Editorial

The Jak-STAT Pathway in Rheumatoid Arthritis

The advent of biologic agents targeted against tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1) receptor has successfully suppressed inflammation in many individuals with rheumatoid arthritis (RA), but 60% of patients have at least some evidence of persistent disease[1]; moreover, the majority relapse when treatment is withdrawn. This has prompted research into alternative ways to suppress disease activity.

The hematopoietin family of cytokines, which includes several postulated to have roles in RA (e.g., interferons, IL-6, IL-2, IL-7, IL-12, IL-15) bind to Type I and II cytokine receptors and signal through the janus kinase-signal transducers and activators of transcription (Jak-STAT) pathway (Table 1)[2,3]. Therefore, a better understanding of Jak-STAT activation within the rheumatoid synovium may allow for development of novel therapeutic agents. In addition, these agents would have potential for oral bioavailability because they are targeted against small proteins[4].

Only 4 mammalian Jak have been identified — Jak1, Jak2, Tyk2, and Jak3. The first 3 are widely expressed, while Jak3 expression is essentially limited to hematopoietic cell lines. The STAT family has 7 identified members (STAT1, 2, 3, 4, 5a, 5b, and 6), and their recruitment is a critical component of inducing cell-specific responses[2].

Briefly, Jak-STAT signaling occurs when hematopoietins bind to cytokine receptors, initiating a conformational change in the receptor. This brings Jak into apposition, resulting in transphosphorylation and subsequent activation. Once activated, Jak mediate phosphorylation of specific tyrosine residues. STAT and other molecules that recognize these phosphorylated sites are recruited to the receptor and undergo activation by Jak-driven tyrosine phosphorylation. The activated STAT then dissociate, undergo dimerization in the cytoplasm, translocate to the nucleus, and bind to members of GAS (γ-activated site) enhancers (Figure 1)[2]. Recent studies have suggested that the predominant cytoplasmic distribution of STAT in resting cells may reflect a steady-state process[5].

STAT activity generally peaks 5–30 minutes after cytokine stimulation and then declines to baseline over the next 1–4 hours, although there are examples of sustained STAT activation. These observations are consistent with a tightly regulated pathway, and constitutive inhibitory mechanisms (proteolysis, dephosphorylation, and protein inhibitors of activated STAT) as well as inducible mechanisms have been described. Inducible mechanisms mediated by downregulation of receptor expression include induction

Table 1. Jak-STAT signaling by cytokines expressed in RA. Adapted from Kisseleva, et al. Gene 2002; 285:1–24[2]; and from Gadina, et al. Curr Opin Immunol 2001;13:363–73[3].

Cytokines	Janus Kinases	STAT
γc family		
IL-2, IL-7, IL-9, IL-15	**Jak1, Jak3**	**STAT5**, STAT3
IL-4	**Jak1, Jak3**	**STAT6**
ßc family		
GM-CSF	**Jak2**	STAT5
Gp 130		
IL-6, IL-11, LIF, OSM	**Jak1**, Jak2, Tyk2	**STAT3**, STAT1
IL-12	Jak2, **Tyk2**	**STAT4**
Receptor tyrosine kinases		
EGF, PDGF	Jak2	STAT1, STAT3, STAT 5
Interferon family		
IFN-α/ß	**Jak1**, Tyk2	**STAT1, STAT2**, STAT3, STAT4, STAT5
IFN-γ	**Jak1, Jak2**	**STAT1**
IL-10	**Jak1**, Tyk2	**STAT3**

Bold type denotes molecules proven to be essential for cytokine signaling. GM-CSF: granulocyte-macrophage colony-stimulating factor; LIF: leukemia inhibitory factor; OSM: oncostatin M; EGF: epidermal growth factor; PDGF: platelet-derived growth factor.
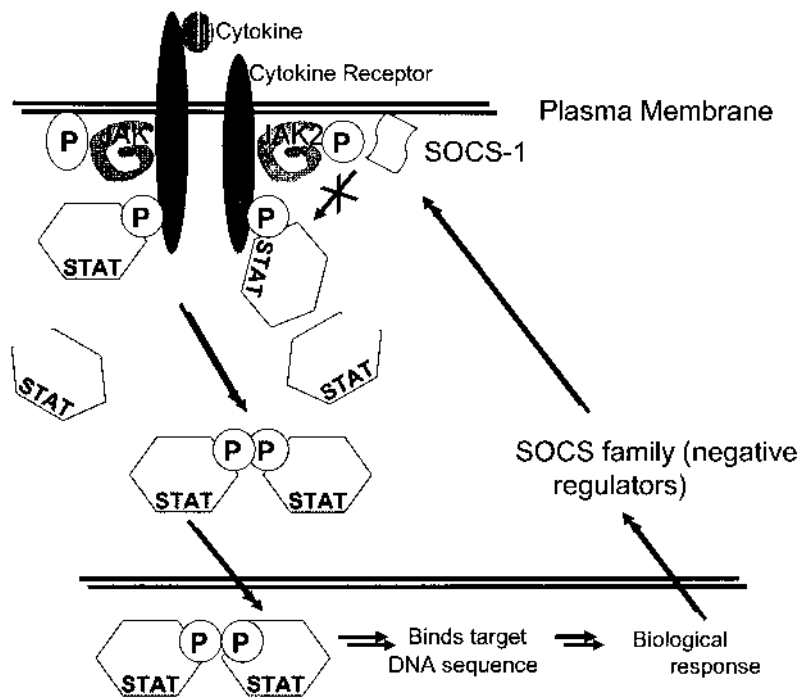
Figure 1. The Jak-STAT signaling pathway. Upon ligand binding, Jak associated with cytokine receptors undergo transphosphorylation and mediate phosphorylation of specific tyrosine residues on the cytokine receptor. Cytoplasmic STAT recruited to these sites undergo phosphorylation and subsequent dimerization in the cytoplasm before translocation to the nucleus. SOCS family members are induced by STAT and regulate signaling by a classical negative feedback loop.

of suppressors of cytokine signaling (SOCS) proteins, and the more rapid modification of preexisting signaling components (e.g., through mitogen-activated protein-kinase)[6]. To date, most research into modulating STAT expression in the field of inflammatory arthritis has centered on the SOCS pathway, of which 8 members have now been identified.

SOCS family members can inhibit Jak-STAT signaling by inhibiting Jak catalytic activity, competing with STAT for receptor docking sites, and targeting cytokine receptors for degradation by proteosomes. SOCS expression is upregulated in response to many types of cellular activation and functions as a classical negative feedback loop, although different family members exert their effects by different mechanisms. For example, SOCS-1 binds directly to phosphorylated Jak2, while SOCS-3 binds to the activated receptor to inhibit Jak activity[7].

Preliminary studies of Jak-STAT expression have been performed in human inflammatory arthritis tissue, and animal models and results support their important role in regulation of the inflammatory response.

Studies in STAT1 –/– mice suggest that STAT1's primary role is in interferon-γ (IFN-γ) signaling[8]. STAT1 mediates both inflammatory and antiviral effects of IFN as well as their antiproliferative and proapoptotic roles. This suggests that STAT1 has the capacity to inhibit, as well as promote, inflammation. STAT1 also has noninflammatory roles,

including the regulation of bone formation and destruction under both homeostatic and pathological conditions[6]. While IFN-γ is the primary activator of STAT1, other candidate cytokines include IL-6, IL-10, and IFN-α/β. Indeed, Yokota, *et al* found that IL-6 was the main activator in synovial fluid cells consisting mainly of neutrophils, suggesting differences in cell-specific activation pathways[9]. Initial reports described upregulation of STAT1 in rheumatoid tissue, but its role and extent of activation remained uncertain. More recently, van der Pouw Kraan, *et al*[10] utilized microarray analysis in 30 patients (21 with RA and 9 with OA) to look at differences in expression of immune-related genes. They found marked heterogeneity in expression, with data suggesting involvement of 2 distinct disease processes in rheumatoid pathogenesis. In the RA subgroup with high levels of inflammation, genes were indicative of an activated STAT1 pathway, with increased expression of STAT1 and genes known to be regulated by STAT1. The pattern of gene activation was most consistent with IFN-γ stimulation. Kasperkovitz, *et al*[11] demonstrated pSTAT1 expression and increased STAT1 expression in patients with RA as compared with osteoarthritis and reactive arthritis. The pattern of expression was predominantly in T and B cells in inflammatory infiltrates and in fibroblast-like synoviocytes in the intimal lining layer. Intriguingly, they found little STAT1 expression in synovial macrophages, even though a recent

study has identified that small amounts of IFN-γ can sensitize macrophages to express STAT1 following appropriate stimulation[12]. Taken together, these findings demonstrate increased activation and expression of STAT1 in RA synovium, possibly as a result of stimulation by IFN-γ.

However, the physiologic role of STAT1 remains uncertain. Krause, *et al* found that STAT1 played a proapoptotic role in RA synoviocytes, suggesting a role in limiting inflammation and synovial hypertrophy[13]. In addition, de Hooge, *et al* have recently reported the outcome of zymosan-induced arthritis in STAT1-deficient mice[14]. They found that this resulted in exacerbation of chronic joint inflammation and granuloma formation. SOCS-1 expression was also markedly reduced, suggesting that expression of SOCS-1 could be the underlying mechanism by which STAT1 limits joint inflammation. In support of this observation, Egan, *et al* have shown that SOCS-1 knockout mice have increased inflammation in an IL-1-dependent model of arthritis[15]. In summary, the evidence in human tissue and rodent arthritis models suggests that STAT1's antiinflammatory effects outweigh any stimulation of proinflammatory genes. Further, they raise the possibility of modulation of STAT1 or SOCS-1 as a method of controlling inflammation.

STAT3 has a variety of seemingly contradictory roles due to recruitment of distinct sets of target genes in different cell types. It is activated by a variety of cytokines including IL-6, IL-10, and IFN-α/γ and is postulated to be the major downstream regulator of gp130-like receptors. In T cells, STAT3 potentiates proliferation through IL-6-mediated suppression of apoptosis. Further, clinical trials of humanized anti-IL-6 receptor antibody have been promising[16], suggesting, indirectly, that suppression of STAT3 activation may alter inflammation. IL-10 signals through STAT3 to exert its antiinflammatory effects on macrophages. STAT3 has also been implicated in malignancy. Constitutive activation of STAT3 can lead to fibroblast transformation[6,17].

These diverse effects suggest that STAT3 may play a significant role in inflammatory arthritis. In 1995, Wang, *et al* reported predominant STAT3 but not STAT1 DNA binding activity in cells isolated from the synovial fluid of patients with inflammatory arthritis[18]. This was soon followed by animal model studies suggesting that dysregulation of STAT3 may alter the course of inflammatory arthritis. Ernst, *et al*[19] generated mice with a gp130$^{\Delta STAT}$ mutation to identify biologic responses to leukemia inhibitory factor/IL-6 mediated by STAT *in vivo*, and observed that these mice developed joint pathology with synovial hyperplasia, chronic inflammation, and secondary cartilaginous metaplasia. Subsequent stimulation studies with cultured synovial fibroblasts from wild-type and mutant mice strongly suggested that the hyperresponsiveness of synovial cells arose from impaired STAT-mediated induction of SOCS[19]. More recently, De Hooge, *et al* showed that STAT3 may contribute to the chronicity of inflammation in a murine zymosan-induced arthritis model[14]. Finally, periarticular SOCS-3 (a negative regulator of IL-6 –gp130-Jak-STAT3 signaling) suppresses murine collagen-induced arthritis[20].

In vitro studies in human tissue support the role of STAT3 in contributing to the chronicity of inflammatory arthritis. Krause, *et al* recently reported on the role of STAT3 in mediating the abnormal growth and survival properties of RA synoviocytes[13]. Many studies have suggested that synoviocytes are pivotally involved in cartilage destruction in RA in addition to producing chemokines, cytokines, and angiogenic factors. Using retroviral-mediated gene transfer of a dominant negative mutant of STAT3 (STAT3-YF) in cell cultures, Krause's group[13] found that ablation of STAT3 function converts epidermal growth factor from a growth/survival factor for RA synoviocytes to a death factor. Further experiments in synovial fibroblasts showed that STAT3 works, at least in part, by suppressing the proapoptotic effects of STAT1. Preliminary findings suggested that STAT3 may suppress additional proapoptotic pathways in RA, but these observations need confirmation. Taken together, these findings identify modulation of STAT3 expression, either by direct suppression or by altering expression of SOCS-3, as a potential therapeutic target in RA.

Less is known about the effects of STAT4 and STAT6 in inflammatory arthritis, although both of these are signal transduction targets for cytokines thought to play a significant role in inflammation. IL-12, a classical Th1-driven cytokine, signals through STAT4, and preliminary work has shown its presence in human rheumatoid synovium[21]. In contrast, IL-4, a Th2-mediated cytokine, signals through STAT6. Altering the balance between Th1- versus Th2-driven processes may alter the clinical course of inflammation. Using a murine proteoglycan-induced inflammatory arthritis model, Finnegan, *et al* found that arthritis severity appeared to be regulated by IL-4 through a STAT6-dependent mechanism. IL-4 –/– mice and STAT6 –/– mice demonstrated a more severe, rapidly progressive arthritis, suggesting that IL-4 mediates inflammation through STAT6 and that its predominant role is antiinflammatory. These changes were associated with elevated levels of the proinflammatory cytokines IL-12, TNF-α, and IFN-γ. In addition, IL-12 was found to regulate the magnitude of IFN-γ expression through a STAT4-dependent pathway, and both STAT4 –/– and IFN-γ –/– mice had reduced arthritis severity[22].

Modulation of STAT1, STAT3, STAT4, and STAT6 may therefore be an alternative means of altering arthritis severity. STAT do not have enzymatic activity and so any therapeutic agent would have to block STAT expression, cytokine receptor binding, dimerization, or DNA binding. While targeting these sites is theoretically possible, the successful design of small agents has proven difficult. An alternative therapeutic measure is to target the regulators of STAT activity such as SOCS-1 and SOCS-3. Work in murine models suggests that modulation in human disease may be effi-

cacious, but it is unclear how such drugs would be designed[23].

STAT activity may also be modulated through Jak, particularly Jak3 and Tyk2, as deficiencies in these kinases are nonlethal. Unlike other members of its family, Jak3 expression is relatively conserved, being largely limited to hematopoietic cell lines. It appears to interact uniquely with the common γ-chain (γc) receptor subunit used by a family of cytokines (IL-2, -4, -7, -9, -15) with important roles in lymphocyte function. An orally bioavailable Jak3 antagonist has been developed that was found to reduce transplant rejection in 2 animal models. As yet, there is no published work documenting expression on Jak3 in inflammatory arthritis. Tyk2 is involved in Type I IFN signaling, and mice with a mutation in Tyk2 have reduced susceptibility to collagen-induced arthritis[23]. Therefore a specific antagonist to Tyk2 may have therapeutic potential.

The Jak-STAT signal transduction pathway is differentially regulated in inflammatory arthritis, with changes already documented in STAT1, STAT3, STAT4, and STAT6 expression. Modulation of STAT expression, either directly or through modulation of Jak or SOCS function, represents an alternative therapeutic target to cytokine antagonists such as TNF-α and IL-1. RA is a pleiotropic disease with inappropriate activation of a number of inflammatory pathways. Longterm disease suppression, at least in more severe cases, is likely to require targeting of a variety of inflammatory cascades. There is growing evidence that modulation of the Jak-STAT pathway may represent a viable alternative therapeutic target in the treatment of rheumatoid arthritis.

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