JAK3 Specific Kinase Inhibitors: When Specificity Is Not Enough

Luk Cox[1,2] and Jan Cools[1,2,*]
[1]Center for Human Genetics, K.U.Leuven, Leuven, B-3000, Belgium
[2]Department of Molecular and Developmental Genetics, VIB, Leuven, B-3000, Belgium
*Correspondence: jan.cools@med.kuleuven.be
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Janus kinases are important signaling proteins implicated in cytokine signaling. In particular, Janus kinase 3 (JAK3) has gained attention as a target for inhibition of the immune system, due to its importance for T and B cell development and function. In this issue however, Haan et al. (2011) show that inhibition of JAK3 activity may not be sufficient for this purpose.

Janus kinases (JAKs) are cytosolic tyrosine kinases that associate with cytokine receptors. Since cytokine receptor proteins lack any enzymatic activity, they are completely dependent on the tyrosine kinase activity of the JAKs to initiate signaling upon binding of the cytokines. Based on different properties, domains, and motifs, the cytokine receptors are divided in five major subgroups, as shown in Table 1.

JAKs are thus critically important for cytokine signaling in the immune system, but are also required to regulate cell proliferation, survival, development, and differentiation of a variety of cells. Given the importance of cytokine receptor signaling for the development and functioning of the hematopoietic system, it is not surprising that both gain-of-function mutations and loss-of-function mutations of JAKs are implicated in human hematological diseases. Activating mutations in JAK1, JAK2, and JAK3 have been identified as causes of hematological cancers, while inactivating mutations of JAK3 and TYK2 are known causes of immune deficiency (Vainchenker et al., 2008). Mutations in the IL7 receptor (IL7R and the common gamma chain)- JAK3 complex are specifically important, as they account for approximately 70% of severe combined immunodeficiency cases (Ghoreschi et al., 2009). Also it was confirmed in mouse models that the absence of JAK3 or the common gamma chain leads to immunodeficiency due to defects in both B cell and T cell development and activation (Pesu et al., 2005). Inactivation of *Jak1* or *Jak2* genes in mice causes severe defects and results in early lethality. In agreement with this, inactivating mutations in *JAK1* or *JAK2* have not been identified in immunodeficiency patients, most likely because the loss of these kinases would cause a plethora of severe defects that would be incompatible with life (Ghoreschi et al., 2009).

From these insights in the role of JAK3 for the development and functioning of the immune system, development of JAK kinase inhibitors was initiated, with possible applications for the treatment of autoimmune diseases, inflammation, and the prevention of organ transplant rejection (Changelian et al., 2003; Kudlacz et al., 2004; Pesu et al., 2005). JAK3-specific inhibitors were hypothesized to be as effective as general JAK inhibitors but less toxic, given the more restricted expression of JAK3 and results from knockout mice.

Previous attempts, however, to design JAK3-specific inhibitors have not been very successful. Initially, the inhibitor CP-690,550 was designed as a JAK3-specific inhibitor, but subsequent analysis of this compound revealed it is also a potent inhibitor of the other JAKs, including JAK1 (Karaman et al., 2008). NIBR3049, a novel JAK inhibitor recently described to be more specific for JAK3, could be a JAK3-specific inhibitor, as it has shown more than 100-fold lower activity on the other JAKs, based on in vitro kinase assays (Thoma et al., 2011). Surprisingly, this compound displayed low activity in cellular assays, measuring cytokine-induced signaling through receptors dependent on the common gamma chain.

Based on these observations, Haan et al. (2011) performed an in-depth analysis of the exact role of JAK3 inhibition in cytokine receptor signaling. This work clearly shows that an intact JAK3 protein is strictly required for functioning of the IL2 receptor (containing the IL2R beta chain and common gamma chain). When JAK3 is absent or when its expression is decreased by siRNA-mediated knockdown, activation of the receptor is not possible. This is in agreement with previous work that showed that mutations

Table 1. Cytokine Receptors and the Involvement of JAK Kinases

Type	Subgroup	Receptors for	JAK Kinase Usage
Type I	Homodimeric	EPO, TPO, GH, G-CSF	JAK2
Type I	Using the common beta chain (CSF2Rβ, CD131)	IL3, IL5, GM-CSF	JAK2
Type I	Using the gp130 chain (IL6ST, CD130)	IL6, IL11, OSM, LIF	JAK1, JAK2, TYK2
Type I	Using the common gamma chain(γc, IL2Rγ, CD132)	IL2, IL4, IL7, IL9, IL15, IL21	JAK1, JAK3
Type II	—	IFNα, IFNβ, IFNγ, IL10, IL19, IL20, IL22, IL24, IL28, IL29	JAK1, JAK2, TYK2

The receptors for EPO, TPO, GH, and G-CSF are homodimers; all other cytokine receptors are heterodimers containing a ligand-specific chain and a common chain used by different receptors. JAK3 is required for signaling of the type I receptors that use the common gamma chain (γc).

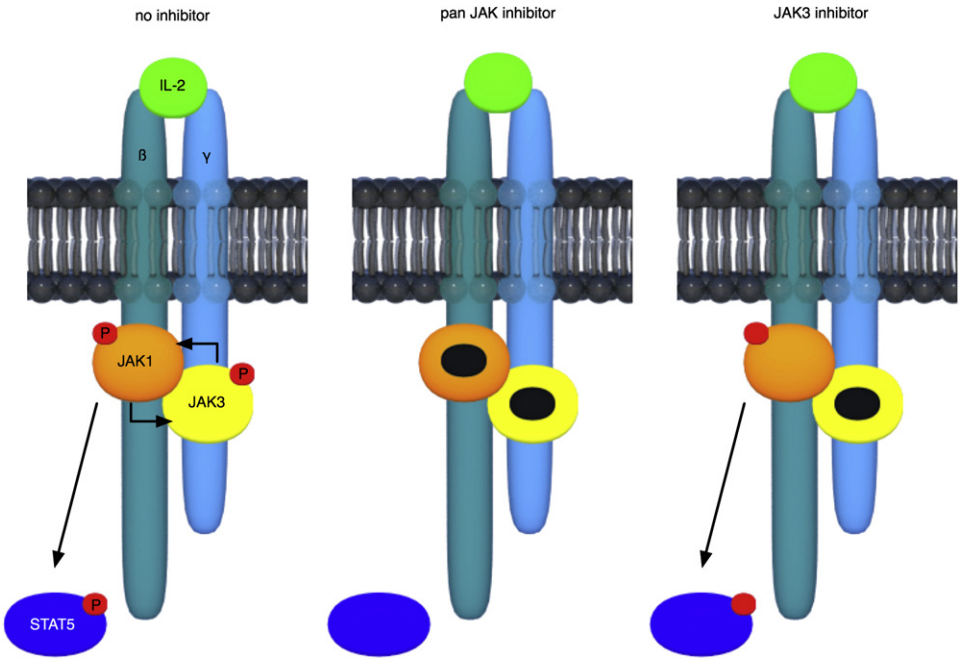

Figure 1. Overview of the effect of JAK inhibitors on IL2 receptor signaling
In the IL2, heterodimeric receptor JAK1 is bound to the beta receptor and JAK3 is bound to the common gamma receptor. Note that the nonsignaling alpha chain of the IL2 receptor is not shown in this graph. IL2 stimulation results in the activation of JAK1 and JAK3 through cross phosphorylation, and JAK1 also phosphorylates STAT5. Work by Haan et al. (2011) shows that inhibition of JAK3 using a JAK3-specific inhibitor only slightly affects JAK1 and STAT5 activity, and that a JAK inhibitor with JAK1 inhibitory activity is needed to fully inhibit JAK1 and STAT5 phosphorylation.

or deletions in JAK3 can affect the trafficking of the common gamma chain, and without the presence of the common gamma chain, the IL2-type receptors (type I cytokine receptors using the common gamma chain) cannot function (Pesu et al., 2005). In contrast to this, Haan et al. found that JAK3 kinase activity is not strictly required for signaling of the IL2 receptor (Figure 1). Expression of a kinase-inactive JAK3 reduced activation of downstream signaling proteins, but did not completely abolish it. In addition, specific inhibition of JAK3 kinase activity did not significantly reduce STAT5 phosphorylation (Haan et al., 2011). While JAK3 kinase activity is thus not essential, JAK1 kinase activity was indispensable, and inhibition of JAK1 or expression of a kinase inactive JAK1 protein completely abolished signaling (Figure 1).

These data, together with early data illustrating the low activity of a JAK3-specific inhibitor, question the potency of JAK3-specific inhibitors for modulating the immune response. In contrast, JAK inhibitors with activity against JAK3 but also JAK1 show potent inhibitory activity on cytokine receptor signaling. It remains to be investigated if these inhibitors will pass or fail in ongoing clinical trials with respect to activity and, importantly, toxicity.

Based on previous studies that showed that the association of JAK3 with the common gamma chain is required for its transport to the plasma membrane and on the data presented here, which confirm that siRNA-mediated knockdown of JAK3 is highly effective in inhibiting IL2-induced signaling on the IL2 receptor, one could speculate that compounds that interfere with the binding of JAK3 to the common gamma chain would be effective and selective for inhibiting gamma chain-dependent cytokine receptor signaling. The development of such inhibitors that target protein-protein interactions is, however, not trivial and may not be needed if the general JAK inhibitors show potent activity and a good safety profile.

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