Abstract. Interleukin-6 (IL-6) is a pleiotropic cytokine with central roles in immune and inflammatory reactions. IL-6 first binds to the IL-6 receptor (IL-6R), this complex then associates with gp130, inducing dimerization and the initiation of signaling through signal transducer and activator of transcription-3 (STAT3). Notably, the combination of soluble IL-6 receptor (sIL-6R) and IL-6 stimulates cells that only express gp130 and not IL-6R, a process known as trans-signaling. In contrast, soluble gp130 (sgp130) serves as a natural inhibitor of trans-signaling. Accumulated evidence strongly supports the hypothesis that the development and perpetuation of inflammatory bowel disease (IBD) relies on IL-6-mediated STAT3 activation on mucosal T-cells. This review looks at therapeutic strategies targeting the IL-6/STAT3 cytokine signaling pathway in patients with IBD, including strategies involving the anti-IL-6 receptor antibody and soluble gp130Fc.

A growing body of evidence suggests that interleukin-6 (IL-6) plays a crucial role in the uncontrolled intestinal inflammatory process responsible for inflammatory bowel disease (IBD) (1-3). IL-6 first binds to the IL-6 receptor (IL-6R), and this complex then associates with gp130, inducing dimerization and the initiation of signaling through signal transducer and activator of transcription-3 (STAT3). Notably, the combination of a soluble form of IL-6R (sIL-6R) and IL-6 stimulates cells that only express gp130 and not IL-6R, a process known as trans-signaling. Moreover, a soluble form of gp130 (sgp130) has recently been found to serve as a natural inhibitor of trans-signaling (4-6). The accumulated evidence strongly supports the hypothesis that the development and perpetuation of IBD relies on IL-6-mediated STAT3 activation on mucosal T-cells (Figure 1) (7). We discuss the predominant role of the IL-6/STAT3 cytokine signaling pathway and the therapeutic efficacy of blocking this pathway in patients with IBD.

IL-6/STAT3 Pathway

Two distinct types of cellular receptors for IL-6 have been identified using molecular cloning: an 80-kDa ligand-binding chain, known as IL-6R (IL-6Rα, CD126), and a 130-kDa signal-transducing chain, gp130 (IL-6Rβ, CD130). IL-6 first binds to IL-6R on target cells. The IL-6/IL-6R complex then associates with gp130, promoting dimerization and the subsequent initiation of intracellular signaling, such as STAT3 phosphorylation by Janus kinase (JAK) (Figure 1A) (8, 9). Almost all the cells in the body express gp130, whereas cognate IL-6R is limited and its expression is predominantly confined to hepatocytes, neutrophils, monocytes/macrophages, and some lymphocytes (10-12). This so-called classical signaling pathway is activated during early immune responses and in turn activates the expression of diverse acute-phase proteins, such as C-reactive protein (CRP).

In addition to membrane-bound IL-6R, a soluble form of IL-6R (sIL-6R) has also been identified (10-12). Interestingly, the combination of sIL-6R and IL-6 stimulates cells that express only gp130 and not IL-6R (13, 14), a process referred to as trans-signaling (13-15) (Figure 1B). Consequently, cells that release sIL-6R protein render cells,
that express only gp130, and which cannot normally be stimulated by the cytokine, responsive to IL-6. sIL-6R has also recently been shown to sensitize target cells strongly (16). Furthermore, trans-signaling has been shown to play a key role in the pathophysiology of chronic inflammatory disorders and most likely some types of cancer as well.

A soluble form of gp130 (sgp130) has also been detected in the circulatory system (17). Strikingly, sgp130 exclusively disorders and most likely some types of cancer as well. (16). Furthermore, trans-signaling has been shown to play a also recently been shown to sensitize target cells strongly that express only gp130, and which cannot normally be stimulated by the cytokine, responsive to IL-6. sIL-6R has also recently been shown to sensitize target cells strongly (16). Furthermore, trans-signaling has been shown to play a key role in the pathophysiology of chronic inflammatory disorders and most likely some types of cancer as well.

IL-6/STAT3 Pathway and IBD

IL-6. Several studies have documented the involvement of IL-6 in the pathophysiology of IBD. The first indications that IL-6 might have a profound influence on the progression of IBD were derived from the documentation of high concentrations of IL-6, which were associated with disease severity, in the circulatory system and the intestines. In an earlier study, an increase in serum IL-6 was noted during active disease. Moreover, serum IL-6 concentrations were higher in patients with Crohn’s disease than in patients with ulcerative colitis (18-22). An analysis of endoscopic biopsy samples from IBD patients revealed that the involved colonic mucosa from patients with active disease contained larger amounts of IL-6 than colonic mucosa from patients with inactive disease or normal controls (23). Reverse transcription polymerase chain reaction (RT-PCR) analysis showed that, of the inflammatory cytokines tested, IL-6 mRNA levels were highest in patients with active IBD (24). Spontaneous and inducible IL-6 production by peripheral blood mononuclear cells was elevated in patients with IBD (25, 26). An analysis of cytokine production in lamina propria mononuclear cells isolated from patients with involved IBD revealed an increased spontaneous production of IL-6 as well as tumor necrosis factor α (TNF-α) and IL-1 (27). In the intestinal mucosa, IL-6 has been detected in macrophages, lymphocytes and intestinal epithelial cells (24, 28, 29). Such changes imply that IL-6 may affect systemic events, such as the synthesis of acute-phase proteins, platelet induction, and antibody production, while localized increases within the inflamed intestines suggest its involvement in gut pathology. Indeed, a previous in vivo study using a murine model of dextran sulfate sodium-induced colitis has endorsed the role of IL-6 in IL-6-deficient mice showing limited pathological signs of disease (30). In another study, the administration of anti-IL-6R monoclonal antibody was shown to ameliorate disease in the CD45RB<sup>high</sup>/SCID (severe combined immuno-deficient) adaptive transfer model of colitis, as evidenced by normal growth, decreased T-cell expansion, and the downregulation of adhesion molecules and inflammatory cytokines (31).

STAT3. Recent studies have highlighted the role of STAT3 in a downstream signaling pathway of IL-6. Notably, dextran sulfate sodium-induced colitis was relatively mild in mice lacking STAT3 in the type I interferon responsive cells, including liver cells, adipocytes, macrophages and gut epithelial cells (32). On the other hand, the disease was more severe in mice that had been genetically manipulated to exhibit hyperactivated STAT3 (30), indicating that STAT3 has a crucial role in the development of intestinal inflammation. Furthermore, higher levels of phosphorylated STAT3 were associated with disease activity in patients with IBD as well as in some animal models of colitis or enteritis (30). In immunohistochemical studies, phosphorylated STAT3 was localized predominantly in the T-cells within the diseased intestine (Figure 2) (33). These results are consistent with recent findings demonstrating constitutive STAT3 phosphorylation in T-cells isolated from the intestine of patients with IBD (34). Thus, STAT3 activation, particularly in mucosal T-cells, is an important inflammatory event in the development of IBD. Suppressor of cytokine signaling (SOCS) genes are involved in the negative regulation of the JAK/STAT pathway induced by cytokine signaling (35). Current observations have shown that both SOCS3 and STAT3 are expressed strongly in human IBD and in animal IBD models (30). The amount of SOCS3 in the inflamed intestine may be insufficient to shut off STAT3 activation, thereby inducing inflammation chronicity. Generally, STAT3 is a double-edged sword, since, in contrast to its proinflammatory effects on T-cells, it may have profound anti-inflammatory effects on macrophages and dendritic cells. In fact, macrophage-specific STAT3-knockout mice spontaneously developed chronic enterocolitis (36). However, STAT3 in whole-tissue extracts was strongly activated in several models of intestinal inflammation of different etiologies, including macrophage-specific STAT3-knockout mice (30), suggesting that STAT3 in cell types other than macrophages may play a role in intestinal inflammation. Thus, STAT3 is probably involved in the progression, rather than the initiation, of disease. Therefore, careful attention must be directed to the targeted cell population when contemplating STAT3 inhibition in humans.

IL-6 trans-signaling. Previous studies have demonstrated a functional role for sIL-6R in IBD. T-cells from IBD intestinal tissue show STAT3 activation and are extremely resistant to apoptosis (37). Remarkably, only a small fraction of lamina propria or peripheral blood T-cells from patients with IBD expressed membrane-bound IL-6R, whereas a large fraction of these T-cells expressed membrane-bound gp130. Surprisingly, the in vitro treatment of these T-cells with sgp130, as well as a neutralizing monoclonal antibody to IL-6R, induced apoptosis, demonstrating that IL-6 trans-signaling
promotes the retention of activated T-cells, a process that is facilitated by the induction of anti-apoptotic regulators like Bcl-xL and Bcl-2. Since the apoptosis of spleen cells can be blocked by hydroxamic acid-based metalloprotease inhibitors that inhibit the shedding of IL-6R, IL-6R shedding can be considered as an active process under this condition. sIL-6R is most likely to be released by either macrophages or neutrophils (37, 38). In further studies, this process was shown to occur upon stimulation by the acute-phase protein CRP in macrophages (39) and also by microbial metalloproteinases in monocytes (40). The levels of not only IL-6, but also of sIL-6R were reportedly elevated in the serum of both Crohn’s disease and ulcerative colitis patients, and the IL-6 found in the circulatory system was complexed to sIL-6R, furthermore, the levels of sIL-6R and IL-6 were associated with the CRP levels (41). Correspondingly, an increase in sIL-6R production in cultured intestinal mononuclear cells from patients with IBD was also noticed (42). Additionally, a positive correlation between the mononuclear cell-produced sIL-6R concentration and disease activity was seen. The presence of elevated sIL-6R levels in patients with IBD strongly suggests that sIL-6R production is coordinated as part of the inflammatory response. These findings have highlighted the need to understand the regulation of IL-6-mediated events and the contribution of sIL-6R to IBD pathophysiology. Figure 3 shows a schematic model of the IL-6/STAT3 cytokine signaling pathway in IBD intestine.

**Therapies for Targeting the IL-6/STAT3 Pathway**

**Anti-IL-6 receptor antibody.** Collective studies have emphasized a central role for IL-6 in governing inflammation, highlighting the therapeutic potential of targeting IL-6 as a strategy for the treatment of chronic inflammatory diseases (43). Significantly, IL-6-deficient mice remained resistant to the induction of a number of experimental inflammatory conditions, including colitis (30, 44-48), while agents that inhibit IL-6 or its receptor have shown considerable promise in phase I and II clinical trials (49-53). Indeed, MRA, a humanized anti-IL-6R monoclonal antibody (also known as Atizumab) that blocks both soluble and membrane-bound IL-6R (Figure 4A), is highly effective in the management of Crohn’s disease as well as rheumatoid arthritis and appears to be well tolerated, with no adverse reports of infection or toxicity (49, 50). Results from these trials not only show an improvement in disease activity, but also highlight the fact that MRA reduces the need for supplementary anti-inflammatory agents like corticosteroids; it is also the only anti-cytokine therapy currently under clinical investigation that normalizes the levels of acute-phase proteins in patients with active disease (49, 50).

**Soluble gp130Fc.** Based on the background outlined above, the preferential targeting of IL-6 trans-signaling may represent a viable alternative strategy for the treatment of IBD. For this purpose, the selective antagonistic properties of sgp130Fc have been used (Figure 4B) (38). sgp130Fc is a recombinant sgp130 protein that has been fused to the Fc region of human IgG1 (38). As a strategy, it offers a number of clear advantages, since the administration of sgp130 can be used to supplement the existing circulating levels seen within all individuals and does not result in a global blockade of all IL-6 responses, which could have more widespread clinical ramifications. In particular, the IL-6 induced hepatic acute-phase response, which is part of the innate immune response, is not blocked by sgp130Fc. The rationale for this approach has emerged from the clinical analysis of serum and intestinal IL-6, sIL-6R, and sgp130 levels in patients with IBD. During active disease, IL-6 and sIL-6R concentrations were significantly elevated in individuals with Crohn's disease and ulcerative colitis (41, 42), while sgp130 levels were unaltered or only mildly altered in both diseases (54, 55), these findings suggest that the regulation of IL-6 trans-signaling may be distorted in IBD. To substantiate the validity of this approach, studies exploiting animal models of intestinal inflammation have been adopted. In three different animal models of colitis, including colitis in IL-10-deficient mice and in mice receiving the hapten reagent trinitrobenzene sulfonic acid, treatment with anti-IL-6R antibodies suppressed or reduced colitis activity to a level similar to that observed after treatment using an antibody against TNF-α (37). Using sgp130Fc, the authors demonstrated therapeutic effects similar to those obtained with anti-IL-6R antibodies, indicating that the blockade of sIL-6R, rather than membrane-bound IL-6R, is important as a therapeutic aim. This result has been confirmed in an animal model of spontaneous enteritis, where sgp130Fc ameliorated the disease and suppressed STAT3 phosphorylation and, conversely, an IL-6/sIL-6R fusion protein termed Hyper-IL-6 exacerbated the disease and enhanced STAT3 phosphorylation (56). These studies directly demonstrate an essential pathogenic function for sIL-6R via IL-6 trans-signaling in an IBD model in vivo. Overall, sgp130Fc may represent a valuable addition to the current arsenal of treatments proven to be effective in the management of IBD. At present, the first clinical study involving the use of sgp130Fc in IBD patients is in the planning stage.

**IL-6/STAT3 Pathway and IBD-associated Colorectal Cancer**

Ulcerative colitis patients are well-known as carrying a higher risk of developing colorectal cancer than the general population, furthermore, evidence of increased risk in
patients with Crohn’s disease has also been accumulating (57, 58). Although the molecular pathogenesis of colorectal cancer associated with IBD is still poorly understood, chronic inflammation has been strongly implicated in the promotion of tumorigenesis in the colon. In one model of inflammatory colon cancer, cross-talk occurs between transforming growth factor β (TGF-β) and IL-6. Using TGF-β transgenic mice and T-cell-specific TGFβ-R-dominant negative mice, Becker and colleagues showed that a reduction in the stimulation of T-cells by TGF-β resulted in an increase in IL-6 production by these T-cells. Surprisingly, membrane-bound IL-6R was not detected on the surfaces of epithelial cells in tumor lesions, and an increase in the cell-surface expression of the metalloprotease ADAM17 (a disintegrin and metalloproteinase domain) was responsible for the cleavage of IL-6R (59). Again, tumor growth could

Figure 1. Schematic outline of IL-6/STAT3 cytokine signaling pathway. The two modes of IL-6 activation are presented as classical IL-6 activation via membrane-bound IL-6 receptors (IL-6R) (A) and soluble IL-6R (sIL-6R)-mediated cell signaling (IL-6 trans-signaling) (B). In both cases, the responses are elicited through engagement with membrane-bound gp130. In addition to membrane-bound IL-6R, sIL-6R can also be generated by proteolytic cleavage or alternative splicing. sIL-6R binds to IL-6 with an affinity comparable to that of membrane expressed IL-6R. The IL6/sIL-6R complex can associate with gp130 expressed on cells with no IL-6R expression, inducing dimerization and initiating signaling. Cells that express only gp130, and not IL-6R, are unable to respond to IL-6. The activation of such cells in the presence of sIL-6R is called trans-signaling, since the sIL-6R generated by one cell type enables a second cell type to respond to the cytokine.

Figure 2. Immunohistochemistry. (A) Immunohistochemical localization of phosphorylated STAT3 in the colonic mucosa of a control subject, a patient with ulcerative colitis, and a patient with Crohn’s disease. Significant quantities of phosphorylated STAT3 expression are detected in lamina propria mononuclear cells and epithelial cells within the diseased intestines of the patients with ulcerative colitis or Crohn’s disease. In contrast, only scattered cells positive for phosphorylated STAT3 are found in normal mucosa. In each case, phosphorylated STAT3 is localized in the nucleus. The brown color represents phosphorylated STAT3. (B) Immunohistochemical localization of phosphorylated STAT3, as shown using double staining. Note that phosphorylated STAT3 is localized predominantly in T-cells within the diseased intestine. The brown color represents phosphorylated STAT3, and the blue color represents CD3+ T-cells. (original magnification x200).
be inhibited not only by a neutralizing antibody directed against IL-6R, but also by sgp130Fc, strongly indicating that the growth of the tumor was regulated by IL-6 trans-signaling, rather than by classical signaling via membrane-bound IL-6R (60, 61). Intriguingly, a similar down-regulation of IL-6R and the up-regulation of ADAM17 on the surface of tumor epithelial cells was observed in human colon cancer patients, implying that a similar mechanism operates in the development of both human and mouse colon cancer (61). Therefore, the interruption of IL-6 trans-signaling with sgp130Fc may be a promising strategy for the treatment of colorectal cancer associated with IBD.

Conclusion

Recent advances in our understanding of the pathogenesis of IBD have permitted the development of agents directed at rational therapeutic targets. The selective blockade of inflammatory cytokines through the introduction of novel biologicals, such as anti-TNF-α agents, has led to considerable clinical benefit for patients with Crohn’s disease (62, 63), as well as those with ulcerative colitis (64, 65). Although this approach has validated the targeting of inflammatory cytokines as a strategy for treating ongoing disease, the long-term safety and efficacy of such therapeutic agents remain uncertain.
Indeed, the increased association of active tuberculosis with anti-TNF-α therapy and the inability of certain individuals to respond to such regimens (66) have highlighted the need to identify alternative IBD therapeutic strategies that do not entirely block cytokine responses. Clinical trials for patients suffering from Crohn’s disease (50) and rheumatoid arthritis (49) have clearly shown that the blockade of IL-6 signaling has effects very similar to those resulting from the inhibition of TNF-α. This similarity can be partly explained by the fact that TNF-α leads to the activation of transcription factor NF-κB, which in turn regulates the transcription of many inflammatory cytokines, including IL-6. As outlined above, IL-6 activity can be completely blocked using the blocking anti-IL-6R antibody MRA. This approach blocks both classical signaling and trans-signaling. In contrast, the application of the sgp130 protein selectively inhibits IL-6 trans-signaling without affecting signals mediated via membrane-bound IL-6R. Unlike with anti-TNF-α agents, the blockade of IL-6 signaling may also inhibit the development of colorectal cancer associated with IBD. In conclusion, the IL-6/STAT3 cytokine signaling pathway may play a role in the development of IBD, and strategies targeting this signaling pathway are expected to lead to the effective treatment of IBD.

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