New strategies for immunosuppression: interfering with cytokines by targeting the Jak/Stat pathway

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Purpose of review

Numerous immunosuppressants are available, but their adverse effects related to actions on nonlymphoid cells is problematic. Cytokines are key regulators of immune and inflammatory responses, and blocking their actions has become an important modality in treating autoimmune disorders. This review will discuss strategies to develop novel immunosuppressants that arise from advances in the understanding of cytokine signaling.

Recent findings

It is now recognized that large number of cytokines exert their effect by binding to receptors that activate the Janus kinase/signal transducer and activator of transcription pathway, so targeting intracellular signaling pathways is a logical strategy. A selective inhibitor of Janus kinase 3 has now been generated and is effective for transplant rejection in nonhuman primates and other models. Advances have also been made in understanding the functions of Stat family transcription factors, and approaches to interfering with the action of these DNA binding proteins are being devised. In addition, the identification of negative regulators of cytokine signaling offers additional therapeutic opportunities.

Summary

A selective inhibitor of Janus kinase 3 has now been generated and likely represents a new class of effective immunosuppressants. Strategies for targeting signal transducers and activators of transcription pathway are being intensively studied at present and hold potential promise. Multiple endogenous mechanisms exist for negatively regulating cytokine signaling; whether novel therapies can be devised that exploit these mechanisms remains to be determined.

Keywords

cytokines, Janus kinase 3, interleukin, protein inhibitors of activated stats, severe combined immunodeficiency, signal transducer and activator of transcription pathway, suppressors of cytokine signaling

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Abbreviations

γc	common gamma chain
IL	interleukin
Jak	Janus kinase
PIAS	protein inhibitors of activated stats
SOCS	suppressors of cytokine signaling
SCID	severe combined immunodeficiency
SH2	src homology 2
STAT	signal transducer and activator of transcription
SUMO	small ubiquitin-like modifier
Tyk2	tyrosine kinase 2
X-SCID	X-linked severe combined immunodeficiency

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Introduction

There is no shortage of effective immunosuppressive drugs that target a variety of intracellular molecules, but many of the most widely used drugs target ubiquitous molecules. Consequently, these drugs frequently have adverse effects unrelated to their immunosuppressive actions; as a result, a major problem at this time is not the lack of effective immunosuppressive drugs but rather the side effects. It seems logical, therefore, to try to identify agents that target molecules with expression restricted to immune and inflammatory cells. The expectation is that such strategies could generate effective new immunosuppressants with fewer systemic side effects.

Overview of signaling by type I/II cytokine receptors

Because cytokines are key regulators of immunity and inflammation, interfering with these factors has emerged as an effective new strategy for immunosuppression [1,2]. The improved understanding of intracellular cytokine signal transduction affords new opportunities for the development of immunosuppressive drugs. Unfortunately, the term *cytokine* encompasses a wide range of factors that can bind to a variety of different receptors; this makes it challenging for the nonspecialist to keep track of this expanding array of mediators and to make sense of the molecular basis of their action.

Cytokines that bind so-called type I and II receptors constitute more than fifty factors that regulate processes ranging from body growth and lactation to adiposity. Members of this class of cytokines, however, are especially important for regulating hematopoiesis and host defense. This class of cytokines includes interferons and many interleukins (IL). It is not possible to review all the

actions of these cytokines in this short review, but suffice it to say that they are important in immunoregulation and inflammation [3]. These cytokines control both the innate and adaptive immunity. They are critical for lymphoid development, homeostasis, and differentiation. A word of caution, though: Not all interleukins bind to this class of receptor; in this respect, the term *interleukin* can lead to confusion. For instance, IL-8 is actually a chemokine, and its receptor is a seven transmembrane G-protein coupled receptor. IL-1, IL-8, IL-17, IL-18, and IL-25 also do not bind to Type I/II cytokine receptors. Additionally, the receptors for tumor necrosis factor and transforming growth factor-β are not included in this family. Signaling by all of these cytokines is distinct from the pathways discussed herein. The known cytokines that bind Type I/II cytokine receptors are summarized in Table 1.

The mechanism involved in signaling by Type I/II cytokine receptors seems to be remarkably straightforward; indeed, the pathway is recognized as a paradigm in signal transduction [4]. These receptors lack intrinsic enzymatic activity, but rather bind to a small family of cytoplasmic protein tyrosine kinases, known as Janus kinases (Jaks) (Fig. 1). There are four mammalian Jaks: Jak1, Jak2, Jak3, and tyrosine kinase 2 (Tyk2) (Table 2). Binding of cytokines to their cognate receptors activates the associated Jak, which in turn autophosphorylates and phosphorylates the receptor. Tyrosine phosphorylation of cytokine receptors provides docking sites for a variety of signaling molecules. The generation of knockout mice and analysis of deficient cell lines have established that Jaks are essential for the initiation of cytokine signaling. By inference, a Jak inhibitor would also block cytokine signaling. As will be discussed, the different Jaks have very distinct functions (Table 2), and this needs to be borne in mind with respect to inhibiting this class of kinases.

One critical family of signaling molecule that binds to phosphorylated cytokine receptors is the group of DNA binding proteins known as the signal transducers and activators of transcription (Stats). These cytosolic proteins bind tyrosine phosphorylated cytokine receptors through their src homology 2 (SH2) domains and then are phosphorylated themselves by Jaks (Fig. 1). The phosphorylated Stats dimerize, translocate to the nucleus, bind DNA at specific elements, and regulate gene expression. There are seven mammalian Stats, which have specific functions (Table 2) [5–7].

Janus kinase 3, γc, and immune cell function

Cytokines that bind Type I/II cytokine receptors can be subdivided according to their use of shared receptor subunits. One subfamily includes the cytokines IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21; all these cytokines use a common receptor subunit termed the common gamma chain (γc) in conjunction with a ligand-specific subunit [8–10]. Mutations of γc underlie X-linked severe combined immunodeficiency (X-SCID) and account for roughly half of all known cases of SCID (Fig. 2) [11–14]. Deficiency of γc blocks signaling by IL-7, IL-15, IL-4, and IL-21. IL-7 is critical for lymphocyte development and homeostasis of mature peripheral lymphocytes [15,16]. IL-15 is essential for natural killer cell development [17–20]. IL-4 is critical for the differentiation of Th2 cells and works in concert with IL-21 to regulate immunoglobulin class switching in B cells [21–23]. Thus, γc mutations result in a phenotype of SCID designated $T^-B^+NK^-$, indicative of the fact that T and natural killer cells are absent. Although B cells are present, they are poorly functional, with defective B cell activation and abnormal class switching.

Intracellularly, γc associates with a specific Jak: Jak3. In contrast to other Jaks, which are widely expressed and bind multiple cytokine receptors, Jak3 is predominantly expressed in hematopoietic cells and uniquely binds γc [24–27]. Accordingly, mutations of Jak3 deficiency also result in $T^-B^+NK^-$ SCID (Fig. 2) [28–33].

The development of a selective Janus kinase 3 antagonist

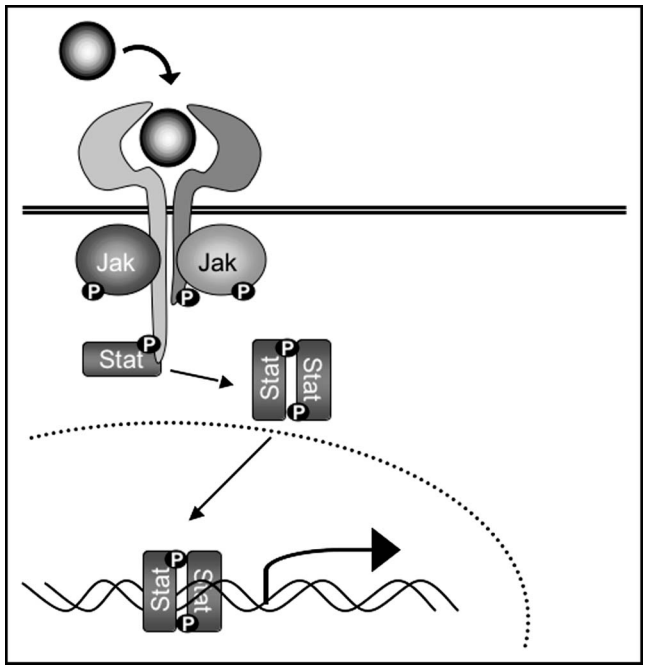
A corollary of the discovery that Jak3 is required for immune cell development is that purposefully interfering with Jak3 activity or function could be the basis for a novel class of immunosuppressants. Moreover, because Jak3 deficiency results in immunodeficiency and not pleiotropic defects, a highly specific Jak3 inhibitor should also have very limited and precise effects. This contrasts sharply with widely used immunosuppressive drugs, which are directed against ubiquitous targets and have diverse side effects. In principle, the selectivity of a Jak3 inhibitor would have advantages over the current agents.

There has been extensive effort to identify Jak inhibitors, and several inhibitors have been reported to have such activity. They include tryphostin (AG-490), dimethyoxyquinazolines (WHI-P154, WHI-P131), undecylprodigiosin

Table 1. Cytokines that bind type I/II receptors

Receptors	Cytokines that bind
Type I cytokine receptors	IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-23, IL-27, IL-31, growth hormone, prolactin, erythropoietin, thrombopoietin, granulocyte colony stimulating factor (CSF), granulocyte-macrophage-CSF, leptin, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, cardiotropin-1
Type II cytokine receptors	IFNα/β, IFNγ, other interferons, IL-10, IL-19, IL-20, IL-21, IL-22, IL-24, IL-26, IL-28A, IL-28B, IL-29, limitin

Figure 1. Critical role for Jak and Stats in cytokine signaling



Binding of a cytokine to its cognate receptor activates the associated Janus kinase (Jak). The Jak in turn phosphorylates the receptor, which provides a docking for signal transducers and activators of transcription (Stats) and other signaling molecules to bind the receptor. Stats also become phosphorylated and translocate to the nucleus, where they regulate gene expression.

antibiotics (PNU156804), octylaminoundecyldimethylxanthine (CT2576, CT5589), leflunomide, cyclic pyridones, and naphthyl ketones [34•,35]. Some of these are not selective for Jak3 and inhibit other Jaks. Other inhibitors affect disparate pathways, including nuclear factor-kB and T cell receptor signaling or inhibit unrelated tyrosine kinases.

However, an orally available, selective Jak3 antagonist has now been developed (Fig. 3) [36]. The drug, designated CP-690,550, has nanomolar potency against Jak3 and is efficacious in preventing transplant rejection in animal models, including a nonhuman primate renal transplant model; in fact, in the primate model, CP-690,550 was more effective as a single agent than cyclosporine A. One critical issue pertaining to a potential Jak3 antagonist is the extent to which other Jaks are inhibited. Interfering with Jak2 would be particularly problematic because Jak2 is essential for signaling by many hematopoietic cytokines, including erythropoietin, thrombopoietin, and GM-CSF (Table 2). Significant inhibition of Jak2, therefore, could result in anemia, and thrombocytopenia [37]. CP-690,550 is approximately 30 to 100 times less potent for Jak2 and Jak1, respectively, and did not cause granulocytopenia or thrombocytopenia. At the highest doses, mild anemia was noted, indicating that Jak2 antagonism is likely not to be an overwhelming concern for CP-690,550. Animals treated with CP-690,550 did show a modest decline in natural killer cells, presumably because of inhibition of IL-15 signaling; whether this will be clinically relevant with respect to viral infections remains to be determined.

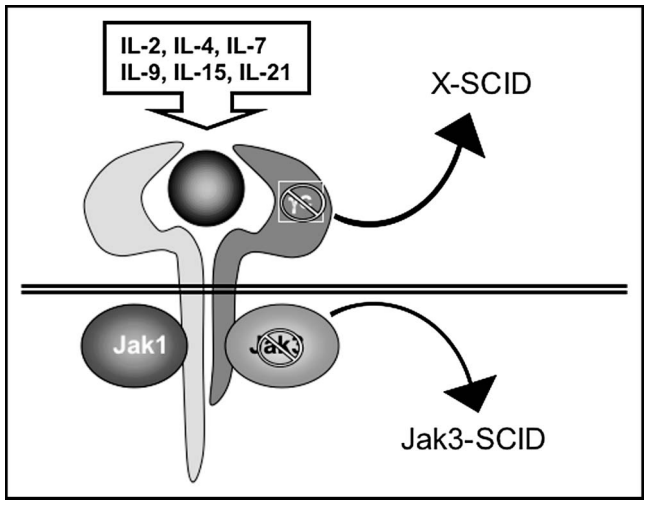
In addition to transplant rejection, clearly CP-690,550 has potential utility in several other clinical settings. The issue of adverse effects is especially important, given that autoimmune disorders occur more frequently in young women in their childbearing years and that treatment is often lifelong. Inhibition of Jak3 might be useful for a range of autoimmune diseases, including psoriasis, psoriatic arthritis, graft-versus-host disease, multiple sclerosis, inflammatory bowel disease, systemic lupus erythematosus, and rheumatoid arthritis. The latter disease, rheumatoid arthritis, is of particular interest because of the role of IL-15 in the pathogenesis of this disorder [38]. For example, targeting of the IL-15R using an antagonistic IL-15-Fc fusion protein prevented the development of arthritis and blocked the disease progression [39]. By inference, attenuating IL-15 signaling by inhibiting Jak3 should also be efficacious. IL-4 and IL-9 promote allergic responses, so a Jak3 inhibitor might also be useful in these disorders [40–42].

A theoretic issue with the use of a Jak3 antagonist relates to inhibition of IL-2 signaling. Because IL-2 deficiency is important for maintenance of peripheral tolerance, it is conceivable that inhibition of IL-2 signaling could

Table 2. In vivo function of Jaks and Stats

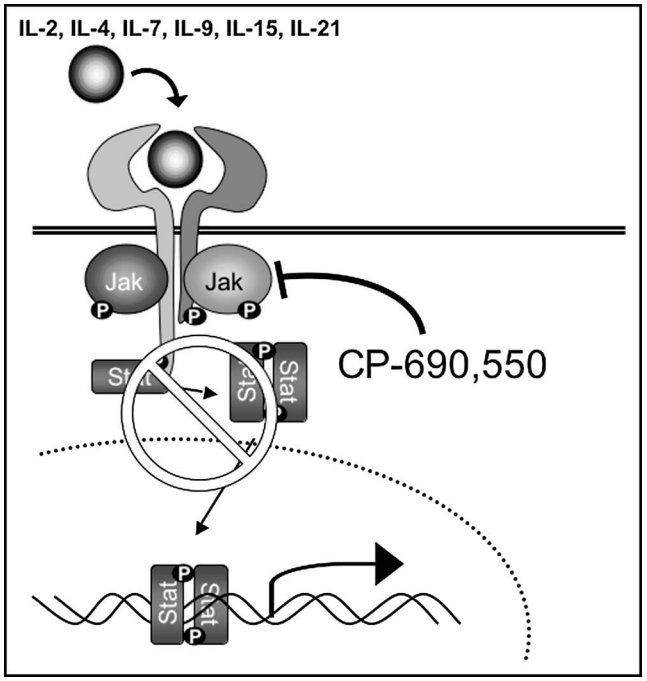
Jak/Stat	Cytokines that activate	Phenotype of knockout
Jak1	gp130 cytokines, Type I IFN, IFN-γ, γc cytokines	perinatally lethal, neurologic defects, SCID
Jak2	erythropoietin, thrombopoietin, prolactin, growth hormone, βc cytokines, IFN-γ, IL-12	embryonically lethal, defective erythropoiesis
Jak3	γc cytokines	SCID
Tyk2	gp130 cytokines, Type I IFNs, IL-12, IL-23	modest viral susceptibility, reduced IL-12 response and resistance to arthritis
Stat1	Type I IFNs	impaired anti-viral response
	IFN-γ	Increased tumors
Stat2	Type I IFNs	impaired anti-viral response
Stat3	Many cytokines especially gp130 cytokines	embryonically lethal
Stat4	IL-12	defective Th1 differentiation
Stat5A	prolactin, other cytokines	defective mammary gland development
Stat5B	growth hormone, other cytokines	impaired sexually dimorphic growth
Stat6	IL-4	defective Th2 differentiation IgE production

Figure 2. Molecular basis of SCID



Mutations of γc are the basis of X-linked severe combined immunodeficiency (SCID) and account for almost half of SCID. In addition, mutations of Janus kinase 3 and interleukin (IL)-7R disrupt cytokine signaling and also cause SCID. Together, mutations of these three genes seem to account for two thirds to three quarters of the cases of SCID.

Figure 3. Inhibition of Jak3 as a novel mode of immunosuppression



A selective Janus kinase 3 antagonist inhibits Janus kinase activity, blocking early cytokine signaling and resultant Stat activation. This abrogates cytokine-dependent gene regulation and subsequent lymphocyte activation. Signal transducers and activators of transcription (Stats) also have critical functions in mediating cytokine signaling; in principle, targeting Stats would also be useful to generate novel immunosuppressants. Strategies being developed included phosphopeptidomimetics that block src homology 2–phosphotyrosine interactions and decoy oligonucleotides.

exacerbate autoimmunity. Monoclonal antibodies against IL-2R-α (CD25, basiliximab, and daclizumab) are used for transplant rejection; however, these agents have not been reported to induce a breakdown in peripheral tolerance and autoimmune disease [43]. A Jak3 inhibitor, which would antagonize all the γc cytokine receptors, would be more immunosuppressive than an IL-2R antagonist. Consequently, the expectation is that such an agent would be even less likely than anti-CD25 antibodies to interfere with tolerance. Obviously though, this possibility will need to be borne in mind in clinical trials.

Targeting other Janus kinases

Tyk2$^{-/-}$ mice have impaired IL-12 signaling, and mice with a mutation in Tyk2, have marked resistance to the development of collagen-induced arthritis [44–48]. Therefore, targeting Tyk2 might be a useful strategy for the treatment of Th1-mediated disorders such as arthritis. It should be noted that IL-23 also uses the IL-12Rβ and activates Tyk2, but the effect of Tyk2 deficiency on IL-23 responses has not been examined [49,50]. Deficiency of Jak1 or Jak2 results in perinatal or embryonic lethality, respectively. Therefore, targeting these kinases could have significant toxicities. One could imagine, however, that in the treatment of cancers or leukemia, a greater level of toxicity might be acceptable, assuming that the drug is efficacious.

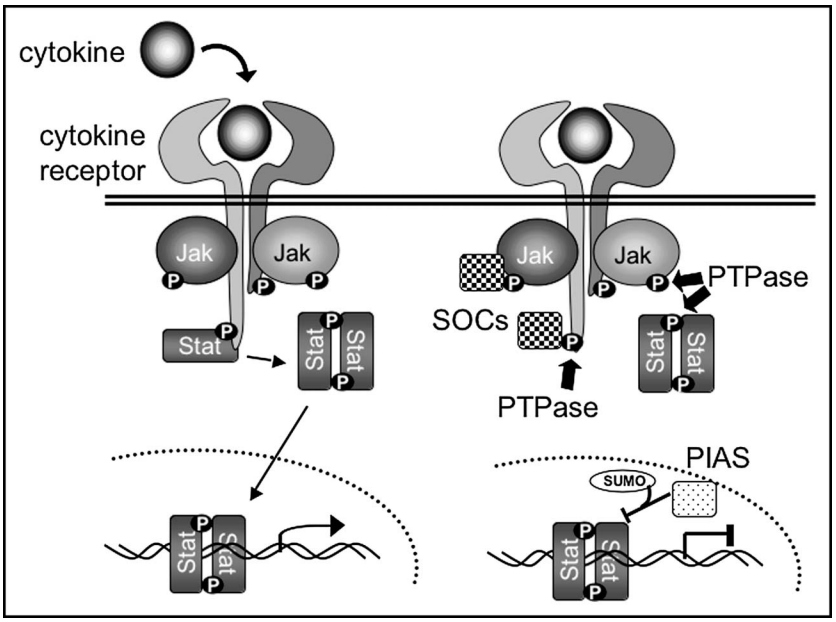
Targeting Stats

Because of their critical and selective functions, Stats are also attractive drug targets. Because they do not have enzymatic activity, one must block Stat expression, recruitment to cytokine receptors, dimerization, or DNA binding. Cytokine recruitment and dimerization are mediated by phosphotyrosine-SH2 interactions, so peptidomimetics have been designed to disrupt these interactions [51•,52]. Although phosphotyrosine-SH2 interactions are important for many aspects of intracellular signaling, the generation of phosphopeptidomimetics has previously met with little success. An alternative strategy is the use of decoy oligonucleotides, which would interfere with Stat binding to endogenous DNA [53–55].

Assuming that Stat inhibitors can be successfully devised, which Stats would be useful to target? In terms of immunoregulation, Stat4 and Stat6 might be useful targets [7,21,22,56]. These Stats are critically important for the differentiation of helper T cells. IL-4 activates Stat6, promoting Th2 cell differentiation and allergic response, whereas IL-12 activates Stat4 and drives differentiation of naïve T cells to Th1 cells. These cells produce interferon-γ, which is critical for host defense against intracellular pathogens but also contributes to many autoimmune diseases. In addition, constitutive activation of Stat3 and Stat5

Figure 4. Negative regulation of cytokine signaling

Tyrosine phosphatases can dephosphorylate activated Janus kinases (Jaks), cytokine receptors, or signal transducers and activators of transcription (Stats) and thereby attenuate cytokine signaling. Suppressors of cytokine signaling (SOCS) proteins bind Jaks or phosphorylated cytokines receptors. Compounds that mimic the effects of SOCS protein might also interfere with cytokine signaling. Protein inhibitors of activated stats (PIAS) also bind to Stats and inhibit Stat-dependent signaling. SOCS and PIAS proteins are shown as a hatched box and a dotted box, respectively.



has been noted in a significant proportion of tumors, and increasing attention is being paid to targeting these Stats in cancer [51•,57•,58,59]. Inhibiting Stat3 may be complicated in that the lack of Stat3 in myeloid cells could promote autoimmune disease [60].

Negative regulators of cytokine signaling

Cytokine signaling can be attenuated by a variety of including tyrosine phosphatases, protein inhibitors of mechanisms activated stats (PIAS) family members, and suppressors of cytokine signaling (SOCS) (Fig. 4) [61•]. SOCS proteins, classic feedback inhibitors of signaling, bind with their SH2 domains to phosphotyrosine residues in Jaks (SOCS1) or cytokine receptors (SOCS2, SOCS3, and CIS), and block signaling. On the basis of the phenotype of knockout mice, different SOCS family members seem to have distinct functions [61•]. There are four family members in PIAS proteins – PIAS1, PIAS3, PIASx, and PIASy – some of which also seem to have restricted functions. Although all PIAS proteins interact with and inhibit Stat proteins, the mechanisms by which this occurs seems to differ between family members [62••]. PIAS1 and PIAS3 inhibit Stat1, Stat3, and Stat5 activity, respectively, by blocking Stat DNA binding [63,64]. Conversely, the inhibition of Stat4 and Stat1 by PIASx and PIASy does not affect Stat DNA binding and must occur by a distinct mechanism [65]. PIAS proteins have also been shown to have E3 small ubiquitin-like modifier (SUMO) ligase activity. Covalent SUMO modification of target proteins is similar to ubiquitylation, but sumoylation is not considered to target proteins for degradation, and the consequence of Stat sumoylation is unknown. [66–68]. Knockout mice lacking PIAS1 and PIASy have been generated. PIAS1 knockout mice had enhanced interferon responses, whereas PIASy knockout mice demonstrated mild defects in interferon signaling [62••,69,70]. In principle, mimics or inducers of SOCS and PIAS proteins would be immunosuppressive in that such agents would be expected to attenuate the effects of cytokines [71•]. Conversely, activators of the tyrosine phosphatases that regulate Jaks and Stats would also inhibit signaling, but it is not yet clear how drugs can be designed or whether these are truly feasible approaches.

Conclusion

In summary, studies in humans with mutations of Jak3 and its associated receptor subunits have predicted that selective Jak3 antagonists could represent a new class of immunosuppressants. In contrast to the targets of existing drugs, Jak3 has limited tissue expression and discrete functions. In principle, a highly selective inhibitor would not be associated with the toxicities seen with existing immunosuppressants. A selective Jak3 antagonist, CP-690,550, has now been developed, and it is not associated with unacceptable toxicities indicative of substantial Jak2 inhibition. The drug is effective in models of transplant rejection, including studies in nonhuman primates. As the drug moves toward clinical trials in humans it will be important to determine other clinical settings ranging from autoimmunity, allergy, and cancer in which this new agent might be useful. The successful generation of a selective Jak inhibitor suggests that targeting other Jaks is feasible; targeting Tyk2 might be another strategy for treating immune-mediated disease. In principle, target

Stats and the negative regulators of cytokine signaling could be of use, and these molecules will surely continue to receive considerable attention as therapeutic targets.

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