapoptotic activity [12]. Research results suggest that IFNγ is the strongest STAT1 activator, at the same time pointing to significant STAT1 activation in the synovial membrane cells of patients with advanced RA [9]. The available research results also suggest that STAT1 activates the expression of the genes coding proinflammatory proteins in some RA patients [13]. The proinflammatory nature of STAT3 in RA pathogenesis is supported by its anti-apoptotic activity profile and the stimulation of the growth of synoviocytes, T cells, and antibody synthesis [14, 15]. Mori et al. showed that STAT3 is the key mediator of both chronic inflammation and joint degradation in RA. They found that the pro-inflammatory cytokines that are highly expressed in RA patients, such as IL-1β, tumor necrosis factor alpha and IL-6, activated STAT3 either directly or indirectly and, in turn, induced the expression of cytokines from the IL-6 family. STAT3 activation also induced expression of the receptor activator of nuclear factor kappa B ligand (RANKL), a cytokine essential for osteoclastogenesis [5].

The present study also demonstrated a statistically significant increase in the expression of STAT1 and STAT3 in peripheral blood leukocytes and synovial fluid cells in RA patients as compared to the controls. This is in line with the results achieved by other authors [4, 16]. Isomaki et al. [4] showed that STAT1 expression was elevated in synovial fluid monocytes in patients with RA. The levels of phospho-STAT3 in resting peripheral blood T cells and monocytes were significantly higher in patients with RA than in healthy volunteers. IL-6 levels were increased in RA plasma and correlated with the level of STAT3 phosphorylation in $CD4^+$ T cells and monocytes. The results suggest that IL-6 induces STAT3 hyperactivation in circulating immune cells in active RA [4].

Like other research, the present study demonstrated an increase in the expression of the STAT4 protein in RA patients [17]. The polymorphism of individual nucleotides for the STAT4 gene may also be associated with susceptibility to RA [18]. Investigation of the role of transcription factor STAT4 in the collagen-induced arthritis (CIA) model showed that STAT4–/– mice were less susceptible to CIA than wild type (WT) mice. Remarkably, the less severe form of arthritis in STAT4–/– mice was associated with decreased

production of IFN-γ in the early stages of the disease, and of TNF-a, IL-6 and IL-17 in the late stages of the disease [19].

Increased expression of STAT4 and -6 proteins in dendritic cells in the synovial membrane may correlate with the presence of the rheumatoid factor in blood serum, which is an unfavorable prognostic factor in the course of RA [17]. In the present study, no correlation was found between the expression of the STAT proteins with the rheumatoid factor or anti-cyclic citrullinated peptide antibodies. However, STAT protein expression was assessed in synovial fluid cells in only a small group of patients, so conclusions cannot be drawn.

Several studies have revealed a correlation between STAT expression and disease activity [8, 12, 16]. These results were mainly associated with STAT expression in synovial membrane cells. The present study revealed increased STAT1 and STAT4 expression in patients with low or moderate disease activity, but due to the small number of individuals studied, the results are of limited value. The authors wish to emphasize the importance of comparing the expression of STATs with disease activity in synovial membrane cells. Peripheral markers of inflammatory disease may not correlate with local pathology and can be an inadequate predictor of disease severity or local joint pathology [13, 20]. Moreover, other diseases and administered drugs may influence the expression of the STAT proteins. Increased STAT protein expression has been described in patients with diagnosed *Helicobacter pylori* infection, as well as STAT4 phosphorylation inhibition by statins [19]. In the current study the patients were not tested for *Helicobacter pylori* infection, but four patients in the group with high RA disease activity and one patient with low disease activity were being treated with statins.

The present study did not show any differences between patients treated with different DMARDs. However, it is not possible to draw any final conclusions on this basis, as the majority of patients were treated with MTX, while the remaining group was not large enough to give conclusive results. Other studies have likewise not demonstrated any correlation between STAT activation and individual DMARDs [16]. It would be interesting to establish the expression of individual STATs before and after treatment using specific classic DMARDs or biologic drugs not only in peripheral blood, but also in synovial fluid and synovial membrane cells. In some studies effective modifying treatment, resulting in decreased disease activity, has been associated with a significant reduction of STAT1, -4 and -6 protein expression in synovial membrane cells [17]. Synovial biopsy specimens were obtained from the same knee joint before DMARD treatment and at six-month intervals after its initiation [17]. The study included 16 patients (seven patients treated with intramuscular gold salts, three with methotrexate, two with sulfasalazine, three with MTX + intravenous gold salts, and one with cyclosporin A). The expression of STAT1 in rheumatoid arthritis synovial tissue was found to be significantly down-regulated after effective DMARD treatment; in contrast, there was no significant change in the overall expression of STAT4 or JAK-3 in rheumatoid arthritis synovial tissue in response to DMARD treatment; and there was no major difference in STAT6 lining expression [17]. Initial studies have already been published concerning the influence of biologic treatment on the expression of the STAT proteins. Adalimumab therapy increases Th2-associated STAT6 phosphorylation and restores activation-induced STAT4 phosphorylation to the levels found in healthy individuals [21].

Glucocorticoids (GCs) are still used in RA treatment. It is, however, emphasized that they should be used at the lowest effective doses and for the shortest period of time possible. In the current study about 2/3 of patients were being treated with GCs. No statistically significant differences in disease activity were found between the patients undergoing GC treatment and the remaining patients. In patients undergoing GC treatement the expression of STAT1 and STAT5 in synovial fluid cells as well as the expression of STAT1 in peripheral leukocytes were reduced. Steroids are anti-inflammatory medications that act through transcriptional or post-transcriptional gene regulation. Steroids have also been shown to inhibit STAT proliferation and differentiation, and the JAK/STAT pathways. GCs physically interact with STAT3, STAT5a, STAT5b and STAT6, and functionally synergize with them to promote STAT-responsive gene transcription. Steroids inhibit Th1 differentiation by blocking the IL-12/STAT4 pathway. This effect may in part be mediated through the suppression of cytokine signaling 1 (SOCS1). SOCS1 inhibits multiple JAK/STAT pathways, which may inhibit cell proliferation and differentiation. The functional interaction of SOCS1 with STAT1 is more complex. Bhattacharyya et al. showed that SOCS1 and type I interferons are critical GCs targets for regulating STAT1 activity and may account for the overall GCs effectiveness in inflammation suppression [22].

The NF-κB proteins are present in cell cytoplasm in an inactive form, combined with their inhibitor IκB. After cell activation, the IκB molecules undergo degradation under the influence of an appropriate kinase, which enables the translocation

of the NF-κB factor to the nucleus, its binding with an appropriate gene sequence, the activation of transcription and the production of pro-inflammatory cytokines [23]. In the current study, a statistically significant increase in NF-κB expression was demonstrated in both peripheral blood leukocytes and synovial fluid cells, as well as a positive correlation with the expression of the STAT proteins.

It is widely believed that influencing the JAK/ /STAT pathway may turn out to be a promising therapeutic target, especially in the case of multidirectional impact on different elements of the signaling cascade [24]. Selective blocking of a single kinase, e.g. JAK3 or a single STAT, will likely be a worse choice than less selective blocking. The currently available JAK1/-3 kinase inhibitor, tofacitinib, is registered by the US Food and Drug Administration (FDA) for the treatment of RA patients after ineffective treatment with classic DMARDs or biologic drugs [25]. Boyle at al. showed that changes in synovial phosphorylation of STAT1 and STAT3 strongly correlated with four-month clinical responses in RA patients treated with 10 mg of tofacitinib twice a day [26].

Migita et al. demonstrated that JAK-3-selective inhibition alone is insufficient to control STAT3-dependent signaling in rheumatoid synovial fibroblasts, and that inhibition of JAKs, including JAK1/-2, is needed to control the proinflammatory cascade in RA. The JAK3-selective inhibitor PF-956980 suppressed STAT1/-5 activation, but did not affect STAT3. The JAK inhibitors CP-690 550 and INCB028050 both suppressed the activation of JAK1/-2/-3 and downstream STAT1/-3/-5, as well as the expression levels of target proinflammatory genes (MCP-I, SAA1/2) in oncostatin-M (OSM)-stimulated rheumatoid synovial fibroblasts [24]. Novel protein kinase specific may be targeted as a dual protein kinase inhibitor, such as the newly described N1-p-fluorobenzyl-cymserine, which is reported to inhibit both p38 kinase and NF-κB [9].

Higher expression of the STATs and NF-κB was observed in the group of patients with RA in comparison with the control group, which corroborates earlier data. The strength of the present work is the assessment of a wide spectrum of transcription factors in one study. In the future, this may contribute to a better understanding of the etiopathogenesis of rheumatoid arthritis and may have important therapeutic implications. Some data, like the n influence of DMARDs and biologic treatment on STAT activity, still need further evaluation on a larger group of patients.

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