New therapeutic strategies for treatment of inflammatory bowel disease

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Although the precise etiology of inflammatory bowel disease (IBD) still remains unclear, considerable progress has been made in the identification of cytokine-mediated signaling pathways involved in the inflammatory process. Recent data have clearly shown that these pathways induce augmented intestinal T-cell activation and thus resistance to apoptosis, which is a central process in disease pathogenesis, as it impairs mucosal homeostasis. Therefore, novel therapeutic strategies aim at restoring activated effector T-cell susceptibility to apoptosis in the gut, based on a pathophysiological rationale. This development is best exemplified by the emergence of agents that target the TNF pathway, IL-6 trans-signaling, and the IL-12/IL-23 pathway. These compounds give hope for the development of new strategies aiming at more effective and less toxic therapies for IBD.

INTRODUCTION

Inflammatory bowel diseases (IBD) comprise Crohn's disease and ulcerative colitis, which are defined as chronically relapsing inflammations of the gastrointestinal tract not due to specific pathogens.[1] While Crohn's disease is a multifocal, transmural inflammatory process that can affect any part of the digestive tract, ulcerative colitis is characterized by a superficial, continuous inflammation, which is limited to the large intestine. Although the precise etiology of IBD still remains obscure, extensive studies in the past decade have gradually unravelled several factors contributing to disease pathogenesis.

Genetic predisposition, environmental factors, infectious agents, impairment of local tolerance, and mucosal imbalance with ongoing activation of the intestinal immune system have been implicated in IBD pathogenesis.[2] Current concepts are based on a genetic susceptibility, which is modulated by various environmental factors,[3] resulting in the deregulation of the host's immune response to the indigenous intestinal flora. This leads to excessive activation of the intestinal immune system, which ultimately causes pathogenic gastrointestinal inflammation and tissue damage.[4]

The exact pathogenic mechanisms involved are nevertheless still incompletely understood and therapeutic strategies thus far have been limited to mostly evidence-based principles. The conventional treatment options for IBD were corticosteroids and immunosuppressive agents like azathioprine, which ameliorate the inflammatory process. However, corticosteroids have been insufficient for a large subgroup of patients, as population-based studies have proven that a significant fraction of corticosteroid-treated Crohn's disease patients develop steroid dependency or even steroid-refractory illness,[5] while the need for surgical intervention has apparently remained unchanged.[6] The moderate therapeutic effect and the possible association with severe side effects of these drugs demonstrated the necessity for a more sustained therapeutic response, which can only be the result of a more comprehensive approach in targeting critical points of signal transduction pathways involved in the inflammatory cascade.[7]

Recent advances in understanding the underlying immunopathogenetic mechanisms of disease in IBD have led to the development of biological therapies, which selectively inhibit crucial mediators of the inflammatory process. These therapeutic strategies are based on the conception that uncontrolled activation of central effector cells in the gut is the pivotal pathogenic mechanism involved in the initiation and perpetuation of the inflammatory reaction.[8,9] There is consensus that CD4 + T cells are the main activated immune cells involved in IBD pathogenesis, which are characterized by enhanced proliferation and trafficking into the intestinal mucosa.[4] This aberrant T-cell activation is also characterized by alterations in cytokine production, resulting in a disturbed balance between pro- and anti-inflammatory cytokines.[10] Deregulated cytokine profiles are currently an important focus of both clinical and basic research in IBD, which reflects their heightened importance for the development of new therapeutic strategies.

Augmented lamina propria T-cell activation, and thus resistance to apoptosis, has been identified as a key factor in IBD pathogenesis, as it impairs mucosal homeostasis and leads to unrestrained accumulation of activated lymphocytes that perpetuate the inflammatory response.[9,11,12] On the basis of these findings, which have elucidated the main cellular and molecular mechanisms involved in gut inflammation, novel therapeutic strategies evolved that specifically modify the aberrant immune response. In the following sections, therapeutic implications of recent advances made in the understanding of the mechanisms that confer resistance to apoptosis in intestinal CD4 + T cells in IBD are discussed and potential targets for future therapeutic strategies are presented.

INDUCING APOPTOSIS BY TARGETING TNF-α

The proinflammatory cytokine tumor necrosis factor-α (TNF-α) is mainly produced by activated macrophages and lymphocytes and exists as a transmembrane protein (membrane-bound TNF (mTNF)), from which the soluble form (sTNF) is released via proteolytic cleavage by the TNF-α-converting enzyme. Secreted TNF exerts its biological functions via two distinct cell surface receptors, tumor necrosis factor receptor 1 (TNFR1) (p55) and TNFR2 (p75).[13,14] mTNF can further activate target cells via cell–cell contact via TNFR2. Moreover, TNFR2 is able to ligate mTNF on the opposing cell and subsequently trigger signal transduction from the mTNF molecule to the nucleus. Due to its bipolar function, mTNF can not only act as a ligand, but also confer reverse signaling into cells expressing this molecule.[14]

In accordance with its implication as a mediator of inflammation in many autoimmune diseases, several studies have demonstrated that the production of the proinflammatory cytokine TNF-α is elevated in the intestinal mucosa and serum of IBD patients.[15–18] Accordingly, this cytokine has been considered as an attractive target for the treatment of IBD, and several anti-TNF agents have subsequently been developed. Among these, infliximab (a chimeric IgG1 monoclonal antibody), adalimumab (a recombinant human IgG1 monoclonal antibody), and certolizumab pegol (a pegylated humanized Fab' fragment monoclonal antibody) have proven their therapeutic effect in several studies of patients with Crohn's disease.[19–24] This therapeutic strategy has meanwhile established itself as an important option to induce remission in steroid-refractory patients with Crohn's disease and in the successful treatment of fistulizing disease.[25] The efficacy of anti-TNF agents has been attributed to multiple effects, but the precise molecular mechanism of action is still unclear. It has been shown that infliximab is able to block soluble TNF and mTNF *in vitro*.[21] But mere neutralization of soluble TNF is not the only therapeutic modality of α-TNF agents,[26] as the inflammatory process in Crohn's disease may depend more on effects mediated via mTNF. Early studies regarding the mechanism of action of infliximab in IBD could demonstrate that it induces apoptosis in circulating peripheral blood monocytes from Crohn's disease patients by caspase activation in a Fas-independent manner. Furthermore, mitochondrial release of cytochrome *c* and transcriptional activation of the proapoptotic proteins Bax and Bak were noticed.[27] These

findings were supported by results from another group showing that infliximab induced apoptosis in a CD3/CD28-stimulated T-cell line (Jurkat T-cells), whereas this effect was not visible in unstimulated cells. Upon anti-TNF treatment the stimulated Jurkat T cells showed heightened levels of Bax expression, resulting in an increased Bax/Bcl2 ratio and subsequent apoptosis.[28] These data are supported by recent findings indicating that adalimumab is also able to induce apoptosis *in vitro* in peripheral blood monocytes.[29]

Further experiments also examined the effect of infliximab on lamina propria T cells at the site of intestinal inflammation. For that purpose, intestinal biopsies were taken from macroscopically inflamed areas of Crohn's disease patients before and 24 h after a single infusion of infliximab. It could be shown that this treatment similarly leads to induction of intestinal T-cell apoptosis.[28] These data were confirmed by other data showing that infliximab induced sustained apoptosis in lamina propria T cells of patients with Crohn's disease, which was still detectable four weeks after the last application. Correspondingly, infliximab also induced apoptosis in cultured intestinal T cells from Crohn's disease patients in a Fas-independent manner.[30,31] These data led to the concept of an apoptosis-inducing effect of anti-TNF-α antibodies in lamina propria mononuclear cells, explaining their clinical efficacy in the treatment of Crohn's disease. In this concept, reverse signaling through mTNF in intestinal T cells, elicited by mTNF-bound α-TNF antibodies, results in the induction of caspase-dependent induction of apoptosis in intestinal T cells in Crohn's disease[27,32,33] (**Figure 1**).

In contrast to this predominant paradigm, other data could not show induction of apoptosis in infliximab-treated peripheral blood monocytes from Crohn's disease patients, thereby contradicting the results of the groups mentioned above.[34] Furthermore, the clinically effective anti-TNF agent certolizumab,[23] in contrast to other anti-TNF-α agents tested (infliximab, adalimumab, and also etanercept), did not mediate increased levels of apoptosis in peripheral blood monocytes or lymphocytes.[35] On the other hand, perhaps the most compelling evidence for the apoptosis-inducing modality of α-TNF agents in Crohn's disease comes from a very recent study imaging apoptosis *in vivo*. This method relies on the use of labeled annexin V, which binds to apoptotic cells and is imaged by single-photon emission computer tomography, which enables non-invasive localization and quantification of apoptotic cells in patients with Crohn's disease. The study by van den Brande *et al.* demonstrated that infliximab induces apoptosis in lamina propria T cells of patients with active Crohn's disease. Even more significantly, they could show that induction of intestinal cell apoptosis related to clinical efficacy of anti-TNF treatment in these patients.[36] Together, these data substantiate that induction of intestinal T-cell apoptosis is a central molecular mechanism of action of anti-TNF agents in IBD, and that this effect also explains the clinically visible rapid induction of remission after anti-TNF antibody application.

Nevertheless, these data still do not fully explain the molecular mechanism of action of anti-TNF antibodies in IBD, because the clinically effective anti-TNF agent certolizumab does not appear
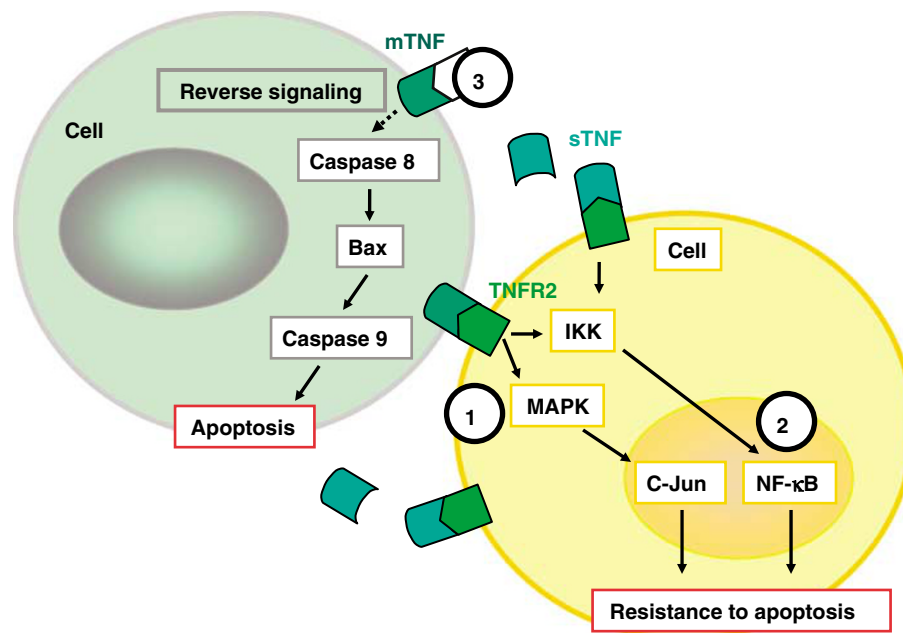
Figure 1 Possible targets of TNF-mediated resistance to apoptosis in IBD. Therapeutic strategies that induce apoptosis through targeting nuclear factor-κB (1) and the mitogen-activated protein kinase (2) that play a role in TNFR2-mediated antiapoptotic pathways. The proposed mechanism of action of anti-TNF antibodies that induce apoptosis through reverse signaling via mTNF in intestinal T cells. Activation of proapoptotic pathways leads to activation of caspase 8 and upregulation of the proapoptotic Bcl-2 family member Bax, which elicits the mitochondrial pathway. This results in the activation of caspase 9 and induction of apoptosis of intestinal T cells in Crohn's disease (3). IBD, inflammatory bowel disease; mTNF, membrane-bound TNF; TNF, tumor necrosis factor; TNFR2, tumor necrosis factor receptor 2.

to induce apoptosis. Therefore, further studies are needed to clear this discrepancy. Furthermore, several important questions remain to be solved, as the exact signaling pathways that lead to the restoration of intestinal T-cell susceptibility to apoptosis in IBD upon α-TNF treatment are incompletely understood. Elucidating the molecular mechanisms of action is crucial, as this might enable us to determine the susceptibility of lamina propria T cells to undergo apoptosis upon α-TNF treatment. Because 30–50% of patients with refractory Crohn's disease have been found to be resistant to α-TNF therapy or lose response to it, this would enable us to stratify suitable patients for treatment beforehand, avoiding futile exposure to the toxicity of this substance.[37] This problem also highlights the present lack of understanding of the pathogenic mechanisms of apoptosis resistance in lamina propria T cells in IBD, as it is currently not known why certain T cells are responsive to apoptotic stimuli while others are not.[38]

The urgency to further characterize the signaling pathways involved in α-TNF-mediated effects is also emphasized by novel therapeutic strategies that induce apoptosis through targeting nuclear factor-κB[39] and the mitogen-activated protein kinase,[40] which also play a role in TNFR2-mediated antiapoptotic pathways (**Figure 1**). These alternative approaches of inhibiting TNF-mediated signaling pathways indicate that further understanding of the local inflammatory process in IBD could be the basis to identify novel therapeutic strategies.

The emergence of α-TNF agents has without a doubt set the standard for future developments of IBD therapies. Nevertheless, a large proportion of refractory Crohn's disease patients do not respond to α-TNF treatment and the long-term use of these agents is hampered by immunogenicity and the risk for severe infectious complications. Therefore, the need for alternative compounds that may be beneficial in specific groups or subgroups of IBD patients is still evident.

TARGETING IL-6 TRANS-SIGNALING-MEDIATED RESISTANCE AGAINST APOPTOSIS

There is accumulating evidence that the proinflammatory cytokine interleukin-6 (IL-6) plays an important role in the pathogenesis of both entities of IBD. It has reproducibly been shown that serum IL-6 levels were substantially elevated in patients with active Crohn's disease.[41–43] Moreover, serum IL-6 levels are considered as a clinically relevant parameter, as they correlate with the inflammatory activity[44] and the frequency of relapses in Crohn's disease patients during remission.[45,46] These data are consistent with the measurement of IL-6 levels in the intestinal mucosa, because IL-6 mRNA levels in colonic mucosal biopsy specimens from patients with active Crohn's disease are elevated.[47] In addition, a positive correlation between high levels of intestinal IL-6 production and the severity of endoscopic and histopathological signs of inflammation in Crohn's disease was postulated.[48,49] Further studies disclosed that lamina propria T cells[50] and macrophages[49,51] are likely to be the main producers of IL-6. These results of elevated IL-6 concentration in the peripheral blood and in the inflamed mucosa of IBD patients imply that IL-6 affects systemic events, such as the synthesis of acute-phase proteins, while simultaneously being locally involved in mucosal disease pathogenesis. This hypothesis was

supported by *in vivo* studies in a murine model of dextran sulfate sodium-induced colitis, where IL-6-deficient mice showed reduced signs of intestinal inflammation.[52]

The biological function of IL-6 in IBD is remarkably not mediated through the membrane-bound receptor for IL-6 (IL-6R), which consist of a ligand-binding subunit (glycoprotein 80, gp80) and signal-transducing subunit (gp130), because most lamina propria T cells of IBD patients do not express this form of receptor.[50] Instead, IL-6 exerts its effects through binding to a soluble form of its corresponding receptor (sIL-6R), which is generated by limited proteolysis of the membrane-bound form (shedding) from the surface of macrophages.[53,54] Consistently, sIL-6R and circulating sIL-6R/IL-6 concentrations in the serum are elevated in IBD, and increased *in vitro* sIL-6R production by intestinal mononuclear cells was also noticed.[50] The complex of IL-6/sIL-6R then activates gp130-positive T cells lacking the IL-6R.[53] As a consequence, IL-6 uses this alternative pathway to activate intestinal T cells in IBD that normally do not express the membrane-bound form of the IL-6R. This process, called trans-signaling, plays a crucial role in IL-6-modulated signal transduction in IBD.[50] IL-6 trans-signaling then leads to translocation of signal transducer and activator of transcription (STAT)-3 and subsequently induction of the antiapoptotic genes Bcl-2 and Bcl-xl in lamina propria T cells in IBD.[50] This pathway has been shown to confer resistance against intestinal T-cell apoptosis in experimental models of colitis, as well as in IBD patients.[50,55] These findings suggest a novel pathophysiological mechanism underlying the uncontrolled intestinal inflammatory process in IBD, providing the basis for potentially new therapeutic strategies.

To substantiate the validity of this approach, an antibody against the IL-6R was tested in diverse murine models of chronic intestinal inflammation. Antibody treatment suppressed or reduced inflammatory activity in all models, thereby confirming the role of the IL-6-mediated trans-signaling in mucosal inflammation *in vivo*.[50,55] Moreover, the curative impact of the antibody was based upon induction of intestinal T-cell apoptosis, confirming the pathogenic role of IL-6 trans-signaling.[50] On the basis of these findings, another therapeutic strategy of targeting the IL-6 trans-signaling pathway has been proposed, using a recombinant fusion protein sgp130Fc composed of a recombinant sgp130 protein fused to the Fc region of human IgG1.[56] The rationale for this approach was to selectively bind the sIL-6R in order to inhibit the IL-6-mediated trans-signaling. In the experimental trinitrobenzene sulfonic acid (TNBS) colitis model, sgp130Fc showed therapeutic effect similar as the IL-6R antibody, indicating that blockade of the sIL-6R is pivotal for therapeutic efficacy.[50] This result was confirmed in another model of spontaneous enteritis, where spg130Fc ameliorated intestinal inflammation and suppressed STAT-3 activation.[57]

Subsequently, an exploratory clinical trial to investigate the safety and efficacy of a humanized anti-IL-6R monoclonal antibody, Tocilizumab (MRA), in patients with active Crohn's disease was performed. The results were promising, as 80% of the patients given MRA infusions biweekly for 12 weeks showed significantly higher clinical response rate than the placebo group.[58] Overall, the anti-IL-6R antibody may represent another therapeutic option for the management of IBD.

Because STAT-3 is important in the downstream signaling pathway of IL-6, further studies have examined the role of this transcription factor, as it is regarded as a crucial regulator of antiapoptotic and proinflammatory effects in IBD pathogenesis. A more severe DSS-induced colitis was present in genetically manipulated mice with heightened STAT-3 expression.[52] The importance of STAT-3 activation in IBD has not only been shown by findings demonstrating constitutive STAT-3 activation in intestinal T cells from IBD patients,[50,59] but also by its association with disease activity.[52] However, inhibition of STAT-3 in IBD might be a double-edged sword, since it not only conveys proinflammatory effects on T cells, but also mediates anti-inflammatory effects in macrophages and dendritic cells.[60] This is evident in macrophage-specific STAT-3-deficient mice, which spontaneously develop chronic enterocolitis.[61] Even more strikingly, mice with tissue-specific disruption of STAT-3 in bone marrow cells die after birth, exhibiting a Crohn's disease-like pathogenesis.[62] Moreover, STAT-3 was also strongly activated in models of intestinal inflammation in macrophage-specific STAT-3-deficient mice, indicating that STAT-3 expression in cells other than macrophages is essential for the inflammatory reaction.[52] Taken together, these data indicate that therapeutic strategies of inhibiting STAT-3 in IBD must carefully take into account the cell population that is targeted, as unspecific inhibition of STAT-3 in macrophages may result in the loss of vital anti-inflammatory effects.

An interesting approach was recently published, in which administration of growth hormones reduced colonic STAT3 activation in experimental colitis and in biopsy samples from patients with Crohn's disease. This effect was attributed to the specific inhibition of gp130 and consequent reduction of STAT-3 activation in T cells.[63]

In summary, these data implicate that specific targeting of the IL-6 trans-signaling pathway could lead to a more selective therapeutic approach in inflammatory bowel disease, resulting in more effective treatment (**Figure 2**).

TARGETING IL-12/IL-23-MEDIATED PATHWAYS

The cytokine IL-12 and especially the recently identified IL-23, which are both able to mediate T-cell differentiation and activation, have been described to play a pivotal role in IBD disease pathogenesis. The heterodimeric protein IL-12 is produced by innate immune cells and is composed of a helical subunit p35, as well as a p40 subunit. It is important for differentiation of interferon-γ-producing T helper 1 (Th1) cells, which are crucial for resistance to intracellular infections. Data from murine experiments indicated that IL-12 inhibits Fas-dependent apoptosis via inhibition of caspase 3 activity in lamina propria lymphocytes,[64] and in accordance antibodies against IL-12, were able to induce apoptosis via the Fas pathway.[65] Furthermore, the antibody was also able to induce apoptosis in cultivated lamina propria mononuclear cells from patients with Crohn's disease.[66] These observations prompted a subsequent clinical trial in patients with active Crohn's disease with a monoclonal
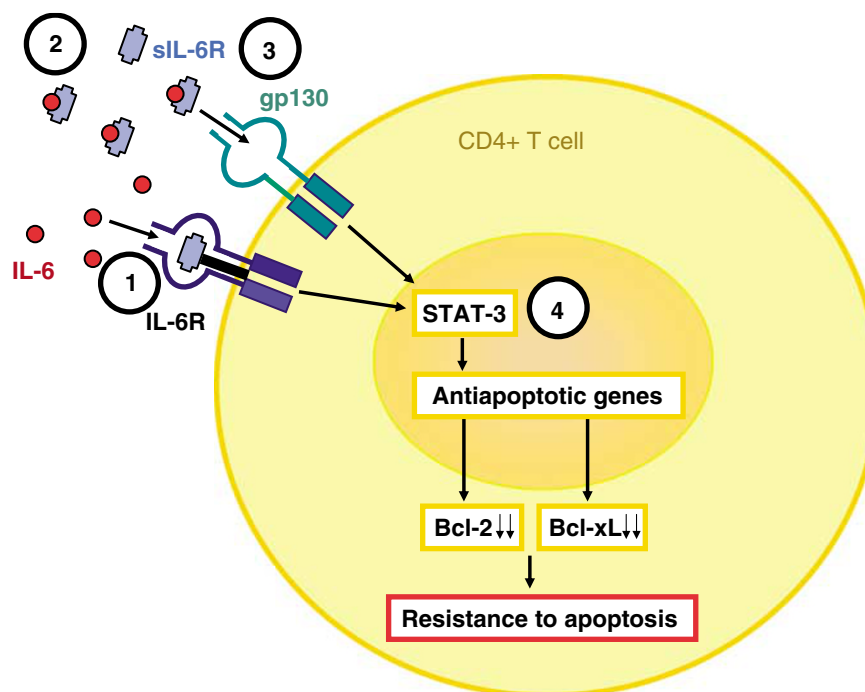
Figure 2 Targeting IL-6 trans signaling mediated resistance to apoptosis: Inhibiting IL-6 trans signaling mediated resistance of CD4+ T cells to apoptosis by blocking the IL-6R (1) and the sIL-6R (2) with the anti-IL-6R antibody. This prevents the translocation of STAT-3 into the nucleus and consecutive activation of anti-apoptotic genes such as Bcl-2 and Bcl-xl. Alternatively the selective blockade of the sIL6R with the sgp130Fc fusion protein (3) also inhibits IL-6 trans signaling mediated resistance to apoptosis of T lymphocytes. Another therapeutic option is targeting STAT-3 with specific antibodies (4). IL, interleukin; IL-6R, interleukin-6 receptor; sIL-6R, soluble form of IL-6R; STAT-3, signal transducer and activator of transcription-3.

anti-IL-12p40 antibody. It could be shown that this antibody was efficacious as it elicited response and remission rates of 75% and 38%, respectively.[67] However, recent data strongly indicate that neutralization of the newly described cytokine, IL-23, which shares the p40 subunit with IL-12, rather than IL-12, is responsible for these therapeutic effects. IL-23 is a member of the IL-12 family of cytokines and is composed of a p19 subunit and the IL-12p40 subunit. IL-23 also has one receptor subunit in common with IL-12, as IL-12Rβ1 together with another specific subunit forms the final signaling complex. IL-23 promotes the expansion and survival of a distinct lineage of T cells, called Th17 cells.[68–70] These cells are initially generated from naive CD4+ T cells in the presence of transforming growth factor-β and IL-6. Subsequently, IL-23 is then required for expansion and survival of these cells.[71,72] Th17 cells are characterized by the expression of the transcription factor RORγt, and stimulate macrophages and other cells to produce multiple proinflammatory cytokines like IL-6 and TNF-α.[73]

The definitive role of IL-23 rather than IL-12 in experimental IBD pathogenesis came from studies using p19- and p40-knockout mice in an experimental colitis model. It could be shown that IL-23p19-deficient mice, but not IL-12p35-deficient mice, were protected against the onset of intestinal inflammation in the IL-10 − / − model.[74] In accordance with these data, IL-17R-deficient mice were significantly protected against TNBS-induced colitis.[75] Further data revealed significant increase in intestinal IL-23 mRNA[76] and IL-17 serum and mucosal levels in IBD patients.[77] Furthermore, in Crohn's disease patients

receiving the α-IL-12p40 antibody, lamina propria mononuclear cells produced IL-23 and T-cell-derived IL-17 concentrations were considerably suppressed after the treatment.[78] These data clearly implicate that activation of the highly pathogenic Th17 cells by IL-23 plays a fundamental role in the inflammatory process in IBD pathogenesis, thereby representing attractive targets for forthcoming therapeutic strategies. Hence, a monoclonal antibody against IL-23p19 was used in the C3H/HeJBir transfer model. This study first demonstrated that transfer of bacterial reactive CD4+ Th17 cells induced a colitis in severe combined immunodeficient mice, which was much more severe than colitis induced after transfer of CD4+ Th1 cells. *In vitro*, the antibody significantly reduced the number of cultured Th17 cells, while not having any effect on Th1 cells. Administration of the antibody was able to both prevent and effectively treat the intestinal inflammation in this model. Moreover, the antibody was able to induce apoptosis in the colitogenic Th17 cells, in that antibody-treated mice had significantly higher proportion of Th17 cells that coexpressed caspase 3 as a marker of apoptosis. Thus, the anti-IL-23p19 antibody exerts its clinical efficacy by preventing the expansion of Th17 cells and also by inducing apoptosis in Th17 cells.[79] These results give reasonable hope for further development of therapeutic concepts that selectively target the components of the IL-23/Th17-mediated pathway in IBD. Thus, further understanding and characterization of this pathway and the numerous biological effects it mediates is needed, as novel therapeutic strategies would have to clarify if it is necessary to inhibit the entire IL-23/Th17 axis through
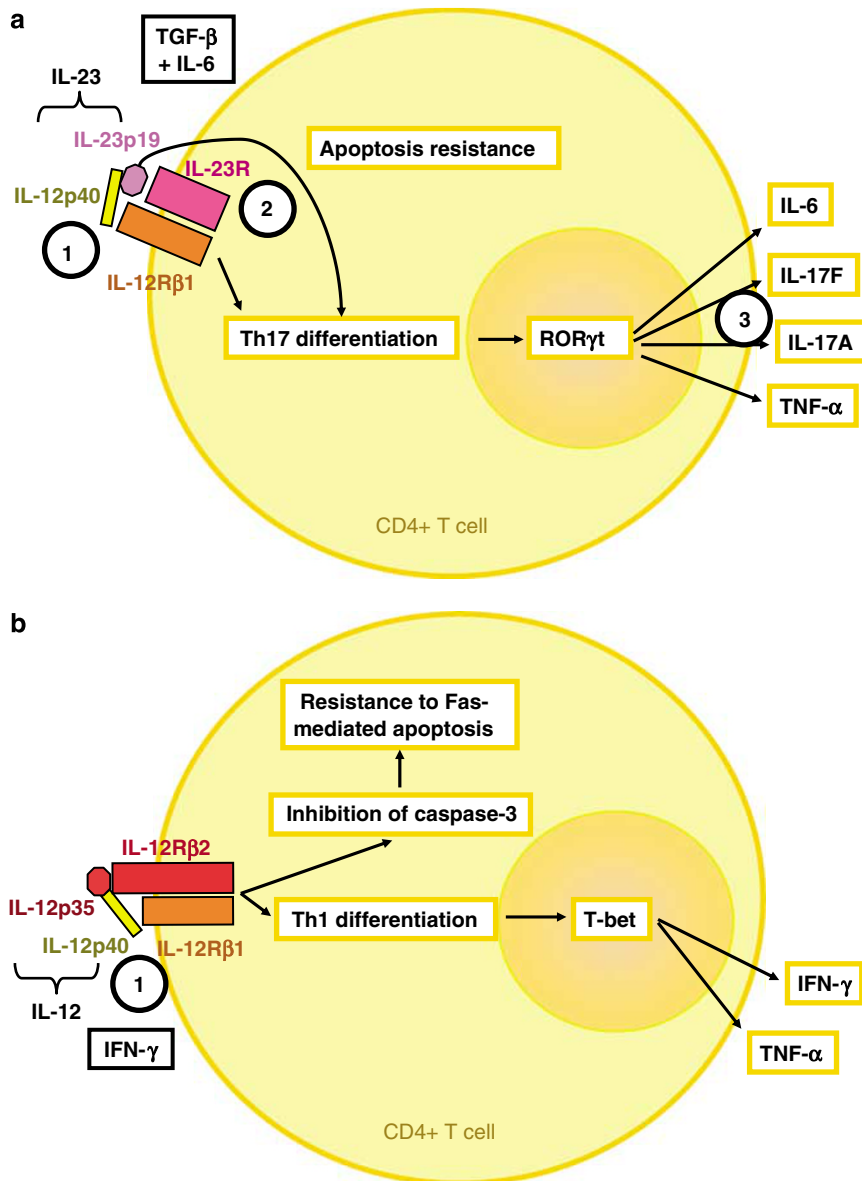
Figure 3 (**a**) Targeting the IL-23-mediated inflammation and resistance to apoptosis in IBD. Inducing apoptosis and blocking generation of inflammatory Th17 cells by inhibiting IL-12p40 (1) or IL-23p19 (2) with specific antibodies. Th17 cells are characterized by expression of the transcription factor RORγt that mediates production of the proinflammatory cytokines IL-17, IL-6, and TNF-α. Another possible treatment option is the blockade of the proinflammatory effects of IL-17 with specific antibodies against IL-17A and/or IL-17F (3). (**b**) Targeting IL-12-mediated resistance to apoptosis in IBD. Inducing apoptosis and blocking generation of inflammatory Th1 cells by inhibiting IL-12p40 (1) with a specific antibody. This inhibits the IL-12-mediated antiapoptotic signaling cascade that conveys resistance to CD4 + T cells to undergo Fas-mediated apoptosis. IBD, inflammatory bowel disease; IL, interleukin; Th, T helper cell; TNF, tumor necrosis factor.

blockade of IL-23, or if it is more efficacious to target products of Th17 cells, like IL-17A or IL-17F (**Figure 3a, b**).

CONCLUSION

The characterization of these signaling pathways that confer resistance to activated intestinal T cells to apoptosis reflect the considerable progress that has been made in unravelling the immunpathogenic mechanisms involved in IBD. The newfound therapeutic agents, whose mode of action is based at least in part on restoring susceptibility to apoptosis in activated intestinal T cells, represent a valuable addition to the current arsenal of treatment options, as conventional, rather unspecific, treatment strategies have proven to be insufficient for a large number of patients.

Moreover, these pathogenic mechanisms that confer heightened resistance to activated effector T cells to apoptosis are not limited to IBD, but instead also seem to be involved in a variety of other immune-mediated disease states. In accordance with this assumption, clinically effective agents in IBD, like α-TNF antibodies, have also established themselves in the treatment of other chronic inflammatory disorders, like plaque psoriasis or rheumatoid arthritis, where impaired apoptosis of inflammatory cells in the joint might contribute to pathogenesis.[80] These existing strategies are highly promising, but it would be desirable

to elucidate the specific characteristics of the signaling cascades utilized in these diseases, as these pathophysiological insights would generate a variety of potential compounds that may be even more beneficial to these patients.

On the other hand, the continuous introduction of new biologicals in IBD will also create significant problems in terms of choosing the most suitable one for each patient, as all existing agents have only been able to prove their clinical efficacy in a subgroup of patients. This indicates that each of the described signaling pathways may have a different importance in each individual IBD patient. Therefore, the forthcoming challenge lies not only in finding new targets for novel therapeutic strategies that are based on a sound pathophysiological rationale, but also to find reliable biomarkers that select the most suitable subgroup of patients for each agent. Therefore, a customized therapeutic approach for each individual would truly enable more effective and less toxic treatment of IBD patients. Lastly, it is tempting to speculate that combinations of agents that alter intestinal T-lymphocyte apoptosis through different mechanisms may be even more effective through synergistic effects. Nevertheless, potential safety liabilities of targeting these pathways should be considered, especially when a cytokine response is entirely blocked, thereby compromising host immune defense and tumor suppression.

DISCLOSURE
The authors declared no conflict of interest.

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