The activity of JAK-STAT pathways in rheumatoid arthritis: constitutive activation of STAT3 correlates with interleukin 6 levels

Pia Isomäki¹,², Ilkka Junttila¹,³, Krista-Liisa Vidqvist², Markku Korpela² and Olli Silvennoinen¹,⁴

Abstract

Objective. Many cytokines involved in RA activate the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways. Therapeutic drugs that inhibit these pathways are being developed for RA. To investigate disease-related alterations in the activity of JAK-STAT pathways in RA, we studied the expression and activation of STAT1 and STAT3 in unstimulated and cytokine-stimulated cells and determined the levels of circulating cytokines.

Methods. The expression of STAT1 and STAT3 mRNA in peripheral blood (PB) and SF T cells and monocytes was studied in RA patients and healthy volunteers by RT-PCR. Basal and cytokine (IFN-γ, IL-6, IL-10)-induced STAT phosphorylation was analysed in PB T cells and monocytes using multicolour flow cytometric analysis.

Results. STAT3 mRNA levels were up-regulated in both PB and SF T cells and monocytes from RA patients. STAT1 expression was elevated in SF monocytes. The levels of phospho-STAT3 in resting PB T cells and monocytes were significantly higher in patients with RA than in healthy volunteers. IL-6 levels were elevated in RA plasma and correlated with the level of STAT3 phosphorylation in CD4⁺ T cells and monocytes. IL-6-mediated STAT3 activation was deregulated in T cells from RA patients. IL-6-induced phosphorylation of STAT3 was decreased in CD4⁺ T cells from patients with high plasma IL-6 levels and constitutive STAT3 phosphorylation.

Conclusion. The results suggest that IL-6 induces hyperactivation of STAT3 in circulating immune cells in active RA, and this subsequently desensitizes the IL-6 response in T cells.

Key words: rheumatoid arthritis, cytokine, IL-6, signal transduction.

Rheumatology key messages

- Expression and phosphorylation of STAT3 are elevated in circulating T cells and monocytes in RA.
- Baseline activation of STAT3 in RA correlates with IL-6 levels.
- A proportion of RA T cells are hyporesponsive to IL-6-mediated STAT3 activation in vitro.

Introduction

T cells, B cells and macrophages contribute to the pathogenesis of RA, in part by secreting cytokines [1, 2]. Many cytokines that are expressed in rheumatoid joints, such as TNF-α, IFN-γ, GM-CSF, IL-1, IL-6 and IL-17 function as pro-inflammatory mediators [1]. In addition, significant quantities of anti-inflammatory cytokines such as IL-10 are present in the joints [3]. The significance of cytokines...
in the pathogenesis of RA has been confirmed by the successful use of biologic agents that suppress the actions of TNF, IL-1 and IL-6 in RA [1, 4].

In addition to the level of expression, the biological effects of cytokines depend on the activation of signal transduction pathways. Although cytokine expression in RA has been extensively studied, there are a limited number of studies investigating the intracellular signals induced by cytokines in patients with RA. Several cytokines that regulate immune responses in RA, such as IFN-γ, GM-CSF, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15 and IL-21, activate Janus kinases (JAKs) and signal transducer and activator of transcription (STATs) transcription factors [5]. The binding of cytokine to its receptor leads to the tyrosine phosphorylation of associated JAKs and to the recruitment, phosphorylation and dimerization of STATs. Finally, STAT dimers translocate to the nucleus, where they regulate the transcription of the target genes. The activity of the JAK-STAT pathway is negatively regulated by suppressors of cytokine signalling (SOCS) proteins [6-8]. Our previous studies have demonstrated up-regulation of SOCS protein expression in peripheral blood (PB) T cells and monocytes from patients with active RA [9]. This could lead to unexpected effects of both pro- and anti-inflammatory cytokines in RA.

Although the currently available anti-cytokine agents provide significant clinical benefit to many patients, not all patients respond to these drugs. In addition, biologic drugs have to be administered parenterally and are expensive to produce. Therefore small molecular weight inhibitors that target cytokine signalling pathways are currently being developed for the treatment of RA [10, 11]. A JAK inhibitor, tofacitinib, was recently approved for the treatment of RA in several countries. For developing other inhibitors of cytokine signalling, it will be important to identify which signalling pathways are perturbed in patients with RA.

To study the activity of JAK-STAT pathways in RA, we examined the expression and phosphorylation of STAT1 and STAT3 in unstimulated T cells and monocytes and their activation by cytokines IFN-γ, IL-6 and IL-10. These cytokines were chosen as they are implicated in RA pathogenesis and their responses are regulated by SOCS1 and SOCS3, which show increased expression in RA [9]. Our present results suggest systemic activation of the IL-6/STAT3 pathway in RA.

**Patients and methods**

**Patient samples**

Patients’ samples were collected from two cohorts of patients with active RA who met the 1987 ACR criteria for RA [12] (Table 1). The first cohort included 23 patients with active RA who generally had long-standing disease (with the exception of two patients who were diagnosed as having RA at the time of sample collection). PB and SF samples were concurrently collected from these patients. PB samples for phosphorylated STAT (pSTAT) analysis were collected from the second patient cohort, which included 15 patients with active RA as defined by a 28-joint DAS (DAS28) ≥ 3.2 (Table 1). This cohort included both newly diagnosed patients (n = 4) and patients with long-standing disease. PB samples from healthy blood donors (Finnish Red Cross Blood Transfusion Service, Tampere) were obtained as controls. Informed written consent was obtained from all patients according to the Declaration of Helsinki, and this study was approved by the Medical Ethics Committee of Tampere University Hospital.

**Cell preparations**

PB mononuclear cells (PBMCs) and SF mononuclear cells (SFMCs) were isolated by Ficoll-Paque Plus (Amersham Biosciences, Buckinghamshire, UK) density gradient centrifugation. T cells and monocytes were further purified from freshly isolated PBMCs and SFMCs using magnetic cell sorting technology (Milteny Biotec, Auburn, CA, USA). CD3+ T cells were purified by negative selection using a cocktail of hapten-conjugated antibodies specific for non-T cells and anti-hapten-coated microbeads (Pan T Cell Isolation Kit; Miltenyi Biotec). Monocytes were isolated by positive selection with anti-CD14-coated microbeads.

**RNA isolation and quantitative RT-PCR analysis**

Total RNA was isolated from the cells using the RNeasy MiniKit (Qiagen, Valencia, CA, USA). Total RNA (1 μg) was reverse transcribed using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and random hexamers (Amersham Biosciences) as a primer.

The forward and reverse primers recognizing separate exons of the STAT and TBP genes were designed using Primer3 software (available at http://primer3.sourceforge.net). The 15 μl real-time PCR reactions were performed in the LightCycler apparatus (Roche Diagnostics, Mannheim, Germany) using the QuantiTect SYBR Green PCR kit (Qiagen) as described [9]. Mean STAT expression values were normalized by dividing them by the mean values obtained for the TBP housekeeping gene.

**Cytokine stimulation and flow cytometric analysis**

To study phosphorylation of STAT proteins, four-colour staining and flow cytometric analysis were performed. First, 100 μl aliquots of fresh blood samples were either left unstimulated or were stimulated by 100 ng/ml recombinant human IFN-γ (PeproTech, London, UK), IL-6 (PeproTech) or IL-10 (R&D Systems, Wiesbaden, Germany) for various time points at 37°C. The activation was terminated by transferring the tubes to ice. The samples were stained with FITC-conjugated anti-CD3 (eBioscience, San Jose, CA, USA) and allophycocyanin (APC)-conjugated anti-CD14 (Miltenyi Biotec) for 20 min on ice. The samples were then incubated in BD Phosflow Lyse/Fix buffer for 15 min at 37°C, washed and permeabilized in ice-cold methanol overnight at −20°C. Following two washes, the samples were stained with
PerCP-Cy5.5-conjugated anti-CD4 and with PE-conjugated anti-phospho-STAT1, anti-phospho-STAT3 or mouse IgG2a isotype control (all from BD Biosciences, San Jose, CA, USA) for 30 min at room temperature. To study IL-6 receptor α (IL-6Rα) chain and gp130 expression, 0.2 x 10^6 cells were stained with FITC-conjugated anti-CD3, PE-conjugated anti-IL-6Rα, anti-gp130 or isotype control (BD Biosciences), PerCP-Cy5.5-conjugated anti-CD4 and APC-conjugated anti-CD14 for 30 min on ice, followed by two washes. The analysis of flow cytometer data was performed using Cytobank software (http://www.cytobank.org/). The CD4+ and CD4⁺/CD0 T cells were gated from the CD3+ lymphocyte population, and CD14+ cells represent monocytes. The results are expressed either as mean fluorescence intensity (MFI) of pSTAT staining or the percentage of pSTAT-positive cells. For the latter analysis, the gating for pSTAT-positive cells was set on the basis of staining with the isotype control. Basal STAT phosphorylation and IL-6R expression are expressed as the MFI ratio (MFIR; MFI of the staining divided by MFI of the isotype control staining).

Cytokine ELISA

Cytokine levels were simultaneously measured using the MILLIPLEX MAP high sensitivity human cytokine panel with magnetic beads (Millipore, Billerica, MA, USA) in plasma samples derived from the second patient cohort. A blocking agent to prevent false positives from RF is present in this assay.

Statistical analysis

Statistical analysis was performed using a Mann-Whitney U-test. Correlations were calculated using Spearman’s rank correlation method.

Results

Increased expression of STAT3 in PB and SF T cells and monocytes and STAT1 in SF monocytes from RA patients

First we examined the levels of STAT1 and STAT3 mRNA in PBMCs as well as in purified PB and SF T cells and monocytes derived from patients with active RA (Table 1, Patients for RT-PCR) and from healthy volunteers. The expression of STAT3 was elevated in PBMCs from RA patients compared with healthy controls, while the expression of STAT1 was unaltered in RA (Fig. 1, left panel). No significant correlation between the expression of STAT3 and the age of patients, disease duration, CRP level or ESR was observed (data not shown). In addition, the type of treatment used by the patients did not significantly influence STAT3 expression levels (supplementary Table S1, available at Rheumatology Online).

STAT3 levels were increased in both PB T cells and monocytes from RA patients when compared with healthy controls, while the expression of STAT1 was unaltered in RA (Fig. 1, left panel). No significant correlation between the expression of STAT3 and the age of patients, disease duration, CRP level or ESR was observed (data not shown). In addition, the type of treatment used by the patients did not significantly influence STAT3 expression levels (supplementary Table S1, available at Rheumatology Online).

STAT3 levels were increased in both PB T cells and monocytes from RA patients when compared with healthy controls (Fig. 1, middle and right panels). The expression of STAT3 was even higher in SF T cells and monocytes. In contrast to STAT3, STAT1 mRNA levels did not differ between RA patients and healthy controls in PB T cells and monocytes. However, STAT1 expression was increased in RA SF monocytes when compared with PB monocytes.

Increased pSTAT3 in PB T cells and monocytes and pSTAT1 in CD4⁺ T cells from patients with RA

Next we wanted to examine whether STAT1 and STAT3 are systemically activated in circulating immune cells in patients with active RA (Table 1, Patients for flow cytometry). To study this we set up a multicolour flow cytometric assay. Since the staining procedures were performed without cell purification or manipulation using

Table 1: Demographic and clinical characteristics of patients with RA

<table>
<thead>
<tr>
<th>Study group</th>
<th>Patients for RT-PCR (n = 23)</th>
<th>Patients for flow cytometry (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample obtained</td>
<td>PB/SF</td>
<td>PB</td>
</tr>
<tr>
<td>Female/male, n</td>
<td>12/11</td>
<td>10/5</td>
</tr>
<tr>
<td>Age, median (range), years</td>
<td>62 (32-86)</td>
<td>64 (35-88)</td>
</tr>
<tr>
<td>Disease duration, median (range), years</td>
<td>15 (0-49)</td>
<td>2 (0-25)</td>
</tr>
<tr>
<td>CRP, mean (s.d.), mg/l</td>
<td>33 (20)</td>
<td>19 (15)</td>
</tr>
<tr>
<td>ESR, mean (s.d.), mm/h</td>
<td>38 (21)</td>
<td>42 (24)</td>
</tr>
<tr>
<td>Number of tender joints, mean (s.d.)</td>
<td>ND</td>
<td>12 (11)</td>
</tr>
<tr>
<td>Number of swollen joints, mean (s.d.)</td>
<td>ND</td>
<td>8 (2)</td>
</tr>
<tr>
<td>DAS28, mean (s.d.)</td>
<td>ND</td>
<td>5.5 (0.7)</td>
</tr>
<tr>
<td>Medication, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral glucocorticoids</td>
<td>15 (65)</td>
<td>11 (73)</td>
</tr>
<tr>
<td>Synthetic DMARDs</td>
<td>19 (83)</td>
<td>9 (60)</td>
</tr>
<tr>
<td>TNF inhibitors</td>
<td>6 (26)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Rituximab</td>
<td>0 (0)</td>
<td>1 (7)</td>
</tr>
</tbody>
</table>

DAS28: 28-joint DAS; ND: not done; PB: peripheral blood.

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STAT3 in RA
fresh whole blood, the results are likely to reflect the phosphorylation status of the cells in vivo.

The phosphorylation of STAT1 was slightly increased in PB CD4+ T cells, but not in CD4− T cells or monocytes, from patients with RA (Fig. 2). In contrast to STAT1, the phosphorylation of STAT3 was strongly up-regulated in PB CD4+ T cells and monocytes, and to a lesser extent in CD4− T cells, from RA patients when compared with controls. The degree of STAT3 phosphorylation differed between individual patients and some patients demonstrated very high level of basal STAT3 phosphorylation (e.g. patient RA71; Fig. 2B). The levels of phospho-STAT3 were not significantly different between RA patients with newly diagnosed or chronic disease, and various treatments did not have a major influence on pSTAT3 levels (supplementary Table S1, available at Rheumatology Online).

In addition to presenting the data as MFIR (Fig. 2A), we also calculated the percentage of pSTAT3-positive cells. The mean percentage of pSTAT3-positive CD4+ cells was 48 (s.d. 22) and 18 (s.d. 12) in RA patients and healthy controls, respectively (P = 0.0006). In monocytes, the mean percentage of pSTAT3-positive cells was 84 (s.d. 16) and 56 (s.d. 22) in RA patients and healthy controls, respectively (P = 0.0010). We also observed significant correlation between pSTAT3 levels in CD4+ T cells and in monocytes (r = 0.80, P = 0.0006), indicating that the same patients demonstrated high phosphorylation of STAT3 in both CD4+ T cells and monocytes.

Basal STAT3 phosphorylation correlates with IL-6 levels in plasma

We then examined whether cytokine levels in plasma could explain the differences in STAT3 phosphorylation between patients. The levels of IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-10 and TNF-α were determined in plasma samples, and correlation analysis was performed with cytokine levels and the percentage of pSTAT3-positive cells.

Of the cytokines tested, only IL-6 levels were significantly elevated in RA patients when compared with controls (Fig. 3A, data not shown). The levels of IL-6 correlated well with the percentage of pSTAT3-positive CD4+ T cells and monocytes (Fig. 3B). In contrast to IL-6, cell surface expression of IL-6Rα and gp130 on T cells or monocytes was not significantly different between RA patients and controls (Fig. 3C) and did not correlate with baseline STAT3 phosphorylation (data not shown).

Statistical analysis also showed that the levels of IFN-γ correlated with pSTAT3 in monocytes (r = 0.57, P = 0.0334), levels of IL-10 with pSTAT3 in CD4+ and CD4− T cells (r = 0.55, P = 0.0428 and r = 0.56, P = 0.0310) and levels of TNF-α with pSTAT3 in CD4+ T cells and monocytes (r = 0.53, P = 0.0492 and r = 0.59, P = 0.0260). However, if the MFIR was used instead of the percentage of pSTAT3-positive cells, then only the correlation between TNF-α level and STAT3 phosphorylation in monocytes remained significant (r = 0.55, P = 0.0428). Therefore the association of STAT3 phosphorylation with
IL-6 level seems most striking, but the relationship with other cytokines cannot be completely excluded. We also studied whether disease activity parameters correlated with pSTAT3 level. However, baseline STAT3 phosphorylation in T cells or monocytes did not correlate with the age of patients, disease duration, DAS28, the number of swollen or tender joints, CRP level or ESR (data not shown). All patients in this analysis had active disease (DAS28 \( \geq 3.2 \)), which may explain the lack of correlation.

Decreased IL-6 signalling in T cells from RA patients

Next we studied whether phosphorylation of STAT1 and STAT3 following stimulation by cytokines IFN-\( \gamma \), IL-6 or IL-10 is also altered in PB T cells and monocytes from RA patients.

Cytokine-induced phosphorylation of STAT1 and STAT3 in T cells and monocytes was seemingly similar in patients with RA and in healthy volunteers (Fig. 4). However, IL-6-induced STAT3 phosphorylation was found to occur only in a certain fraction of T cells from RA patients.
RA patients (two-peaked histograms), and in this case MFI is not an accurate measure of STAT phosphorylation. We therefore calculated the percentage of pSTAT3-positive CD4+ and CD4− T cells in response to 15 min of IL-6 stimulation. There were significantly fewer pSTAT3-positive CD4+ cells following IL-6 stimulation in RA samples when compared with healthy controls (Fig. 5A and B). In certain patients, IL-6 unresponsiveness was very clear (up to 50% of the cells remained pSTAT3 negative after stimulation), while in some patients the response to IL-6 stimulation was comparable to that in healthy controls. Similar results were observed with shorter (5 min) and longer (30 min) stimulation time points (data not shown).
The basal pSTAT3 levels in RA CD4+ T cells were elevated and therefore the percentage increase in pSTAT3-positive cells after IL-6 stimulation was clearly lower in RA patients compared with controls (Fig. 5C). Similarly, in CD4-CD8 T cells, fewer cells became pSTAT3 positive after IL-6 stimulation and the increase in pSTAT3-positive cells mediated by IL-6 was lower in RA patients than in controls (Fig. 5A and C). In newly diagnosed untreated patients, hyporesponsiveness to IL-6 stimulation was more pronounced than in patients with established disease (supplementary Table S1, available at Rheumatology Online). In contrast, patients treated with biologic DMARDs showed the strongest STAT3 activation in response to IL-6 (supplementary Table S1, available at Rheumatology Online).

T cells from patients with RA responded normally to IL-10 stimulation (Fig. 5A and B). The hyporesponsiveness to IL-6 was specific for STAT3 since STAT1 activation in response to IL-6 stimulation was more pronounced than in patients with established disease (supplementary Table S1, available at Rheumatology Online).

Plasma IL-6 level and baseline STAT3 phosphorylation negatively correlate with IL-6-induced STAT3 phosphorylation in CD4+ T cells

Next we examined whether plasma cytokine levels would correlate with the degree of IL-6-mediated STAT3 phosphorylation in T cells. No correlation was observed between cytokine levels and the percentage of pSTAT3-positive CD4+ or CD4- T cells following IL-6 stimulation. In contrast, there was a strong negative correlation between plasma IL-6 level and the IL-6-mediated increase in the percentage of pSTAT3-positive CD4+ T cells (Fig. 5D). The levels of IL-10 (r = -0.55, P = 0.0351) and TNF-α (r = -0.58, P = 0.0228) were also negatively correlated with the induction of STAT3 phosphorylation by IL-6, but this finding was not as significant as that observed with IL-6.

In addition, there was a strong negative correlation between the percentage of unstimulated, pSTAT3-positive CD4+ T cells and the IL-6-induced increase in the percentage of pSTAT3-positive cells (r = -0.78, P = 0.0006). Similar negative correlation was not observed for CD4- T cells (r = -0.21, P = 0.4520). Cell surface levels of IL-6Rα or gp130 did not correlate with IL-6-mediated phosphorylation of STAT3 in CD4+ or CD4- T cells (data not shown).

Finally, we investigated whether disease characteristics could explain the differences in IL-6-induced STAT3 phosphorylation between patients. The increase in pSTAT3-positive CD4+ T cells following IL-6 stimulation did not correlate with the age of patients, disease duration, DAS28, the number of tender or swollen joints, CRP level or ESR (data not shown). The increase in pSTAT3-positive CD4- T cells induced by IL-6 negatively correlated with the age of the patients (r = -0.57, P = 0.0253), but not with other parameters. Taken together, these results indicate that RA CD4+ T cells that are exposed to high levels of IL-6 in circulation and demonstrate high basal phosphorylation of STAT3 do not respond normally to IL-6 stimulation.

Discussion

The present results show that in RA the STAT3 pathway is systemically activated in circulating T cells and monocytes, both in terms of the expression and phosphorylation of STAT3. Although there is previous evidence for
activation of the STAT1 pathway in RA synovial tissue [13–15] and in PB [16], our present results show increased STAT1 expression only in SF macrophages, and only slightly enhanced phosphorylation of STAT1 in PB CD4+ T cells from patients with RA. Finally, we show that IL-6-induced STAT3 phosphorylation is down-regulated in a proportion of RA T cells.

IL-6 is an important pro-inflammatory cytokine that has pathogenic effects on multiple cell types in RA [17], and neutralization of IL-6 is effective in the treatment of
patients with RA [18]. Levels of IL-6 are elevated in the serum and SF of patients with RA [17, 19, 20]. In our study, IL-6 was the only cytokine that showed significantly increased levels in plasma samples from RA patients. Our present results suggest a scenario where circulating T cells and monocytes are chronically exposed to elevated levels of IL-6, resulting in constitutive phosphorylation of STAT3 in these cells. Whether IL-6 functions by increasing STAT3 expression or by inducing STAT3 phosphorylation cannot be concluded on the basis of the present study. A proportion of CD4+ T cells demonstrating constitutive STAT3 phosphorylation then become hyporesponsive to further IL-6-mediated activation, although further studies are needed to characterize the T cell subset to which this applies. Since the absolute number of phospho-STAT3-positive T cells following IL-6 stimulation did not correlate with IL-6 level, it is likely that additional factors besides IL-6 are also involved in T cell hyporesponsiveness to IL-6.

There is limited knowledge on the expression and activation of the STAT3 pathway in RA. Immunohistochemistry and western blotting analysis have suggested that STAT3 and pSTAT3 levels are higher in the synovial tissues and SFMCs of patients with RA when compared with patients with OA [21, 22]. A more quantitative analysis was performed by Huang et al. [23], who used flow cytometric analysis to study phosho-STAT levels in patients with SLE and in nine RA patients as controls. In this study, pSTAT3 levels were elevated in PB T cells in both SLE and RA patients, but only in SLE PB monocytes. The latter result is not consistent with the present results demonstrating significantly increased levels of STAT3 mRNA and phospho-STAT3 in RA PB monocytes. Since only RA patients were included in the present study, further studies are needed to see whether constitutive STAT3 phosphorylation is a general finding of inflammatory arthritis.

Our results show increased basal pSTAT3 levels in both T cells and monocytes in RA, but IL-6-induced STAT3 phosphorylation was deregulated only in RA T cells. According to our data, this was not due to changes in IL-6 receptor expression in T cells. SOCS3 is a major inhibitor of IL-6-induced STAT3 phosphorylation [24], and we previously demonstrated increased levels of SOCS3 in RA PB T cells [9]. However, RA PB monocytes showed even higher levels of SOCS3, suggesting that IL-6/STAT3 signalling in monocytes would be equally decreased. It is thus possible that the sensitivity of the IL-6/STAT3 pathway to inhibition by SOCS3 is different in RA T cells and monocytes. To address this question in the future, it will be important to analyse SOCS3 expression and IL-6 signalling in the same patients.

Although STAT1 and STAT3 are both activated in response to IL-6 stimulation, STAT3 is the main mediator of IL-6 functions [25–27]. The pathogenic potential of IL-6/STAT3 signalling in RA is illustrated by recent findings demonstrating that activation of this pathway in CD4+ T cells predicts development of RA in a cohort of undifferentiated early arthritis [28]. Our results showing constitutively activated STAT3 in PB CD4+ T cells in active RA patients are in line with these results. These findings are not surprising, since IL-6/STAT3 signalling has important effects on T cells that are pathogenic in the context of RA. First, IL-6 enhances T cell proliferation and protects T cells from activation-induced cell death via STAT3 activation [29–31]. Second, activation of STAT3 by IL-6 is essential for the generation of Th17 cells, which are considered pro-inflammatory in RA [32], and mice that lack STAT3 in T cells are resistant to models of autoimmunity that involve Th17 cells [33, 34]. In addition, STAT3 activity prevents the generation of Tregs [35]. Using CD4+ T cells from RA PB or SF, Ju et al. [22] recently showed that inhibiting STAT3 in these cells prevented Th17 differentiation but increased the proportion of Tregs. It is tempting to speculate that increased phospho-STAT3 expression in RA CD4+ T cells favours Th17 cell differentiation and prevents the generation of induced Tregs.

The results of this study show that the IL-6/STAT3 pathway is constitutively activated in circulating T cells and monocytes from patients with active RA. Previous results have identified that the activity of the IL-6/STAT3 pathway in CD4+ T cells predicts progression of undifferentiated arthritis to RA [28]. Together, these results suggest the involvement of this pathway in the initiation and progression of RA. Given the present results and the pathogenic potential of IL-6 in RA, a synthetic inhibitor that would specifically target the IL-6/STAT3 signalling pathway might have beneficial therapeutic characteristics in RA.

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Supplementary data

Supplementary data are available at Rheumatology Online.

References


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Clinical vignette

18F]fluorodeoxyglucose positron emission tomography/computed tomography can reveal subclinical prostatitis in a patient with IgG4-related disease

An 82-year-old man was admitted to our hospital because of left exophthalmos. Head MRI showed a left orbital mass with low intensity on the T2-weighted image (Fig. 1A), while the whole body [18F]fluorodeoxyglucose (FDG)-PET/CT revealed abnormal uptake at the same site [maximum standardized uptake value (SUVmax 6.65)] (Fig. 1B) and also in the left lobe of the prostate (SUVmax 6.74) (Fig. 1C). Laboratory examination revealed a normal level of prostate-specific antigen (2.84 ng/ml) and a high level of IgG4 (856 mg/dl). The US did not show any focal lesions in the prostate and his urinary disturbance was not apparent. A needle biopsy of the left lobe of the prostate was performed and the pathological examination revealed lymphoplasmacytic infiltration with abundant IgG4+ plasmacytes (IgG4+:IgG+ plasma cell ratio was >80%) and showed neither malignancy nor infection (Fig. 1D). Thus the patient was diagnosed as having IgG4-related disease (IgG4-RD) accompanied by prostatitis and orbital inflammation according to the 2011 comprehensive diagnostic criteria for IgG4-RD [1]. This is the first report indicating that FDG-PET/CT can reveal subclinical prostatitis in patients with IgG4-RD.

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Reference


![Image of clinical vignette](https://example.com/image.png)

(A) Axial T2-weighted MRI of the head. The arrow indicates an orbital mass with low intensity. (B) FDG-PET/CT of the head. The arrow indicates abnormal FDG uptake in the orbital mass (SUVmax 6.65). (C) FDG-PET/CT of the pelvis. The arrow indicates abnormal FDG uptake in the left lobe of the prostate (SUVmax 6.74). (D) The pathological findings from the left lobe of the prostate (left: H&E staining; middle: immunohistochemical staining for IgG; right: immunohistochemical staining for IgG4). The IgG4+/IgG+ plasma cell ratio was >80%. The scale bar indicates 20 μm. FDG: [18F]fluorodeoxyglucose; H&E: haematoxylin and eosin; SUVmax: maximum standardized uptake value.

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