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Review

JAK and STAT Signaling Molecules in Immunoregulation and Immune-Mediated Disease

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The discovery of the Janus kinase (JAK)-signal transducer and activator of transcripton (STAT) signaling pathway, a landmark in cell biology, provided a simple mechanism for gene regulation that dramatically advanced our understanding of the action of hormones, interferons, colony-stimulating factors, and interleukins. As we learn more about the complexities of immune responses, new insights into the functions of this pathway continue to be revealed, aided by technology that permits genome-wide views. As we celebrate the 20th anniversary of the discovery of this paradigm in cell signaling, it is particularly edifying to see how this knowledge has rapidly been translated to human immune disease. Not only have genome-wide association studies demonstrated that this pathway is highly relevant to human autoimmunity, but targeting JAKs is now a reality in immune-mediated disease.

The importance of interferons (IFNs) and hormones such as erythropoietin, growth hormone, and prolactin has been recognized for more than half a century. With the advent of the molecular biology era came the discovery of a plethora of other cytokines, which we now know regulate all aspects of cell development and differentiation. Cytokines represent a collection of structurally distinct ligands that bind to different classes of receptors. A major subgroup of cytokines, comprising roughly 60 factors, bind to receptors termed type I and type II cytokine receptors. Cytokines that bind these receptors include type I IFNs, IFN-γ, many interleukins, and colony-stimulating factors. From an immunology perspective, these cytokines are important for initiating innate immunity, orchestrating adaptive immune mechanisms, and constraining immune and inflammatory responses.

As discussed by Stark and Darnell (2012) in this issue, the discovery of Janus kinases and of signal transducers and activators of transcription (JAKs and STATs) stemmed from attempts to understand how IFNs exerted their effect. However, we now know that all type I and II cytokine receptors selectively associate with JAKs (JAK1, JAK2, JAK3, or TYK2). For these receptors, activation of the receptor-bound JAKs is critical for initiating phosphorylation of the cytokine receptor and subsequent recruitment of one or more STATs. Over the past two decades, multiple lines of evidence have clearly established the roles of different JAKs and STATs in mediating the effect of cytokines that use type I and type II cytokine receptors in immunoregulation, host defense, and immunopathology (Darnell et al., 1994; Leonard and O'Shea, 1998; O'Shea and Murray, 2008).

As our understanding of these processes have become more sophisticated, additional roles for this signaling pathway have been recognized. For instance, with the identification of "newer" T helper (Th) cell subsets comes the appreciation of important roles of STATs in these subsets as well as unexpected roles for STATs in recognized subsets. As our understanding of the mechanisms involved in innate immunity expands, previously unrecognized roles of STATs in these processes become evident. In addition, new technologies also allow comprehensive views of STAT action whereas insights from genome-wide association studies clearly implicate JAKs and STATs in human autoimmunity. Finally, the possibility of targeting the JAK-STAT pathway in autoimmune disease has now become a reality.

Recent Insights into the Immunoregulatory Roles of JAKs and STATs

When the STATs were first discovered, the palette of helper T cells was simple: Th1 and Th2 cells. TYK2, JAK2, and STAT4 were found to be critical for interleukin-12 (IL-12) signals and Th1 cell differentiation whereas JAK1, JAK3, and STAT6 were key for IL-4 signaling (Darnell et al., 1994; Leonard and O'Shea, 1998; O'Shea and Murray, 2008). In various models of infectious disease and immune-mediated disease, deficiency of STAT4 and STAT6 had the expected outcomes.

It is now appreciated, however, that Th2 cell responses can occur in the absence of STAT6 (van Panhuys et al., 2008). In fact, early Th2 cell differentiation can by driven by IL-2, which upregulates the transcription factor GATA3 and enhances IL-4 receptor expression (Paul, 2010). Activated by IL-2, STAT5A and STAT5B can directly bind the *Il4ra* gene and promote its expression (Liao et al., 2008); however, STAT5A and STAT5B can also enhance Th1 cell responses by regulating *Tbx21* and *Il12rb2* (Liao et al., 2011b). Interestingly, STAT3 is also a contributor to Th2 cell differentiation and binds Th2 cell-associated gene loci (Liao et al., 2008; Stritesky et al., 2011). Thus, in contrast to the previous views equating STAT6 with Th2 cell differentiation, it appears that this process involves more subtle and complex interactions of STAT3, STAT5, and STAT6 with the relevant genetic loci.

Along with the cytokine TGF-β, IL-2 is a key regulator of differentiation of regulatory T (Treg) cells in the thymus and the

periphery. As mediators of IL-2 signaling, STAT5A and STAT5B are critical for the differentiation of Treg cells. Their effect is very direct in that STAT5A and STAT5B directly bind the *Foxp3* gene and drive expression of this key gene (Yao et al., 2006, 2007; Zorn et al., 2006). In addition, STAT5A and STAT5B regulate *Il2ra*, expression of which is also a critical for Treg cells. Surprisingly, STAT3 also has an important role in Treg cell function (Chaudhry et al., 2009). Deletion of STAT3 in Treg cells results in lethal gastrointestinal disease, but the effect is selective and does not globally impair Treg cell function. Treg cells retain the ability to limit T cell proliferation but have impaired ability to block Th17 cell-mediated pathology. Of interest, STAT3 physically associates with Foxp3.

With the recognition of a multiplicity of fates for T cells, it has become clear that STATs are also key elements for these "new" subsets. We now know that STAT3 is critical for Th17 cell differentiation both in mouse and humans, mediating signals by IL-23 and IL-6 (Chen et al., 2006; Milner et al., 2008). STAT3 regulates Th17 cell differentiation by directly binding *Il17a* and *Il17f*, *Rorc*, and *Il23r*, as well as other genes involved in Th17 cell differentiation (Durant et al., 2010).

Interestingly, IL-2, acting via STAT5A and STAT5B, is an important negative regulator of Th17 cell differentiation (Laurence et al., 2007). In this case, the actions of STAT5A and STAT5B are very direct as they compete with STAT3 binding to the *Il17a-f* locus (Yang et al., 2011). Intriguingly, by sequestering IL-2, regulatory T cells promote Th17 cell differentiation (Chen et al., 2011b; Pandiyan et al., 2011).

One of the newest "lineages" of $CD4^+$ T cells is the follicular helper T cell, which provides help to B cells in germinal centers. Cytokines like IL-6 and IL-21 signal via STAT3 and promote expression of Bcl6 and other molecules that contribute to the phenotype and function of this subset. However, IL-12 and STAT4 also turn out to be drivers of Tfh cells (Nakayamada et al., 2011; Schmitt et al., 2009). STAT4 directly binds many genes involved in Tfh cell differentiation, including *Bcl6* and *Il21*. Conversely, IL-2 inhibits Tfh cell differentiation and once again, the action of STAT5 appears to be very direct. It competes with STAT3 binding to the *Bcl6* locus and also promotes expression of *Prdm1*, which encodes Blimp1 (Johnston et al., 2012; Oestreich et al., 2012).

Perhaps less surprising given its role in transmitting IL-4 signals, STAT6 is also an important regulator of the recently defined Th9 cells (Goswami et al., 2012).

IL-7 and IL-15 are important for $CD8^+$ T cell memory formation and accordingly STAT5A and STAT5B are also important (Hand et al., 2010; Tripathi et al., 2010). STAT5A and STAT5B are essential for the survival of viral-specific $CD8^+$ T cells and expression of Bcl-2. In contrast, in the setting of viral infection, the numbers of $CD4^+$ effector T cells are unaffected by the absence of STAT5A and STAT5B. However, STAT5A and STAT5B are not the only family members important for CD8 cell function; STAT3 is also important, mediating signals by IL-10 and IL-21 (Cui et al., 2011). Expression of such key molecules as Eomes, Bcl-6, Blimp-1, and Socs-3 are all reduced in STAT3-deficient CD8 T cells. Defects in $CD8^+$ T cell memory also occur in patients with hyperimmunoglobulin E syndrome and dominant-negative *STAT3* mutations, which are associated with viral infection (Siegel et al., 2011).

IL-7, acting via STAT5A and STAT5B, is important in B lymphopoiesis, controlling survival and development (Malin et al., 2010). Conversely, the B cell adaptor BLNK antagonizes IL-7 signaling via inhibition of JAK3, and absence of BLNK leads to constitutive JAK-STAT activation and leukomogenesis (Nakayama et al., 2009).

In summary, as we learn more about T and B cells, critical roles for STATs continue to be revealed. Essential functions have been identified in recently recognized subsets of $CD4^+$ T cells. In addition, other roles for STATs in classic subsets are also now recognized.

STATs and Innate Immunity

STATs also have numerous functions in innate immunity—too many to review in detail here, but summarized in detail elsewhere (Murray, 2007; O'Shea and Murray, 2008). The importance of STAT1 in mediating IFN effects has long been recognized, as has the role of STAT3 in IL-6 signaling and the acute phase response. Colony-stimulating factors and cytokines like granulocye macrophage-CSF, granulocyte-CSF, and IL-5, which regulate myeloid development, also signal via STATs. Consequently, STATs have key functions for neutrophils and macrophages (Nguyen-Jackson et al., 2010; Panopoulos et al., 2006; Zhang et al., 2010a). GM-CSF inhibits Flt3L-mediated plasmacytoid DC production and conventional DC growth and STAT5 is important in this process (Esashi et al., 2008). In contrast, STAT3 is important for the expansion of DC progenitors.

The importance of IL-22, acting via STAT3, in regulating the barrier function of epithelial cells and wound repair is a topic of considerable interest (Sonnenberg et al., 2011). Like IL-10, IL-22 is produced by and acts on innate immune cells and has critical anti-inflammatory properties. Precisely how STAT3 promotes inflammation in some circumstances and inhibits in others is an important but challenging question (El Kasmi et al., 2006). STAT3 can negatively regulate IFN responses and has been proposed to inhibit TLR signaling either by inducing anti-inflammatory molecules or by a direct suppression of NF-κB (Wang et al., 2011). Nonetheless, a clear understanding of the pro- and anti-inflammatory actions of STAT3 remains elusive.

Recently, the role of innate immune cells in promoting Th2 cell responses has become increasingly apparent. Thymic stromal lymphopoetin (TSLP) in particular is an important type I cytokine that promotes allergic responses. It acts on multiple cells, especially basophils, which are major producers of IL-4 (Siracusa et al., 2011; van Panhuys et al., 2011). The identity of the JAKs responsible for signaling had been enigmatic, but we now know that TSLP signals via JAK1 and JAK2 to activate STAT5 (Rochman et al., 2010).

In addition to the classical mode of activating macrophages via IFN-γ, the appreciation of the importance of Th2 cytokines in generating alternatively activated macrophages (AAMs) is now recognized. AAMs appear to be important in a range of processes including host defense, fibrosis, metabolic regulation, obesity, and cancer. As IL-4 and IL-13 are major drivers of the AAM, STAT6 is a key player for these cells. STAT6 is important in regulating insulin action, lipid metabolism, and expression of proliferation-activated receptor isoforms (Ricardo-Gonzalez et al., 2010; Szanto et al., 2010). Very recently, AAMs and STAT6 have been implicated in the mammalian thermogenic

response (Nguyen et al., 2011). Intriguingly, AAMs secrete catacholamines in a STAT6-dependent manner and induce thermogenic gene expression in brown adipose tissue and lipolysis in white adipose tissue. Beyond their role as transcription factors, a direct role of STATs in mitochondrial function makes the argument for key roles in metabolism even more compelling (Gough et al., 2009; Potla et al., 2006; Wegrzyn et al., 2009).

Although it has long been recognized that viruses can disrupt IFN signaling by disrupting STAT signaling (Ramachandran and Horvath, 2009), recent work shows that *T. gondii* alters host response by injecting the kinase ROP16 and activating both STAT3 and STAT6 (Butcher et al., 2011; Saeij et al., 2007). In macrophages, the effect is downregulation of proinflammatory cytokine signaling and deviation to an alternatively activated phenotype. Viruses can also activate STAT6 and can do so apparently in a JAK-independent manner (Chen et al., 2011a). In this case though, Stat6 activation is protective in terms of host response.

Toward a Genomic View of STAT Action: Transcriptional and Epigenetic Roles

The advent of chromatin precipitation and massive parallel sequencing (ChIP-Seq) has permitted the understanding of STAT action on a global scale. Analysis of the genome-wide targets of STATs via ChIP-Seq analysis for all the STATs has now been obtained, albeit in a limited number of tissues with relatively few stimuli and time points. Gene expression is dramatically influenced by chromatin organization and until recently, the importance of STATs in regulating epigenetics has been implicated only by analysis of selected regions of certain genes. However, recent technologies in measuring cell-specific transcriptomes and epigenomes, coupled with the use of gene-targeted mice, allows assessments of the global impact of STAT-dependent signaling. What emerges is that STATs have thousands of genomic targets and have major effects on transcription and epigenetic modifications (Durant et al., 2010; Elo et al., 2010; Good et al., 2009; Liao et al., 2011a; Wei et al., 2010). In the case of STAT6, about half of its target genes are affected in terms gene expression, epigenetic modifications, or both when STAT6 is lacking in polarized Th2 cells (Wei et al., 2010). The impact of STAT4 in Th1 cells is less, but this is expected because both STAT4 and STAT1 contribute to Th1 cell differentiation (Schulz et al., 2009).

In addition to their roles in driving transcription, it is also clear from genomic studies that a major role of STATs is to act as functional repressors (Mandal et al., 2011a; Wei et al., 2010; Yang et al., 2011). In B cells, IL-7-mediated activation of STAT5 maintains proliferation and represses Igk germline transcription. Recently it has been shown that STAT5 binds the *Igk* intronic enhancer as a tetramer. This results in the recruitment of the histone methyltransferase Ezh2, which in turn induces histone H3 lysine 27 trimethylation, a repressive mark (Mandal et al., 2011a). Genome-wide analyses showed a STAT5 tetrameric binding motif is frequently associated with transcriptional repression. As indicated above, in T cells STAT5 displaces STAT3 and inhibits IL-17 expression (Yang et al., 2011). In Th1 and Th2 cells, STAT4 and STAT6 binding is frequently associated with repression. However, the mechanism of inhibition is not necessarily mediated by competition; in a large number of cases they bind distinct sites (Wei et al., 2010). Thus, it is clear that STATs can both enhance and repress gene expression depending upon the complexes they recruit.

Equally intriguing is evidence that aside from phosphorylating STATs, JAK can have a direct role in regulating chromatin (Dawson et al., 2009). JAK2 has been found in the nuclei of hematopoietic cells, where it phosphorylates histone H3 tyrosine 41. Phosphorylation of this residue prevents heterochromatin protein 1alpha binding, and thereby counteracts gene silencing (Li, 2008; Shi et al., 2006).

Evidence for Genetic Links between Cytokines and Cytokine Signaling and Human Autoimmune Disease

Although data from numerous animal studies have implicated type I and II cytokine receptors and the JAK-STAT pathway in autoimmune disease, these are models that may or may not reflect actual human disease. However, the study of human genetics provides the ability to directly link genes to human disease. The field has moved rapidly from candidate gene to genome-wide investigation of single-nucleotide polymorphisms (SNPs), and systematic interrogation of the entire genome through next-generation sequencing is also now feasible (Mardis, 2011). Genome-wide association studies (GWAS) have led to an explosion of loci associated with risk of immune-mediated diseases. Importantly, these data show that inherited variation in genes encoding cytokines, type I and type II cytokine receptors, JAKs, and STATs are associated with these disorders (Figure 1).

Among the strongest evidence is work showing that multiple genes in the IL-23 signaling pathway are involved in human autoimmunity. One of the first variants to be identified was a non-synonymous variant of the IL-23R (Arg381Gln) (Duerr et al., 2006), which is associated with reduced risk of inflammatory bowel disease (IBD), psoriasis (Cargill et al., 2007; Nair et al., 2009), and ankylosing spondylitis (Burton et al., 2007). More recently, additional coding variants have been found to influence disease susceptibility to Crohn's and Behcet's disease (Momozawa et al., 2011; Remmers et al., 2010). Subsequently, polymorphisms of the genes encoding both subunits of IL-23 (*IL23A* and *IL12B*), *JAK2*, *TYK2*, and *STAT3* have all been linked to autoimmunity (Bowes et al., 2011; Chu et al., 2011; Franke et al., 2010; Jakkula et al., 2010).

STAT3 is also activated by IL-6 and its receptors, IL-6R and gp130 (encoded by *IL6R* and *IL6ST*, respectively), which have also been implicated in immune-mediated disease (Alloza et al., 2011; Ferreira et al., 2011; Stahl et al., 2010). *IL6R* is also associated with cardiovascular disease (Elliott et al., 2009; Sarwar et al., 2012; Hingorani et al., 2012) and a disease-associated missense allele correlates with serum CRP concentrations (Dehghan et al., 2011; Melzer et al., 2008).

Multiple genes in the IL-12 pathway have also been implicated by GWAS. *IL12A* and *IL12RB2*, which are unique to IL-12 and not shared by IL-23, and STAT4 are associated with multiple autoimmune diseases (Hirschfield et al., 2009; Mells et al., 2011; Radstake et al., 2010; Remmers et al., 2007, 2010; Trynka et al., 2011; Zhernakova et al., 2011). It needs to be borne in mind that STAT4 not only is activated by IL-12, but also can be activated by IL-23 and type I IFNs.

Polymorphisms of genes encoding cytokines, cytokine receptor, JAKs, and STATs are relevant not just to autoimmune

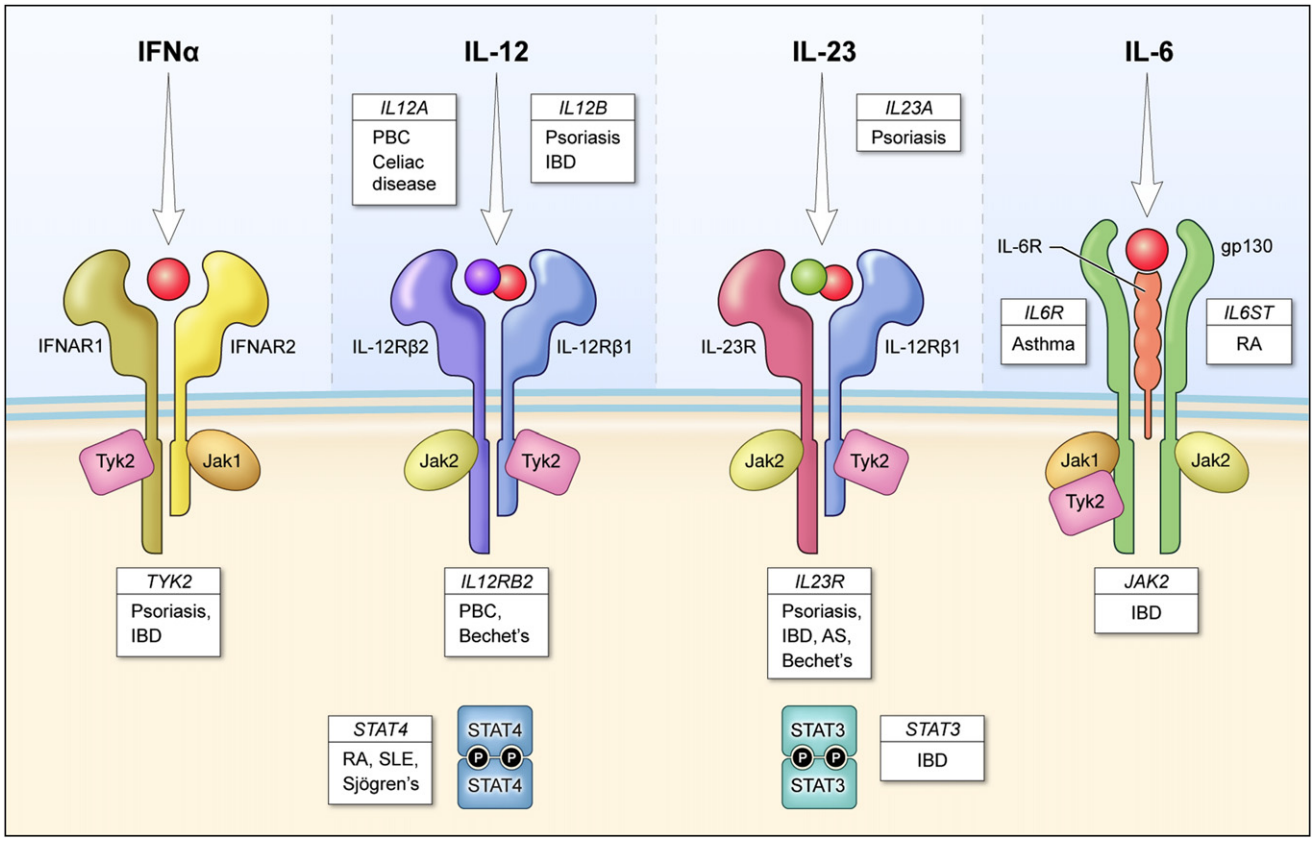

Figure 1. Genetic Links of Cytokine Signaling with Human Autoimmune Disease
Although various animal models have implicated cytokines, their receptors, JAKs, and STATs with autoimmune disease, genome-wide association studies (GWAS) now show that these factors are truly relevant to human disease. This work shows that pathways that lead to STAT3 and STAT4 activation lie at the heart of many common autoimmune diseases. Adapted from Cho and Gregersen (2011). AS, ankylosing spondylitis; IBD, inflammatory bowel disease; PBC, primary biliary cirrhosis; SLE, systemic lupus erythematosus.

disease; they are also pertinent to allergic disease. Specifically, polymorphisms of *STAT6* and *IL13* are associated with elevation of IgE titers and increased risk of atopic dermatitis (Granada et al., 2012; Paternoster et al., 2012). Polymorphisms of *IL6R* are associated with asthma (Ferreira et al., 2011).

Despite these exciting leads, there are challenges of interpreting the biological function of genetic association data. Most disease-associated SNPs fall outside of protein-coding regions, and several genes may be in the region of linkage disequilibrium (LD) surrounding the SNP. The best biological candidate gene in the region is assumed to be the causal gene, but this may not be the correct assumption. For instance, although there is an association of RA and multiple sclerosis with a SNP near the *IL6ST* gene (Alloza et al., 2011; Stahl et al., 2010), there is no direct evidence that the disease-associated variant disrupts *IL6ST* function. Similarly, *IL12RB2* and *IL23R* are adjacent to each other in the genome, and it is not clear whether the associated Behcet's risk allele influences one gene or the other.

Another challenge is inferring function when a gene product can be involved in multiple pathways. STAT4 is one example, but Tyk2 is another—both are involved in signaling by IL-12, IL-23, and type I IFNs. Exactly which signaling pathway is involved in disease pathogenesis? Bioinformatic methods have been developed to search for relationships across genetic risk loci in order to find patterns that might otherwise be difficult to decipher. Future studies aimed at functional integration of genetic risk loci are a major effort to follow up GWAS findings. Regardless, the data clearly implicate the JAK-STAT pathway and cognate cytokines in human immune-mediated disease.

Targeting Cytokine Signaling

The role of cytokine and cytokine signaling in mediating immune-mediated disease, now supported by GWAS data, has made these attractive pharmacological targets (Plenge, 2010). In fact, monoclonal antibodies directed against specific cytokines and cytokine receptors (e.g., ustekinumab, tocilizumab, mepolizumab, lebrikinumab, and daclizumab) have already shown efficacy in a variety of clinical settings. Additionally, the prospect of targeting intracellular signaling by these cytokines is also now a reality.

As discussed by Casanova et al. (2012) in this issue, the unequivocal in vivo importance of the JAK-STAT pathway was established by the identification of patients with severe combined immunodeficiency with *JAK3* mutations. The profound but selective phenotype associated with JAK3 deficiency led to the proposition that targeting JAKs would represent a new class of immunomodulatory drugs (Ghoreschi et al., 2009).

Tofacitinib, formerly designated CP-690,550, has been the most widely studied JAK inhibitor (JAKinib) to be studied in

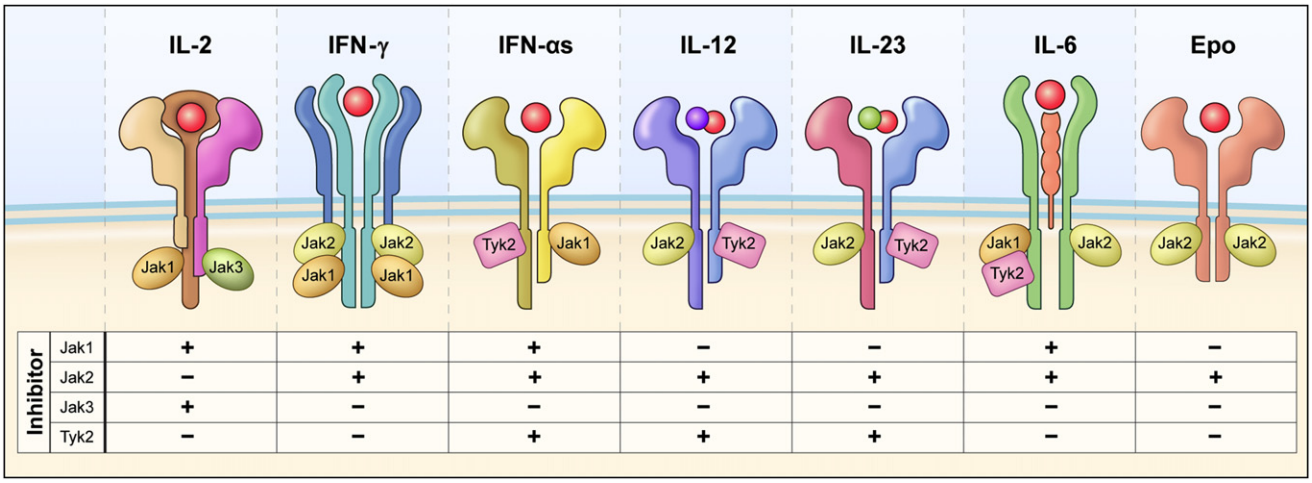

Figure 2. Consequence of Jak Inhibition on Signaling by Key Immunoregulatory Cytokines
A variety of JAKinibs have been developed with varying degrees of specificity for the different JAKs. At present, most inhibitors in clinical use inhibit more than one JAK and examples of the cytokines blocked (as indicated by the plus sign) are shown. Consequently, the first generation of JAKinhibs block multiple cytokines. Selective JAKinibs are in development, but the relative efficacy of drugs that block a single JAK and therefore potentially fewer cytokines versus drugs that inhibit multiple JAKs and many cytokines is not known. A selective TYK2 inhibitor has yet to be reported.

humans. It inhibits JAK3 and JAK1 and to a lesser extent JAK2. Consequently, tofacitinib potently inhibits common γ chain cytokines but also blocks IFN-γ, IL-6, and to a lesser extent IL-12 and IL-23 (Figure 2; Ghoreschi et al., 2011). Functionally, tofacitinib affects both innate and adaptive immune responses (Ghoreschi et al., 2011). Remarkably, tofacitinib has little activity on kinases other than JAKs (Karaman et al., 2008).

Tofacitinib was effective in preclinical models (Changelian et al., 2003) and has shown efficacy in a variety of phase II and III trials in rheumatoid arthritis, as monotherapy and in combination with other drugs (Fleischmann et al., 2012; Kremer et al., 2009, 2012; Tanaka et al., 2011). Importantly, tofacitinib is effective in patients who have failed one or more biologic monoclonal antibody therapies and also prevents destruction of arthritic joints. Tofacitinib is under investigation for the treatment of psoriasis, inflammatory bowel disease, and Sicca syndrome and in the prevention of transplant rejection.

Other JAK inhibitors are also rapidly moving ahead in preclinical assessment and clinical trials (Table 1; Fridman et al., 2010; Lin et al., 2010; Lu et al., 2011; Stump et al., 2011). The JAK1 and JAK2 inhibitor ruxolitinib is efficacious in the treatment of polycythemia and myelofibrosis, disorders as a result of gain-of-function *JAK2* mutations. As might be expected, based on its ability to block cytokines that use JAK1 and JAK2, this drug is also efficacious in arthritis (Fridman et al., 2010). Conversely, drugs that have relative selectivity for individual JAKs (JAK1, JAK2, and JAK3) also appear to have utility in preclinical and early clinical trials (Table 1).

Adverse effects associated with JAKinibs appear to be largely related to their mode of action. Infections are among the common adverse effects, but opportunistic infections are uncommon. Anemia and neutropenia, presumably related to JAK2 inhibition, and interference with signaling by erythropoietin and other colony-stimulating factors can also occur. Increases in serum LDL also occur, as has been seen with the IL-6 blocker tocilizumab. Little reduction in $CD4^+$ T cells has been noted in nonhuman primates treated with tofacitinib, but more substantial reduction in NK cells and $CD8^+$ T cells can occur. Whether this will be pertinent and clinically relevant in humans remains to be determined. A decline in functional Treg cells has not been noted in human subjects in a renal transplant study (Sewgobind et al., 2010).

Given the profound role of cytokines in disorders ranging from malignancy to autoimmunity, JAKinibs have enormous potential utility. The extent to which JAK inhibitors will be used as steroid-sparing agents or even supplant the use of steroids in diseases like the vasculitides or systemic lupus erythematosus remains to be seen. A surprise in the field is that targeting multiple kinases is not necessarily detrimental, especially in circumstances in which multiple cytokines drive pathogenesis. Conversely though, it is conceivable that more selective JAK inhibitors (e.g., selective JAK1 and JAK3 inhibitors) might have efficacy with reduced adverse effects related to JAK2 inhibition. It is likely that we will soon see whether this is the case given the intense interest in JAKinibs.

Given their importance and circumscribed functions, it would also seem logical to target STATs—especially if different STATs could be selectively targeted. A number of STAT inhibitors have been described (Nelson et al., 2011; Yue and Turkson, 2009); however, to date, there is no STAT inhibitor that is near clinical development. Conceptually, one might target STATs by (1) blocking STAT phosphorylation, (2) disrupting STAT binding to phosphorylated receptors or dimerization (both of which are mediated by the STAT Src homology (SH)2 domain), or (3) interfering with DNA binding. Phosphopepitidomimetics continue to be designed that interrupt phosphotyrosine-SH2 binding (Mandal et al., 2011b; Zhang et al., 2010b; Zhao et al., 2010); however, the challenge will be to generate compounds with in vivo efficacy and selectivity. Targeting of the N-terminal domain has also been proposed as a strategy (Timofeeva et al., 2007). Screening of libraries has revealed that small molecules like pimozide, nifuroxaide, and pyrimethamine may also be useful STAT inhibitors (Nelson et al., 2011). Whether any of these strategies ultimately

Table 1. Selected JAKinibs

Agent	Targets	Clinical Indications and Extent of Clinical Trials
tofacitinib	JAK3, JAK1, and JAK2	RA, phase III
		psoriasis, phase II
		IBD, phase II
VX-509	JAK3	RA, phase II
R-348	JAK3	RA, phase I
ruxolitinib	JAK1, JAK2	FDA approved for MF and PV
INCB-28050	JAK1, JAK2	RA, phase II
GLPG-0634	JAK1	RA, phase II
AC-430	JAK2	RA, phase I
		lymphoma, phase I
lestaurtinib	FLT3, TrkA, JAK2	AML, phase III
		psoriasis, phase II
		pancreatic cancer, phase II
CEP-33779	JAK2	preclinical

generate orally available drugs that have efficacy with acceptable safety remains to be determined. However, given the prominent role of STATs in cancer, it is likely that work will continue in this area.

Concluding Remarks

The elegance of the JAK-STAT pathway is that it provides a simple membrane-to-nucleus mechanism for rapidly inducing gene expression. As complexities of immune cell function continue to be unraveled, JAKs and STATs remain central players in all of the key immune cells, ranging from the "newest" $CD4^+$ helper cell subset to alternatively activated macrophages. Curiously, there is still a paucity of information on conditional gene targeting of JAKs and STATs. Although some mouse models were quickly generated and extensively studied, we are still surprisingly ignorant about tissue-specific functions of other JAKs and STATs (e.g., JAK1, JAK2, JAK3, TYK2, STAT1, STAT4, and STAT6).

In addition, although the simplicity of the pathway is appealing, some subtleties have become apparent. For instance, in contrast to a simplistic linear view, most cytokines activate more than one STAT. Precisely what this means in terms of the molecular basis of cytokine action is still being unraveled. However, technologic advances have certainly facilitated a broader understanding of the function of STAT proteins. It is now clear that STATs activate and repress gene expression and serve to organize the epigenetic landscape of immune cells. Nonetheless, our understanding of how this occurs is still in its infancy. Despite the gaps in our knowledge, it is clear that this pathway is directly relevant to human disease and that the pathway can be successfully targeted. For all these reasons, the next twenty years are likely to be just as exciting as the first.

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