REVIEW

Differential activation of JAK enzymes in rheumatoid arthritis and autoimmune disorders by pro-inflammatory cytokines: potential drug targets

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Abstract: Although several pro-inflammatory cytokines including interleukin-6 (IL-6), IL-7, IL-12/IL-23, IL-17, IL-2, interferon, and the anti-inflammatory cytokines, IL-4/IL-13, IL-10, and IL-22, all activate the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway, in autoimmune disorders, a skewing of the cytokine repertoire in favor of pro-inflammatory cytokines results in amplifying the effects of pro-inflammatory cytokines. An apparent deficiency of anti-inflammatory cytokines to counterbalance the 'ramping up' of pro-inflammatory cytokine-mediated activation of JAK/STAT is also significant, while endogenous negative regulators of cytokine signaling and JAK/STAT activation may also be compromised. In addition, JAK/STAT pathway activation can result in activation of stress-activated protein/mitogen-activated protein kinase (SAP/MAPK) and phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin pathways that are instrumental in promoting matrix metalloproteinase gene expression, aberrant cell survival, and osteoclast differentiation. The critical role played by pro-inflammatory cytokines in differentially activating JAK/STAT and parallel signal transduction pathways resulted in the development of several cytokine/cytokine receptor neutralizing monoclonal antibodies and fusion proteins that are currently employed for treating rheumatoid arthritis, Crohn's disease, and psoriasis. Small molecule inhibitors (SMIs) that target specific JAK enzymes have led to the development of CP690550, a JAK3-specific SMI, which is the first JAK-specific SMI to reach phase III in a rheumatoid arthritis clinical trial.

Keywords: autoimmunity, cytokines, inflammation, Janus kinase, signal transducers and activators of transcription, small molecule inhibitors

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Introduction

Recent and quite compelling evidence has implicated activation of the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway by pro-inflammatory and/or anti-inflammatory cytokines as a key regulatory step in the pathogenesis and progression of inflammation associated with autoimmune-mediated diseases such as rheumatoid arthritis (RA)[1–9] and other autoimmune disorders, systemic lupus erythematosus (SLE)[8,10] and Crohn's disease.[11] Thus, the pro-inflammatory cytokines, interleukin-6 (IL-6),[12–14] IL-7,[15–19] IL-12/IL-23,[4,20–23] IL-17,[24–27] the interferon (IFN) family of proteins,[28–31] as well as the macrophage-derived cytokine IL-2[32] via their interaction with specific cytokine receptors (CyRs) result in the phosphorylation (ie, activation) of JAK enzymes.[33] In this regard, cytokine/CyR binding regulates a host of immune cell regulatory functions including activation of antigen-presenting

cells such as plasmacytoid dendritic cells (DC)[6,34–37] as well as the genesis and modulation of two T-lymphocyte subpopulations and the T_h17[20] and T-regulatory (T_{reg}) cell subsets,[37–40] the activity of the latter T-cell subset being crucial for instituting and maintaining immune tolerance. In that regard, dysfunctional T_{reg} cell activity has been attributed to a breakdown in immune tolerance in RA[37–39] perpetuation of immune-mediated inflammation[40] and immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome,[41] a rare autoimmune disorder originating from mutations in the FoxP3 transcription factor that resulted not in defective $CD4^+ CD25^+ T_{reg}$ cell production but rather in dysfunctional T_{reg} cell and effector T-cells.[42]

Counterbalancing the overproduction of pro-inflammatory cytokines in autoimmune-mediated inflammatory disorders should occur by activation of JAK/STAT with the anti-inflammatory cytokines IL-4, -13,[43] -10,[44] and -22 (an IL-10-related cytokine).[45] Of note, deficient levels of these anti-inflammatory cytokines do not appear to be responsible for blunted anti-inflammatory responses in autoimmune-mediated disorders. In actuality, higher levels of IL-13 were found in the synovial fluid of patients with RA, SLE, and Sjögren's syndrome,[46] as well as in sera and synovial fluid samples from patients with psoriatic arthritis[47] where the levels of IL-13 in the patient group was comparable to IL-13 levels in a control group. Additionally, no significant relationship was found to exist between IL-13 levels and antinuclear antibody titers.[46] However, in RA, dysfunctional IL-10/IL-4/IL-13 could serve to blunt potential anti-inflammatory responses. Reportedly, this can occur by suppressing the synthesis of suppressor of cytokine signaling (SOCS) proteins[48–51] or by the reduced production of IL-10 by T_{reg} cells.[43] Interestingly, IL-2 was reported to enhance the synthesis of IL-10 in the T-cell line, HOZOT.[52]

In RA, the potential for modulating JAK/STAT terminating signals mediated by SOCS[48–51] protein tyrosine phosphatases (eg, SHP-1, -2), protein inhibitor of activated proteins, and signal transducing adaptor protein (STAM)[33,53] must also be considered. Thus, in RA, defective regulation of these negative regulatory proteins may account for any significantly elevated levels of constitutively activated STAT proteins.

Cytokine-mediated activation of the JAK/STAT pathway

Activation of specific STAT proteins (see below) via JAK enzyme activation converts STAT proteins into potent transcription factors.[54] In this conversion step, activated STAT proteins function to up regulate the expression of pro-inflammatory and anti-inflammatory cytokine genes,[1,33,53–55] modify the survival signaling pathways of activated monocytes, lymphocytes, and synoviocytes in the inflamed synovial joint[56–60] and in a positive feedback loop setting down regulates JAK/STAT and cytokine signaling by activating SOCS gene expression.[61] Of note, 'cross talk' between activated JAK/STAT, SAP/MAPK, and the phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTor) pathways may all be involved in promoting the survival and/or apoptosis of activated immune cells, activated synoviocytes, and chondrocytes. Thus, maintaining activated immune cell, synovial cell, and chondrocyte vitality in RA joints appears to be crucial for regulating the chronic inflammatory response[33,60–63] and for promoting chondrocyte-mediated articular cartilage repair.[64]

The results of recent experiments conducted in the Arthritis Research Laboratory at Case Western Reserve University shed some light on the mechanism by which inhibition of JAK enzyme activation could induce human chondrocyte apoptosis. Thus, incubating juvenile human chondrocyte macroaggregate pellet cultures[65,66] with 50 µM of the JAK2 tyrosine kinase inhibitor, tyrphostin (AG-490),[67] induced apoptosis after 60 min as evidenced by the increased frequency of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive nuclei in 5-µm histologic cross sections compared to untreated chondrocyte macroaggregate pellet cultures (unpublished data). These results suggested that induction of apoptosis by AG-490[67,68] could reflect the down regulation of JAK/STAT and MAPK pathways as was previously reported when AG-490 was incubated with an IL-6-dependent myeloma cell line.[69] The down regulation of the synthesis of the gp130 signaling subunit, but not JAK2 or STAT-3, was also found in rat schwannoma RT4 cell cultures but only after treatment with recombinant human IL-6.[70]

Although dampening JAK/STAT signaling is a laudable clinical goal designed to regulate chronic inflammation, inhibition of parallel SAP/MAPK[60,62,71,72] and PI3K/Akt/mTor signaling pathways,[53] Toll-like receptor (TLR) activation,[62] and the immunoreceptor tyrosine-based activation motif pathway[73,74] may also have to be considered as a way to fully ameliorate autoimmune-mediated inflammation. Nevertheless, recently, development of novel therapies with the capacity to inhibit or promote cytokine-mediated activation of the JAK/STAT pathway has been considered, which focuses on designing novel agents with the capacity to inhibit or promote cytokine-mediated activation of the

JAK/STAT pathway for treating human myeloproliferative diseases (MPDs)[75,76] and inflammatory arthritis.[14,19,56,77,78] Interestingly, the JAK2V[617]F mutation found in several lymphoproliferative disorders, which gives rise to constitutively activated STATs, was proposed as a link between B-cell dysfunction in MPDs and the B-cell hyperactivity associated with RA.[76]

Cytokine activation of STAT proteins

The earliest steps in the activation of JAK/STAT signaling by IFNs and other extracellular signaling molecules previously held that under basal conditions 'inactive' or unphosphorylated-STAT proteins (U-STATs) were found in the cytoplasm where they existed as free monomeric proteins.[79] After the appropriate stimulation is achieved via cytokine/CyR binding, JAK enzymes are activated and the U-STAT proteins are recruited from the cytoplasmic compartment to the CyR complex using Src homology 2 (SH2) regions that are homologous between specific JAK enzymes and their respective STAT proteins.[3,33] This proved to be the case for IFN-γ where the specificity of p-STAT accumulation via IFN-γ-mediated JAK1/JAK2 activation was found to reside in those interactions that occurred between SH2 homologous domains and specific phosphotyrosine motifs found in the JAK enzyme and STAT protein.[79,80] The next step in STAT protein activation was shown to occur when phosphorylated STAT proteins (p-STATs) dissociated from the cytokine/CyR/JAK complex leading to the formation of STAT hetero/homodimers whose interaction was also stabilized via reciprocal p-STAT/SH2-phosphotyrosine motifs. The formation of p-STAT dimers facilitated their transport to the nucleus where they bound to STAT-target genes to regulate the initiation of transcription.[33,81]

Recent studies, however, have resulted in a paradigm shift in the interpretation of these early steps in STAT protein activation. Thus, it is now recognized along with other components of the 'revised' understanding of JAK/STAT signaling[82] that U-STAT proteins exist primarily as U-STAT dimers and as high molecular mass 'statosome' complexes. Further, U-STAT proteins can also activate target genes that are distinct from those genes which are the targets for p-STAT proteins. Two examples of this phenomenon include findings that IL-6-stimulated p-STAT-3 also activated U-STAT-3 where U-STAT-3 drove the expression of both nuclear factor-κB (NF-κB)-dependent and NF-κB-independent gene targets[83] and the finding that IFN which drives p-STAT-1 accumulation in human mononuclear cells in vitro also resulted in a significant increase in U-STAT-1, which persisted for several days, leading to the expression of many immune-regulated genes that were distinct from those gene targets activated by p-STAT-1.[84]

Do cytokine/CyR interactions result in specific STAT protein activation?

Seven mammalian STAT proteins, STAT-1, -2, -3, -4, STAT5α, STAT5β, and STAT6 have been described,[1] and specific STAT protein activation has been reported based on various cytokine/CyR interactions. For example, STAT-3 is the main STAT mediator of IL-6/IL-6/gp130 signaling,[44,85,86] STAT-2 for IFN-α,[87] and STAT-1 and -6 for IFN-γ[88,89] and IL-4,[90] respectively. However, STAT-1 can also be activated by IL-6.[33] Moreover, IFN-α/β can act cooperatively with other T-cell mitogens including IL-2, -4, -7, and -12 to activate STAT-1 and -2, but not STAT-3.[91] IL-23, a member of the IL-6/IL-12 protein family was shown to induce the formation of STAT-3/STAT-4 heterodimers and activate STAT-1, -3, -4, and -5,[23] whereas IL-17 and -33 have been reported to activate STAT-3 and -5, respectively.[92]

Each specific cytokine/CyR interaction is also known to produce a different downstream gene response. In that regard, studies in STAT gene knockout mice have been instructive because specific STAT gene ablation resulted in variable downstream physiological responses.[43,93,94]

Specific gene responses initiated by cytokine/CyR interactions
The IL-6/IL-6R/gp130 pathway

IL-6 belongs to a family of pleiotypic pro-inflammatory cytokines including IL-11, leukemia inhibitory factor (LIF), oncostatin M, ciliary neurotrophic factor, epidermal growth factor, granulocyte colony-stimulating factor (G-CSF), leptin, and cardiotrophin-1 that signal through a common gp130 receptor subunit.[52,95,96] The binding of IL-6 to its specific receptor (IL-6R) in conjunction with the gp130 signaling subunit initiates the activation of JAK/STAT signaling[33,53] with the phosphorylation of mainly p-STAT-3.[95] In contrast, oncostatin M was found to mainly induce activation of JAK2/STAT-5.[97] A variety of transcriptional coactivator proteins some of which belong to a group of histone acetyltransferases but also including, CBP/p300 and CR6-initiating factor-1, were shown to improve access of p-STAT proteins to the transcription initiation complex.[33] Steroid receptor coactivator-1 which at one time was considered as performing a similar function as CBP/p300 and CR6-initiating factor-1 was recently found to be dispensable for the transcriptional

control by STAT-3, but the results of this study also confirmed the critical role of CBP/p300 in STAT-3-mediated target gene transcription.[98]

Gene expression profiling microarrays have systematically cataloged those target genes which are likely to be relevant to some aspect of the pathogenesis and progression of RA and other autoimmune-mediated arthritic disorders following IL-6, oncostatin M, or LIF-mediated STAT protein activation. In that regard, Andreas et al[99] recently defined 100 RA-related genes expressed by human chondrocytes after stimulation with the conditioned medium from SV40-T-antigen immortalized human synovial fibroblast cultures. One of these genes, for example, *Cyr61*, which encodes a cysteine-rich heparin-binding protein, was known to be a STAT-3 target gene (Table 1).

The IL-7/IL-7Rα pathway

The expression of the IL-7Rα gene also known as CD127 is likely to be important in the pathogenesis and progression of immune-mediated arthritis because of the central role played by IL-7R in T-cell survival, the maturation of B-cells, interactions of T-cells with DCs, and as a lymphoid tissue inducer cytokine.[15,16,18,106,107] Additionally, suppressed activity of the pro-apoptosis proteins Bad and Bim[108] and the levels of anti-apoptosis proteins Bcl-2 and Bax[109] have been implicated in activation of the IL-7/IL-7R regulatory pathway which in all likelihood is a contributor to defective apoptosis exhibited by activated T-cells in RA.[57] Also critical to this expanded understanding of the role of IL-7/IL-7Rα-mediated signaling in RA were analyses showing elevated levels of IL-7 in RA synovial fluid.[110] Additionally, the F759 mutation in gp130/IL-6R subunit resulted in elevated levels of IL-7 and activated STAT-3,[111] suggesting a strong correlation between defective gp130 signaling and increased IL-7 levels. Furthermore, Kim et al[110] also showed that IL-1β and tumor necrosis factor alpha (TNF-α) increased IL-7 levels by stromal cells in vitro. This result was coupled to the finding that IL-7 markedly induced osteoclastogenic cytokine production by T-cells, which was receptor activator of NF-κB ligand (RANKL)-dependent while being TNF-α independent. This result strongly suggested a critical link between IL-7/IL-7Rα-dependent signaling and STAT protein activation playing a role in the destruction of subchondral bone, which is a characteristic feature of chronically progressive RA. Of note, glucocorticoids that are often employed in the initial therapy of RA inhibited IL-7-mediated signaling (along with IL-4 and -15) in primary human T-cells which was accompanied by inhibition of STAT-5 activation.[112]

Although STAT-5-mediated signaling via IL-7/IL-7Rα was crucial for promoting naïve T-cell survival, the forced expression of constitutively active STAT-5 failed to rescue CD4+ T-cells in SOCS1 transgenic mice, implying that STAT-5 was necessary but not sufficient for survival of naïve T-cells.[106] Moreover, employing T-lymphoid cells, Jahn et al[113] showed that Kit, the transmembrane protein receptor tyrosine kinase for stem cell factor and IL-7R could act synergistically with partial complementation of γ-C or IL-7-mediated signaling occurring via the Kit signaling pathway so that Kit-mediated activation of JAK-3 became IL-7R-dependent. Of note, deficient STAT-5 activation was also found in the Kit mutant YY567/569FF which lacked intrinsic Src activation capacity. This defect could be partially reconstituted in the presence of IL-7R and JAK-3.[113]

IL-17

IL-17 exists in six isoforms among which the bioactivity of isoforms IL-17A and -17F is the most thoroughly studied. IL-17 is a T-cell-derived cytokine that is overexpressed in the synovial tissue of RA patients.[24,114] IL-17 can drive the progression of arthritis in well-validated animal models of RA which was found to be independent of the presence and/or activity of IL-1β[57] and/or TNF-α.[115,116] Noteworthy was the finding

Table 1 IL-6 protein family member-regulated genes

Cytokine	STAT-regulated genes	Cell target	References
GM-CSF	Survivin	CD34+ hemoprogenitor cells	100
IL-6, sIL-6R	Type II collagen, link protein, aggrecan, Sox9	Articular chondrocytes	101
IL-6	*BCL-3*	Multiple myeloma cell lines	102
Oncostatin M	*c-fos, TNF-R, Pcnt, Bcl-3, Peg10, Cdo, Cin6, Perq1, Smad9, Boc, CBP, Ect2, FasL*	NIH3T3 STAT3-dependent	103
Oncostatin M	*CH13L1, PLAU, MTA2, EPAS1*	U1242MG glioma cell line	104
LIF	*Dact1, Klf4, Klf5, Rgs16, Smad7 Ccrn1, Ocln, Ier3, Pim1, Cyr61, Sgk*	Mouse embryonic stem cells STAT3-dependent	105

Abbreviations: GM-CSF, granulocyte macrophage-colony-stimulating factor; IL, interleukin; LIF, leukemia inhibitory factor.

that a T-cell subset, T_H17, isolated from RA peripheral blood mononuclear cell preparations had several characteristics that made them distinct from the T_H1/T_H2 T-cell subsets with TNF-α, GM-CSF, and IL-6 among the most prominent pro-inflammatory cytokines produced by T_H17 cells.[25] Importantly, IL-23[21,22,117] and over-expression of the retinoic acid receptor-related orphan receptor-γ (ROR-γ)[118] were shown to be potent inducers of IL-17 synthesis and T_H17 cell differentiation. Moreover, ROR-α t and ROR-γ t coexpression acting in synergistic fashion was found to further drive the production of T_H17 differentiation. T_H17 cells in vitro also secreted IL-21 and -22.[119] In that regard, Korn et al[120] showed that the robust production of T_H17 cells only occurred in the presence of transforming growth factor-β, IL-6, -21, -23, and ROR-α t and ROR-γ t which also drove the activation of STAT-3. T_H17 cells specifically lacking gp130 and STAT-3 failed to differentiate into T_{reg} cells or express the ROR-γ t phenotype indicating that activation of the JAK/STAT-1, -3 signaling pathway was critical for the differentiation of T_{reg} cells from T_H17 cells.[121–123] Not unexpectedly, two genome-wide analyses determined that STAT-dependent targets (eg, IRF8-STAT-3 and NFAT-STAT-3) were regulated at the level of transcription either directly by IL-17,[124] indirectly by the up regulated expression of IL-6 in response to IL-17, or by 'cross talk' via activation of NF-κB and PI3K/Akt/mTor pathways.[125,126] Conversely, IL-17 expression was blocked by IFN-γ and IL-4 signaling.[127]

IL-12/IL-23

IL-23 is a heterodimeric cytokine composed of IL-12p40 and the IL-12p35 cytokine.[128,129] IL-23 binds to the IL-12 receptor-β_1 (IL-12R β_1), which strongly activates JAK2/STAT-1, -3, -4, and -5 but with a weaker STAT-4 activation profile.[130] Interestingly, different patterns of DNA-binding complexes emerge in response to IL-12 and -23.

Serum IL-12 and -23 levels are elevated in RA sera and synovial fluid,[20,131] but neither of these cytokines were decreased in response to conventional therapy of RA with corticosteroids.[131] At the cellular level, IL-17[129] and IL-1β[132] stimulated IL-23 synthesis, whereas IL-23 up regulated IL-8 and -6 expression[132] in cultured RA synovial fibroblasts. The stimulatory effects of IL-17 and IL-1β on IL-23 production were both dependent on activation of the p38 MAPK, PI3K/Akt/mTor, and AP-1 pathways.[131,132] Conversely, IL-12-induced IFN-γ production by human T-cells was also found to be regulated by mTor.[133]

Xu et al[134] showed that DCs that were engineered (ie, 'silenced') to not produce IL-12p35 were capable of blunting T-cell responses. This occurred by dampening the activation of T-cell JAK2, Tyk2, STAT-3, and -4. STAT-4 induced target genes following stimulation of human T-helper cells with IL-12, which included macrophage inflammatory protein-1α and -3α, IL-1-receptor antagonist, IFN-regulatory factor, v-ets erythroblastosis virus E26 oncogene homolog 1 (Ets)-related transcription factor, CCR5 and the IL-18 receptor.[135] Of note, Kageyama et al[136] showed that a significant decrease in serum IL-23 and macrophage inflammatory protein-3α, but not IL-17, occurred after 3- and 6-month therapy of RA patients with etanercept, an anti-TNF-α fusion protein. Additionally, in a Phase II clinical trial in RA patients, the orally administered IL-12/IL-23 small molecule inhibitor (SMI), STA-5326, down regulated IL-12p35 and IL-12/IL-23p40 at the transcriptional level with decreased T_h1 responses.[130]

The interferon-mediated pathway

An extensive analysis of the critical role played by the IFN protein family and the interferon-regulated gene (IFG) pathway in RA,[137,138] including the emerging role of two newly identified IFN-λ protein family members, IL-28A,B and IL-29[139] as well as the role of IFN/IFG-dependent pathways in other autoimmune disorders such as SLE[140,141] and Sjögren's syndrome[142] is beyond the overall scope of this review. Sufficed, the IFN/IFG-mediated pathway has been extensively studied in these autoimmune diseases and was shown to play an important regulatory role in mediating autoimmune-dependent inflammation.

The following patterns of IFN/IFG-regulated gene responses have emerged in these disease states. Despite the fact that most of the previous studies have focused on the capacity of IFN to activate the JAK/STAT pathway resulting in IRG-mediated responses,[33,143,144] recent studies have also shown that i) non-STAT-dependent pathways, including, those signaling pathways involving activation of the MAPK components, p38 kinase, and ERK1/2 as well as activation of PI3K/Akt/mTOR signaling were important in transmitting cellular signals that were critical to IRG-mediated metabolism originating from IFN/IFN-receptor-(IFNR)-associated complexes;[145–147] ii) that Akt activity was crucial for the up regulation of key IFN-α,-γ responses[148] with these IFN-α,-γ-mediated responses also reflecting the direct control of IRG activity by mTOR[149] at the initiation of translation level;[150] and iii) that Akt/mTOR substantively regulated the initiation of translation of three IRGs pertinent to autoimmune-mediated inflammation in RA, SLE, and psoriatic arthritis, namely, IFN-induced 17kDa protein (Isg15), Cxc110, and IFN-regulatory factor-7 (Irf7).[151–155]

Of note, JAK/STAT rather than Akt/mTOR was shown to regulate IFN-α-mediated translation of irf9 and the antiproliferative effect of IFN-α against the ovarian carcinoma cells line, OVCAR3.[147] However, although irf9-RNAi inhibited the activity of TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in OVCAR3, STAT-1-RNAi did not, indicating that TRAIL-mediated apoptosis was ifr9-dependent rather than STAT-1-dependent.[147] In contrast, irf3 was shown to regulate IFN-stimulated response element promoter activity as well as IFN-β, irf5, irf7, RANTES (regulated upon activation normal T-cell expressed and presumably secreted), IFN-inducible protein-10, MCP-1, and MIP-1α in response to poly (I-C). Interestingly, irf3 knockdown also blocked the activity of some genes in RA-synovial fibroblasts not generally thought to be mediated by an IRSE such as matrix metalloproteinase (MMP)-3, -9, IL-6, and -8, the latter which required JNK and AP-1 activity.[156]

Finally, Österlund et al[157] showed that the binding of an irf to the Type III IFN promoter/IRSE was the critical step regulating the transcription of IRSE-regulated genes. In this regard, IFN-λ-1 gene responses could be induced by virally-activated irf3 and irf7 thus resembling IFN-β gene activation, whereas IFN-λ2/3 gene expression was mainly controlled by irf7 and thus resembled an IFN-α gene response. The results of this study[157] also demonstrated that IFN genes could be regulated by both TLR-dependent as well as TLR-independent pathways.

Signaling by the IL-10 protein family

The IL-10 protein family of cytokines consists of IL-10, -19, -20, -22, -24, -26, and the IFN-λ group (IL-28A, -28B, and -29) all of which bind to a shared class II of CyRs to form heterodimeric complexes which activate JAK/STAT signaling and thus play a potential role in cell survival and proliferation.[158–162] During the past decade or so, there has been an increased interest to restore anti-inflammatory-mediated events associated with the IL-10-type cytokines that may have become dampened during autoimmune-mediated events. This approach has often taken a somewhat overly simplistic approach. For example, in one study, IL-22 was shown to act synergistically with TNF-α, IL-1β, and IL-17.[159] However, under most other conditions, the biological activity of IL-22 did not require cooperation with any of these cytokines so that modifying the biological activity of TNF-α, IL-1β, and IL-17 would not be expected to abrogate the effects of IL-22 on inflammatory responses.

As discussed previously, autoimmune-mediated disorders such as RA and SLE are characterized by a skewing of T_h1

and T_h2 T-cell subsets in favor of T_h1. Thus, development of T_h1 and T_h2 T-cell subsets may simply be related to the biological activity of specific transcription factors; T-bet for T_h1[163] GATA3 for T_h2,[164,165] RORγt for Th17 cells,[166] and the *Schnurri* (*Shn*) zinc-finger proteins for memory and resting T-cells.[167] These transcription factors all appear to play a fundamental role in modulating the ratio of memory T_h1 and T_h2 T-cell subsets. Therefore, regulation of the biological activity of these transcription factors may have significant effects on regulating levels of IL-10 family cytokines. How this phenomenon is actually controlled at the molecular level still remains to be fully elucidated. However, recent evidence indicated that *Shn-2* was responsible for promoting memory T_h cell survival, that suppression of additional transcription factors was required for the development of memory T_h cells and resting T_h cells, and for that matter naïve CD4+ cells could be modulated by repressor proteins such as *Shn-2*.[167] Another factor that may explain reduced IL-10 function and the dysregulation of STAT protein activation in RA and SLE could be related to the recent results reported by Hermann et al[168] who showed that an IL-10R1 loss of function $G^{330}F$ mutation cloned into HeLa cells resulted in weak induction of both SOCS and STAM after stimulation with IL-10.

Over a decade ago, Riley et al[169] demonstrated that IL-10 was responsible for suppressing macrophage-derived TNF-α. Therefore, macrophages that were STAT-3- or JAK1-deficient could not respond to induction of TNF-α by lipopolysaccharide (LPS). Furthermore, Riley et al[169] demonstrated that two redundant STAT-3 recruitment sites located at $^{427}YQKQ^{430}$ and $^{477}YLKQ^{480}$ were required for all IL-10-dependent effects on B-cells or macrophages. Of note, IL-10-mediated anti-inflammatory effects required the intracellular domain of the IL-10R at the COOH-terminus which contained at least one functional serine phosphorylation site. Thus, it was likely that the progression of auto-immune-mediated arthritis could go on unabated if some or all of these IL-10 functional requirements were lost during the disease process. These results were later confirmed by Ahmed and Ivashkiv[170] and Herrero et al,[171] who showed that IL-10-(and IL-6)-mediated signaling could be blunted by activation of pro-inflammatory and stress-activated pathways involving p38 MAPK, JAK-1, and STAT-3. Further, modulation of IFN-γ regulated the 'on/off' switch which controlled IL-10-mediated STAT activation and macrophage responses to IL-10.

Several other possibilities that may account for the loss of functional IL-10 in RA were also proposed by Ji et al[172] and van Roon et al[173] whereby macrophages become refractory

to the anti-inflammatory effects of IL-10 when macrophages are continuously exposed to immune complexes in vivo. In addition, macrophage activation by IFN-γ required F_c receptor activation to mediate the suppression of IL-10 signaling, and diminished STAT-1- and IL-10-inducible gene activity with concomitant suppression of pro-inflammatory cytokine production was also dependent on the activity of protein kinase C-δ. More recently, it was shown that IL-27, a member of the IL-12 cytokine family, was capable of priming monocytes to respond to TLR stimulators which were STAT-1 dependent and which altered IL-10 signaling.[174] Further, IL-27 was found to strongly suppress TLR-induced IL-10 production by human monocytes suggesting that the elevated levels of IL-27 mRNA produced by the macrophages recovered from the synovial fluid of RA patients compared to control macrophages[175] could be responsible, in part, for abrogating the potential anti-inflammatory effects of IL-10. Importantly, IL-27 mRNA levels did not differ between RA and control macrophages after TLR2 ligation suggesting a mechanism for modulating the effects of IL-27 on IL-10-mediated signaling.

IL-4/IL-13

IL-4 and -13 are produced primarily by T_h2 cells, mast cells, and basophils. IL-4-mediated signaling is initiated via the binding of IL-4 to two receptors, type I and type II, whereas IL-13-mediated signaling is activated only through binding of IL-13 to the type II receptor.[176] Both IL-4 and -13 activate the JAK/STAT pathway,[176] but evidence has also shown that PI3K/Akt/mTor,[43,176] Fes tyrosine kinase, insulin receptor substrates, and inositol phosphatases are also activated by IL-4/IL-13.[43] Additionally, IL-4 was shown to induce phosphorylation of Syk, p38, ERK 1/2, JNK, as well as JAKs-1 and -2, STAT-1 and -6 in neutrophils with IL-4 also increasing the expression of SOCS3 at the mRNA and protein level.[177]

Wang et al[178] showed that IL-4/IL-13 activated STAT-1/STAT-6 in multiple cell types, including smooth muscle, epithelium, endothelium, fibroblasts, and lymphoid cell lines. IL-4 and -13 utilized a common receptor for activation composed of IL-4Rα and IL-13Rα1, but IL-4 also used IL-4Rα and the common γ chain for activation of JAK/STAT suggesting a common mechanism among various cell types that regulate IL-4/IL-13-mediated signaling.

The role played by IL-4 and -13 in regulating inflammatory responses in arthritis has also been systematically analyzed. Thus, Morita et al[179] showed that IL-4 and -13 (as well as IL-10) inhibited the production of IL-1β, TNF-α,

IL-6, and IL-8 in freshly isolated synovial tissue cells in vitro. IL-4 and -13 also increased the production of IL-1R antagonist protein. However, IFN-γ production was suppressed by IL-4 (and IL-10), but not by IL-13. Finally, IL-1β-induced RA synovial fibroblast proliferation was inhibited by IL-4 and -13, but not by IL-10, a result which suggested that if IL-4 and -13 could retain their potency in RA synovial joints, IL-4 and -13 could potentially suppress aberrant synoviocyte proliferation induced by the elevated levels of IL-1β.

Proof-of-concept studies to show that IL-4 could ablate arthritic changes were performed by Woods et al[180] who showed that adenovirally-directed IL-4 administered to rats with adjuvant-induced arthritis (AIA) showed reduced joint inflammation compared to the empty vector-control group. The reduction in joint inflammation was accompanied by lower levels of IL-1β, TNF-α, macrophage inhibitory protein-2 (MIP-2), and RANTES chemokine. A reduction in synovial tissue cellularity, vascularization, and bone destruction was also noted. In contrast, Nabbe et al[181] showed that local IL-13 gene transfer when prophylactically administered to rodents prior to the development of immune-complex-mediated arthritis had a lower frequency of apoptotic chondrocytes and reduced MMP-mediated cartilage proteoglycan degradation, despite the fact that IL-13 gene transfer had little or no effect on inflammatory responses or on MMP-3, -9, -12, and -13 mRNA levels.

The results of other studies have also indicated that IL-4 and -13 (but not IL-10) protected human synoviocytes or synovial tissue explants from apoptosis induced by sodium nitroprusside in a dose-dependent manner.[182] In addition, elevated levels of IL-13 were found in RA sera and synovial fluid compared to normal sera or synovial fluid from OA patients.[183] Further, IL-13 recovered from RA samples promoted DC maturation and IL-13 production by DC. Of note, DC growth activity could be inhibited by etanercept in vitro which was also associated with diminished IL-13 activity. Lastly, etanercept-treated RA patients who demonstrated noticeable clinical improvement also showed concurrent increases in circulating macrophage-colony stimulating factor (M-CSF), a non-DC, monocyte-specific growth factor. M-CSF is known to promote monocyte/macrophage differentiation, to act as a survival factor for osteoclasts[184] and to activate STAT-5 during myeloid cell differentiation.[185] However, it remains to be determined if clinical improvement by RA patients in response to etanercept was totally independent of changes in the level of M-CSF.

Three other studies[186–188] were noteworthy because they have focused on the putative role of IL-4 and -13 in

suppressing neoangiogenesis in rat AIA. In one study,[186] intraarticular administration of adenovirus-producing IL-4 reduced synovial tissue vascularization in rat AIA. The reduction in blood vessel density was accompanied by decreased synovial joint inflammation that was also characterized by lower levels of IL-18, CXC chemokine ligand 16 (CXCL16), and LPS-induced CXC chemokine (CXCL5) but with higher levels of endostatin. The antiangiogenic effects induced by adenovirus-producing IL-4 occurred despite persistently high levels of vascular endothelial growth factor in the joints of rats with AIA. In a follow-up study, Haas et al[187] produced an almost identical result using rats with AIA treated with adenovirus-producing IL-13. The reduction in joint inflammation was characterized by down regulation of IL-18, cytokine-induced neutrophil chemoattractant-1 (CXCL1), CSCL5, and up regulation of endostatin as well as a decrease in the activities of MMP-2 and -9. Interestingly, neither study examined the extent to which adenovirus-producing IL-4 or -IL-13 altered JAK/STAT or other signaling pathways in rat AIA, although Ruth et al[188] previously showed that intragraft injection of human CLCL16 which mediated the recruitment of human mononuclear cells to RA synovial tissue implanted in SCID mice was inhibited by antisense oligonucleotides directed toward ERK1/2 MAPK, suggestive of an effect of CLCL16 on the SAP/MAPK pathway.

IL-2-signaling activates STAT proteins

Mice deficient in IL-2 or its receptor, IL-2R showed an elevated level of lymphocytic proliferation coupled to an autoimmune disorder.[189] This finding together with the knowledge that IL-2 plays a critical role in regulating T-cell proliferation in vitro ultimately led to the conclusion that constitutive expression of IL-2Rα on CD4$^+$CD25$^+$ T-cells as well as IL-2/IL-2Rα binding was critical for maintaining normal T-cell proliferation and homeostasis. Prior to this discovery, it was already known that IL-2/IL-2Rα signaling activated the JAK/STAT pathway with STAT-5a and -5b, the principal STAT proteins activated in this process, and that SOCS was the negative regulator of STAT-5a, -5b protein activation by IL-2/IL-2Rα.[190] In this regard, Murawski et al[191] showed that up regulation and sustained activity of FOXP3 were required for the sustenance of mouse and human T$_{reg}$ cells which were also dependent on STAT-5 activation by IL-2. Moreover, Taylor et al[192] showed that the STAT-5-driven activation in response to IL-2/IL-2Rα was critical for lymphocyte homeostasis and could, in fact, 'supersede' the general requirement for T-cell receptor engagement and

cytokine stimulation with other cytokines such as IL-15 as a T-cell proliferation activator. Thus, chronic cellular stressors that limited STAT-5 phosphorylation might be expected to suppress CD4$^+$CD25$^+$FOXP3$^+$ T$_{reg}$ cell activity. Such was the case with CD4$^+$CD25$^+$FOXP3$^+$ T$_{reg}$ cells from patients that were chronically infected with hepatitis C virus. In this particular case, inadequate levels of CD4$^+$CD25$^+$FOXP3$^+$ T$_{reg}$ cells were accompanied by up regulation of the interaction between programmed death-ligand-1 (PD-L1) and B7.1[193] suggesting that PD-L1 negatively regulated T$_{reg}$ cell activity by blocking STAT-5 activation at sites where chronic inflammation was persistently present.

Is epigenetic status a mechanism for regulating STAT-protein expression?

Recent studies have focused attention on the distinct possibility that epigenetic modifications, including chromatin methylation and histone post-translational alterations, play a critical role in the pathogenesis and progression of RA.[194] Thus, Karouzakis et al[195] showed that histone hyperacetylation and elevated microRNA expression were a characteristic of RA synovial fibroblast cultures. Moreover, normal synovial fibroblasts grown in a culture milieu that supported hypermethylation resulted in normal cultured synoviocytes acquiring an RA-like phenotype. One possible interpretation of these results was that aberrant DNA methylation altered the progression of RA by inducing abnormal synoviocyte proliferation and activation. These findings were extended to show that similar epigenetic alterations affecting chromatin and DNA supercoiling occurred in other autoimmune disorders, such as SLE and multiple sclerosis,[196] whereby epigenetic modifications affected autoreactive lymphocyte development and neural demyelination, respectively. As previously indicated, in RA, DNA methylation and histone modifications were also found to be strong promoters of aberrant synoviocyte proliferation.[195,196]

STAT proteins appear to be particularly sensitive to epigenetic modifications.[197] For example, Shin et al[198] showed using normal human T-cells that STAT-4 expressional regulation was associated with the hypermethylated state and STAT-4 gene expression was strongly increased in human T-cells following treatment with a DNA methyltransferase inhibitor. Moreover, methylation exhibited a stronger association with STAT-4 protein expression than that associated with promoter polymorphisms. A similar result was found for the regulation of STAT-6 signaling in human T-cells.[199]

Of note, loss of functioning JAKs resulted in an enhancement of heterochromatin gene silencing and the over-expression of heterochromatin protein-1 which correlated with the growth of oncogenic JAK-induced tumors in the absence of any alterations in JAK/STAT-mediated signaling.[200] These results suggested that epigenetic status was critical for disrupting heterochromatin-mediated tumor suppression characterized by overactivation of JAKs which may have particular relevance for establishing a role for altered JAK activity in autoimmunity.

Finally, Nile et al[201] showed using LPS-stimulated macrophages that methylation of a single CpG site in the IL-6 promoter region altered IL-6 gene regulation. Moreover, the IL-6/CpG motif in monocytes from RA patients was undermethylated compared to the IL-6/CpG motif from control monocytes. Although the relevance of these findings to RA requires further study, they may be associated with the elevated levels of IL-6 found in RA synovial fluid and sera as well as the altered responsiveness of IL-6-induced STAT activation[2,6,8,14] found in human RA synoviocytes and monocytes ex vivo.

Potential drug targets

The majority of the newer drug treatment strategies for RA and other autoimmune disorders have focused on neutralizing pro-inflammatory cytokine/CyR interactions, especially those initiated by IL-1, TNF-α, and IL-6.[4,5,13,14] Other pro-inflammatory cytokines that include IL-17,[202,203] IL-12/IL-23,[204–206] IL-7,[17,19] and IL-7Rα[207] have also been considered as promising targets for drug development for the medical therapy of various forms of inflammatory arthritis, Crohn's disease, and psoriasis. Manipulating DC activity deemed to be critical to the progression of RA has also been contemplated as a viable form of 'cell' therapy.[208] Drug development strategies for improving the potential disease-modifying effects of anti-inflammatory cytokines such as IL-4/IL-13 and IL-10 must also be formulated but at the present time, the development of experimental strategies to counteract the effects of pro-inflammatory cytokine-induced responses in RA using anti-IL4/Il-13 or anti-IL-10 have lagged behind other experimental approaches. However, in the context of the overall theme of this review, there have been experimental protocols designed to directly target JAK/STAT-mediated signaling.[209,210] Indeed, several drugs in various current stages of development as specific JAK and/or STAT protein inhibitors are shown in Table 2. In addition to RA, several of these JAK/STAT-specific SMIs are contemplated for use in the treatment of MPDs, renal carcinoma, and malignant glioma.

Table 2 Drug targeting of the JAK/STAT signaling pathway

Inhibitor	JAK selectivity	Potential indication	References
CP690550	JAK3	RA	211,212
MS-0120	JAK3	Hodgkin's lymphoma	213
INCB018424	JAK1/JAK2	MPD	214
INCB028050		RA	215
420999	JAK1/2/3 (?)	Osteosarcoma	216
CYT387	JAK2	Hematologic malignancy	217
TG101348	JAK2	Polycythemia vera	218
TG101209	JAK2		219
JS-124	JAK1/2/3 (?)	Glioblastoma multiforme, Malignant glioma	220
WP1066		Renal carcinoma	221
CPA-7		Malignant glioma	222

Abbreviations: JAK, Janus kinase; MPD, myeloproliferative disease; RA, rheumatoid arthritis; STAT, signal transducers and activators of transcription.

Conclusions

Activation of the JAK/STAT signaling pathway initiated by the interaction of pro-inflammatory and/or anti-inflammatory cytokines with their respective CyRs regulate a host of immune-mediated inflammatory responses that are highly relevant to the pathogenesis and progression of RA and other autoimmune disorders. Although the importance of STAT protein activation in RA is undeniable and has resulted in the development and testing of several JAK-specific SMIs in well-validated animal models of inflammatory arthritis and now in RA clinical trials, there are other potential targets for RA intervention that should not be overlooked. Thus, circulating hormonal axes relevant to chondrogenesis and cartilage repair involving insulin-like growth factor binding protein and the growth hormone/IGF-1 nuclear receptor peroxisome proliferator-activated receptor pathway, the latter participating in the maintenance of normal cartilage homeostasis, can also initiate STAT protein activation.[223,224] The extent to which non-cytokine mediators should be further studied to determine whether or not they promote or inhibit JAK-specific activation or if they affect cartilage repair in RA via JAK/STAT or any of the other parallel signaling pathways seems appropriate and worthwhile.

There had been strong implications based on the results from well-validated animal models of inflammatory arthritis that suppression of the SAP/MAPK pathway and p38 kinase, in particular, dampens the severity of bone loss in experimental arthritis.[225] However, there has been little clinical efficacy obtained when p38 kinase isoform-specific SMIs were employed in human RA clinical trials.[226,227] In the meantime,

significant progress has already been made in determining the efficacy, safety, and tolerability of the orally administered JAK3-specific SMI, CP690550 in RA clinical trials. The use of CP690550 for future RA therapy will hinge on data forthcoming from the currently ongoing phase III clinical trial.

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Disclosure

The author reports no conflict of interest in this work.

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