JAK inhibition using tofacitinib for inflammatory bowel disease treatment: a hub for multiple inflammatory cytokines

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Submitted 4 September 2015; accepted in final form 21 November 2015

INFLAMMATORY BOWEL DISEASE (IBD) is a chronic, idiopathic condition with two main forms: ulcerative colitis (UC) and Crohn’s disease (CD). A recent review of the global epidemiology of IBD showed increasing incidence and prevalence of both forms of the disease worldwide; for UC, the highest reported prevalence values were 505, 168, and 249 cases per 100,000 people for Europe, Asia and the Middle East, and North America, respectively; and for CD, 322, 68, and 319 cases per 100,000 people, respectively (36). In addition to a small associated increase in mortality (7), the impact of IBD on patient quality of life is high (24), with alternating periods of relapse and remission a feature of both UC and CD (3, 47).

UC is associated with diffuse mucosal inflammation affecting the colon (47), while, in CD, inflammation may be transmural and can affect any region of the gastrointestinal tract (3). The exaggerated immune response observed in affected tissues, characteristic of both forms of IBD, has a multifactorial pathogenesis driven by the imbalanced production of proinflammatory cytokines (53). The purpose of this article is to discuss the cytokine pathways central to IBD pathogenesis and, subsequently, to review the evidence and hypotheses for the mechanism of action of Janus kinase (JAK) inhibitors for the treatment of IBD.

CURRENT TREATMENTS AND GUIDELINES FOR IBD

Appropriate choice of therapy for IBD depends on multiple factors, including disease severity, response to previous treatment, and comorbidities, with the goals of therapeutic intervention being to prevent intestinal damage and to induce and maintain steroid-free remission of symptoms (63, 64). More recently, the promotion of mucosal healing has emerged as a new goal of treatment and predicts sustained clinical remission without surgical intervention (45, 57).

Current Therapies and Unmet Needs for the Treatment of IBD

Existing conventional therapies for the treatment of IBD include aminosalicylates, corticosteroids, and immunosuppressive agents, such as azathioprine, mercaptopurine, and methotrexate (29, 35). Advances made during the 1990s in understanding the inflammatory cascade and the role of cytokines and cell adhesion molecules in IBD pathogenesis led to the introduction of the first disease-modifying biological drugs for the treatment of the disease. Tumor necrosis factor inhibitors
(TNFi) infliximab, adalimumab, and golimumab are available for the treatment of UC (29), whereas infliximab, adalimumab, and certolizumab are indicated for the treatment of CD (73). TNFi therapy inhibits signaling elicited by TNF-α, one of the key mediating cytokines of intestinal tract inflammation in IBD, and is effective in the management of acute disease for many patients. Therapies that target integrin molecules (responsible for mediating cell-cell interactions between endothelial cells and leukocytes) represent a novel approach to the treatment of IBD. Vedolizumab, a humanized monoclonal antibody against the α4β7-integrin, was efficacious in UC and CD (14, 65), and further agents within this class of drug are in clinical development (11).

The efficacy of combination therapy for the treatment of IBD has been demonstrated in two recent clinical trials. In the UC SUCCESS (49) and SONIC trials (10), combination therapy with infliximab and azathioprine was superior to azathioprine monotherapy in inducing steroid-free remission in patients with moderate to severe UC and CD, respectively.

Despite the range of treatment options available, induction and maintenance of remission remains problematic with both conventional and biological therapies (56). In randomized controlled trials of infliximab, aminosalicylates, and thiopurines, maintenance of remission was observed in 33, 53, and 60%, respectively, of patients with UC (23, 62, 72). Similarly, 43, 45, 70, and 71% of patients with CD maintained remission in randomized controlled trials of adalimumab, infliximab, methotrexate, and azathioprine, respectively (52, 55, 59). Furthermore, ~20% of patients with CD or UC still require surgical treatment, despite biological therapy (15, 56, 70).

Hence, there remains a need for new therapies, resulting in better tolerability and greater long-term efficacy.

Currently approved TNFi therapies target inflammation through inhibition of a single cytokine, with long-lasting effect. Given the involvement of multiple cytokine-driven inflammatory pathways in the pathogenesis of IBD, a therapeutic approach blocking multiple cytokines could be effective (16, 37). Such an approach could be achieved with a JAK inhibitor.

The discovery of the JAK family of intracellular tyrosine kinases (TYKs), reviewed by Ghoreschi et al. (22), and elucidation of their role in cytokine signaling pathways has led to their identification as potential therapeutic targets in the treatment of IBD. Successful development of JAK inhibitors will rely on further understanding of their mode of action based on a sound understanding of the pathogenesis of IBD.

INFLAMMATORY CYTOKINES INVOLVED IN THE PATHOGENESIS OF IBD

The pathogenesis of IBD is multifactorial and comprises genetic, environmental, microbial, and immune response components (79). In health, the gut microbiota and immune cells resident in the lining of the gastrointestinal tract exist in equilibrium. The intestinal mucosa forms a barrier layer mediating this equilibrium by preventing microorganisms within the gut lumen from entering host tissue, with intact barrier function necessary for the prevention of uncontrolled inflammation. The pathogenesis of IBD is related to altered barrier function in the form of structural changes to intestinal epithelial cells (44). Subsequently, substantial changes in the composition of gut microbiota are observed, termed “dysbiosis,” eliciting an inflammatory response from the immune system. The initial innate immune response in IBD involves activation of immune cells, including macrophages, dendritic cells, neutrophils, and natural killer cells, in reaction to the presence of microbial antigens. Subsequent antigen presentation to T cells stimulates the adaptive immune response, which varies between UC and CD, according to the prevailing T helper (Th) cell profile in each disease (69, 79). Th cells mediate the continued inflammatory response in both cases, with Th17 cells having been identified as key effectors in the immunopathogenesis of IBD. They are involved in the production of proinflammatory cytokine interleukin (IL)-17 and also display functional plasticity, exhibiting a regulatory Th17/Th1 phenotype in which IL-17 production is suppressed and interferon-γ (IFN-γ) is secreted (18, 34). Thus there is a balance between the proinflammatory Th17 response and anti-inflammatory regulatory response, which may be important when considering treatment with IL-17-suppressing agents.

Dysfunction of both innate and adaptive immune response pathways contributes to the inflammatory response (20, 71). IL-17-producing cells are central to the mediation of the cell-cell interactions at the heart of these responses (20, 71). A continuing immune response to commensal microbes results in the cytokine-mediated chronic cycle of inflammation characteristic of IBD, challenging mucosal homeostasis (38, 53). In the late stage of the disease process, continued accumulation of myofibroblasts at locations where barrier function has been compromised may lead to intestinal fibrosis and the formation of fistulae (38). The optimal timing of intervention with a JAK inhibitor with respect to disease stage remains to be studied in the clinic, but may be an important concept, given the potential for variation in IBD immunophenotypes between early and late-stage disease (31). The immunopathogenesis of IBD is detailed in Fig. 1, and cytokines involved in the innate and adaptive immune responses in the disease are summarized in Fig. 2. Key cytokines in the immune response include TNF-α, IFN-γ, IL-1β, IL-6, IL-12, IL-13, IL-17, and IL-2 (1, 25, 69), as well as IL-15 (48). Thus therapeutic approaches targeting multiple cytokines in combination may be a viable new approach for the treatment of IBD (5).

JAK INHIBITION OF MULTIPLE INFLAMMATORY PATHWAYS

A large number of cytokines operate by activating JAK-signal transducers and activators of transcription (JAK-STAT) pathways (46). The JAK family comprises four intracellular protein TYKs: JAK1, JAK2, JAK3, and TYK2 (22). Cytokine binding to its receptor at the surface of a cell leads to intracellular activation of JAKs, followed by phosphorylation and activation of STATs. Phosphorylated dimerized STATs then translocate to the cell nucleus, where they modulate gene transcription that ultimately alters aspects of cellular function, including growth, maturation, differentiation, and survival, in addition to inflammatory and immune responses (39). Cytokines signal through activation of JAKs in pairs, with each JAK associated with different intracellular domains of individual cytokine receptor chains. As a consequence, different combinations of JAKs are associated with different cytokine receptors. Figure 2 shows cytokine signaling pathways in IBD and the associated JAK combinations.
Cellular functions associated with the immune response in IBD are regulated by multiple different cytokines, which, in turn, signal through different combinations of JAKs. IL-12 and IL-23 signal through the combination of JAK2 and TYK2 and regulate innate immune responses, differentiation of Th1 and Th17, and production of proinflammatory cytokines (32). IFN-γ is an important cytokine in the intestinal response against bacterial pathogens and activates the JAK1/JAK2 combination. The γ-common cytokines, IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, all signal through the activation of JAK1/JAK3 and are associated with modulation of the adaptive immune function, including B-cell maturation and Th1, Th2, and Th17 differentiation (27). IL-13 signals through JAK1 and either JAK2 or TYK2 and is an important effector cytokine associated with impaired barrier function in IBD (25). In addition to direct inhibition of cytokine signaling, JAK blockade may also achieve downstream inhibition of cytokine synthesis by reducing the production of activator cytokines; an important example in the IBD disease process is the IL-23-stimulated production of IL-17 (28). As a result, JAK pathways are central to both innate and adaptive immune responses in IBD, playing critical roles in T-cell differentiation, B-cell development, and production of mucus and antibodies required to maintain antiviral and anti-bacterial defenses at the mucosal surface (26).

Accordingly, a blockade of JAKs results in modulation of the adaptive and immune response found in IBD and may, therefore, be effective in interrupting the chronic cycle of gastrointestinal inflammation (8, 16, 21, 66).

Owing to the role that JAKs play in functions such as cell growth, maturation, differentiation, and hematopoiesis, it is necessary to balance the inhibition of JAK pathways involved in disease pathogenesis with the potential negative effects of JAK inhibition. Each of the protein kinases is responsible for various aspects of the innate and adaptive immune responses. As such, JAK inhibition may lead to increased risk of infection adverse events. Inhibition of JAK2 may potentially lead to neutropenia, anemia, and thrombocytopenia due to the involvement of JAK2 in erythropoiesis (41). JAK3 associates only with a single cytokine receptor chain (the common γ-chain) and appears to have the most discrete function, having no role

Fig. 1. The immunopathogenesis of inflammatory bowel disease (IBD) showing the initial immune response and amplification of the immune response leading to the phenotypic expression of the disease and tissue destruction. Recruitment of leukocytes is mediated by interactions between adhesion molecules known as integrins and the intestinal endothelium. Structural changes to the epithelial barrier result in microbial antigens from the gut lumen entering the host tissue. The initial immune response to the presence of these antigens involves production of interleukin (IL)-7, IL-12, and IL-15, and activation of immune cells, including dendritic cells and macrophages. This results in a T helper (Th) 1 cell response and production of tumor necrosis factor (TNF)-α and interferon (IFN)-γ. The subsequent adaptive immune response results in an amplification of the initial inflammatory response characterized by high levels of IL-6 and IL-23 and the Th17-mediated production of IL-17A, IL-21, and IL-22. The accumulation of myofibroblasts in response to the proliferation of multiple inflammatory cytokines leads to an imbalance in the normal epithelial healing process, resulting in fibrosis and the formation of fistulae. Treg, T regulatory cell.
Table 1. Key cytokines and Janus kinase (JAK) combinations for cytokines that depend on JAK pathways for signaling.

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Fig. 2. Key cytokines in IBD and Janus kinase (JAK) combinations for cytokines that depend on JAK pathways for signaling. The pairing of JAKs is significant to their role in cytokine signaling, with each member of the JAK family exhibiting preferential binding to the intracellular domains of individual cytokine receptor chains. JAK1 pairs with JAK3 to control signaling of the γ-common cytokines, including IL-2, IL-7, IL-15, and IL-21. Type II cytokine receptors, such as those for the gp130 subunit-sharing receptors, include IL-6 signal mainly through JAK1, but are also associated with JAK2 and tyrosine kinase (TYK) 2. IL-13 signals are through receptors associated with JAK1 and either JAK2 or TYK2. JAK2 is unique in pairing with itself and controls signaling for IL-5. Other key cytokines in IBD that signal using JAKs include IFN-γ (JAK1/JAK2), IL-22 (JAK1/TYK2), and IL-12 and IL-23 (JAK2/TYK2). IL-1β, IL-8, IL-17, IL-18, and TNF-α are other important cytokines in IBD, but do not signal directly using JAKs. An important indirect effect of JAK2/TYK2 blockade for IBD is the downstream inhibition of IL-17 production achieved through direct inhibition of IL-23 signaling. Type II cytokine receptors, such as those for the gp130 subunit-sharing receptors, including IL-6, signal mainly through JAK1, but are also associated with JAK2 and TYK2. IL-13 signals are through receptors associated with JAK1 and either JAK2 or TYK2.

outside of hematopoietic cells. As a consequence, targeting JAK3 may be advantageous in suppressing the inflammatory response associated with the γ-common cytokines, with less scope for side effects resulting from the inhibition of cellular processes associated with other JAKs (54).

Existing biological therapies inhibit cytokine signaling by binding and neutralizing either the cytokine itself or its receptor (61). None of the currently approved biologic therapies targets the intracellular components of cytokine signaling pathways. Thus targeting JAK-STAT pathways offers an alternative approach to combination therapy, with the potential to block multiple cytokine signals by inhibition of a common signal transduction pathway. In addition to targeting multiple cytokines, a downstream approach to cytokine inhibition is characterized by its ability to achieve partial and reversible inhibition through appropriate dosing. In contrast to biological therapies, which effect near-complete inhibition of cytokine signaling during a dosing period, intracellular inhibition with a small molecule affords the ability to modulate JAK-dependent signaling downstream, allowing the potential for greater control of the inflammatory and immunomodulatory response throughout the dosing period (9). This partial and reversible inhibition of multiple cytokine pathways with a small molecule, compared with inhibition of a single cytokine pathway with a biological therapy, could offer a differentiated therapeutic profile.

**JAK Inhibition with Tofacitinib**

Tofacitinib is an oral, small-molecule, ATP-competitive reversible inhibitor of JAKs that is being investigated as a targeted immunomodulator for IBD. It has a short pharmacokinetic half-life of ~3 h and is rapidly absorbed (time to peak concentration is ~0.5 h) and eliminated (13). In a cellular setting, tofacitinib demonstrates preferential inhibition of signaling pathways associated with JAK1 and/or JAK3 (43). With tofacitinib 5 mg twice daily (bid), average inhibition of preferentially inhibited cytokines is 50–60%, declining to 10–30% at trough concentration during each dosing period (12). Reversibility of pharmacodynamic effects is generally seen within 14 days of tofacitinib discontinuation (19). Unlike biological therapies, tofacitinib does not elicit neutralizing antibodies through immunogenicity, and data for tofacitinib in rheumatoid arthritis (RA) are available, showing sustained responses for up to 7 yr (77). It is likely, therefore, that tofacitinib may have utility in patients experiencing inadequate response to biological therapies, as well as those not yet exposed to biologics. It is currently approved in many territories for the treatment of adults with moderate to severe RA and is being investigated for a number of other inflammatory diseases, including psoriasis, psoriatic arthritis, UC, and CD.

As with IBD, the pathogenesis of RA is mediated by a number of inflammatory cytokines (40). Inhibition of JAK1 and JAK3 with tofacitinib blocks signaling for IL-2, IL-4, IL-6, IL-7, IL-9, IL-15, IL-21, and IFN-γ cytokines, some of which are integral to the activation of the immune response in RA pathophysiology (22, 42). The efficacy of tofacitinib, either as monotherapy or in combination with nonbiological disease-modifying antirheumatic drugs, for the treatment of RA has been demonstrated across phase 3 and long-term extension studies (6, 17, 30, 33, 75, 76, 78).

In psoriasis, JAK inhibition is expected to attenuate T-cell function and differentiation and the production of IL-6, IL-21, IL-23, and IFN-γ cytokines, which play a key role in the activation of Th1 and Th17 cells, characteristic of the inflammatory psoriatic phenotype (60). One phase 2 study (50) and four phase 3 studies (2, 4, 51) have demonstrated the efficacy of oral tofacitinib compared with placebo for the treatment of
plaque psoriasis, highlighting the importance of JAKs as therapeutic targets in this immune-mediated inflammatory disease.

Based on the modulation of the cytokine-mediated immune response observed in other inflammatory diseases, targeting the JAK pathway in IBD with tofacitinib represents a promising therapeutic approach, and initial clinical trials investigating tofacitinib treatment for UC are encouraging and, taken together with the potential signals of efficacy observed with tofacitinib in CD, provide a rationale for the importance of the ongoing clinical development program in IBD (UC: NCT01465763, NCT01458951, NCT01458574, NCT01470612; CD: NCT01470599). In an 8-wk placebo-controlled phase 2 trial of 194 patients with moderate to severely active UC (66), patients received tofacitinib at a dose between 0.5 and 15 mg bid. The primary endpoint of clinical response was defined as an absolute decrease of at least three points from baseline total Mayo score, or relative decrease of at least 30%, with an accompanying decrease in rectal bleeding subscore of at least 1 point or absolute subscore of 0 or 1. Significant improvement vs. placebo in the primary endpoint and secondary endpoints (clinical remission, endoscopic response, and endoscopic remission) were observed with tofacitinib 15 mg bid. Tofacitinib 10 mg bid demonstrated significantly greater efficacy in the majority of secondary endpoints. Reductions in fecal calprotectin and C-reactive protein, and improvements in the IBD therapeutic targets in this immune-mediated inflammatory disease.

CONCLUSIONS

IBD is a chronic, inflammatory disease regulated by multiple cytokine signaling pathways central to the innate and adaptive response of the immune system. Rates of induction and maintenance of remission in IBD indicate that there is still a need for new therapies with alternative modes of action that are able to demonstrate long-term efficacy and allow steroid-free remission. JAK-STAT pathways play an important role in the innate and adaptive cytokine signaling pathways central to the immunomodulatory mode of action, evaluation of the long-term safety of tofacitinib has been a focus of its clinical development program. In patients with moderate to severe RA, consistent safety up to 72 mo has been demonstrated in open-label extension studies of tofacitinib dosed at 5 mg bid and tofacitinib 10 mg bid (77, 78). Incidence rates for safety events of special interest, including serious infections, opportunistic infections, tuberculosis, cardiovascular events, malignancy, and mortality, were stable over the duration of phase 3 and open-label extension studies and were consistent with rates observed with other biological disease-modifying antirheumatic drugs (6, 17, 30, 33, 75, 76, 78). Changes in clinical laboratory parameters, including neutrophil, lymphocyte and platelet counts, hemoglobin, creatine kinase, serum creatinine, and serum lipids (low-density lipoprotein and high-density lipoprotein cholesterol), were generally stable during long-term treatment and reversible with appropriate medical management (78). In phase 2 and phase 3 studies of up to 52 wk of tofacitinib for patients with moderate to severe chronic plaque psoriasis (2, 4, 50, 51, 58), tofacitinib 5 mg bid and tofacitinib 10 mg bid have been well tolerated, with no new safety findings observed with respect to the RA program. In phase 2 studies of UC and CD (66, 67), dose-dependent increases in low-density lipoprotein and high-density lipoprotein cholesterol levels were observed with tofacitinib, consistent with observations in tofacitinib RA and psoriasis studies; however, the UC and CD studies had relatively short durations and sample sizes.

Other JAK Inhibitors in Development

The current stage of development and associated clinical programs of other JAK inhibitors are summarized in Fig. 3. As the clinical development programs of tofacitinib and other JAK inhibitors progress, they will provide further mechanistic information regarding the role of JAK pathways in cytokine-mediated diseases. Comparisons of their relative efficacy, tolerability, and safety will be important in determining optimal dosing and the specificity of their effect.
importantly, JAK inhibition targets multiple cytokine pathways. Potential benefits of JAK inhibition relating to the intracellular mechanism of action include the ability to achieve inhibition with a small molecule, offering the potential for oral dosing, and the ability to modulate the extent of cytokine inhibition during a dosing period. Potential side effects of JAK inhibitors arising from the pleiotropic nature of JAK-STAT signaling pathways include increased rate of infections and malignancies and changes in laboratory parameters, including neutrophil and lymphocyte cell counts, platelets, and liver enzymes. Initial results from clinical development programs investigating JAK inhibitors have demonstrated signals of efficacy in both UC and CD, with safety profiles generally similar to existing therapies, and no new safety signals with respect to their use in other indications. With continued clinical development, it is hoped that new therapies will move IBD treatment forward and provide additional clinically meaningful benefits to patients with IBD. Key areas to consider in the future development of JAK inhibitors for IBD will be the potentially disparate effect of JAK inhibitors between UC and CD, a greater understanding of the safety and tolerability of JAK inhibitors specific to their use in IBD indications, and the positioning of JAK inhibitors within treatment guidelines.

GRANTS

M. Grisham is grateful for support provided by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-091269 and Department of Defense Grant PR000317.

DISCLOSURES

J.-B. Telliez and J. Hodge are employees of Pfizer Inc. M. Grisham has worked on contract projects for Fibrogen Inc. Medical writing support was provided under the guidance of the authors by Daniel Binks, PhD, of Complete Medical Communications and was funded by Pfizer Inc.

AUTHOR CONTRIBUTIONS


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