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REVIEW ARTICLE

Th17-cytokine blockers as a new approach for treating inflammatory bowel disease

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Abstract
Anti-cytokine therapies, including the anti-TNF-α antibody-based therapies, have largely transformed the management of patients with inflammatory bowel diseases (IBD). However, benefit is seen in nearly 50% of patients, and response can wane with time. Moreover, patients treated with anti-TNF-α antibodies can develop severe side-effects and new immune-mediated diseases. Therefore enormous effort has been made by the research community to elucidate new inflammatory networks in the IBD tissue and to develop novel anti-cytokine compounds, which may act in patients who do not respond to or cannot receive anti-TNF-α therapies. In this article we review the available data supporting the pathogenic role of Th17 cytokines in IBD, and discuss whether and how inhibitors of these inflammatory mediators may enter into the therapeutic armamentarium of IBD.

Key words: *Th17, IL-17, IL-23, IL-21, IBD*

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), as the main inflammatory bowel diseases (IBD) in humans, are chronic relapsing inflammatory disorders of the intestine. The aetiology of IBD is still unknown, but evidence suggests that, in both CD and UC, genetic and environmental factors interact to promote an excessive and poorly controlled mucosal inflammatory response directed against components of the normal microflora of the gut (1,2). Studies in experimental models also indicate that IBD-related tissue damage results from a dynamic interplay between immune and non-immune cells and that cytokines are crucial mediators of this cross-talk (1,2). The pathogenic relevance of these observations is supported by the innumerable descriptions of elevated cytokine levels in inflamed IBD tissue (3–6) and by translational studies showing that cytokine blockers (e.g. anti-tumour necrosis factor (TNF)-α antibodies) are of therapeutic benefit in patients (7,8). However, more than one-third of patients treated with the three licensed anti-TNF-α antibodies do not respond to the treatment (7–9). Moreover, response can wane with time due to the development of antibodies against anti-TNF-α compounds that reduce trough serum concentrations of the drug (10,11). Loss of responsiveness could also be secondary to the activation of new inflammatory signals, which could be triggered by anti-TNF-α therapy (12). These observations, together with the possibility that TNF-α blocker therapy may associate with severe side-effects (13–15) and with the development of new immune-mediated diseases, such as psoriasis (16), suggest the necessity of novel anti-inflammatory agents for IBD patients. Therefore, a major challenge for the research community is to identify new targets for therapeutic intervention and to clarify which patient will benefit from which therapy. A valid approach to address this issue is to look more in detail at cellular components of the inflammatory process.

Th1- and Th17-related cytokines in CD

CD has long been considered as a T helper type 1 (Th1)-related disease, given that CD mucosal

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Key messages

- Both Th1 and Th17 cytokines are supposed to play key roles in the inflammatory bowel disease (IBD)-related tissue damage.
- Simultaneous inhibition of Th1 and Th17 cell responses can be useful in the management of IBD patients.

CD4+ cells produce large quantities of interferon (IFN)-γ (6) and express high levels of Th1-driving transcription factors, such as T-box expressed in T cells (T-bet) and signal transducer and activator of transcription (Stat) 4 (17,18). In the same tissue, there is also an exaggerated production of interleukin (IL)-12, the major Th1-inducing factor in humans (5). However, the anti-IFN-γ antibody fontolizumab (HuZAF) appears to be only partially effective in patients with active CD (19,20). This could be explained by the fact that when CD patients are saturated with the anti-IFN-γ antibody the mucosal lesion is mediated by other inflammatory mediators, such as Th17 cytokines, which are not inhibited by HuZAF (Figure 1). Th17 cytokines (i.e. IL-17A, IL-17F, IL-21, IL-22, and IL-26) are produced by a distinct subset of Th cells, termed Th17 cells, in response to stimuli that activate the transcription factors retinoic acid-related orphan receptor (ROR)-γt and RORα (21). It is not yet fully understood how human Th17 cells differentiate *in vivo*, even though IL-23 seems to be crucial for expanding Th17 cell responses (21). The inflamed gut of CD patients contains high levels of IL-17A, IL-17F, IL-22, and IL-26 (22–24) (Figure 1). Some of the IL-17A-producing cells are not, however, typical Th17 cells since they co-express IFN-γ (25). In CD tissue there is also excessive production of IL-21 (26). Studies in murine systems have convincingly shown that IL-21 is made by Th17 cells and that IL-21 promotes the expansion of Th17 cell responses (27–29). However, we have recently shown that, in human IBD, IL-21 is preferentially made by

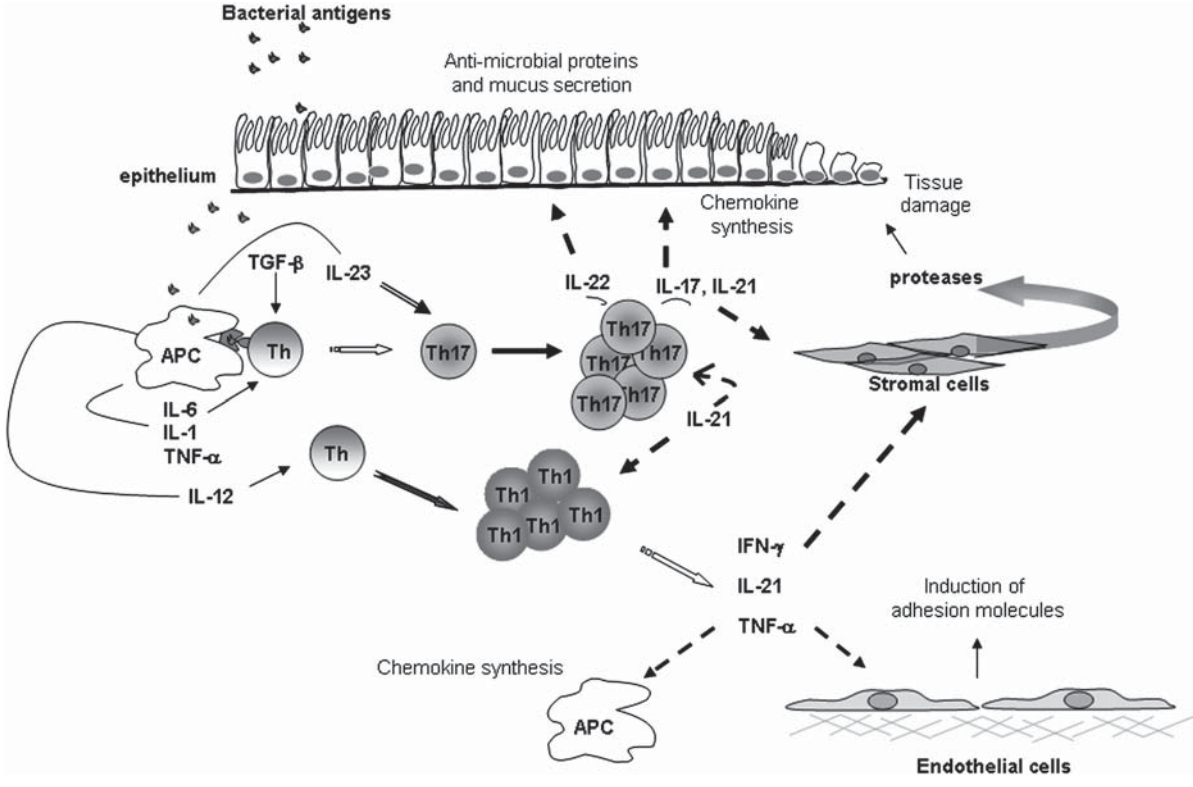

Figure 1. The scheme illustrates some of the pathways involved in the differentiation of Th1 and Th17 cells and the role of Th1 and Th17 cytokines in the gut. Following bacterial antigen stimulation, antigen-presenting cells (APC), such as dendritic cells, produce interleukin (IL)-1β, IL-6, and TNF-α, which in concert with transforming growth factor (TGF)-β1 promote the differentiation of naive T helper (Th) cells in Th17 cells. IL-23, a heterodimeric cytokine produced by APC, acts on and promotes the expansion of Th17 cells. Activated Th17 cells release IL-17 (both A and F) and IL-21, which act on different cell types, thus contributing to expanding the mucosal inflammation and facilitating tissue damage. Th17 cells also produce IL-22, a cytokine that exerts anti-inflammatory effects in the gut due to its ability to stimulate anti-microbial peptides and mucus secretion by epithelial cells. If naive Th cells are activated in the presence of IL-12, another cytokine made by APC and sharing the p40 subunit with IL-23, polarize along the Th1 pathway, a phenomenon which can be expanded by IL-21. Th1 cytokines can amplify the on-going inflammatory process by stimulating APC to make chemokine and up-regulating the expression of adhesion molecules on endothelial cells. Like Th17 cytokines, Th1 cytokines stimulate stromal cells to make extracellular matrix-degrading proteases, thereby contributing to the tissue damage occurring in IBD patients.

IFN-γ-producing CD4+ T cells rather than IL-17A-expressing cells (30), thus suggesting that Th1 and not Th17 cells are major sources of IL-21 in the human gut. This idea is supported by the fact that stimulation of CD4+ T lymphocytes from the normal gut with anti-CD3 and exogenous IL-12 increases the number of IL-21-secreting Th1 cells, while blockade of endogenous IL-12 in cultures of CD lamina propria mononuclear cells (LPMC) reduces IL-21 production (26).

Very little is known about the functional role of Th17-related cytokines in human IBD, even though it is plausible that these molecules can contribute to amplifying the on-going mucosal inflammation (Figure 1). Indeed, IL-17A and IL-17F enhance the expression of various inflammatory cytokines, chemokines, and adhesion molecules (21). IL-17A also stimulates the synthesis of extracellular matrix-degrading proteases (21). IL-21 stimulates intestinal epithelial cells to produce macrophage inflammatory protein (MIP)-3α (31), a chemokine that is up-regulated on the inflamed gut epithelium of IBD patients and involved in the recruitment of Th17 cells in the gut mucosa (32,33). Consistently, blockade of IL-21 in cultures of IBD mucosal explants reduces MIP-3α synthesis by epithelial cells (31). IL-21 also enhances the secretion of extracellular proteases by gut myofibroblasts as well as cell activation and cytolytic activity in peripheral blood-NK cells derived from IBD patients (34,35).

Involvement of Th17 cytokines in the pathogenesis of experimental colitis

The role of Th17-related cytokines in the control of gut inflammation has been evaluated in various murine models of IBD. Studies in IL-17 receptor A (IL-17RA)-deficient mice have demonstrated that IL-17 is necessary for development of acute colitis induced by intrarectal administration of trinitrobenzenesulfonic acid (TNBS) (36). Consistently, administration of IL-17RA IgG1 fusion protein attenuates TNBS-colitis in wild-type mice (36). Since IL-17RA mediates the functional activities of both IL-17A and IL-17F (37), the exact contribution of each of these cytokines in the pathogenesis of TNBS-colitis remains unknown. Studies in dextran sulfate sodium (DSS)-induced colitis showed that IL-17F deficiency results in reduced colitis (38), whereas IL-17A-knock-out mice develop more severe disease (39). Therefore, data from studies in chemically induced colitis models indicate that IL-17A has both pro-inflammatory and tissue-protective properties depending on the model in which it is studied.

IL-17A seems to be involved in the pathogenesis of colitis induced by transfer of the caecal bacterial antigen-specific C3H/HeJBir (C3Bir) CD4+ T cell line to C3H/HeSnJ SCID mice (40). Indeed in this model, gut inflammation is associated with enhanced production of IL-17A, and adoptive transfer of IL-17-secreting T cells to SCID recipients results in a more severe colitis than that induced by transfer of Th1 cells. Optimal expansion of Th17 cell responses requires IL-23 (41), a heterodimeric cytokine composed of a specific subunit, termed p19, and the p40 subunit shared with IL-12 (42). Giving SCID mice a monoclonal anti-IL-23p19 antibody attenuates C3BirT cell transfer-induced colitis, down-regulates the synthesis of inflammatory cytokines and chemokines in the colon, and promotes apoptosis of colitogenic Th17 cells (40).

Adoptive transfer of naive CD8+ T cells into syngeneic RAG-deficient mice results in severe colitis, similar to that seen after transfer of naive CD4+ T cells (43). Analysis of CD8+ T cells in the mesenteric lymph nodes of such mice show the existence of IL-17A and IFN-γ-double-positive cells. Notably, transfer of naive CD8+ T cells derived from either IL-17- or IFN-γ-knock-out mice is associated with less severe colitis, raising the intriguing possibility that IL-17 and IFN-γ can co-operate to cause pathology (43).

Another demonstration that IL-17A has pathogenic effects in the gut is provided by studies in mice colonized with enterotoxigenic *Bacteroides fragilis* (ETBF), a human colonic bacterium. Following ETBF colonization, mice develop a marked Th17 cell response in the colon, which is accompanied by the development of colitis and tumours (44). Antibody-mediated blockade of IL-17A and IL-23R inhibits ETBF-induced colitis and tumour formation (44).

A somewhat different picture emerges from studies conducted by O'Connor and colleagues (45). These authors showed that adoptive transfer of CD45RBhiCD25-CD4+ T cells from IL-17A-deficient mice to recipient immunodeficient mice results in severe colitis. Transfer of IL-17R-deficient T cells to recipient mice induces the same aggressive disease, indicating that IL-17 exerts its protective effects directly on T cells. The greater severity of colitis induced by transfer of IL-17A-deficient T cells is not due to their enhanced migratory and infiltration capacity but is instead related to enhanced Th1 cell effector function, raising the possibility that the anti-inflammatory effect of IL-17A in this model relies on the negative regulation of Th1 cell responses. In contrast to these findings, Leppkes and colleagues showed that transfer of IL-17A-, IL-17F-, or IL-22-deficient T lymphocytes into RAG1-null mice induces severe colitis that is indistinguishable from that caused by wild-type cells (46). In contrast, transfer of RORγ-null T cells, which associates with no induction of IL-17 cytokines in the intestine, does not induce colitis. Treatment of RAG1 mice that received IL-17F-null T cells with a neutralizing anti-IL-17A antibody

suppresses disease, indicating redundant biological effects of IL-17A and IL-17F (46). Similarly Noguchi et al. showed that colitis induced in RAG mice by transfer of naive CD4+ T cells prepared from IL-17-knock-out mice does not differ in terms of severity from that induced by transfer of wild-type cells (47). The reason why these studies provided us with different results remains unknown. However, it is increasingly apparent that the Th17 subset is not a homogeneous cell population, and that, based on the cytokine milieu during differentiation, IL-17-expressing cells can be inflammatory or protective depending on the co-expression of other cytokines, and particularly IL-10 (48).

IL-21 is up-regulated in wild-type mice with chemically induced colitis, such as DSS- colitis and TNBS-relapsing colitis, and administration of IL-21R/Fc to DSS-treated mice attenuates the on-going colitis and reduces the production of Th17-related cytokines (49). Consistently, IL-21-deficient mice are largely protected against DSS- and TNBS-colitis, and this protection is associated with a marked decrease in IL-17A and IL-17F (49).

Pathogenic role of IL-23 in the gut

A considerable amount of work has been done to clarify the role of IL-23 in intestinal inflammation. By back-crossing IL-10-deficient mice, which spontaneously develop a mild colitis, with mice lacking either the IL-12/p35 or the IL-23/p19 subunit, Yen et al. showed that IL-23 was responsible for manifestation of chronic intestinal inflammation, whereas IL-12 was not (50). RAG mice reconstituted with naive CD4+ T cells developed a more severe colitis when injected with recombinant IL-23. Such a colitis was associated with enhanced production of IL-6 and IL-17 and preventable by treatment of mice with blocking IL-6 and IL-17 antibodies (50). Subsequent studies by Powrie and co-workers confirmed the pathogenic effects of IL-23 in the gut and contributed to showing that IL-23-driven colitis is not strictly dependent on IL-17 (51,52).

A mouse model of T cell-independent intestinal inflammation is that caused by infection of 129Sv EvRAG-deficient mice with the bacterium *Helicobacter hepaticus*. In this model the development of chronic colitis is mediated through activation and accumulation of innate immune cells, including granulocytes and monocytic cells, and is characterized by induction of IL-23/p19 and IL-12/p40 but not IL-12/p35 (51). Treatment of mice with a neutralizing p19 antibody ameliorates colitis. Analysis of inflammatory cytokines in the inflamed colons of infected mice shows that, concomitant with the increased IL-23 expression, there is a marked increase in IL-17 expression, indicating that IL-23 induces the secretion of IL-17 by non-T cells (51).

Infection of IL-10-knock-out mice with *Helicobacter hepaticus* causes a severe colitis that is T cell-dependent and associates with enhanced production of IL-17A and a marked Th1 response. However, in the absence of IFN-γ, IL-17A is not sufficient to induce maximal colitis (52), raising the possibility that, at least in some circumstances, Th1 and Th17 cytokines can synergize to elicit intestinal pathology. IL-23 can facilitate colitis not only via direct effects on inflammatory mediators but also indirectly by counteracting regulatory mechanisms, such as the differentiation of Foxp3-expressing T regulatory cells in the intestine (53). Although the above observations underline the critical role of IL-23 in mediating intestinal pathology, studies in the CD-like TNBS-colitis model have demonstrated that IL-23 negatively regulates the mucosal inflammation (54). How this finding relates to CD inflammation is not clear, because IL-23 is up-regulated in CD tissue (55), where it is supposed to control the expression of various inflammatory molecules, including IFN-γ and IL-17A (55,56).

Neutralization of Th1 and Th17 cytokines as a strategy to combat IBD

The findings described in this review indicate that Th1 and Th17 cells coexist in IBD tissue, and that cytokines made by these cells can co-operate in driving inflammatory pathways in the gut. So, we can speculate that compounds that neutralize both Th1 and Th17 cytokines might actually have advantages over a more targeted approach aimed exclusively at one or the other. A first strategy to reach this goal is to inhibit the activity of IL-12 and IL-23, the master regulators of Th1 and Th17 cytokine responses, respectively (Figure 1). Indeed, ABT-874 and ustekinumab, two monoclonal antibodies directed against the p40 subunit of IL-12 and IL-23, have already been tested in patients with active CD (57,58). Clinical response and remission rates after 7 weeks of treatment with ABT-874 were 75% and 38%, respectively, as compared to 25% and 0% for the group receiving placebo (57). Treatment with ABT-874 was associated with decreases in the secretion of IL-12, IFN-γ, and TNF-α by mucosal cells and with significant improvement in mucosal histologic scores (59). By contrast, a phase II exploratory trial failed to show that induction therapy with ustekinumab, a fully human IgG1 monoclonal antibody, was superior to placebo in patients with moderate-to-severe CD (58). However, ustekinumab appeared to be more effective than placebo in inducing a clinical response in patients who did not respond to TNF-α blockers (58). Thus, one of the potential advantages

of targeting IL-12/IL-23 p40 is that it may help patients who are unresponsive to TNF blockade. However, a more recent study has demonstrated that apilimod mesylate (formerly known as STA-5326), an oral inhibitor of IL-12/IL-23p40 gene transcription, is not superior to placebo in inducing clinical response and remission in patients with moderate-to-severe CD (60). The reason why apilimod mesylate failed in this trial despite successful results with anti-IL-12/IL-23/p40 antibodies remains unknown. A possibility is that the drug does not reach sufficient concentrations in the intestinal tissue to exert anti-inflammatory effects after oral administration.

Other attractive strategies for targeting both Th1 and Th17 cytokines production and/or function could involve IL-21 blockers. Indeed, we have previously shown that when mucosal T cells from CD patients are activated *in vitro* with anti-CD3 in the presence of either a neutralizing anti-IL-21 antibody or an IL-21R-IgG fusion protein, IL-17 and IFN-γ production is approximately halved (27,49).

Therapeutic benefit could derive from the use of inhibitors of intracellular kinases, such as the Janus kinases, and signal transducers and activators of transcription, which translate signals triggered by cytokine binding into intracellular responses involved in the regulation of Th1 and Th17 cell differentiation (61,62). Inhibitors of the prostaglandin 2 EP(4) receptor signalling could also be useful, since these compounds inhibit the synthesis of both Th1 and Th17 cytokines and have already been used with success in murine models of immune-mediated diseases (63).

Conclusions

In the last years, progress in basic and translational research has led to a better understanding of the role of Th1 and Th17 cells in the pathogenesis of IBD. Theoretically, targeting simultaneously these molecules could help attenuate the activation of multiple inflammatory pathways in IBD, thus facilitating the resolution of tissue damaging-immune responses. Various approaches for inhibiting molecules that govern Th1/Th17 cell activity or Th1/Th17 cytokines (e.g. AIN457, an anti-IL-17 monoclonal antibody) have already been developed and tested in patients or are now ready to move into the clinic. Therefore, it is likely that the near future will tell us if patients can really benefit from these new biological compounds. In designing clinical interventions around Th1 and Th17 cytokines, it would be relevant to take into consideration some potential risks that could derive from the blockade of these molecules, as both Th1 and Th17 cytokines have been reported to play a key role in the control of immune responses against infectious agents and cancers (21,42). In this context, it is also noteworthy that IL-22 has been reported to exert beneficial effects in the gut (24,64) (Figure 1). Therefore, inhibition of IL-22 could bear unwanted side-effects. Further experimentation would be necessary to identify biomarkers which predict responsiveness to this anti-cytokine therapy, as well as to ascertain which biological therapy will be effective in an individual patient. To this end, progress could derive from genetic studies. Polymorphisms for IL-12B, IL-23R, and IL-21 have been described in IBD patients (2,65–67), and preliminary evidence suggests that genetic changes could control cytokine production and function (68,69). If expression of these cytokines is genetically predetermined, one could speculate that through genotyping we can identify which IBD patients could benefit from specific anti-Th1 and/or Th17 cytokine therapies.

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