Jaks and Stats as therapeutic targets

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Abstract

Cytokines have critical functions in regulating immune responses. A large number of these factors bind related receptors termed the Type I and Type II families of cytokine receptors. These receptors activate Janus kinases (Jaks) and Stat family of transcription factors. The essential and specific function of Jaks and Stats is particularly well illustrated by human and mouse mutations. The possibility that these molecules could be targeted to produce novel immunosuppressive compounds is considered in this review.

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Many of the cytokines involved in immune and inflammatory responses bind to receptors designated as Type I cytokine receptors. For instance, the receptors for interleukins (IL) 2 to IL7, IL9 to IL13 and IL15, all belong to this family. Also included in this family are the receptors for ciliary neurotrophic factor, leukaemia inhibitory factor, oncostatin M and cardiotropin 1. The receptor family also binds hormones, like erythropoietin (EPO), thrombopoietin, prolactin (PRL), growth hormone (GH) and leptin, and colony stimulating factors (CSF), such as granulocyte CSF and granulocyte/macrophage CSF. Closely related are the Type II cytokine receptors that bind interferons (IFNs) and IL10. Both Type I and II receptors have no intrinsic enzymatic activity. However, the membrane proximal segment of these receptors is conserved and is responsible for binding Jaks. Indeed, it has become clear through a variety of studies that Jaks play a pivotal part in signalling via this family of cytokine receptors. Upon ligand binding, Jaks are activated and initiate signalling by phosphorylating cytokine receptors. The phosphorylated receptors, in turn, are recognised by various signalling molecules, one important class of which is the Stat (signal transducer and activator of transcription) family of DNA binding proteins. Stats also have specific and essential functions in cytokine signalling. Consequently, it is of interest to consider these molecules as potential therapeutic targets.

Janus kinases

The Janus kinase family is a small family of protein tyrosine kinases; only four vertebrate Jaks have been identified (Jak1, Jak2, Jak3 and Tyk2). The essential functions of the Jaks in signalling by interferons and cytokines were first established using a panel of mutagenised cell lines made resistant to the effects of interferons.[1] Through reconstitution experiments, it was first shown that Jaks are required for interferon signalling and it is now recognised that all type I and II cytokine receptors activate various Jaks.[2][3] Some Jaks (Jak1, Jak2 and Tyk2) are used by a variety of cytokine receptors, whereas Jak3 is used only by cytokines whose receptors comprise the common cytokine γ chain, γc. The pivotal function of the Jaks is best illustrated in mice or humans deficient in these kinases. The first deficiency of a mammalian Jak was identified in a human disease.[4][5] Severe combined immunodeficiency (SCID) comprises various disorders manifested by T and B lymphocyte abnormalities, associated with severe infections early in life. The most common form of SCID, X-SCID, is attributable to mutations of γc, which results in impaired signalling via all the cytokines that utilise this receptor subunit (IL2, IL4, IL7, IL9 and IL15). As Jak3 associates with γc, the possibility of Jak3 mutations was investigated in selected SCID patients and we demonstrated that mutation of either γc or Jak3 leads to the same functional defects. Shortly thereafter Jak3 knockout mice were generated, and they, too, have defects of T, B and NK cells; no other defects have been reported.[6–9] The T and B lymphocyte abnormalities in γc and Jak3 deficient mice and humans are principally attributable to the failure of IL7 signalling, as mice and humans with IL7R mutations also have SCID.[10] In contrast, defective NK development in SCID patients is probably the result of defective IL15 signalling.[11][12]

Unlike Jak3, deficiency of Jak1 and Jak2 results in more diverse abnormalities.[13–15] Jak1 -/- mice die perinatally, apparently because of impaired neurological development.[13] Like Jak3 -/- mice, Jak1 knockout mice also have SCID. This is explained by the fact that Jak1 binds the ligand specific subunit of γc using receptors. Other cytokine receptors that require Jak1 include: gp130 cytokine receptors (for IL6, LIF, OSM, CNTF, and IL11) and Type II cytokines receptors (for IL10, IFNα, IFNβ, and IFNγ).

In contrast with Jak3 and Jak1 deficient mice, Jak2 knockouts die as embryos because Jak2 is essential for EPO signalling and Jak2 -/- mice do not form blood.[15]

The critical function of Jaks is supported by another body of evidence. Specifically, chromosomal translocations involving the 3' region of Jak2 gene and the 5' region of the Tel transcription factor gene have been associated with leukaemia.[16–19] Experimentally it has been established that uncontrolled Jak2 signalling produced by this fusion is transforming.

At present, the three dimensional structure for any of the Jaks is still lacking, so our understanding of Jak structure is quite limited. Overall conservation of Janus kinases has been

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noted and the regions of homology are termed Janus homology (JH) domains. The C-terminal tyrosine kinase or JH1 domain is the catalytically active portion of the molecule. Amino terminal to the kinase domain is a segment homologous to a kinase domains but which lacks catalytic activity. This segment is termed the pseudo kinase or JH2 domain and is unique; no other mammalian tyrosine kinase has such a domain. While it lacks catalytic activity, this domain seems to a have regulatory function.[20–22] The association with cytokine receptors is mediated by the N-terminus of the Jaks. For Jak3, a relatively small portion of the molecule confers most of its ability to bind γc.[23 24] For other Jak/receptor interactions, the segments mediating this interaction may be more extended than those of Jak3.[25 26]

As Jaks are essential elements in cytokine signalling, it has been widely recognised that these kinases might be reasonable targets for the development of novel immunosuppressants.[27] Because Jak3 deficiency has such specific defects (that is, limited to lymphoid cells), it has been suggested that this kinase might be a particularly good target.[27] Conversely, based on the phenotypes of the knockout mice, Jak1 and Jak2 would not be expected to be good targets; the toxicity of targeting these kinases might be substantial.

Before answering the question of whether the development of a Jak3 inhibitor is feasible, it is reasonable to ask whether the development of any specific protein kinase inhibitor is attainable. The answer, though, seems clear; Bcr-Abl, EGFR, HER2, and protein kinase C (PKC) inhibitors are currently in Phase I and II clinical trials.[28] Moreover, delineating the three dimensional structure of various tyrosine kinases should facilitate the development of specific inhibitors.[29–31]

A number of compounds have been reported to be Jak inhibitors.[32–41] Exactly how specific they are for Jaks and in particular, Jak3 in vitro and in vivo, has yet to be firmly established. None the less, given their unique structure, it should be possible to generate Jak specific inhibitors. However, developing a Jak3 specific inhibitor may be more challenging. For instance, the most widely tested compound, the tyrphostin AG-490, inhibits both Jak2 and Jak3. The likelihood that a Jak2 inhibitor would cause unacceptable toxicity (for example, cytopenias), seems considerable.

Stats

After Jaks are activated by cytokine binding to cognate receptors, they phosphorylate receptor subunits, creating docking sites for various signalling molecules. Signal transducers and activators of transcription (Stats), latent cytosolic transcription factors, bind to phosphorylated cytokine receptors via their SH2 domains; different Stats bind to specific cytokine receptors.[1 2 42 43] Stats in turn, are phosphorylated by Jaks, effecting their dimerisation via reciprocal SH2-phosphotyrosine interactions. This leads to their nuclear translocation where they regulate gene transcription. Stats bind two types of DNA motifs, ISREs (IFN stimulated response elements, consensus AGTTTNCNTTTCC) and GAS elements (γ activated sequence, consensus TTCNNNGAA). Thus, as the name implies, Stats serve essential functions in rapidly transducing signals from cytokine receptors to the nucleus, where they bind DNA and regulate gene transcription. These functions are readily explained by Stat structure; indeed in the case of the Stats, we are more fortunate than with the Jaks, as the three dimensional structure of this family of transcription factors has been solved. Stat molecules consist of a central DNA binding domain flanked by a coiled-coiled domain that binds other transcription factors and coactivators.[44 45] They also have an N-terminal domain involved in dimer-dimer interactions.[46] C-terminal of the DNA binding domain is a linker domain followed by the SH2 domain and a conserved site of tyrosine phosphorylation. The C-terminus of the Stats is variable and contains the transcriptional activation domain. Some Stats are also phosphorylated on a conserved serine residue in the transcriptional activation domain; this seems to be mediated by a MAPK family member. Recently several studies have pointed to the role of p38 as the kinase responsible for this modification[47–50] (Visconti *et al* submitted data). It is possible that pharmacological inhibitors of p38 might be therapeutically useful to interfere with Stat activation via this mechanism.

There are seven mammalian Stats: Stat1, Stat3, Stat4, Stat5a, Stat5b and Stat6. The critical functions of Stat1 and Stat2 in transmitting cytokine dependent signals were established through the use of mutagenised cell lines defective in IFN responses; reconstitution of these cell lines with the missing Stat was shown to restore signalling.[1] Subsequently various knockout mice have been produced, which also substantiate the specific and essential functions of the Stats. Stat1 -/- mice were found to develop normally but had extreme susceptibility to viral and some bacterial infections[52 53]; entirely consistent with the defects seen in IFNαR and IFNγR -/- mice and IFNγR deficient humans. Stat1 also seems to be important in regulating apoptosis and its absence is associated with tumorigenesis.[54]

In contrast, gene targeting of Stat3 leads to early embryonic lethality.[55] Using conditional knockouts, it has been shown that targeting of Stat3 in myeloid cells has a dramatic effect; these mice have an exaggerated inflammatory response, which seems to be attributable to the failure of IL10 signalling.[56] Stat4 -/- mice develop completely normally but have defective cell mediated immune responses and T helper (Th)1 differentiation and augmented Th2 development.[57 58] This phenotype is entirely consistent with the abnormalities seen in IL12, IL12R -/- mice, and IL12R deficient humans, demonstrating an important role for Stat4 in IL12 signalling and Th1 differentiation. Stat6 was originally purified as factor induced by IL4. Accordingly, in contrast with Stat4 knockouts, Stat6 -/- mice have defective Th2 development.[59–61] They also have defective

IgE response after infection with parasites. Importantly, they have attenuates experimentally induced allergic and asthmatic disease. The cytokine IL13 shares a receptor subunit with IL4; IL13 also activates Stat6 and its responses are abrogated in Stat6 -/- mice.

Stat5a and Stat5b are 91% identical and can be activated by the same cytokines; none the less they have specific functions. Stat5a knockout mice have impaired mammary gland development and failure of lactation, whereas Stat5b knockout mice have defective sexually dimorphic growth and growth hormone dependent regulation of liver gene expression.[62 63] In doubly deficient, Stat5a/5b -/- mice, T cells develop but they are very abnormal; although the cells fail to proliferate in vitro, the mice develop lymphoproliferative disease, suggesting a defect in apoptosis.

From these knockouts, the two most useful targets would seem to be Stat4 and Stat6; inhibitors could block cell mediated immunity and allergic responses respectively. But what would one target in the Stats? Unlike the Jaks, they do not have enzymatic activity. Their major function, of course, is to bind DNA and activate gene transcription and so these properties could be targeted. The crystal structure of the Stats demonstrates that the phosphotyrosine-SH2 interaction is the major contributor to their structure bound to DNA. While logical, attempting to disrupt this interaction seems formidable. Moreover, the precedents for developing such an inhibitor are less clear than those for a kinase inhibitor.

Much progress has been made in the identification of the molecular basis of cytokine action. The elucidation of the Jak/Stat pathway provides a solution to some of the problems of intracellular signalling, comprising surprisingly specific and essential functions. Much remains to be learned about the mechanisms by which cytokine dependent gene regulation occurs and we still have a limited understanding of the complex interplay among the various signalling pathways and the means by which different transcription factors work in concert to regulate gene expression. None the less, the numerous recent advances enable us to define the targets for the development of novel therapies.

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