New targets for mucosal healing and therapy in inflammatory bowel diseases

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Healing of the inflamed mucosa (mucosal healing) is an emerging new goal for therapy and predicts clinical remission and resection-free survival in inflammatory bowel diseases (IBDs). The era of antitumor necrosis factor (TNF) antibody therapy was a remarkable progress in IBD therapy and anti-TNF agents led to mucosal healing in a subgroup of IBD patients; however, many patients do not respond to anti-TNF treatment highlighting the relevance of finding new targets for therapy of IBD. In particular, current studies are addressing the role of other anticytokine agents including antibodies against interleukin (IL)-6R, IL-13, and IL-12/IL-23 as well as new anti-inflammatory concepts (regulatory T cell therapy, Smad7 antisense, Jak inhibition, Toll-like receptor 9 stimulation, worm eggs). In addition, blockade of T-cell homing via the integrins $\alpha_4\beta_7$ and the addressin mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) emerges as a promising new approach for IBD therapy. Here, new approaches for achieving mucosal healing are discussed as well as their implications for future therapy of IBD.

INTRODUCTION

Inflammatory bowel diseases (IBD; Crohn's disease (CD), ulcerative colitis (UC)) are chronic relapsing diseases that lead to structural damage with destruction of the bowel wall.^{1–3} Clinically, IBD patients can suffer from chronic diarrhea, malabsorption, weight loss, rectal bleeding, abdominal pain, stenoses, abscesses, and fistula formation, and many patients require surgery over time.⁴ In addition, various extraintestinal manifestations (primary sclerosing cholangitis, ankylosing spondylitis, iritis/uveitis, pyoderma gangrenosum, erythema nodosum) and immune-mediated diseases (asthma, psoriasis, rheumatoid arthritis) are associated with IBD.⁴ Finally, there is an increased risk of colitis-associated neoplasias in IBD, particularly in patients with ulcerative pancolitis and colonic CD.^{5,6} Given the clinical relevance of these IBD-related problems, it is no surprise that IBD has a major impact on quality of life including fatigue, relationships, and employment.⁷

Although clinical IBD studies in the past have mainly focused on improvement of diarrhea and abdominal pain, mucosal healing on endoscopy has recently emerged as a key treatment goal in IBD.^{8,9} Although there is still no general consensus on the definition of the term *mucosal healing*, mucosal healing on endoscopy usually refers to resolution of ulcers in CD and erosions and ulcers in UC.^{10,11} Interestingly, mucosal healing in

IBD was associated with more effective disease control, more frequent steroid-free remission of disease, lower rates of hospitalization and surgery, and improved quality of life as compared with conventional treatment goals.¹² These findings highlight the role of mucosal healing for therapy of IBD. In spite of the fact that not all groups agree on the relevance of mucosal healing in the absence of large prospective intervention studies,¹³ mucosal healing has become a key end point in clinical trials and predicts sustained clinical remission and resection-free survival of IBD patients.^{8,12,14} New treatment options thus will have to demonstrate their potential to induce mucosal healing in IBD.

THE STRUCTURAL BASIS OF MUCOSAL HEALING

Various studies in recent years have highlighted a crucial role of molecular signaling events for mucosal healing in IBD. It was found that mucosal healing relies on the coordinated activity of intestinal epithelial cells (IECs), goblet cells, and Paneth cells for improvement of intestinal barrier function.^{15–17}

After surface injury, IECs surrounding the wounded area lose their columnar polarity and quickly migrate to the lesion to initiate wound healing. This process is called “epithelial restitution,” which begins within minutes after injury and has been identified as a crucial step in resealing of the injured

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surface.¹⁷ Epithelial restitution was found to be independent of cell proliferation and regulated by cytokines. Tumor growth factor (TGF)- α , epidermal growth factor, interleukin (IL)-1 β , and interferon (IFN)- γ augmented epithelial restitution in wounded IEC monolayers by inducing the production of bioactive TGF- β 1 in epithelial cells.¹⁸ However, TGF- β -independent pathways to induce epithelial restitution exist. In fact, it has been shown that secreted trefoil peptides, galectin-2 and galectin-4, promote IEC restitution independent of TGF- β .^{16, 19} Furthermore, changes of the actin cytoskeleton induced by small GTPases of the Rho family are required for epithelial reconstitution²⁰ and this process is modulated by chemokines including CXCR4 and CXCL12.²¹ In addition, CCL20 and human β -defensin 2 have been shown to favor epithelial restitution by inducing cell migration of IECs without inducing cell proliferation.²²

Epithelial restitution is followed by additional steps in wound healing such as increased epithelial cell proliferation and differentiation. These processes start after epithelial restitution within hours or days after injury.^{23,24} Cell proliferation is important to increase the pool of IECs available to resurface the defect, but requires tight control mechanisms to prevent uncontrolled proliferation. Accordingly, IECs receive signals via growth factors such as epidermal growth factor, cytokines such as IL-6 and IL-22, and Toll-like receptor (TLR) ligands such as lipopolysaccharide (LPS) and CplusG DNA to induce activation of master transcription factors (e.g. nuclear factor- κ B, signal transducer and activator of transcription 3 (STAT3)), cell survival, and cell proliferation.^{8,25,26} For instance, both IL-6 and IL-22 are known to boost proliferation of IECs by activation of the transcription factor STAT3.^{27,28} The relevance of such regulation was highlighted by the finding that inactivation of STAT3 in IECs suppresses experimental wound healing *in vivo*. In contrast to IL-6 and IL-22, the proinflammatory cytokines IFN- γ and tumor necrosis factor (TNF) were found to block IEC proliferation and impaired wound healing by favoring IEC apoptosis.^{29,30} However, growth hormone was shown to induce activation of epithelial STAT5 and support wound healing. Consistently, STAT5 knockout in IECs led to impaired experimental wound healing by activation of epithelial myosin light-chain kinase with subsequent alterations of tight junctions and barrier function.^{31,32} In addition to cytokines, prostaglandin E2 contributes to survival of IECs in tissue injury and prevents IEC apoptosis. Importantly, regulatory factors for epithelial proliferation are not only derived from IECs themselves but also from immune and nonimmune cells in the local microenvironment of the gut. Although macrophages and T cells are known to produce IL-6 and TNF to regulate IEC proliferation and survival, fibroblasts were found to produce hepatocyte growth factor to induce IEC proliferation.^{28,33,34} Furthermore, granulocyte-macrophage colony-stimulating factor-induced dendritic cells were found to regulate wound healing of IECs.³⁵

TLRs are additional key regulator of wound healing.³⁶ In the healthy mucosa, TLRs act as innate receptors for pathogen-associated molecular patterns in commensal bacteria. The

involvement of specific TLRs in mucosal wound healing has been documented by numerous studies in recent years. TLR2 has been shown to selectively induce synthesis of trefoil factor TFF3, thereby favoring survival of IECs and mucosal wound healing.³⁷ In these studies, TLR2-deficient mice demonstrated increased morbidity and mortality during acute colonic injury and this phenotype could be rescued by administration of recombinant TFF3.³⁸ Additional studies indicated a regulatory role of the LPS ligand TLR4 in wound healing.³⁹ In acute mucosal injury during dextran sulfate sodium colitis, TLR4 - / - deficiency was associated with reduced infiltration of inflammatory cells compared with wild-type mice. TLR4-deficient mice showed impaired neutrophil recruitment with diminished macrophage inflammatory protein-2 expression by lamina propria macrophages. Furthermore, TLR4 was found to limit bacterial translocation during mucosal injury, suggesting that TLR4 controls mucosal wound healing through several mechanisms. Finally, recent studies suggested that TLR9 has a crucial role in mucosal wound healing. TLR9 serves as a ligand for CplusG motifs in bacterial DNA and thus acts as a link between the commensal microflora and the innate immune system in the gut. TLR9 expression was found on the apical and basolateral membranes in IECs.^{40,41} However, although apical TLR9 stimulation induced intracellular tolerance to subsequent TLR challenges and restricted inflammation, basolateral TLR9 activation resulted in proinflammatory nuclear factor- κ B responses. TLR9-deficient mice were found to be more susceptible to acute dextran sulfate sodium injury and demonstrated delayed wound repair highlighting the protective role of TLR9 in wound healing. Mechanistically, TLR9 deficiency caused reduced gene expression of *hairy enhancer of split 1*, an epithelial differentiation factor, and vascular endothelial growth factor, a growth factor controlling epithelial cell restitution.⁴² The potential therapeutic relevance of these observations was highlighted by the finding that a TLR9 agonist ameliorated mucosal injury and inflammation by inducing type I interferons.⁴³ Moreover, immunostimulatory DNA and probiotics appear to be mediated by their protective effects on barrier function, mucosal healing, and intestinal inflammation by activation of TLR9 signaling.^{44,45} Thus, in contrast to other TLR ligands such as LPS that may induce overwhelming immune responses upon administration, TLR9 ligands selectively induced anti-inflammatory immune responses and strengthened barrier function.

Cell proliferation, expansion, migration, and differentiation ultimately lead to closure of erosions and ulcerations. This process is supported by antimicrobial peptides and products released by Paneth cells such as defensins and REG proteins (regenerating protein family) that have an important role in intestinal homeostasis^{3,46} as well as by mucins produced by goblet cells that prevent translocation of commensal bacteria with subsequent immune cell activation. Recently, the cysteine-rich domains of the intestinal mucin mucin-3 (MUC3) were found to promote IEC migration, inhibit IEC apoptosis, and accelerate experimental wound healing.⁴⁷ In some cell lines, human β -defensin 2 augmented mucin production (MUC2,

MUC3) and prevented TNF-mediated IEC apoptosis, suggesting that defensins have pleiotropic protective functions in experimental wound healing in addition to their effects on epithelial restitution.⁴⁸

Given the clinical relevance of mucosal healing for patients with IBD, it is of major relevance to identify new targets for therapy that may induce mucosal healing. Thus, in the following paragraphs, we will discuss novel approaches for therapy of IBD and their potential impact on mucosal inflammation.

MODULATION OF MICROBIOTA, BARRIER FUNCTION, AND INNATE IMMUNITY IN IBD

The commensal microflora in healthy humans is estimated at 10^{13} to 10^{14} with a complex microbial community.⁴⁹ Given the high numbers of antigen-presenting cells and lymphocytes in the normal intestine, it is clear that an intact barrier function is essential for prevention of uncontrolled mucosal inflammation.²⁶ The gut barrier comprises IECs with tight junctions, adherens junctions, and desmosomes as well as antimicrobial peptides and mucins.^{17,50}

Numerous studies in recent years have described alterations in barrier function in IBD that favor diarrhea and uptake of potentially noxious antigens^{50,51} (Figure 1). For instance, alterations of IECs in IBD comprise reduced numbers of horizontal tight junction strands and altered tight junction protein expression. Furthermore, IECs in IBD have been described to be more susceptible to undergo apoptosis, leading to structural defects of the bowel wall with erosions and ulcerations. The structural changes of IECs in IBD are likely due to proinflammatory cytokines such as TNF, IFN γ , and IL-13, which are upregulated in the inflamed mucosa (Figures 2 and 3).^{50,52} These cytokines favor IEC apoptosis and may thus have an important role in the altered barrier function in IBD. For instance, TNF favors alterations of tight junctions and cell death of IEC via TNFR1 signaling, indicating that this cytokine induces barrier alterations in IBD.⁵³ Indeed, anti-TNF antibody therapy has been shown to improve IEC-controlled barrier function in IBD patients, suggesting that this treatment may have a profound effect on IEC function *in vivo*.⁵⁴ Furthermore, IL-13 is highly produced by T cells in patients with UC and impairs barrier function.⁵⁵ Thus, antibodies against IL-13 might be a new approach for UC therapy and clinical studies addressing this concept are currently ongoing (Figure 4).

In addition to IECs, changes of barrier function in IBD comprise impaired mucin production.^{56,57} This problem is particularly relevant for the intestinal barrier, as mucins prevent a direct contact of the commensal microflora with IECs. In the normal human intestine, the mucus is organized around glycosylated MUC2 produced by goblet cells. It has been suggested that O-glycans on MUC2 may serve as nutrients for bacteria and have a role in the selection of the local bacterial strains. However, there are important regional differences with regard to the structure of the mucus barrier. In the murine large intestine, two mucus layers have been identified. Although the inner, dense mucus layer is devoid of bacteria and firmly adherent to IECs, the outer nonattached mucus layer contains

Figure 1 Signaling pathways controlling wound healing and intestinal inflammation. Intestinal epithelial cells, goblet cells, and Paneth cells receive signals from the local microenvironment of the gut. In particular, cytokines, Toll-like receptor (TLR) ligands and growth factors induce cellular signaling events, leading to apoptosis or proliferation and cell expansion, respectively. Defects in goblet cells may result in impaired production of antimicrobial peptides such as defensins. AIEC, adherent invasive *Escherichia coli*; GH, growth hormone; LPS, lipopolysaccharide; EGF, epidermal growth factor; FGF, fibroblast growth factor; GH, growth hormone; IL, interleukin; IFN, interferon; NF- κ B, nuclear factor- κ B; PGE2, prostaglandin E2; REG, regenerating protein family; STAT, signal transducer and activator of transcription.

commensal bacteria.^{58,59} In contrast to the large bowel, the mucus layer in the small intestine is discontinuous and thinner.

Severe alterations of the inner mucosal mucus layer have been noted in IBD, particularly in the colon of patients with UC. In these patients, expression levels of secretory MUC2 and transmembrane MUC3 and MUC4 proteins were found to be low or absent.⁶⁰ Thus, it is potentially interesting for therapy of IBD to overcome the impaired barrier function by modulation of mucin function or expression. One approach to circumvent the loss of mucin-controlled barrier function in UC that has been clinically tested consists of phosphatidylcholine (lecithin).⁶¹ Whereas the mucus on the surface of the large intestine normally contains high concentrations of phosphatidylcholine in lamellar structures, phosphatidylcholine is substantially reduced in UC but not CD patients and this reduction may favor bacterial translocation. This finding has led to the concept to treat patients with phosphatidylcholine.⁶² Indeed, in controlled clinical studies, delayed release phosphatidylcholine given daily was significantly superior to placebo in induction of remission in both patients with non-steroid-treated active UC and steroid-dependent active UC. Future phase 3 studies will have to confirm these observations to underline the relevance of the concept that phosphatidylcholine strengthens barrier function and prevents translocation of commensal bacteria with subsequent immune cell activation in UC.

Various studies in recent years have attempted to suppress chronic intestinal inflammation by inducing anti-inflammatory pathways via antimicrobial peptides. One example consists of the induction of defensins as antimicrobial peptides. Defensins are produced by Paneth cells in the terminal ileum

Figure 2 Hypothetical pathogenesis of inflammatory bowel disease (IBD). Although ulcerative colitis (UC) affects the mucosa and submucosa, inflammation may affect all layers of the bowel wall in Crohn's disease (CD). Pathogenesis of IBD is related to an altered barrier function in a genetically susceptible host allowing translocation of bacteria and bacterial antigens into the mucosa. Antigens are taken up by antigen-presenting cells such as dendritic cells and macrophages, followed by antigen presentation to T cells. T lymphocytes enter into the lamina propria via homing from the blood stream and undergo T helper cell polarization upon antigen presentation. Although T helper type 1 (Th1) T cells expressing T-bet predominate in CD,^{121,140,193} T cells producing interleukin (IL)-5 and IL-13 are found in UC. Th17 cells expressing RAR-related orphan receptor A/C (ROR α /C) have been described in UC and CD.^{124,194} Finally, a cell population expressing both Th17 and regulatory T cell (Treg) markers has been recently been described in CD.¹²⁹ MadCAM-1, mucosal vascular addressin cell adhesion molecule 1; TNF, tumor necrosis factor.

and these cells are markedly reduced in active CD because of TNF-mediated necroptosis.⁶³ Furthermore, reduced expression of the α -defensins HD5 and HD6 by Paneth cells has been found in ileal CD, whereas production of the β -defensin HBD1 by goblet cells was suppressed in colonic CD.⁴⁶ These studies have raised expectations that modulation of defensin levels might be used for treatment of gut inflammation in CD, particularly for maintenance of remission. Although treatment with recombinant defensins has not been tested in clinical trials, it was found that overexpression of HD5 in transgenic mice results in resistance against infection with orally administered *Salmonella typhimurium* and experimental colitis.⁶⁴ This finding is at least consistent with the idea that defensins might be used for IBD therapy. Furthermore, the probiotic strain *Escherichia coli* Nissle has been shown to induce defensin production and was as effective as 5-aminosalicylates in maintaining remission in patients with UC.^{65,66} It is thus possible that induction of defensin production contributes to the clinical efficacy of *E. coli* Nissle in UC. However, in contrast to this disease, probiotic strains such as *E. coli* Nissle have not been very effective in CD so far.

As bacteria use CplusG sequence motifs to induce immune cell activation via TLR9 signaling, modulation of such innate immune responses is another new approach for IBD therapy.

As CpG DNA has been successfully used for therapy of experimental colitis,^{45,67} this concept has been recently tested in a small study for steroid-dependent or steroid-refractory UC patients by topical administration of a CpG oligonucleotide (DIMS0150).⁶⁸ However, future studies will have to unequivocally prove the efficacy of this treatment in UC.

In addition to probiotics, antibiotics have been used for modulation of the commensal microflora in IBD. In contrast to other antibiotics, pilot studies suggested that oral administration of the antibiotic agent rifaximine induces remission in CD^{69,70} and that metronidazole and ornidazole suppress postoperative relapses in this disease.⁷¹ However, large phase 3 studies are missing and antibiotics are therefore not yet standard of care for IBD therapy in the absence of systemic infections.

Recent evidence suggests that IBD patients may be exposed to uncontrolled immune cell activation upon translocation of bacteria because of the defects in autophagy.⁷² In fact, single-nucleotide polymorphisms in *ATG16L1* and *IRGM* genes have been identified in CD patients and both genes have been shown to have a crucial role in autophagy/xenophagy during innate immune responses.⁷³ For instance, *ATG16L1* and *IRGM* both control antibacterial autophagy of *Salmonella typhimurium* and adherent invasive *E. coli* (AIEC). This finding is of potential

Figure 3 Signaling events in inflammatory bowel diseases. **(a)** Cytokine signaling events that have major effects of T-cell activation and survival in inflammatory bowel disease (IBD) patients. Although the interleukin (IL)-12/IL-23 pathway is relevant for Crohn's disease (CD) pathogenesis, the IL-6/soluble IL-6R (sIL-6R) pathway is additionally being explored for IBD therapy. Finally, the membrane-bound tumor necrosis factor (mTNF)/tumor necrosis factor receptor 2 (TNFR2) signaling pathway is important for the pathogenesis of both CD and ulcerative colitis (UC). **(b)** The IL-6 signaling pathway in IBD. Membrane-bound IL-6R (mIL-6R) undergoes shedding via proteases such as TACE (tumor necrosis factor-converting enzyme) and ADAM17 (ADAM metalloproteinase domain 17), resulting in the production of the sIL-6R.¹³⁴ IL-6 together with the sIL-6R activates the gp130 target molecule on both T cells and epithelial cells. This results in signal transducer and activator of transcription 3 (STAT3) activation and cell activation and survival. In this way, IL-6 signaling favors T-cell resistance against apoptosis in IBD. In addition, such signaling favors growth of epithelial neoplasias in IBD (modified according to refs. 28,131,132). **(c)** Potential mechanism of action of anti-TNF antibodies in IBD. Anti-TNF antibodies block the interaction between mTNF on CD14+ antigen-presenting cells with TNFR2 on lamina propria T cells. Accordingly, this treatment suppresses TNF receptor-associated factor 2 (TRAF2) and nuclear factor-κB (NF-κB) activation with IL-6 production and T-cell survival. Subsequently, anti-TNF antibodies induce programmed cell death (apoptosis) of T cells in the inflamed gut and such apoptosis correlated to the clinical efficacy of anti-TNF therapy (modified according to ref. 112). EGFR, epidermal growth factor receptor.

interest, as AIECs are thought to have an important regulatory role in CD.⁷⁴ Additional CD susceptibility genes such as nucleotide oligomerization domain 2 may also control autophagy/xenophagy in the inflamed intestine.⁷⁵ As defects in xenophagy may cause impaired clearance of bacteria and inappropriate immune responses, it is likely that these findings contribute to changes in innate immunity in IBD. Prevention of inappropriate handling of translocated bacteria may thus be relevant for IBD therapy. In fact, AIECs present in inflamed CD mucosa in the terminal ileum require TNF to favor their replication in infected lamina propria macrophages.⁷⁶ Anti-TNF treatment has been shown to suppress AIEC replication in infected immune cells and this observation might open new avenues for therapy in the future.

Taken together, substantial progress has been made in our understanding of the coordinated interplay between the commensal microflora, antimicrobial peptides, and innate immunity in IBD. It has become increasingly clear that loss of barrier function is a key phenomenon in IBD that favors secretory diarrhea and translocation of potentially noxious bacterial antigens.^{50,51} The latter point is of particular relevance, as translocation of commensal bacteria into the mucosa is followed by expansion of antigen-presenting cells and T cells that produce proinflammatory cytokines to induce and maintain chronic intestinal inflammation. However, with the important exception of probiotic therapy with *E. coli* Nissle,

these approaches have not reached clinical routine as yet. Future studies will have to demonstrate the relevance of modulation of the microflora for IBD therapy.

MODULATION OF ADAPTIVE IMMUNITY IN IBD: CLASSICAL IMMUNOSUPPRESSIVE DRUGS

Most established therapies for IBD have major effects on the adaptive immune system. For instance, corticosteroids that are used for induction of clinical remission in both CD and UC strongly suppress proinflammatory cytokine production by macrophages and T cells.⁷⁷ Furthermore, cyclosporin A and tacrolimus that are effective for induction of remission in severely active UC^{78,79} and methotrexate that is used for chronically active CD^{80,81} are known to suppress proinflammatory cytokine production by T cells.^{82–85} The former drugs are known to bind to cyclophilins in T lymphocytes to interfere with Ca signaling and inhibit activity of NFAT (*nuclear factor of activated T cells*) transcription factors (NFATc1–4). NFATc2 in turn has been shown to control IL-6 production and T-cell survival in colitis.^{86,87} Finally, azathioprine and 6-mercaptopurine are effective in maintenance of remission in both CD and UC by blocking T-cell activation.^{88,89} Specifically, it was found that these thiopurine drugs block T-cell costimulation by interfering with the vav1/Rac1 signaling pathway in T cells. Azathioprine- and 6-mercaptopurine-derived 6-thio-GTP has been shown to bind to the small GTPase Rac1 to block

guanosine exchange activity of vav1 on this protein. Consecutively, Rac1 activity is blocked over time, leading to inhibition of ERM (ezrin–radixin–moesin)-dependent immunological synapse formation between antigen-presenting cells and T cells. Furthermore, suppression of Rac1 activation induces T-cell apoptosis (via blockade of STAT3 activation). The clinical relevance of this observation was highlighted by the finding that clinical responsiveness to thiopurine therapy correlates to the induction of T-cell apoptosis in the inflamed intestine in IBD.⁸⁸ Collectively, the above findings indicate that classical immunosuppressive drugs that are successfully used for IBD therapy specifically interfere with the adaptive immune system. As not all IBD patients respond to this type of therapy and as these drugs may cause severe side effects in some patients, it is of high clinical relevance to define novel drugs that block specific signaling pathways in cells of the adaptive immune system. In the following part of the review, the development of anti-TNF antibody therapies for IBD as well as some novel approaches to attack key proinflammatory pathways in chronic intestinal inflammation are discussed.

ATTACKING KEY PROINFLAMMATORY PATHWAYS IN IBD: ANTI-TNF ANTIBODIES AND BEYOND

Macrophages and T lymphocytes produce large amounts of the proinflammatory cytokine TNF- α in IBD and this cytokine has a pivotal role in IBD pathogenesis.² These cells produce TNF as a transmembrane protein (mTNF) whose soluble form (sTNF) is released by proteolytic cleavage via the protease TACE (tumor necrosis factor-converting enzyme)/ADAM17 (ADAM metallopeptidase domain 17).⁹⁰ Thus, two forms of TNF exist in the inflamed gut and these forms appear to exert different functions in IBD. Although sTNF is released by immune cells to deliver signals in the local environment or systemic circulation by binding to TNF-R1 on target cells, mTNF is mainly expressed on the surface of CD14 + mucosal macrophages, and preferentially binds to TNF-R2 on lamina propria T cells (Figure 3).⁹¹

The clinical development of neutralizing antibodies against TNF has been a crucial milestone for IBD therapy. In fact, randomized controlled trials found that the anti-TNF antibodies adalimumab, certolizumab pegol, and infliximab were effective for induction and maintenance of remission in both CD and UC.^{92–96} Furthermore, anti-TNF therapy was found to induce fistula closure in CD⁹⁷ and induced mucosal healing in both CD and UC.^{10,98} On the basis on these landmark studies, anti-TNF therapy has been approved for treatment of patients with moderate-to-severe IBD. Although infliximab is a chimeric anti-TNF antibody with murine sequence parts (intravenous administration), certolizumab is a humanized, pegylated anti-TNF Fab fragment and adalimumab is a fully human anti-TNF antibody (subcutaneous administration). In addition to these antibodies, there are numerous new developments in the anti-TNF field. This includes the fully human anti-TNF antibody golimumab that has recently successfully passed phase 3 studies in UC (subcutaneous administration) (Figure 4).^{99,100} Furthermore, small anti-TNF molecules given

orally, biosimilars, and nanobodies are currently being developed for clinical use.¹⁰¹ In this context, one interesting concept consists of genetically modified bacteria-producing anti-TNF nanobodies.¹⁰² Daily oral administration of nanobody-secreting *Lactobacillus lactis* resulted in local delivery of anti-TNF nanobodies in the gut and led to significantly reduced inflammation in mice with dextran sulfate sodium-induced experimental chronic colitis. Finally, active immunization with a therapeutic vaccine against TNF is another alternative as anti-TNF targeting strategy that is potentially devoid of some treatment limitations of current anti-TNF-blocking agents.¹⁰³ Indeed, active immunization with the so-called anti-TNF kinoid has recently been tested in patients with CD and induced polyclonal anti-TNF antibodies in these patients. Future clinical trials will determine the relevance of this strategy for anti-TNF therapy in IBD patients.

In spite of the clinical efficacy of anti-TNF antibody treatment in IBD, however, a subgroup of patients do not respond to anti-TNF therapy within 4–12 weeks after initiation of therapy.^{104–106} These patients show little or no changes of clinical symptoms and no mucosal healing upon anti-TNF therapy but are potentially exposed to side effects of this type of therapy, such as infections, reactivation of tuberculosis, allergic reactions, skin disorders, demyelinating disorders, and lupus-like autoimmunity.¹⁰⁷ Potential reasons for failure of anti-TNF antibody therapy include the development of inhibitory antibodies (human antichimera or human antihuman antibodies) as well as insufficient antibody trough levels in the serum due to underdosing.¹⁰⁸ Furthermore, there is the possibility of mechanistic failure of antibody therapy caused by TNF-independent gut inflammation. It is thus of interest to understand the mechanism of action of anti-TNF antibodies in IBD to identify reasons for therapy failure.

Although several potential mechanisms of action have been proposed for anti-TNF therapy in IBD (e.g. barrier improvement, direct cytotoxicity, induction of regulatory macrophages, and T cells),^{54,109,110} several lines of evidence suggest that the induction of T-cell apoptosis is a key mechanism of action of anti-TNF antibodies. First, in an elegant series of studies involving *in vivo* imaging with radiolabeled annexin V antibodies, it was found that T-cell apoptosis occurs within the first days after the initiation of anti-TNF therapy in IBD.¹¹¹ Moreover, the induction of apoptosis was directly correlated to the rapid clinical response of the patients and patients without the induction of T-cell apoptosis showed little or no response to anti-TNF therapy. Additional studies revealed that clinically effective anti-TNF antibodies do not directly induce T-cell apoptosis of purified primary lamina propria CD4 + T cells in IBD patients, although these antibodies are capable of inducing T-cell death directly in tumor cell lines via induction of bax and bak.^{112–114} Instead, it was found that the anti-TNF antibodies infliximab, adalimumab, and certolizumab pegol bind to mTNF, block the interaction between mTNF on CD14 + macrophages and TNFR2 on lamina propria T cells, and suppress TNF receptor-associated factor 2 and nuclear factor- κ B activation in T cells followed by T-cell apoptosis (Figure 3).

Thus, it appears that the clinically effective anti-TNF antibodies adalimumab, certolizumab pegol, and infliximab mainly work by blocking mTNF signaling to induce T-cell death. This concept is supported by the clinical observation that the fusion protein etanercept that mainly blocks sTNF rather than mTNF signaling was not effective for the therapy of IBD.¹¹⁵ Furthermore, there is recent direct evidence that expression of mTNF on mucosal target cells is important for clinical efficacy of anti-TNF antibodies. In a phase 1 study of 25 in patients with active CD, mTNF expression on gut immune cells was visualized by topical administration of a fluorescent anti-TNF antibody (adalimumab), followed by confocal laser endomicroscopy for mTNF imaging. Interestingly, subsequent clinical therapy with adalimumab revealed that patients with high amounts of mTNF+ cells in the mucosa were significantly more likely to respond to anti-TNF therapy than patients with low amounts of mTNF+ cells.¹¹⁶ This observation opens new avenues for individualized adalimumab therapy in IBD and suggests that failure to anti-TNF therapy may occur in patients with low amounts of mTNF+ lamina propria cells due to TNF-independent gut inflammation. If this concept holds true, it might be of interest to target the mTNF/TNFR2 interaction specifically in the future rather than total TNF (mTNF plus sTNF). This approach might also avoid some potential side effects of anti-TNF therapy such as the reactivation of tuberculosis that is likely related to the blockade of the sTNF/TNFR1 interaction. In any case, the key role of the mTNF/TNFR2 pathway has also been highlighted in animal models of IBD¹¹⁷ and is an important difference to other immune-mediated diseases such as rheumatoid arthritis, where sTNF appears to be very important for disease pathogenesis and where etanercept is clinically highly effective.¹¹⁸

The findings that anti-TNF therapy is not effective in a relevant subgroup of IBD patients and that response to this therapy is lost in an additional subgroup of patients (up to 60% during the first year in CD patients) have led to an intensive search for novel therapeutic approaches. One concept derived from these studies was that combination therapy of the anti-TNF antibody infliximab plus azathioprine was significantly better than monotherapy with infliximab or azathioprine in both CD and UC.⁹² Thus, a combined top-down therapy with these agents might be useful in patients with severely active disease to prevent further complications. Furthermore, additional neutralizing antibodies against proinflammatory cytokines, chemokines, or costimulatory pathways have been clinically tested in IBD to identify new targets beyond TNF. In the following paragraphs, some of the recent new clinical and experimental approaches for therapy of chronic intestinal inflammation are reviewed.

TARGETING NEW CYTOKINES, CHEMOKINES, AND COSTIMULATORY PATHWAYS IN IBD

The exciting results of the anti-TNF trials have raised expectations that selective targeting of other cytokines in IBD might be as good or even better than targeting of TNF. Indeed, targeting of other cytokines beyond TNF has been

effective in other diseases such as rheumatoid arthritis and psoriasis.^{119,120} As CD is associated with a mixed T-helper type 1 (Th1) and Th17 cytokine profile,^{121–124} targeting of cytokines from these T-cell subsets appeared to be of potential relevance for CD therapy (**Figures 1 and 3**). Unfortunately, anti-IFN- γ therapy with the humanized antibody fontolizumab did not result in major improvement of CD symptoms in moderate-to-severe disease activity, although a decrease of CRP levels was noted.^{125,126} This led to the hope that targeting of Th17 cytokines might be more effective than targeting IFN- γ . However, although ROR γ -expressing Th17 cells producing IL-17A/F have been shown to control experimental colitis in mice,¹²⁷ targeting of IL-17A alone (via the monoclonal antibody secukinumab) did not improve but rather aggravated clinical symptoms in CD.¹²⁸ Similarly, anti-IL-17R antibody therapy did not show clinical efficacy in CD, suggesting that targeting of IL-17A or IL-17R signaling is not suitable for CD therapy. The reasons for these disappointing results are not clear. It is possible that targeting of Th17 cytokines boosts Th1 immune responses in CD. Furthermore, anti-IL-17 therapy might affect regulatory T (Treg) cells as a cell population expressing both Th17 and Treg markers has been recently described in CD.¹²⁹ However, as ROR γ deficiency in T cells caused an impressive reduction in the activity of experimental colitis,¹²⁷ targeting of Th17 cells themselves rather than targeting individual Th17 cytokines might be an option for CD therapy in the future. Similarly to IL-17R signaling, targeting of IL-2R signaling in UC with the monoclonal antibody basiliximab did not result in marked improvement of disease activity, indicating that targeting of IL-2R signal transduction is not effective in this disease.¹³⁰

Another interesting cytokine for IBD therapy consists of IL-6. IL-6 signaling via the sIL-6 receptor and gp130 has been shown to cause T-cell activation and mediates T-cell resistance against apoptosis in IBD patients (**Figure 3**).^{131,132} Furthermore, antibodies against the IL-6R were effective for therapy in animal models of colitis and induced T-cell apoptosis in cell culture experiments with lamina propria cells from IBD patients. Finally, IL-6 signaling was found to be crucial for the development of colitis-associated neoplasias in mouse models,²⁸ suggesting that targeting of IL-6 signaling might be useful for IBD therapy. Interestingly, an initial study using the humanized anti-IL-6R antibody MRA showed a clinical response in 80% of the CD patients given biweekly MRA as compared with 31% in the placebo group.¹³³ However, no effects on mucosal healing were noted after 12 weeks. As no phase 3 studies on the use of MRA in CD were subsequently performed, the clinical efficacy of this approach needs to be confirmed by future studies. Another concept for targeting of IL-6 function in IBD consists of a recombinant gp130-Fc protein for suppression of sIL-6R signaling.¹³⁴ It is expected that this approach will enter clinical testing soon.

Studies in experimental colitis have shown that additional proinflammatory cytokines might be new targets beyond TNF that could be explored for IBD therapy. Among these cytokines are IL-18, IL-21, IL-27, and IL-13.^{135–139} The targeting of IL-13

has recently gained particular attention, as blockade of IL-13 expression and function was highly effective in the treatment of experimental colitis. In addition, IL-13 is increasingly produced in UC and targets epithelial cell survival and barrier function in this disease.^{55,140,141} Current phase 2 studies will clarify whether targeting of this T-cell-derived cytokine will be beneficial in UC therapy (**Figure 4**).

In addition to targeting of IFN- γ and IL-17A, blockade of the CD28/B7.1-2 interactions with the monoclonal antibody abatacept was not successful for CD and UC therapy.¹⁴² Furthermore, the anti-CD3 antibody visilizumab was not effective for the treatment of corticosteroid-refractory UC.¹⁴³ Similarly, blockade of the chemokine IP-10 by monoclonal antibodies (BMS936557) was not effective in a recent UC trial.¹⁴⁴ Given these disappointing findings, new concepts for suppression of proinflammatory cytokines and T-cell activation are needed. As many studies targeting single cytokines have failed, it could be of interest to develop novel approaches for the blockade of several cytokines in IBD. Such concepts might involve small molecules for cytokine inhibition, bispecific antibodies targeting two cytokines simultaneously, Jak inhibitors or antibodies targeting cytokine subunits that affect several cytokines.^{145,146} With regard to the latter possibility, antibodies against the p40 subunit of IL-12 and IL-23 have been successfully used in animal models of colitis^{147–149} and appear to affect Th1 and Th17 cells simultaneously in chronic intestinal inflammation.^{123,150,151} Although IL-12 favors Th1 T-cell polarization in colonic inflammation via the transcription factor STAT4, IL-23 induces STAT activation in Th17 cells and stabilizes their phenotype (**Figure 2**).^{152,153} On the basis of promising results in phase 2 studies with anti-p40 antibodies,^{154–156} a recent phase 3 study has tested the efficacy of the anti-p40 antibody ustekinumab in moderate-to-severe CD (**Figure 4**).¹⁵⁷ It was found that ustekinumab induces clinical responses in CD patients, especially in those previously given the anti-TNF antibody infliximab. Thus, ustekinumab may be a new treatment option for anti-TNF refractory CD.

In addition to anti-p40, recent studies have looked at the effects of Jak inhibition in IBD. Interestingly, a recent phase 2 study on the use of the Jak1/Jak3 inhibitor tofacitinib in UC showed impressive results with significantly higher response and remission rates in tofacitinib-treated patients as compared with placebo (**Figure 4**).¹⁵⁸ Tofacitinib treatment also induced mucosal healing on endoscopy in many patients. If these results are confirmed in phase 3 studies, it is likely that tofacitinib will emerge as a new treatment option for UC. Tofacitinib targets both Jak1 and Jak3 and will thus block the action of numerous cytokines dependent on Jak1/Jak3, such as IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (**Figure 4**).^{158,159} In addition, this drug may affect the function of cytokines dependent on Jak1/Jak2, such as IL-6, IL-11, IFN- γ , and IFN- α /IFN- β . Given these pleiotropic effects on multiple cytokines, the safety profile of tofacitinib will require further analysis in future studies.

Collectively, substantial progress has been made in recent years with regard to targeting of proinflammatory cytokines. In

spite of some disappointments, several agents such as ustekinumab for CD and tofacitinib for UC may find their way to the clinic and offer significant therapeutic alternatives for the management of IBD.

INDUCING TREG CELLS AND ANTI-INFLAMMATORY CYTOKINES IN IBD

On the basis of the finding that IL-10-deficient animals spontaneously develop T-cell-dependent colitis and colitis-associated neoplasias,^{160,161} treatment with recombinant anti-inflammatory cytokines was tested for IBD therapy. Although treatment with recombinant anti-inflammatory cytokines was successful for the therapy of experimental colitis in mice, unfortunately, treatment of humans with systemic recombinant IL-10 or recombinant IL-11 did not result in improvement of clinical symptoms in CD.^{162,163} Another strategy consisted of topical delivery of IL-10 to the inflamed gut by transgenic bacteria (*L. lactis* LL-Thy12) producing IL-10. An initial phase 1 study in 10 CD patients showed that this concept is safe but studies on clinical efficacy have not been performed.¹⁶⁴ Another alternative could be the administration of bacterial strains that induce IL-10 production by host cells. In this context, *Clostridium butyricum* has been recently shown to prevent experimental colitis in mice by inducing IL-10 production of F4/80+ CD11b+ macrophages via the TLR2/MyD88 pathway.¹⁶⁵ Collectively, these results suggested that systemic administration of the recombinant anti-inflammatory cytokines IL-10 and IL-11 might not be a good approach for the therapy of IBD. However, local administration of IL-10 could be an alternative as well as a treatment with bacterial strains that induce intestinal IL-10 production, but these approaches require further development.

Additional interesting anti-inflammatory cytokines for IBD therapy are IL-22, IL-35, and TGF- β . IL-22 induces STAT3 activation in IECs and favors IEC proliferation and colonic wound healing *in vivo*.²⁵ Furthermore, IL-22 protects the mucus barrier through stimulation of mucin 1 production under intestinal inflammatory conditions and enhances the epithelial regeneration with goblet cell restitution.¹⁶⁶ Interestingly, IL-22-producing T cells are markedly reduced in UC patients with epithelial alterations,¹⁶⁷ suggesting that treatment with recombinant IL-22 might improve barrier function in UC by induction of STAT3-mediated proliferation in IECs and production of antimicrobial peptides. However, this concept has not been clinically tested so far. Similarly, IL-35 therapy has not been performed, although administration of recombinant IL-35 suppressed the activity of experimental colitis in mice by reducing Th1 and Th17 cytokine responses.¹⁶⁸

The cytokine TGF- β is produced by Treg cells and suppresses T-cell-mediated colitis in animal models.¹⁶⁹ In mice, recombinant TGF- β as well as administration of naturally occurring or inducible Treg cells have been successfully used for the treatment of experimental colitis, raising the possibility that this concept might be of relevance for treatment of IBD.^{170–172} With regard to Treg cells, a recent phase 1/2a study in 20 patients showed that a single injection of antigen-specific (ovalbumin)

Figure 4 New therapeutic concepts in inflammatory bowel diseases. **(a and b)** New therapeutic strategies in Crohn's disease (CD) and ulcerative colitis (UC) that are currently tested in phase 1 to phase 3 trials. Targets, drugs, and antibodies are shown for both diseases. Clinically approved drugs are indicated at the bottom of both panels (Natalizumab is not approved for CD therapy in Europe). Red dots indicate cytokine targets, whereas blue dots represent other targets. One dot is on the circle because it represent a phase 1/2 study rather than a pure phase 1 or phase 2 study. **(c)** Cytokine receptors targeted by the Jak1/Jak3 inhibitor tofacitinib in inflammatory bowel disease. This Jak inhibitor has shown promising results in patients with UC and is currently under further clinical development. A modified schematic diagram of cytokine signaling via Jak proteins according to Liao *et al.*¹⁵⁹ is shown. IL, interleukin; MAdCAM-1, mucosal vascular addressin cell adhesion molecule 1; STAT, signal transducer and activator of transcription; TGF, tumor growth factor; TNF, tumor necrosis factor.

Treg cells is safe in CD and it was found that about 40% of the patients show a clinical response after treatment.¹⁷³ This interesting study is consistent with the possibility that treatment with Treg cells might become available for IBD therapy, although larger phase 2/3 studies are clearly required to further develop this concept.

Treatment with recombinant TGF- β has not been considered for IBD therapy as yet, mainly because of concerns regarding the well-known fibrogenic and cancer-supporting effects of this cytokine.¹⁷⁴ In addition, TGF- β effects in IBD T cells are inhibited by the protein Smad7 and Smad7 is markedly overexpressed in IBD patients.¹⁷⁵ Inhibition of Smad7 via antisense DNA restored TGF- β sensitivity in IBD T cells and was effective in murine models of experimental colitis.^{176,177} On the basis of these findings, a recent phase 1 study on Smad7 antisense DNA in CD patients has yielded impressive results that are currently extended in a larger phase 2 study.¹⁷⁸ It is thus possible that targeting of TGF- β effects is a realistic option for IBD therapy in the future. Furthermore, TGF- β has been implicated in the clinical effects of worm ova therapy in IBD.¹⁷⁹

As IBD are uncommon in underdeveloped areas of the world where many people suffer from worm infections, ingestion of live *Trichuris suis* ova has been tested for IBD therapy. Treatment with 2500 live ova every 3 weeks for 24 weeks was found to be effective in phase 2 studies in both CD and UC.^{180,181} On the basis of these findings, larger phase 3 studies are currently under way to confirm the therapeutic efficacy of this approach. Interestingly, transcriptome analysis of *T. suis* predicted secreted molecules that are related to TGF- β ,¹⁸² suggesting that this therapy might induce local immunosuppression by induction of Treg cells. Although further studies on this concept are needed, these findings suggest that therapy with *T. suis* ova might be a new option for the treatment of IBD.

TARGETING CELL ADHESION AND HOMING IN IBD: A WHOLE NEW WORLD

Active IBD is dependent on the recruitment of mononuclear cells and leukocyte populations from the blood stream into the bowel wall. Recruitment is dependent on a series of steps known as rolling, tight binding/adhesion to endothelial cells,

diapedesis, and migration of immune cells. This process is coordinated by selective adhesion molecules on the surface of immune cells and mucosal addressins on endothelial cells.¹⁸³ Selective adhesion molecules include cell-surface integrins that form heterodimers by various combinations of α - and β -subunits. For gut homing of leukocytes, the interaction between α_4/β_7 -integrins on T cells and the mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) addressin on endothelial cells appears to be of crucial relevance.

A proof-of-concept study was carried out in 1993, in which spontaneous colitis in cotton top tamarins was treated with a neutralizing antibody against the α_4 -integrin subunit.¹⁸⁴ This therapy led to a significant reduction of mucosal inflammation and suggested further avenues for clinical development. Indeed, later clinical studies observed that the neutralizing humanized α_4 antibody natalizumab has clinical efficacy in maintaining remission in CD, although the primary efficacy end point on induction of clinical responses at week 10 was not achieved in phase 3 studies in this disease.¹⁸⁵ The remarkable data on maintenance of remission were associated with an improvement in quality-of-life parameters in CD. However, the use of natalizumab in CD led to rare serious adverse events caused by polyoma JC virus infection with progressive multifocal leukoencephalopathy.¹⁸⁶ The induction of this progressive, fatal infection was probably due to the fact that natalizumab blocks α_4/β_1 integrins in addition to α_4/β_7 heterodimers and that the former integrins are important for T-cell homing to the central nervous system. Therefore, natalizumab might have caused severe immunosuppression in these patients with consecutive development of progressive multifocal leukoencephalopathy based on the blockade of the interaction between α_4/β_1 -integrin and its ligand vascular cell adhesion molecule. To circumvent this problem, subsequent clinical trials (phase 2/3) used a second humanized monoclonal antibody, denoted vedolizumab (MLN-02/LDP-02), that specifically targets the α_4/β_7 heterodimer in IBD.¹⁸⁷ Intravenous administration of vedolizumab led to significantly higher response and remission rates in UC patients as compared with placebo.¹⁸⁸ Moreover, vedolizumab induced mucosal healing compared with placebo. Further studies showed that vedolizumab favors maintenance of remission in UC, suggesting that this substance will soon be available for clinical routine therapy in this disease. Importantly, vedolizumab also appeared to be effective for the treatment of anti-TNF refractory UC.

On the basis of these promising results, additional anti-adhesion strategies are currently being tested in IBD, including the anti- β_7 antibody etrolizumab.¹⁸⁹ Etrolizumab therapy was significantly more effective for induction of remission in a recent phase 2 study in UC than placebo and further phase 3 development is expected soon (**Figure 3**). In addition, a MAdCAM-1 antibody targeting the α_4/β_7 ligand is now in clinical trials in UC. Moreover, a selective small-molecule antagonist of the CCR9 chemokine receptor that targets the interaction between CCR9 on leukocytes and its ligand CCL25 on endothelial cells is currently tested in CD of the small bowel.¹⁹⁰ This antagonist targets a tissue-specific

lymphocyte-trafficking molecule that controls T- and B-cell homing to the small intestine.¹⁹¹ Finally, a new concept to target lymphocyte traffic in UC consists of the sphingosine-1-phosphate receptor agonist fingolimod (FTY-720).¹⁹² This substance induces retention of leukocytes in lymph nodes and thereby prevents their entry into the gut mucosa. Clinical data will have to demonstrate whether this substance has a role in IBD therapy.

Collectively, these findings suggest that targeting of leukocyte homing is an emerging new concept for IBD therapy. It is likely that further neutralizing antibodies or small molecules for the blockade of adhesion molecules will soon enter clinical development for IBD therapy. Additional studies will have to determine the clinical relevance of this new concept in comparison to established immunosuppressive strategies including anti-TNF therapy.

SUMMARY

In recent years, remarkable progress in IBD therapy has been made. The anti-TNF era has shown that mucosal healing is a key goal for therapy that predicts clinical remission and resection-free survival in IBD patients. Combined immunosuppression with azathioprine plus anti-TNF antibodies is a new concept for patients with severely active disease. As many patients do not respond to anti-TNF therapy, many new targets have been recently tested for induction of mucosal healing as well as for induction and maintenance of remission in IBD. It is expected that these new studies will have a marked impact on future therapy of IBD patients.

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DISCLOSURE

M.F.N. has served as an advisor to MSD, Pentax, Giuliani, 4SC AG, Teva, and Abbott.

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