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Comment on Boggon et al, page 996

Structure of a Janus kinase: molecular insights and prospects for optimizing a new class of immunosuppressants

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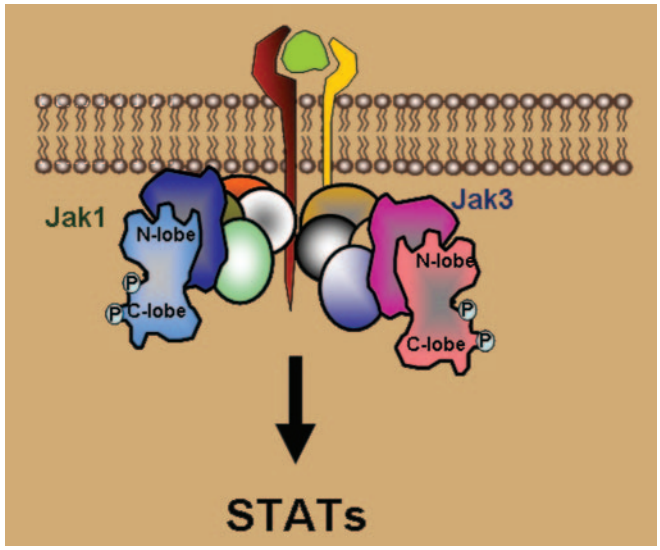
Jaks, in general, are critical for cytokine signaling and Jak3 is crucial for key immunoregulatory cytokines. The study by Boggon and colleagues provides the first insights into the structure of these key tyrosine kinases.



Janus kinases, multidomain proteins consisting of a catalytic or kinase domain associated with pseudokinase, SH2, and FERM domains, associate with cytokine receptors. Upon ligand binding, Janus kinases are activated resulting in their phosphorylation. According to Boggon et al, the Jak3 kinase domain has a typical bilobed structure. Phosphorylation of tyrosine 981 in the activation loop allows interaction with arginine residues in the C-helix of the N lobe, allowing the opening of the binding site cleft. Activation of the kinase results in phosphorylation of the receptor and subsequent activation of the cytosolic family of transcription factor signal transducers and activators of transcription (STATs). Boggon et al also identified other unique structural features of the Jak3 kinase domain, which may mediate interactions of the kinase domain with other regulatory domains.

Cytokines, key regulators of cell development and homeostasis, are especially important for hematopoiesis and immunoregulation. A subset of cytokines bind receptors denoted as Type I and II cytokine receptors, and these receptors associate with a family of cytoplasmic protein tyrosine kinases known as Janus kinases, or Jaks.[1,2] While we know from various genetic approaches that Jaks have distinct but essential functions in initiating cytokine signaling, a precise understanding of their regulation has been hampered by the absence of 3-dimensional structural information. In this issue of *Blood*, Boggon and colleagues begin to fill this gap by providing the first glimpse of the structure of a Jak kinase domain. This work reveals interesting structural similarities and differences with other protein tyrosine kinases. Equally important, this new information has the potential for optimizing the selectivity of Jak antagonists.

There are 4 members of the Janus kinase family: Jak1, Jak2, Jak3, and Tyk2.[3] Each has distinct functions based on its ability to bind different cytokine receptors. The present study focuses on Jak3, which specifically associates with the common gamma chain γc.[4] This is a shared receptor subunit for interleukin (IL)–2, IL-4, IL-7, IL-9, IL-15, and IL-21. These cytokines are critical for immunoregulation, with IL-7 being especially crucial for lymphoid development. Importantly, mutations of the IL-7 receptor, γc, or Jak3 underlie the majority of cases of severe combined immunodeficiency (SCID).[5] This discovery led to the idea that Jak3 inhibitors might represent a new class of immunosuppressant drugs. In fact, a potent, selective, orally available Jak3 inhibitor has now been generated.[5] This drug is efficacious in preclinical models of transplant rejection.[6]

The new study reports the successful solution of the crystal structure of the Jak3 kinase domain with a nonselective kinase inhibitor. Not surprisingly, the work reveals that this domain has the classic bilobed (N- and C-lobe) composition seen in all other protein kinases. Furthermore, the overall conformation is similar to well-studied tyrosine kinases such as lymphocyte-specific kinase (Lck), the insulin receptor kinase, and Ableson kinase (Abl). However, it appears that the active state of the kinase is directly coupled to the tyrosine-phosphorylated activation loop. In addition to the kinase domain, Jaks comprise band 4.1, ezrin, radixin, moesin (FERM)–, src homology 2 (SH2)–, and pseudokinase domains; the latter domain is unique to Jaks. Boggon et al noted additional unique structural features of the Jak3 kinase domain, which they suggest may mediate interdomain contacts. They identified an additional helix in the C-lobe, referred to as

the αFG helix. They also noted that the structure of the loop between β2 and β3 is distinct and creates a pocket in the posterior on the N-lobe. The authors predict, based on primary structure, that these features may be conserved in other vertebrate Jaks. This is of interest, as we know from several lines of evidence that pseudokinase and FERM domains have essential regulatory functions with respect to catalytic activity. Mutations in the Jak3 pseudokinase domain result in SCID, whereas mutations in the Jak2 pseudokinase domain have recently been shown to underlie polycythemia vera and related disorders.[7]

In summary, the study by Boggon and colleagues is an important advance that should facilitate the development of Jak inhibitors. Exploiting the unique aspects of Jak3 structure can hopefully help in the generation of more selective inhibitors. Among the Jaks, Jak3 is most closely related to Jak2, which is important for erythropoietin and thrombopoietin signaling. Minimizing the effect of a Jak3 inhibitor on Jak2 could be of clinical benefit. Conversely, this information might be helpful in generating selective inhibitors of other Jaks. For instance, Tyk2, which is critical for IL-12 signaling, may be a useful target in diseases characterized by T-helper 1 (Th1)–mediated pathology, whereas targeting mutant Jak2 molecules could be of use in the treatment of myeloproliferative disorders. While the solution of the structures of the individual Jak domains is important, defining the structure of an intact Jak molecule will ultimately be needed to understand how these critical kinases are regulated. Understanding precisely how the pseudokinase domain regulates catalytic activity might offer unique opportunities for intervention. ■

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NEOPLASIA

Comment on Moreaux et al, page 1021, and Nishio et al, page 1012

APRIL showers cause CLL and myeloma to flower

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There is growing evidence that tumor cell growth and survival are intimately associated with signals derived from the microenvironment and, as such, these signals represent therapeutic targets.

Two studies in this issue of *Blood* expand on this concept by providing evidence that dysregulated B cell–activating factor of the tumor necrosis factor (TNF) family/a proliferation-inducing ligand BAFF/APRIL signaling exists in B-chronic lymphocytic leukemia (B-CLL) and multiple myeloma (MM). B-lymphocyte stimulator (BlyS)/BAFF and APRIL are members of the tumor necrosis family of membrane-bound ligands expressed by cells of the monocyte lineage that bind B-cell–specific receptors BAFF-R, B-cell maturation antigen (BCMA), and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) to control B-cell maturation and proliferation. A clear link between APRIL signaling and B-cell malignancies came from the observation that mice transgenic for *APRIL* develop B-1 cell–associated neoplasms.[1] Multiple studies have now reported that APRIL and BAFF levels are elevated in the sera of patients with CLL and myeloma and that both autocrine and paracrine APRIL and BAFF signaling exists in these related malignancies.[1-5] The 2 papers in this issue focus on the role of accessory cells and paracrine signaling in these diseases. Burger et al originally reported that blood-derived nurse-like cells (NLCs) protect CLL cells from spontaneous apoptosis through stromal cell–derived factor-1 (SDF-1).[6] This same group has now discovered that NLCs express BAFF and APRIL and that these molecules can promote CLL cell survival via a paracrine pathway that is distinct from that of SDF-1. Nisho and colleagues discovered that NLCs expressed significantly higher levels of BAFF and APRIL than monocytes and B-CLL cells. The viability of CLL B cells cultured with NLCs was significantly reduced when cultured with a decoy receptor of BCMA, which binds APRIL, and BAFF, but not BAFF-R:Fc, which only binds BAFF. Importantly, the effect of these molecules on survival was additive and distinct from that of SDF-1α, which may be explained by differences in activation of downstream signaling cascades. An important finding is that CLL cells likely use multiple nonoverlapping survival signaling pathways as a means to escape death. It will be interesting to see which other factors NLC produce to promote CLL growth and survival.

In the second report, the Klein laboratory expands on previous work in which they showed that BAFF and APRIL can protect myeloma cells from apoptosis induced by IL-6 deprivation and/or dexamethasone.[5] In the current study, Moreaux and colleagues show that the main site of BAFF and APRIL production is in the bone marrow and that, not unexpectedly, this is derived mainly from osteoclasts. Obviously this is of great relevance given that osteoclast numbers are increased in myeloma bone marrow. They also showed that *TACI* expression varied dramatically in malignant plasma cells. Using supervised hierarchical clustering of global gene expression profiles from 65 primary myeloma samples, they demonstrated that differences in *TACI* expression could distinguish tumors with a microenvironment-interacting signature versus a plasmablastic signature, suggesting that myeloma expressing high levels of *TACI* are microenvironment dependent. As with the plasmablastic gene expression signature, patients with the