Recent advances in cytokines: Therapeutic implications for inflammatory bowel diseases

Guillaume Bouguen, Jean-Baptiste Chevaux, Laurent Peyrin-Biroulet

Abstract

Inflammatory bowel diseases (IBDs) are complex and chronic disabling conditions resulting from a dysregulated dialogue between intestinal microbiota and components of both the innate and adaptive immune systems. Cytokines are essential mediators between activated immune and non-immune cells, including epithelial and mesenchymal cells. They are immunomodulatory peptides released by numerous cells and these have significant effects on immune function leading to the differentiation and survival of T cells. The physiology of IBD is becoming a very attractive field of research for development of new therapeutic agents. These include cytokines involved in intestinal immune inflammation. This review will focus on mechanisms of action of cytokines involved in IBD and new therapeutic opportunities for these diseases.

INTRODUCTION

Ulcerative colitis (UC) and Crohn’s disease (CD) are immune-mediated disorders of the intestine[1]. Accumulating data suggests that inflammatory bowel disease (IBD) results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host[2]. Emerging evidence suggests that disease development implicates a dysregulated dialogue between the intestinal flora and components of both the innate and adaptive immune systems[3-4].

Active IBD is defined as an infiltration of the lamina propria by innate immune cells [neutrophils, macrophages, dendritic and natural killer (NK) T cells] and adaptive immune cells (B and T cells). Increased numbers and activation of these cells in the intestinal mucosa enhance local levels of tumor necrosis factor-α (TNF-α) and several proinflammatory interleukins (IL)[5]. Cytokines are essential mediators of the interaction between activated immune cells and non-immune cells, including epithelial and mesenchymal cells[6-7].

Recent advances in the study of key cytokines during major forms of IBD promise the development of more effective mechanism-based therapies[8]. Given that many of these involve regulation of dynamic biological processes, it is likely that the most effective agents will fall within the broad rubric of biologic therapy.
The prototypic example of the ability of a biologic agent to effectively change the therapeutic landscape is provided by anti-TNF-α, first demonstrated through clinical validation of the prototypic agent infliximab[8]. The advent of anti-TNF-α agents has changed the way of treating IBD refractory to standard medications[3,9].

Advances in the understanding of IBD pathophysiology have become a very active area for the development of novel therapeutic agents. New targets for biologies include cytokines involved in intestinal immune inflammation that have led to new therapeutic opportunities[10,11]. Although IBD etiology is unknown, some molecules which are involved in the physiopathology have been identified and can be targeted by biological therapies[12]. This review will focus on cytokines involved in the dysregulated inflammatory response in IBD and targeted by biological therapies.

**CYTOKINE NETWORK AND IMMUNITY**

Cytokines (from Greek cyto: cell; kinos: movement) are substances that are secreted by specific cells of the immune system and carry signals locally between cells, with extensive use in cellular communication. The term “cytokine” encompasses a large and diverse family of polypeptide regulators that are produced widely throughout the body by cells of diverse embryological origin. Basically, the term “cytokine” has been used to refer to the immunomodulating agent. Interferon was the first cytokine to be described in 1957[13]. The clinical efficacy of targeting TNF-α indicates that cytokines are potential therapeutic targets in IBD[6].

Cytokines have profound effects on immune functions[16]. Beyond the classical T helper Th1/Th2 paradigm indicating predominant Th1-mediated responses dominated by the production of interferon-γ (IFN-γ) in CD and an exaggerated Th2-like inflammation in UC characterized by an increased production of IL-13[14], there has been a surge of information with regard to the role of innate immunity in IBD pathogenesis. Thus new data on adaptive immunity are emerging, indicating that: (1) the mucosal Th1 and Th2 responses of CD and UC may be actually secondary to defects of the innate immune response; (2) the dysfunction of regulatory T cells may be contributing to mucosal immune abnormalities; and (3) the newly described Th17 cells are also prominently involved in the gut inflammatory response in both forms of IBD[5,15].

The differentiation and survival of T cells depend on the relative amount of key regulatory cytokines produced mainly by macrophages and dendritic cells[12]. In the presence of IL-12 and IFN-γ, naïve CD4+ T cells adopt a Th1 phenotype which then activate macrophages that release IL-1, IL-6 and TNF-α. Thus this creates a positive feedback loop[15,16]. In the presence of IL-4, naïve CD4 T cells adopt a Th2 phenotype[17,18]. The Th17 development is triggered by both IL-6, IL-21, IL-23 and transforming growth factor-β (TGF-β), leading to secretion of the IL-17 cytokine family and IL-22[19]. Although the function of Th17 cells is not clearly known, there is probably an important part of this T cell population which expresses IL-23 receptors. This has been recently demonstrated as an IBD susceptibility gene in genome-wide association studies.

In contrast, TGF-β and IL-10 modulate differentiation of naïve T cells to T regulatory cell subgroups leading to high amounts of IL-10 and TGF-β, and are able to suppress bystander T cell activation. This could be defective in IBD[17-20]. There is a complex network between these different cell populations in the case of inflammation as, for example, in the negative crossregulation of the differentiation of Th17 cells by Th2 cells (IL-4, IL-27) and Th1 cells (IFN-γ) (Figure 1)[21].

**PROINFLAMMATORY CYTOKINES**

**TNF family: TNF-α and TNF-like factor 1A**

**Mechanisms of action:** TNF-α is a major mediator of inflammation in the gut[22-31]. It is synthesized by several cells including intestinal epithelial cells but predominantly by cells of the monocyte line and T lymphocytes[14]. TNF-α is a homotrimeric protein that mediates its diverse biologic effects through 2 distinct receptors known as TNF-α receptor type I expressed on all nucleated cells and TNF-α receptor type II restricted to cells of hematopoietic lineage[21,22,24]. Through the activation of nuclear factor-κB (NF-κB), TNF-α induces the expression of various genes such as urokinase plasminogen activator, cyclooxygenase II (COX II) and vascular endothelial growth factor (VEGF)[20]. By this method, TNF-α has multiple biological effects such as increasing leukocyte recruitment (induction of leukocyte adhesion molecules)[24-28], modulation of nitric oxide (NO) production (increasing the vascular permeability)[28,30], induction of secretion of proinflammatory cytokines[21], and the proliferation and differentiation of immune cells[20,31]. TNF5F15 encodes TNF-like factor 1A (TL1A), which is a TNF-like molecule that mediates co-stimulation of Th1 and Th17 cells. It is required for optimal differentiation of

![Figure 1 Overview of T cell differentiation and interleukin pathways](www.wjgnet.com)
Th17 cells. Variants in the TNFSF15 gene contribute to overall CD susceptibility and an increased production of TLR1A has been observed in CD. Interestingly, in mice, colitis was prevented and attenuated by an anti-TLR1A antibody.

**Results of clinical trials (Table 1):** Three anti-TNF agents, namely infliximab, adalimumab and certolizumab pegol have been approved by the US Food and Drug Administration for the treatment of luminal CD. In Europe, certolizumab has not yet received approval for IBD. Infliximab has also been approved for fistulizing CD and UC. In luminal CD, infliximab was effective in inducing clinical remission in 33% of patients compared with only 4% of a placebo group at week 4 \( (P = 0.005) \), and in maintaining clinical remission (45% in the infliximab group vs 21% in the placebo group, \( P < 0.005 \)). Adalimumab was also significantly more effective than placebo in inducing clinical remission (36% vs 12%, \( P < 0.001 \)) and more effective than placebo in maintaining clinical remission at week 56 (36% vs 16%). Infliximab and adalimumab have also been shown to be more effective than placebo in maintaining steroid-free remission at 1 year. Regarding certolizumab pegol, results from large randomized, placebo-controlled trials are more controversial, with no improvement at week 6 and different long-term response rates between trials. In fistulizing CD, 55% of the patients who received 5 mg/kg infliximab had complete fistula closure, as compared with only 13% of the patients assigned to placebo \( (P = 0.001) \). In UC, 2 large randomized, placebo-controlled studies, namely the ACT 1 and ACT 2 trials, evaluated the efficacy of infliximab for induction and maintenance therapy in UC. In both trials, at week 8, nearly two-thirds of the patients in the group receiving 5 mg of infliximab had had a clinical response, as compared with one-third of patients in the placebo group \( (P < 0.001) \).

Regarding the safety of anti-TNF agents, the Crohn’s Therapy, Resource, Evaluation, and Assessment Tool registry, including 3179 CD patients who received infliximab, demonstrated that this agent was not an independent predictive factor of serious infections. In a meta-analysis of 21 placebo-controlled trials enrolling 5356 individuals, anti-TNF therapy did not increase the risk of death, malignancy or serious infection when compared to control arms. However, a longer duration of follow-up and a larger number of patients are required to better assess the safety profile of anti-TNF agents in CD.

Mechanisms of action of anti-TNF-α agents remain poorly known. Neutralization of TNF-α in the inflamed mucosa is unlikely to be a sufficient explanation. Antibody-dependent cytotoxicity also induces apoptosis or lysis of TNF-α-producing cells. This mechanism involves the Fe portion of antibodies that increases the pro-apoptotic factor caspase-3.

**IL-12, p40/IL-23, p40**

**Mechanisms of action (Figure 2):** IL-12 is a key cytokine that drives the inflammatory response mediated by Th1 cells. As such, it underlies both normal host responses to a variety of intracellular bacterial, fungal and protozoan pathogens, and abnormal inflammatory responses linked to many autoimmune diseases, such as CD. Indeed CD is characterized by increased production of IL-12 by antigen-presenting cells in intestinal tissue. IL-23, secreted by antigen-presenting cells, is also a central cytokine involved in the differentiation and function of Th17 cells. The IL-23-Th17 interaction mediates microbial defenses and intestinal inflammation. Individual properties of IL-23 are also underscored by identification of the gene encoding the receptor for this cytokine as modifying host susceptibility. These 2 most potent Th1- and Th17-activating cytokines, IL-12 and IL-23 are both composed of a p40 subunit and therefore, a p40 antibody may have therapeutic potential in inhibiting both Th1-activating IL-12 and Th17-activating IL-23.

**Results of clinical trials (Figure 2 and Table 2):** IL-12 and IL-23 are targeted by one humanized IL-12/23 antibody, ABT-874. It has shown promising results in a phase II dose-ranging study comprising 79 patients with CD. Seven weeks of uninterrupted treatment with 3 mg/kg ABT-874 resulted in higher response rates than placebo (75% vs 25%, \( P = 0.03 \)). Another dose-ranging study comparing efficacy, safety and pharmacokinetic of intravenous infusions of ABT-874 at placebo in subjects with active CD is ongoing. A double-blind, placebo-controlled, parallel-group, crossover study, assessing ustekinumab in 104 patients with CD has been completed. The clinical response to ustekinumab was significantly greater than the group given placebo at weeks 4 and 6 (52%-54% vs 22%-39%, \( P < 0.05 \)) but not at week 8 (49% vs 40%, \( P = 0.34 \)). Interestingly, the effect was most prominent in patients treated previously with infliximab at weeks 4, 6 and 8 (59% in the ustekinumab group vs 25%-26% in the placebo group, \( P < 0.05 \)). A phase 2, randomized,

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**Table 1  Clinical efficacy and marketing approval for anti-tumor necrosis factor-α agents**

| Drug name                      | Efficacy (% of induction of remission/% sustained remission) | Approved (FDA/Europe) |
|-------------------------------|-----------------------------------------------------------|------------------------|
|                               | Luminal CD | Fistulizing CD | UC | Luminal CD | Fistulizing CD | UC |
| Infliximab (Remicade®)        | 33/45      | 55/36         | 38.8/23.1 | Yes/Yes | Yes/Yes | Yes/Yes |
| Adalimumab (Humira®)          | 36/36      | No RCT        | No RCT   | Yes/Yes | No/No | No/No |
| Certolizumab (Cimzia®)        | 35/48      | No RCT        | No RCT   | Yes/No | No/No | No/No |

CD: Crohn’s disease; UC: Ulcerative colitis; FDA: US food and drug administration; RCT: Randomized controlled trial.

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double-blinded, placebo-controlled study has evaluated the efficacy of apilimod mesylate, an oral IL-12 and IL-23 inhibitor in treating 220 patients with moderate-to-severe CD. The enrollment was closed early because it did not demonstrate efficacy over placebo.

**IL-6**

**Mechanisms of action:** IL-6 is produced by various cells such as T cells, B cells, monocytes, fibroblasts, osteoblasts, keratinocytes, endothelial cells, mesangial cells and some tumor cells. This cytokine specifically binds to the IL-6 receptor (IL-6R) or a soluble IL-6R, forming the IL-6/IL-6R complex that binds to gp130 and activates intracellular pathways including JAK/STAT signaling, tyrosine phosphatase SHP2 and NF-κB. Many cells express gp130, hence IL-6 is a pleiotropic multi-functional cytokine acting as both a proinflammatory and an antiinflammatory cytokine. It is involved in terminal differentiation of B cells, differentiation and activation of T cells, induction of a hepatic acute-phase response, hematopoiesis and fever. Thus activated IL-6 plays a major role in its own amplification and then in the chronic phase of inflammation helped by mononuclear cell accumulation at the site of injury, through continuous monocyte chemoattractant protein-1 secretion, angioproliferation and ant apoptotic functions of T cells. Plasma soluble IL-6R is increased in patient with CD and IL-6 plasma concentrations increase in active CD.

**Results of clinical trials (Table 2):** Tocilizumab binds to both the membrane-bound and the soluble forms of human IL-6R with high affinity and specificity. Tocilizumab has shown promising results in a small phase I / II study (n = 36) that met its primary endpoint. At 12 wk, the response rate was higher in patients given an 8 mg/kg infusion of tocilizumab every 2 wk than in those given placebo (80% vs 31%, P = 0.019) and is accompanied by a decrease in C-reactive protein concentration. However, only 2 of 10 patients went into remission, compared with none of 13 in the placebo group (P = 0.092), without significant improvement in mucosal healing. Improvement in disease activity in a patient with UC associated with Takayasu arteritis has been reported after treatment with tocilizumab. A placebo-controlled phase I study on the safety and biological effects of c326, an inhibitor of IL-6, in CD is ongoing.
IFN-γ
Mechanisms of action: Type II IFN, also called IFN-γ, is a proinflammatory cytokine secreted by Th1-cells. IFN-γ drives expression of major histocompatibility complex class II on antigen-presenting cells, modulates lipopolysaccharide responsiveness in intestinal epithelial cells, and increases chemokine secretion. It also activates macrophages, Th1 lymphocytes in a positive feedback loop, NK cells and endothelial cells. Concentrations of IFN-γ are increased both in UC and CD.

Results of clinical trials (Table 2): Fontolizumab has been assessed in 3 phase I/II dose-ranging studies enrolling a total of 374 patients with moderate to severe CD. Fontolizumab at doses of up to 4 mg/kg improved endoscopic lesions and decreased concentrations of C-reactive protein, but no study met its primary endpoint, which was defined as induction of clinical response at 1 mo; thus the development of fontolizumab for CD has been stopped.

IL-2 family
Mechanisms of action: IL-2 is produced mainly by activated T cells. In addition to promoting T cell proliferation and activation, IL-2 increases cytokine production and modifies the functional properties of B cells, NK cells, and macrophages. Thus, it improves the activated macrophage microbicidal and cytotoxic activities and promotes secretion of hydrogen peroxide, TNF-α and IL-6. IL-2 signals through a heterodimeric αγ trimeric αβγ high-affinity receptor complex. Studies have proved a role for IL-2 in IBD pathogenesis, for example the calcineurin inhibitor cyclosporin, which inhibits IL-2 production, is effective in the treatment of severely active UC. IL-21, an IL-2 cytokine family member expressed by activated CD4+ T cells and NK T cells, is a key regulator in production of Th17 cells. It also increases the proliferation of Th1 cells, CD4+ and CD8+ lymphocytes and regulates the profile of cytokines secreted by these cells. Indeed, IL-21-deficient mice are protected from experimental colitis, possibly through the failure to generate the Th17 response. Furthermore, blockade of endogenous IL-21, with an antagonistic IL-21R/Fc, ameliorated dextran sulphate sodium colitis in mice. No studies have been performed in humans as yet.

Results of clinical trials (Table 2): Two antibodies against the α-chain of the IL-2 receptor (CD25), namely daclizumab and basiliximab, have been studied to mimic the activity of cyclosporine. Despite promising response rates observed in an uncontrolled trial, a randomized, double-blind, placebo-controlled, dose-ranging trial failed to demonstrate an increased remission of clinical response both at high (2 mg/kg intravenously at weeks 0, 2, 4, and 6) and low doses (1 mg/kg intravenously at weeks 0 and 4) in 159 treated patients with daclizumab for active UC.

ANTIINFLAMMATORY CYTOKINES

IL-10
Mechanisms of action: IL-10 is secreted by a wide variety of cells and has pleiotropic effects on T cells, B cells, myeloid cells, and other cell types. IL-10 has suppressive antiinflammatory activity on T cells, macrophages, and dendritic cells (among other cells) in humans, as well as in animal models of inflammatory diseases. In particular, mice deficient in IL-10 or the IL-10 receptor undergo spontaneous development of intestinal inflammation, similar to human disease. Even though IL-10 effectively treats colitis in mouse models and suppresses inflammatory cytokine production in vitro in intestinal cells from IBD patients, unfortunately clinical trials using recombinant IL-10 to treat IBD in humans have been largely disappointing.

Results of clinical trials: A placebo-controlled study was conducted in 329 patients with moderate-to-severe CD and in 94 patients with UC and did not demonstrate any significant improvement in response and remission rates compared to placebo. Also, no evidence of prevention of endoscopic recurrence in CD by subcutaneous IL-10 injection was observed in a placebo-controlled trial of 65 CD patients. Animal studies showed that local administration of IL-10 to the colon via genetically engineered Lactococcus lactis bacteria administered orally allowed for the achievement of high colonic mucosal concentrations of IL-10, potentially resulting in increased efficacy.

IL-11
Mechanisms of action: IL-11 is a pleiotropic cytokine from mesenchymal cell origin. It exhibits potent antiinflammatory activity on macrophages and T cells by inhibiting the secretion of pro-inflammatory cytokines and has shown beneficial effects on intestinal mucosa in several animal IBD models. However one study suggested that the expression of the IL-11 receptor α-chain in the mucosa was restricted to epithelial cells, and although reducing apoptosis, it had no antiinflammatory effects on these cells.

Results of clinical trials: In a placebo-controlled study in 76 active CD patients, subcutaneously administered recombinant human IL-11 was shown to be safe and well tolerated. In a second placebo-controlled study in 148 patients comparing 2 doses of subcutaneously administered recombinant human IL-11, it was significantly superior in inducing remission after 6 wk when compared to placebo. In contrast, a recent trial showed significant inferiority of recombinant human IL-11 when compared to prednisolone in inducing remission in active CD and in obtaining a clinical response.

Type I IFNs
Mechanisms of action: Type I IFNs consist of 14 α
isomers and β, ε, η, and κ isoforms. Immunoregulatory therapy with type I IFNs such as IFN-α or IFN-β can inhibit production of TNF-α and IFN-γ, antagonize the IFN-γ signaling pathway and increase production of the antiinflammatory cytokine IL-10. It has also been shown to be immunoregulated by enhanced regulatory T lymphocyte and NK cell activity.

**Results of clinical trials:** Several type I IFNs have been studied in UC. A phase 2 placebo-controlled, dose-ranging trial, studied IFN-β1a in 194 patients with moderately active UC. Clinical outcomes, including the proportion of patients achieving endoscopically confirmed remission, were not statistically significantly superior in the IFN-β1a treatment groups over placebo. A randomized, placebo-controlled trial of pegylated IFN-α in 60 patients with active UC did not show any efficacy in clinical response and response rate despite a significant decrease in levels of C reactive protein.

**WHERE DO WE GO FROM HERE?**

In 2010, infliximab represents the pinnacle of the therapeutic pyramid of IBD treatment. However, this anti-TNF agent has several limitations. First, despite its widespread use in IBD, 20% of patients still require surgery. Second, about 10% of patients are primary non-responders to infliximab and only one-third of IBD patients are in clinical remission at 1 year. Third, the annual risk of loss of response is 13% per patient-year. Finally, infliximab treatment optimization with combination therapy can be considered, but this must be weighed against the increased risk of serious infections and perhaps lymphoma. These data underscore the urgent need to develop new drug classes.

Humanized IL-12/23 antibodies seem the most promising therapy for the future: (1) IL-23 is an essential mediator for the differentiation and amplification of the proinflammatory Th17 pathway; (2) its role is underscored by the increased host susceptibility for IBD in cases of polymorphism of the gene encoding the receptor for this cytokine; and (3) the effective results observed in a recent randomized, controlled trial, particularly in cases of infliximab withdrawal. Phase III trials are ongoing in IBD patients.

Recent advances in the pathophysiology of IBD have led to the identification of additional cytokine pathways representing potential therapeutic targets. Numerous other cytokines are currently under investigation: IL-27, produced mainly by dendritic cells, acting in the differentiation of both Th1 and Th2 cells; IL-32, produced by NK cell-activated lymphocytes and epithelial cells, providing a proinflammatory amplification pathway in the innate immune responses to bacterial infection; IL-31, preferentially produced by T cells skewed towards a Th2 phenotype, playing a role in the acute phase of inflammation by maintaining proliferation of B and T cells. Further studies are needed to fully explore their different roles in human IBD, and their biological significance, to eventually determine the therapeutic implications (Figure 3).

To overcome anti-TNF therapy failure in IBD, one way would be to develop more targeted therapy. A humanized TNF receptor-1 specific antagonistic antibody for selective inhibition of TNF action has shown interest. Another way would be to use cytokine therapy in association with other anti-cytokine agents. The efficacy of TNF-α antagonist agents alone reflects probably the pleiotropic effects of TNF-α. An effective treatment strategy for patients might therefore involve the blockade of multiple cytokines in order to intervene in several pathways.

Animal studies in rheumatoid arthritis showed that anti-CD4 therapy acts synergistically with anti-TNF-α in improving established collagen-induced arthritis. In IBD, a safety study suggested several positive trends in improving efficacy when natalizumab was added to infliximab treatment. Further investigations are necessary to better evaluate the cost-effectiveness and long-term safety profile of these associations.
CONCLUSION
Despite recent advances in the pathophysiology of IBD, leading to the identification and understanding of several cytokine pathways, anti-TNF-α agents still represent the pinnacle of the therapeutic pyramid of IBD treatment. The humanized IL-12/23 antibodies appear to be the most promising therapy. Future directions could include the development of more targeted therapy or therapeutic blockade of multiple cytokines in order to intervene in several pathways.

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