Inflammatory bowel disease: Exploring gut pathophysiology for novel therapeutic targets

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Running head: Exploring gut pathophysiology for new targets in IBD

Abbreviations: UC=Ulcerative Colitis; CD=Crohn's Disease; IL=Interleukin; GALT=Gut Associated Lymphoid Tissue; ADA=Adenosine Deaminase; APC=Antigen Presenting Cells; NF-κB=Nuclear Factor Kappa B; IFN= Interferon; DSS=Dextran Sodium Sulfate; STAT=Signal Activator of Transcription; SMAD-7=Mothers against decapentaplegic homolog 7; ECP=Eosinophil Cationic Protein; EPX=Eosinophil Protein X; EPO=Eosinophil Peroxidase; CARD=Caspase Recruitment Domain Family Member; TLRs=Toll-Like Receptors; TNF-α=Tumor Necrosis Factor Alpha; TGF-β1=Transforming growth factor beta 1; iv=Intravenous; sc=Subcutaneous; il=Intralesional; ic=Intracolonic; CAM=Cell Adhesion Molecule; ICAM=Intercellular Adhesion Molecule; MAdCAM=Mucosal Vascular Addressin Cell Adhesion Molecule; LPS=Lipopolysaccharides; FcRn=Neonatal Fc Receptor; NKG2D=Natural killer activating receptor 2D; HSP=Heat Shock Proteins; IP-10=Interferon-γ-Inducible-Protein-10; MCP=Monocyte Chemoattractant Protein; MIP=Macrophage Inflammatory Protein; MMP=Matrix Metalloproteinases; GLP=Glucagon-like Peptide; FMT=Fecal Microbiota Transplantation; HSC=Haematopoietic Stem Cell; MSC=Mesenchymal Stem Cell; PDLIM2=PDZ and LIM Domain 2; PTEN=Phosphatase and Tensin Homolog

ABSTRACT

Ulcerative colitis (UC) and Crohn's disease (CD) are the two major phenotypes of inflammatory bowel disease (IBD), which are influenced by a complex interplay of immunological and genetic elements, though the precise aetiology still remains unknown. With IBD developing into a globally prevailing disease, there is a need to explore new targets and a thorough understanding of the pathophysiological differences between the healthy and diseased gut could unearth new therapeutic opportunities. In this review, we provide an overview of the major aspects of IBD pathogenesis and there after present a comprehensive analysis of the gut pathophysiology leading to a discussion on some of the most promising targets and biological therapies currently being explored. These include various gut proteins (CXCL-10, GATA-3, NKG2D, CD98, microRNAs), immune cells recruited to the gut (mast cells, eosinophils, toll-like receptors 2, 4), dysregulated proinflammatory cytokines (interleukin-6, -13, -18, -21), and commensal microbiota. We also evaluate some of the emerging non-conventional therapies being explored in IBD treatment focusing on the latest developments in stem cell research, oral targeting of the gut associated lymphoid tissue, novel anti-inflammatory signaling pathway targeting, adenosine deaminase inhibition, and the beneficial effects of anti-oxidant and nutraceutical therapies. In addition, we highlight the growth of biopharmaceuticals and their targets in IBD by providing information on the pre-clinical and clinical development of over 60 biopharmaceutical molecules representing the state of the art in UC and CD drug development.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic relapsing idiopathic inflammatory disorder of the gastrointestinal tract leading to long term impairment of gastrointestinal structure and function [1]. Ulcerative colitis (UC) and Crohn's disease (CD), the two main forms of IBD, share several pathological and clinical symptoms but also have markedly distinct features. In CD, the inflammation and damage to the mucosa can occur throughout the gastrointestinal tract but occurs more commonly in the terminal ileum and colon. It is transmural in nature and can affect all layers of the intestinal tissue. UC on the other hand is confined to the colon, most commonly affecting the rectum and distal colon (often extending from distal to proximal areas as disease progresses) and is characterized by inflammation restricted to the mucosal layer without affecting the deeper layers of the intestinal tissue [2].

Approximately 1.4 million patients in the United States suffer from IBD, of whom around half have UC. Approximately 2.2 million people in Europe suffer from UC and CD [3]. Several factors have been proposed as possible causes of IBD but no single agent or mechanism can fully explain all aspects of the disease aetiology. Some of the proposed factors include environmental, genetic and/or psychological factors, as well as microbial infections and impaired mucosal immune system, which all appear to interact in a way to trigger a dysregulated mucosal immune response leading to chronic inflammation and potential irreversible damage to the gastrointestinal mucosal tissue.

The more conventional therapies for IBD treatment involve aminosalicylates and corticosteroids, generally indicated in mild-moderate condition, and immunosuppressive agents that are indicated in moderate-severe IBD cases. Mesalazine and corticosteroids are the first in line treatment in

IBD, especially UC, but stable, long term clinical and mucosal healing have not been observed with these agents which have also been associated with adverse effects. The immunosuppressive agents such as azathioprine or 6-mercaptopurine have been implemented to maintain steroid-free treatment, but are not effective in inducing remission and require careful monitoring for adverse effects that include anemia, neutropenia, liver toxicity and pancreatitis [4]. Due to the limitations of efficacy and potential toxicity associated with these drugs, a new generation of biopharmaceuticals such as monoclonal antibodies infliximab, adalimumab, golimumab, certolizumab pegol, natalizumab and vedolizumab have now been introduced in IBD management as more selective therapeutic agents, particularly in moderate to severe cases where the conventional therapies have failed. However, there is a potentially increased risk of malignancies, such as non-Hodgkin's lymphoma and non-melanoma skin cancers and loss of response over time, seen in up to about 50% of patients on anti-tumor necrosis factor alpha (TNF-α) antibodies [5]. These limitations highlight the therapeutic gaps in IBD treatment and provide a clear impetus to explore new targets and inflammatory pathways that can potentially direct the development and translation of more efficacious and safer therapeutic agents.

The aim of this review is to give an overview of the pathophysiological changes that occur throughout the gastrointestinal tract in IBD patients and their potential to be exploited as novel targets for the treatment of IBD.

PATHOGENESIS OF IBD

Immune response

Both UC and CD have been associated with a defective innate and adaptive immune response, related to responses generated against the commensal microbiota. Activation of macrophages and dendritic cells in the lamina propria stimulates a proinflammatory response by secretion of cytokines such as interferon-gamma (IFN-γ), IL-1β, IL-6, IL-8, and IL-18. IL-12 and IL-23 are produced by inflammatory myeloid cells and influence the development of Th1 and IL-17 producing Th17 responses respectively, predominantly being observed in CD pathogenesis [6].

UC pathogenesis has been more associated with an atypical Th2 response characterized by the production of TGF-β and IL-5 [7]. Enhanced IL-13 production by an invariant natural killer T cell population in the lamina propria has been shown to be a prominent feature of the inflamed gut, driving inflammation in UC [8, 9]. Studies in dextran sodium sulfate (DSS)-induced colitis models and pediatric UC patients have also demonstrated the mucosal overexpression of Th2 signaling molecule GATA-3, a T-cell specific transcription factor, which mediates the subsequent increased mucosal expression of IL-4 and IL-13, and Th1 signaling molecule signal transducer and signal activator of transcription (STAT)-4 proteins, suggesting their involvement in the pathogenesis of UC [10, 11]. Pronounced infiltration of eosinophils in the mucosal exudates and lamina propria has also been proposed as a potential cause of UC and CD [12]. Eosinophils secrete toxic proinflammatory proteins such as eosinophil cationic protein (ECP), major basic protein, eosinophil protein X (EPX), eosinophil derived neuroendotoxin and eosinophil peroxidase (EPO). Elevated levels of these proteins in IBD patients damage intestinal tissues, insert pores into membranes of target cells and increase smooth muscle reactivity by generating toxic oxygen radicals [13-17].

Genetic factors

Many studies based on animal models induced with colitis or transgenic knockout model studies have shed considerable light on the role of multiple genetic mutations as a trigger for IBD. Figure 1 highlights some of the key genes that are involved in the pathogenesis of UC and CD. Mutation of caspase recruitment domain family member 15 (CARD15; formerly NOD2) gene has been shown to be involved in the development of CD. Leucine rich repeat region of CARD15 gene has been identified to have a sole ligand called muramyl dipeptide, a specific motif of peptidoglycan [18], binding to which activates the NF-$_K$B pathway, which regulates the secretion of proinflammatory and protective molecules involved in the homeostasis of intestinal epithelium. Mutations Arg702Trp, Gly908Arg and 1007fs found in the leucine rich repeat domain of CARD15 lead to defective binding with muramyl dipeptide and causes disregulation of NF-$_K$B activation and bacterial recognition. This leads to increased levels of luminal bacteria, decreased clearance of invasive bacteria from epithelial cells and reduced levels of antimicrobial peptides such as α-defensins due to the expression of CARD15 in Paneth cells [6]. Mutations in disks large homolog 5 gene, involved in encoding scaffolding proteins which maintain epithelial barrier integrity, has been shown to be associated with CARD15 mutations and ultimately in development of CD [19]. Multi drug resistance gene 1 variants, associated with encoding of P-glycoprotein 170 transporter, have been associated in the pathogenesis of both UC and CD as shown by development of colitis in multi drug resistance gene 1 deficient mice [6, 20].

Multiple gene effects in IBD pathogenesis have also been highlighted by several studies that have investigated putative genetic mutations with candidate genes coding for inflammatory cytokines. Polymorphism in the G(-308)A and C(-511)T site of the TNF-α and IL-1β promotor regions respectively leads to impaired cytokine expression and has been suggested to play a role in IBD pathogenesis [21]. Genome-wide association studies have identified and confirmed many

susceptibility loci for IBD. The most recent and largest study involving genome-wide association data for 75,000 IBD patients identified 163 susceptibility loci, covering ~300 potential candidate genes. Of the 163 loci, 110 were a risk to both forms of IBD, while 30 loci were unique to CD and 23 to UC [22]. Further studies are required to understand the involvement of these multiple genetic loci in immunity and inflammation in susceptible individuals.

Genetic factors contribute only partially to general disease variance. It has been suggested that the complex interaction between the human genome, immune system and the intestinal microbiota with external environmental factors such as food, smoking, drugs, breastfeeding and many others plays an important role in IBD pathogenesis. Epigenetics refers to the mitotically heritable modifications in gene expressions, potentially reversible changes in DNA methylation and/or chromatin structure, without alterations in the DNA sequence [23]. DNA methylation is the most studied epigenetic modification correlated to IBD pathogenesis with significant differences in DNA methylation patterns of healthy and inflamed tissues of UC and CD patients. Epigenetic factors may also play a role in the regulation of TNF-α and IL-1β gene expression due to the location of the polymorphism site within a transcription factor AP2 binding site that is sensitive to DNA methylation [21]. Further studies and research in the field of genetic mutations, interaction between genes and epigenetics could not only provide new insights into IBD pathogenesis but may also lead to the development of new medical therapies.

Commensal microbiota

Bacterial components such as lipopolysaccharides (LPS), peptidoglycans, flagellin and nonmethylated DNA can bind to innate immune cell receptors such as toll-like receptors (TLRs), intestinal epithelial cells (IECs) and mesenchymal cells leading to activation of NF-$_K$B and

macrophages, stimulating the transcription of proinflammatory cytokines IL-1β, IL-6, IL-8, IL-12, IL-23, IL-18, TNF-α, ROS, nitric oxide and leukotrienes triggering inflammation [6]. Several studies have shown reduced mucosa-associated colonic microbiota diversity in IBD (Table 2). A 50% and 30% less diversity has been associated with active CD and UC respectively, confirmed by 16S ribosomal RNA gene-based single strand confirmation polymorphism analysis [24]. This state of microbial imbalance and impairment of its functions is called "dysbiosis" and has been proven to play an important role in IBD pathogenesis and complications. The microbial dysbiosis is a result of complex interactions with environmental factors such as diet, smoking, infections and geographical regions as well as genetic modifications associated with IBD susceptibility gene pathways that include microbiota recognition (CARD15 and TLR4), microbial clearance (autophagy genes-ATG16L1, IRGM), immune response (IL-23R, JAK2, TNFSF15) and mucosal barrier function (IBD5) [25]. The impaired bacterial recognition and clearance due to defects in the innate immune response, including neutrophil dysfunction, allows the entry of microbial species into the epithelial cells and a breach in the mucosal integrity forming a major hypothesis for IBD pathogenesis. The uncleared bacteria are walled off in the tissue and a granuloma forms to restrict dissemination of the infective agents. The granulomas secrete proinflammatory cytokines and presents the antigens to adaptive immune system leading to T-cell activation [26]. This drives an inflammatory response which is a hallmark of CD. The defects in bacterial clearance pathway also results in an intense Crohn's like disease in humans called chronic granulomatous disease. The disease is characterized by disorder of the NADPH oxidase system leading to the inability of the phagocytes (neutrophils, macrophages, dendritic cells, mast cells and monocytes) to generate superoxide and consequently result in impaired microbial clearance.

The repeated bacterial and fungal infections, as well as the formation of granulomas in the tissues are a feature of the disease [27].

There is increasing evidence suggesting the critical role of the interaction between the host's defense system and the commensal microbiota in the pathogenesis of UC and CD, as well as the presence of certain bacterial species such as *Escherichia coli*, that can bind to the epithelium, and the role of *Bacteroides* species [28, 29]. Genetic mutations (impaired CARD15, ATG16L1 and IRGM expression) followed by an impaired microbial clearance leads to increased levels of proinflammatory, gram negative adherent-invasive *E.coli* strains that are able to invade, survive and replicate in host cells, a phenomenon frequently observed in ileal CD patients (Figure 2) [24]. Adherent-invasive *E.coli* strains adheres to the epithelial cells via the interaction between the type-1 pili on the surface of the bacteria and carcinoembryogenic antigen-related adhesion molecule 6 which is expressed at the apical surface of enterocytes [30].

Mucus barrier impairment

The gut bacteria can also interact with the host cells in the small intestine via penetration through the mucus layer, majority of which is removable and very thin and discontinuous in nature, leading to an aberrant immune and inflammatory response in the small intestine [10]. In the colon however, the enormous amount of commensal microbiota resides in a non-adherent outer 'loose' mucus layer, while the firmly 'adherent' inner mucus layer is impermeable to bacteria and thus acts as a protective barrier to the underlying epithelial cell surface and is fundamental in maintaining the homeostasis of the colon [31, 32]. $MUC2^{-/-}$ mice, as well as colonic biopsies from UC patients, showed lower expression levels of MUC2 glycoprotein [33]. This facilitates bacterial permeation through the inner mucus layer coming in direct contact with the epithelial

cells to be found deep in the crypts [32, 34]. Modulations in mucin glycoprotein production and impairments in the colonic inner adherent mucus layer could be one of the key features in UC pathogenesis. CD however is associated with an enhanced expression of mucin glycoproteins MUC2 and MUC3, leading to a thicker mucus layer barrier. Hence the possibility of mucus layer impairment in pathogenesis of CD is not yet well studied. The pathophysiological comparisons of mucus layer in healthy, UC and CD state is shown in Table 2.

B cell activity imbalance

B cells play an important role in the maintenance of human gastrointestinal immune homeostasis by secreting IgA and IgM that protect the epithelial barrier from commensal and pathogenic bacteria, avoiding their permeation into the tissue to cause local and systemic infections [35]. Dysregulations in B cell activity have been shown to exacerbate inflammation by inhibiting regulatory T cell activity and producing epithelial cell-specific autoantibodies, suggesting that B cell activity imbalance plays a role in mucosal inflammation [36, 37]. Noronha and colleagues have demonstrated an increased tissue B cell activity, elevated surface TLR2 expression and spontaneous IL-8 secretion in CD patients [38]. The increased TLR2 expression in CD positively correlated with disease activity and this could result in patients experiencing higher levels of microbial ligands exposure and enhanced bacterial translocation in the tissue, resulting in the transmural nature of inflammation that is characteristic of CD. However, the increase in B cell activity was not observed in UC patients with increase in disease severity and the properly regulated B cell activation may actually help in reducing clinical symptoms in UC. Therefore, the TLR2+ B cell mediated responses to gut microbiota may play an important role in the

pathogenesis of CD-associated inflammation and the regulation of its activity might prove to be beneficial in curbing disease symptoms**.**

Immunoglobulin secretion

The luminal bacterial antigens also play a central role in the induction and progression of high levels of colonic mucosal immunoglobulins (Ig), mainly IgA and IgG, in patients with UC and CD compared to healthy state. IgA is the predominant antibody isotype produced at the intestinal mucosal surfaces and is a critical mediator of mucosal immune response. Recognition of pathogenic bacteria by the intestinal immune system results in production of high-affinity IgA which is transcytosed into the lumen. These IgA can bind and coat the pathogens to neutralize them, thus protecting the intestine against bacterial penetration and infection [39]. Indigenous bacteria can also stimulate IgA production and can become coated with IgA. However, the induced IgA response is of low-affinity and specificity, resulting in lower levels of coating compared to pathogenic bacteria. This property of IgA to coat pathogenic bacteria has been exploited by Palm and colleagues to identify members of the gut microbiota that drive inflammation in IBD [40]. Analyses of the fecal microbiota of UC and CD patients identified 35 bacterial species that were highly coated by IgA, but not in healthy subjects, paving the way for potential targeted antimicrobial therapies in IBD. But maturation defects in B-cells and reduced J-chain expression lead to reduced secretory dimeric IgA and could cause a breakdown in the mucosal homeostasis leading to bacterial invasion and contributing to pathogenesis of IBD [41]. Mucosal secretory IgA concentration has been shown to decrease in IBD patients, suggesting the lack of its barrier protective role in IBD-associated epithelial damage [42]. Ferreira and colleagues also demonstrated that genome-wide association studies of selective IgA deficiency

showed genes that were also linked to IBD pathogenesis, namely ORMDL3, REL and PTPN22 [43].

CD, associated with a Th1 response, shows an increased level of IgG1, IgG2 and IgG3 subclasses, whereas UC, associated with a Th2 response, shows an increased level of IgG1 and IgG3 subclasses [44, 45]. These increased mucosal IgGs are specifically directed against cytoplasmic, but not to membrane proteins, of commensal microbiota [44]. A specific bacterial antigen flagellin has been shown to induce a pathogenic response in a mouse colitis model mediated by efficient APC activation which requires FcRn-mediated antigen presentation of immune-complexes [46]. However, the mechanisms involved in the interaction between commensal bacteria and our gut immunoglobulins (mainly IgA and IgG) are still very complex and the knowledge of bacterial contribution on the pathogenesis of chronic gut inflammation is still relatively scarce.

External environmental contributors

The increase in the incidence and prevalence of IBD in the 21st century has led to considerable interest in external environmental factors as important pathogenesis factors in IBD. These factors include diet, smoking and stress [47]. Smoking confers a twofold increase in the risk of CD and is associated with increased reoccurrence after surgery and poorer response to medical therapy. On the contrary, smoking appears to have a protective effect against UC with a halving of the risk in current smokers compared to never smokers. Smoking cessation however is associated with an increased risk of developing UC in susceptible individuals, with the effect lasting up to 10 years post cessation [47]. While the exact mechanism by which smoking exerts its differential effect on UC and CD patients is unclear, recent evidence has suggested the impact of smoking on

inducing alterations in the microbiota, intestinal permeability and innate and adaptive immune response [48]. Tobacco smoke contains high levels of dioxins, a diverse group of halogenated hydrocarbons, that can have an immunomodulatory effect in humans [49]. These include toxic compounds such as 2,3,7,8-tetrachlorodibenzodioxin and 6-formylindolo (3,2b) carbazole [48]. The aryl hydrocarbon receptor is the only known dioxin receptor and has been recently postulated to play a role in linking environmental factors to host immune system in IBD [49]. However further work is required to fully understand the exact role of this receptor and the chemical complexity of tobacco smoking in the development and progression of IBD.

The role of diet on UC and CD pathogenesis remains one of the most challenging associations to study due to its variability with time and difficulty in tracking diet patterns to name a few. Studies conducted by Persson *et al.* and Reif *et al.* in 152 CD patients, 145 UC patients and 305 healthy control patients, found an increased risk of CD with high sucrose consumption and lower risk for high fiber consumption [50, 51]. A significant change in diet has been the increased level of sulphur content. It has been observed in active UC patients that sulphides inhibit the oxidation of n-butyrate analogues which maintains altered intestinal epithelial barrier leading to the development of UC symptoms [52]. UC patients have been shown to have increased numbers of sulfate-reducing bacteria which can interact with luminal substrates to generate sulphoxides that can be highly damaging to the colonic mucosa, emphasizing the hypothesis that sulphur containing diets lead to increased risk of UC [53].

CURRENT AND EMERGING BIOLOGICAL THERAPEUTICS FOR IBD

Biopharmaceuticals are fast gaining priority in the pharmaceutical industry compared to small molecules in both clinical utility and market share. Rapid scientific developments in genomics, proteomics, cell culture and antibody development technologies, as well as increasing knowledge of genetics and cell biology are leading the shift in focus from small molecule therapeutics to biologicals. This is signified by the number of biopharmaceutical patent applications that now exceeds those for small molecules. Reflecting this, of the top 10 highest selling drugs in the world in 2014, 7 were biologicals. Their market is estimated to reach $497.9 billion by 2020, growing at 13.5% compound annual growth rate [54].

Biologicals offer the ability to interact with challenging targets which have thus far eluded small molecule drugs. The emergence of monoclonal antibody therapeutics in particular have revolutionized the treatment of IBD with 4 antibodies against TNF-α and 2 antibodies against cell adhesion molecules currently in the market. However, the increased risk of systemic toxicity and the loss of response over time is forcing scientists to investigate alternative therapeutic targets and biological agents with improved efficacy and safety profile. Some of these emerging biological therapies for IBD in the market and in clinical development, along with their targets and development status have been summarized in table 1.

EXPLORING GUT PATHOPHYSIOLOGICAL CHANGES FOR NOVEL TARGETS IN IBD TREATMENT

The gastrointestinal tract is a highly complex environment that undergoes physiological changes under diseased condition. Analyses of these changes not only improves our understanding of the disease pathogenesis, but also presents novel targeting opportunities that can be exploited for the development of new medical therapies. The pathophysiological differences between a healthy and IBD gut have been comprehensively analyzed and summarized in Table 2. Some of the most promising physiological changes being exploited as potential therapeutic targets are discussed thereafter.

Gut proteins and cytokines

An important pathophysiological difference in healthy and inflamed intestine is an enhanced cellular immune response which, in general, is an increased presence of immune cells such as T-cells, M-cells, neutrophils and macrophages (Table 2). The strong cellular immune response translates into an increased level of secretory proinflammatory cytokines that can be inhibited to elicit a potential therapeutic response in IBD. Currently, there are four approved TNF-α cytokine inhibitors available for the treatment of IBD. However, not all IBD patients respond to anti-TNF-α molecules potentially due to polymorphism in the Fcγ receptor IIIA gene or formation of anti-drug antibodies, resulting in loss of response over time [55]. Currently there are several new biopharmaceutical agents in clinical stages whose mode of action is inhibition of proinflammatory cytokines and proteins.

UC

IL-13

Overexpression of proinflammatory cytokine TNF-α is one of the key pathophysiological features of IBD, and the most targeted cytokine in terms of the currently available and in clinical stage biopharmaceutical therapies in IBD (Table 1). However, other cytokines of the inflammatory cascade that are differentially expressed in UC patients are now being explored as potential new targets. IL-13 from lamina propria mononuclear cells is increased and highly expressed in colonic epithelial cells from UC patients compared to active CD patients and healthy controls, and presents a potential therapeutic target for treatment of UC [56]. NK-T cell type II have been associated with the secretion of IL-13 cytokine as the major source [57], and elimination of NK-T cells along with direct inhibition of IL-13 has been shown to prevent colitis and can be of therapeutic benefit in treatment of IBD [58]. Therapeutic benefit has also been demonstrated in UC by inhibition of IFN-β downregulating the production of IL-13 [59]. At present, there are three monoclonal antibodies against IL-13 that have completed phase II clinical trials in UC patients. Tralokinumab, a fully humanized IgG4 antibody against IL-13 did not significantly improve clinical response in UC patients; however, the higher clinical remission rate compared to placebo suggests it may benefit some UC patients [60]. Anrukinzumab, a fully humanized IgG1 antibody to IL-13, did not show statistically significant therapeutic effect in patients with active UC [61].

IP-10 (CXCL-10)

Overexpression of IP-10 (CXCL-10) in the colonic mucosa of IBD patients, mediated by TLR-3, is being explored as a possible therapy in UC, after IP-10 expression blockade was shown to prevent development of asymptomatic colitis and achieve remission in IL-$10^{-/-}$ mice [62]. A fully

human IP-10 antibody (BMS-936557) has completed a phase II clinical trial in moderate-to-severely active UC patients and showed a significantly higher rate of clinical response compared to placebo along with histological improvements, thus representing a potential candidate for therapy in UC [63]. But significant adverse events and serious infections observed in the trial warrants more safety and tolerability studies for anti-IP-10 therapy.

GATA-3

GATA-3 is a transcription factor which is involved in Th2 mediated immune response process of activation of Th2 cells, signals and cytokine expression, and has been shown to play a role in the pathogenesis of acute phase UC in children [11]. This transcription factor is being explored as a novel therapeutic strategy in UC with an investigative molecule, SB012, currently in clinical phase I/II in UC patients. The product is based on DNAzymes which are chemically synthesized to cleave GATA-3 mRNA and reduce cytokine production, thereby reducing the key features of mucosal inflammatory response in UC [64].

MicroRNAs

MicroRNAs (miRNAs) are endogenous noncoding RNAs, ~22 nucleotides in length that play a role in gene-regulation by binding to the 3'-untranslated regions of the messenger RNAs (mRNAs) of protein-coding genes and destabilize the mRNAs to reduce the target protein output [65]. They are essential regulators of inflammatory signaling pathways like NF-$_K$B [66], and have shown to be differentially expressed in inflammatory conditions like UC [67, 68]. Wu *et al.* showed 3 miRNAs (miR-192, miR-375, and miR-422b) were significantly down-regulated in active UC tissues whereas 8 miRNAs (miR-16, miR-21, miR-23a, miR-24, miR-29a, miR-126,

miR-195, and Let-7f) were significantly up-regulated in active UC tissues, as compared to healthy tissues [67]. A study by Polytarchou *et al.* showed an >8-fold increased expression of miRNA-214 in particular, in UC colonic tissues, compared to CD and uninflamed tissues [69]. The increased expression was correlated to the disease state with enhanced expression in active UC tissues, while no change in inactive UC tissues. The same study also investigated the role of miR-214 on NF-$_K$B phosphorylation activity and the anti-inflammatory effect induced by chemical inhibition of miRNA-214. The miRNA-214 inhibitor was able to significantly suppress NF-$_K$B phosphorylation levels (>90%) and the chemical inhibition of miRNA-214 showed suppression of inflammation in a DSS-colitis mouse model as well as in colonic biopsies from UC patients [69], suggesting the therapeutic potential of miRNAs as a novel treatment for IBD. To understand the molecular link between miRNA-214 and UC, 2 genes were identified as direct targets for miRNA-214 in their 3'-untranslated regions, PDZ and LIM domain 2 (PDLIM2) and phosphatase and tensin homolog (PTEN). PDLIM2 is nuclear ubiquitin E3 ligase targeting the p65 subunit of NF-$_K$B and inhibiting its activity [70]. PTEN is a suppressor of AkT signaling pathway that is shown to intervene with NF-$_K$B activation [71], resulting in increased severity of colitis [72]. Overexpression of miRNA-214 suppresses the expression of PDLIM2 and PTEN mRNA and protein levels and induces activation of NF-$_K$B phosphorylation and subsequent IL-6 secretion [69]. The STAT-3 transcription factor is able to bind to the promotor region of the miRNA-214 and the presence of IL-6 leads to IL-6-STAT3-dependent miRNA-214 expression and regulation of PDLIM-NF-$_K$B and PTEM-AkT pathways. These results demonstrated the direct involvement of the 2 genes in miRNA-214 regulation of the NF-$_K$B inflammatory response [69].

Identification of both UC and CD-associated miRNAs which are differentially expressed in the tissues, as well as understanding of the regulatory role of these miRNAs in acute and chronic inflammatory processes, may lead to the development of miRNAs as effective therapeutic targets for treatment of chronic inflammatory diseases such as IBD.

TNF-α and CD98

Small interfering RNA (siRNA) are double stranded RNA segments (~20-25 nucleotides in length) which can downregulate the expression of a specific gene at mRNA level, in this case TNF-α, a highly expressed proinflammatory cytokine in IBD. Gene silencing and siRNA therapy is currently an exciting area of research exploring its therapeutic role in IBD. One of the major advantages of this therapy could be the local targeting of the siRNA molecules against specific genes in the GI tract via several promising oral delivery systems such as nanoparticles-in-microspheres oral system (NiMOS) [73], thioketal-nanoparticles [74], and cationic cyclodextrin and polyethyleneimine (PEI) vectors [75]. These studies have shown *in-vivo* in the murine colitis model the ability to deliver the encapsulated siRNA against TNF-α locally, leading to subsequent reduction in TNF-α mRNA levels as well as other proinflammatory mediators (IL-6, IL-1β, IL-5, IFN-γ and GMCSF) in the colitis tissue via the gene silencing mechanism.

CD98 is a 125 kDa type-II transmembrane protein composed of an 80 kDa heavy chain and a 40kDa light chain, involved in amino acid transport, integrin and fusion regulation [76]. CD98 also plays a crucial role in regulating intestinal homeostasis and innate immune responses in the gut. CD98 is highly expressed in the IECs and macrophages, potentially upregulated by proinflammatory cytokines [77], and an increased expression has been shown in IBD colonic tissues from mice [78] and humans which can be correlated to the disease state [79]. One

plausible mechanism underlying the contribution of CD98 to intestinal inflammation is the IEC-specific CD98 overexpression resulting in intestinal barrier dysfunction, disruption of homeostatic regulation of cell proliferation and survival and increased intestinal permeability [80]. Thus maintaining low levels of CD98 in IECs in inflamed tissue could have beneficial effects on improving the mucosal barrier function, preventing further tissue damage and could represent a potential therapeutic target for prevention and treatment of IBD. Laroui *et al.* exploited PEI siRNA molecules to inhibit CD98 expression in DSS-colitis mice colonic tissue via oral delivery in polylactic acid (PLA) nanoparticles [81]. The strategy showed decrease in colitis proving that siRNA-mediated knockdown of CD98 expression could therefore be a promising therapeutic strategy for the treatment of IBD.

The studies have shown that there is clinical potential in local treatment of IBD by gene silencing therapy by not only targeting TNF-α, but several other overexpressed proteins. However, further studies will be needed to overcome the gastrointestinal luminal, mucosal and cellular barrier in humans.

OX40

OX40 (CD134) is a member of TNF receptor family expressed by T cells after ligation of T cell receptor. OX40 ligand (OX40L) is expressed on APCs, vascular endothelial cells, mast cells, natural killer cells and some T cells. The OX40-OX40L interaction between T cell and APC contributes to optimal T cell function and the generation of memory T cells, implying its role in sustaining immune response [82]. However, in pathological conditions, as demonstrated in colitis mice models, OX40-OX40L interaction on endothelial cells has been thought to contribute to T cell migration and tissue infiltration, leading to secretion of proinflammatory cytokines via a Th2

response [83]. OX40 also starts to suppress regulatory T cells leading to imbalances in tolerance and immunity. $OX40^+$ T cells expression has been shown in the lamina propria of colitis mice, compared to normal mice which showed $OX40^+$ T cells only in lymphoid tissue, including Peyer's patches of the gut [84]. $OX40^+$ T cells have also been shown to be highly expressed in the lamina propria of the colon from UC and CD patients [85]. These findings support the role of OX40 signaling in the pathogenesis of IBD. Administration of anti-OX40L monoclonal antibody in colitis mice model has shown to decrease T cell infiltration into the colon and reduce production of proinflammatory cytokines TNF-α, IFN-γ and IL-2 in the lamina propria [86]. However, the outcomes of altering the OX40-OX40L interactions requires further in-depth investigations before being studied in humans as it can potentially confer serious adverse effects owing to its immunoregulatory role. It is also not clear whether OX40 or OX40L inhibition will lead to the desirable effects in humans. The results of the ongoing phase II study of an anti-OX40 monoclonal antibody (KHK4083) to determine the safety in moderate to severe UC patients would answer some of the questions surrounding the potential of OX40 as a therapeutic target in IBD [87].

Guanylate cyclase-C

Guanylate cyclase-C is a heterodimeric transmembrane enzyme expressed at the apical, brush-border membranes of intestinal epithelial cells and is distributed along the crypts, villi and mucosal surfaces [88]. Guanylate cyclase-C receptor activation by ligands uroguanylin and guanylin plays an important role in maintaining intestinal mucosal homeostasis and epithelial barrier function by stimulation of cyclic guanosine-3',5'-monophosphate production through cystic fibrosis transmembrane conductance regulator to induce secretion of sodium chloride and

bicarbonate [89]. These electrolytes are critical in the maintenance of the mucus barrier and its interaction with the microbiota. Studies in guanylate cyclase-C and uroguanylin knockout mice model demonstrated a breakdown in the intestinal barrier homeostasis and decreased production of goblet cells and mucin [90]. Guanylate cyclase-C and ligands guanylin and uroguanylin gene expression has also been shown to be downregulated in colon biopsies of UC and CD patients, suggesting a role of guanylate cyclase-C signaling in pathogenesis of IBD [91]. Therefore, guanylate cyclase-C agonists can be exploited as a unique class of therapeutics in IBD management and UC in particular. Shailubhai and colleagues recently reported the therapeutic utility of orally delivered mucosally active guanylate cyclase-C agonist peptides plecanatide and dolcanatide in colitis mice model [92]. The peptides were able to ameliorate inflammation and restore colonic mucosal integrity and homeostasis. Dolcanatide recently completed a phase Ib study in UC patients and was found to be well tolerated. Further studies are warranted to elucidate the precise mechanism by which guanylate cyclase-C agonists promote intestinal barrier function and exert their anti-inflammatory effect.

CD

IL-18

IL-18 has been shown to be elevated in Th1 mediated CD [93], and an anti-IL-18 strategy has also been explored in murine colitis models where inhibition of IL-18 was shown to suppress IFN-γ synthesis and subsequently IL-1β converting enzyme (ICE) synthesis that cleaves IL-1β and IL-18 and converts them into active cytokine. Anti-IL-18 strategy also shows suppression of proinflammatory cytokine TNF-α by inhibiting its synthesis and shows potential for a rational strategy in treatment of IBD [94].

IL-6

IL-6 is a pleiotropic cytokine that plays a central role in biological activities such as immune regulation, hematopoiesis, inflammation and oncogenesis. IL-6 induces the formation of Th17 cells from naïve T cells together with TGF-β, and inhibits TGF-β induced regulatory T cell differentiation [95]. The proinflammatory cytokine IL-6 has been shown to bind to cells lacking IL-6 receptor when it forms complexes with the soluble IL-6R. This so-called trans-signaling prevents mucosal and lamina propria T-cell apoptosis and contributes to inflammatory conditions such as observed in CD [96]. This suggests that IL-6 blockade by monoclonal antibodies could prove to be an innovative treatment of CD-associated inflammation. Anti-IL 6 receptor monoclonal antibody has been shown to illicit a clinical effect in active CD [97], and a phase II clinical study is currently ongoing by Pfizer.

IL-13

Between 17% - 50% of CD patients have been shown to develop fistulae, a pathological connection between two epithelium-covered organs. Scharl and colleagues demonstrated the association of fistulizing CD with epithelial to mesenchymal transition along with elevated levels of TGF-β, IL-13 and its receptor IL-13Rα_1 in transitional cells lining the fistulae and in the epithelial cells of deformed crypts adjacent to the fistulae [98]. The findings were somewhat unexpected due to the known association of IL-13 with Th2 cell response linked to UC pathogenesis [99]. TGF-β induced epithelial to mesenchymal cell transition by disrupting

epithelial cell formation and IL-13 enabled the epithelial to mesenchymal transition cells to penetrate deeper into the tissue layers. This suggested that a dysregulation of TGF-β/IL-13-induced effects could play a major role in the pathogenesis of CD-associated fistulae. An anti-IL-13 antibody was shown to block IL-13 induced events such as STAT6 phosphorylation and SLUG mRNA expression, since both these proteins are strongly expressed in and around CD-associated fistulae and are associated with the invasive potential of transformed epithelial cells [98]. Thus inhibition of IL-13 could provide a novel and successful approach for the treatment of fistulizing CD. An anti-IL-13 monoclonal antibody dectrekumab (QAX-576) developed by Novartis for the treatment of perianal fistulas in CD patients has completed a phase II trial but the results have not yet been published at the time of this review's preparation [100].

IL-21

IL-21 is overexpressed in the inflamed gut of IBD patients (both UC and CD) [101], and targeting of this cytokine can possibly have an impact on both Th1 and Th17 cytokines which are upregulated by IL-21. Fina and colleagues demonstrated amelioration in experimental colitis in wild type mice by targeting IL-21 with a neutralizing IL-21R/Fc fusion protein [102, 103]. An anti-IL-21 antibody developed by Novo Nordisk completed phase II clinical trials in active CD patients to assess safety and efficacy [104]. The results of the trial have not been disclosed yet, however, the company has discontinued further development of the IL-21 antibody therapy for CD.

NKG2D

NKG2D is a known activating receptor on natural killer cells, natural killer T cells, activated $CD8^+$ T cells, activated macrophages and γδ T cells, which are involved in response to cellular stress such as inflammation and infection. $CD4^+$ T cells expressing NKG2D have been shown to be increased in the lamina propria of CD patients with an elevated Th1 cytokine profile leading to inflammatory and cytotoxic responses via interaction with ligand MICA or MICB (MHC class I polypeptide-related sequence A or B) [42]. A novel therapeutic target could be the use of specific monoclonal antibodies in blocking of MICA and NKG2D interaction, which has been shown to prevent murine $CD4^+$ T-cell mediated colitis in mice [105], and inhibition of NKG2D receptor, which attenuated disease state in mild but not in severe colitis [106]. However, the results in animal models could not be translated into humans after a phase II study using a human IgG4 anti-NKG2D monoclonal antibody (Novo Nordisk A/S) failed to meet the primary endpoint (reduction in disease activity at week 4 at 2mg/kg dose) [107]. The company has discontinued further development of anti-NKG2D as a treatment for CD. Overall the findings suggest a possible role of NKG2D receptor in CD pathogenesis and how its functional inhibition is insufficient to completely inhibit the inflammatory process. However, further clinical development, including dose optimization and frequency for anti-NKG2D therapy is needed.

IL-12/23

It has been suggested that IL-12 and IL-23 play a role in UC and CD pathogenesis. IL-12 and IL-23 are heterodimeric proteins that share a common p40 subunit, IL-12/23p40 [108]. Targeting of the p40 subunit by monoclonal antibodies has been shown to abrogate colitis in animal models [102, 109, 110]. Elevated intestinal mucosal levels of IL-12p40 have been observed in IBD patients [109]. Genome-wide association studies have confirmed single nucleotide

polymorphism in IL-12B and IL-23R genes (encoding for IL-12p40 and IL-23, respectively) to be linked with both UC and CD pathogenesis [109, 111]. Due to the genetic association of IL-23 pathway in CD, monoclonal antibodies directed against IL-12/23p40 subunit (Ustekinumab, Briakinumab) and IL-23p19 (BI655066) have been investigated as potential therapeutic agents. The safety and efficacy of IL-12/23p40 antagonists has been shown in previous clinical trials for the induction of clinical remission and response in CD patients [112]. However, the inability of briakinumab to meet the primary end-point led to the termination of a phase II trial in CD patients, thus proving there is still much to learn about the pathogenesis of CD [113]. Close attention also needs to be paid to future study designs evaluating IL-12/23p40 antagonists to ensure that suitable end points are chosen to evaluate their true therapeutic potential in CD. Monoclonal antibody against IL-23p19 has also been shown to be effective in both the prevention and treatment of active colitis in mice [114]. Hence, IL-23 has been proposed as an attractive therapeutic target, not only for CD but also in UC treatment. An IL-23 antibody (LY3074828) is currently being tested in a phase II study for clinical efficacy in patients with moderate to severe UC [115].

IL-17A

IL-17 subtype A (IL-17A) is a dimeric glycoprotein with biological functions bridging innate and adaptive immunity [116]. Antibodies to IL-17A (Secukinumab) and IL-17A receptor (Brodalumab) have been evaluated as potential therapeutics [117]. IL-17A and IL-17RA antagonists have proven to be surprisingly ineffective with high rates of adverse effects observed in trials in CD patients, mainly disease worsening [118]. The therapies failed to meet the primary

end point of the trials and had to be terminated. The negative results could be explained by the blocking of IL-17A interfering with its protective function in the perpetuating chronic inflammation by activated T cells in the intestine, as shown in animal models [119]. Problems with trial design, lack of response and persistent gut inflammation associated with polymorphism in tumor necrosis factor-like ligand gene (TL1A) that encodes for cytokine driving pathogenic T cells, were also thought to be the reasons for the unsuccessful trial results [118]. Colombel *et al.* also hypothesized that worsening of CD could be linked to *C. albicans* thriving in the gut induced by the loss of control by IL-17 [120].

IFN-γ

Elevated mucosal levels of cytokine IFN-γ, a type II interferon, have been observed in CD in humans [121, 122]. Strategies aimed at neutralizing IFN-γ in CD were therefore explored. Fontolizumab, a humanized form of murine anti-human IFN-γ antibody, showed no significant difference in response and remission compared to placebo in a phase II study [123]. Unlike TNF-α, IFN-γ does not exist as a membrane bound protein with no *in vitro* evidence suggesting that target binding by IFN-γ antagonists, such as fontolizumab, would result in acute apoptosis of mucosal lymphocytes. IFN-γ targeting therapy might have a more gradual onset of action and, with a favorable side effect profile, long half-life and low immunogenicity. Therefore, multiple and more sustained dosing should be considered for maximum benefit [123].

These therapeutic failures more importantly highlight the gaps in our understanding of gut pathogenesis of CD and the limitations in the successful translation of positive data in animal models into effective therapies in humans.

294

295 ***Mast cells in IBD***

296 Mast cells have long been suspected to play a key role in a variety of chronic inflammatory
297 processes, including the inflammation seen in IBD [124]. Markedly increased number of mast
298 cells have been reported by a number of studies in the mucosa of ileum and colon from IBD
299 patients as compared to healthy volunteers [125, 126]. Mast cell degranulation has been
300 suggested due to higher levels of histamine, tryptase, chymase and carboxypeptidase A levels, as
301 well as proinflammatory cytokines such as TNF-α, IL-3, IL-4, IL-5, IL-16 and substance P, both
302 spontaneously and in response to epithelial cell antigens, than do mast cells obtained from
303 normal tissues in healthy subjects or uninflamed tissues from IBD subjects [127, 128]. This
304 significant elevation of mucosal mast cells can be utilized as a potential therapeutic target using
305 pharmacological agents against numerous biologically active molecules secreted by mast cells
306 such as mast cell stabilizer agent cromolyn, ketotifen, H_1 receptor antagonists, serotonin 5-HT3
307 receptor antagonist, leuprolide and octreotide acetate [129]. Corticosteroids have also been
308 shown to decrease the number of mast cells [130].

309

310 ***Eosinophils in IBD***

311 Histological findings have shown that the healthy gut mucosa contains and secretes low
312 concentrations of functionally active eosinophils and eosinophil cationic protein (ECP) that
313 protect the host from infectious agents like bacteria, fungi, viruses or parasites [131]. Both
314 inflamed and uninflamed gut mucosa of CD patients have been found to contain significantly
315 higher levels of ECP as compared to healthy gut mucosa. It is interesting to note that the ECP
316 levels in the healthy gut mucosa decrease moving from the terminal ileum to the ascending colon

and the distal colonic segments. However, it has been found that in the gut mucosa of CD affected patients, there is an increase in the levels of ECP towards the distal colonic segments, with peak levels in the transverse colon [131]. Carlson *et al.* showed the mucosal release of ECP, EPX and EPO to be increased by 10-20 fold while granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-8 levels were 7 fold greater, in patients with UC and proctitis as compared to healthy controls [12]. This pathophysiological difference provides opportunities for new therapeutic interventions in UC and CD by the potential use of electrostatically charged microparticulate delivery systems such as anionic microparticles, nanoparticles or liposomes, which can bind to the elevated levels of positively charged eosinophilic proteins in the gut mucosa of IBD patients. However, the negatively charged mucus layer in the small intestine and colon will also need to be considered during the designing of electrostatically charged delivery systems which need to overcome the mucus barrier.

Novel signaling pathway inhibitors in IBD

Various anti-inflammatory molecules apart from the biopharmaceuticals currently in clinical phase are being investigated to elicit a potential therapeutic response in IBD by inhibition of signaling pathways which in turn leads to reduced expression of proinflammatory cytokines. Some of these promising molecules have been summarized in Table 3. Probiotic approaches showing promise in inhibition of signaling pathways and potential anti-inflammatory response in IBD have been discussed separately.

Microbiota

Probiotics

Several studies have suggested qualitative changes in indigenous microbiota in the small and large intestine in IBD patients, especially a decrease in the bifidobacteria count. With the help of PCR-assays, quantitative differences in intestinal microbiota have been observed in healthy controls and IBD patients. The variation in microbial species was dependent on the IBD symptoms prevailing in patients. Exploiting these variations by the use of probiotic bacteria like lactobacilli and bifidobacteria has attracted a lot of attention and interest in the field of gastroenterology, especially in inflammatory conditions like IBD. Yan *et al*. reported two purified proteins, p75 (75 kDa) and p40 (40 kDa) from probiotic bacterium *Lactobacillus rhamnosus GG* which promote cell growth and inhibit TNF-induced epithelial cell apoptosis in cultured and *in vivo* colon models [132]. *Lactobacillus rhamnosus GG* has shown to induce remission in IBD patients and animal models. However, in a clinical trial designed to test its efficacy as an adjunct to standard maintenance therapy of aminosalicylates, 6-mercaptopurine and corticosteroids in pediatric CD, no significantly beneficial effect was observed [133].

VSL#3 is another probiotic therapy consisting of 450 billion freeze-dried bacteria from 8 different strains (*Streptococcus thermophilus, Bifidobacterium longum, B. breve, B. infantis, Lactobacillus acidophilus, L. plantarum, L. casei, L. bulgaricus*) [134]. VSL#3 was able to induce significant clinical improvement in relapsing mild-moderate UC patients by protection of epithelial barrier function and reducing apoptosis [134, 135]. Further studies to investigate its effect on microbiota, metabolic profile, cytokine and chemokines expression in inflammatory conditions are currently ongoing (Table 1) [136].

Table 4 summarizes some of the promising probiotic strategies that have shown positive results for treatment of IBD. However, there are certain issues related to the delivery, dosing and

362 survival of probiotic agents that need to be investigated along with further studies in humans to
363 evaluate the true potential of probiotics as a safe and effective treatment option.

364

365 ***Fecal microbiota transplantation***

366 Fecal microbiota transplantation (FMT) is another strategy being researched that involves the
367 infusion of fecal material from a healthy donor into the gut of a recipient for the treatment of a
368 particular disease. The administration of fecal microbiota is by the upper route
369 (nasogastric/nasojejunal tube or upper endoscopy) or the rectal route (enema or colonoscopy)
370 [137]. The aim of FMT is to restore the microbial balance by introduction of bacteria into the
371 intestinal ecosystem and correction of the dysbiosis. FMT has been shown to be very effective
372 for refractory and recurrent *Clostridium difficile* infection [138], and could be a promising
373 approach for IBD if the therapy is able to restore the essential components of the microbiota and
374 reverse the inflammatory process. Several case studies have been reported on the success rates of
375 FMT but the assessment of the effectiveness is complicated due to the small number of patients
376 analyzed as part of the study and the lack of detailed analysis of the microbial changes occurring
377 with FMT [139, 140]. A review by Cammarota and colleagues has collectively presented the
378 FMT studies conducted in UC and CD [141]. The first randomized, placebo-controlled phase II
379 study evaluating the efficacy of FMT in a large population of active UC patients showed that
380 FMT induced remission was significantly greater than placebo, with no difference in adverse
381 effects [142]. Contrary to this result, Rossen *et al.* showed no clinical and endoscopic remission
382 between UC patients receiving FMT from healthy donors and those receiving their own
383 microbiota. In another study by Vermeire and colleagues, FMT showed higher success rates in

UC (2 out of 8 patients in prolonged remission) than in CD patients (no clinical efficacy observed at week 8) [143]. FMT was suggested to be more efficacious in patients with a more recent diagnosis of UC, as restoring microbial dysbiosis in early disease state might be more plausible. The results were intriguing and the lower response in CD could be explained by the importance of mucosa-adherent bacteria and transmural nature of inflammation, potentially requiring longer treatment cycles. The milder nature of microbial dysbiosis in UC than in CD patients could also lead to easier restoration of the microbial balance [144]. Only a few case series have been described for the management of CD by FMT and in small patient groups. Borody and colleagues showed complete resolution of symptoms in some CD patients treated with rectal FMT [145], while Cui and colleagues demonstrated that FMT input through mid-gut might be the safer and efficient approach in refractory CD [140]. However, in another pilot study in CD patients, no clinical or endoscopic efficacy was observed [146]. Despite FMT being a new and promising research area for IBD treatment, the results are still too fragmentary. Further clinical trials are needed to investigate the clinical efficacy, frequency and duration of administration, dosing regimens, route of delivery, effect of donor on success rates and standardization of microbiota analysis would be needed [25].

Toll-like receptors

TLR2

Toll-like receptors (TLRs) comprise a class of transmembrane pattern recognition receptors which are involved in pathogen recognition, induction of anti-microbial genes, control and initiation of inflammatory and adaptive immune responses [147]. TLRs are expressed by many

cells throughout the gastrointestinal tract such as the IECs [148], and the dendritic cells [149]. TLR2 is a member of the TLR family which recognizes the molecular patterns associated with both gram-positive and gram-negative bacteria like lipoproteins and peptidoglycans [149]. TLR2 stimulation selectively enhances transepithelial resistance of IECs, inhibits cell apoptosis and preserves the innate immune barrier equilibrium through regulation of tight junctions which maintains mucosal homeostasis against inflammation in IBD. TLR2 expression is barely detectable in a healthy human colon [150], and also remains unchanged in IBD [150]. This loss of function of human TLR2 could be due to selective mutation in TLR2 response of the intestinal epithelium to commensal TLR2-ligands [151]. The Arg753Gln polymorphism silencing TLR2 has recently been associated with a pancolitis phenotype in UC patients [152]. The downregulated TLR2 expression in UC patients can be used as an opportunity for developing new therapeutics targeting modulation of barrier-protective TLR2 using exogenous TLR agonists. Cario *et al.* showed the effect of TLR2 ligand on mucosal inflammation and regulation of epithelial barrier function in DSS-induced murine colitis model [151]. Oral administration of TLR2 ligand PCSK efficiently restored the tight junction integrity of the colitis intestinal epithelium, preventing the entry of luminal antigens and neutrophils and preventing the abnormal immune response and mucosal intestinal inflammation [151]. Ongoing studies may help to understand the regulatory effects of PCSK ligand on the stability and arrangement of tight junction proteins involved in intestinal barrier homeostasis. Further studies on the safety and efficacy of TLR2 agonists in humans would be needed to understand their potential as a therapy in IBD.

TLR4

TLR4 is known to recognize LPS present in the cell wall of gram-negative bacteria through its co-receptor myeloid differentiation factor-2, which triggers the translocation of NF-κB pathway and secretion of proinflammatory response [153]. In non-IBD state, TLR4 is expressed at low levels throughout the small and large IECs, but is highly expressed in IBD [126]. This increased expression suggested a potential role of TLR4 in initiation and prolongation of intestinal inflammation, and was thus investigated as a therapeutic target in IBD. An anti-TLR4 antibody therapy was investigated in DSS induced colitis mice model. TLR4 inhibition resulted in amelioration of colitis by decrease in recruitment of antigen presenting cells (APCs) to the lamina propria and reduction in the expression of proinflammatory cytokines TNF-α, IL-6, CCL2 and CCL20. However, TLR4 blockade led to defective mucosal healing and impaired epithelial proliferation and is a significant limiting factor in the development of TLR4 antagonists in IBD therapy [154]. Further studies are warranted to evaluate the safety and clinical outcomes of TLR4 inhibition due to its regulatory role in maintenance of intestinal homeostasis before studies can be carried out in humans.

EMERGING NON-CONVENTIONAL STRATEGIES IN IBD THERAPY

Restoring pro-regulatory mechanisms

Transforming growth factor-beta 1 (TGF-β1) is a regulatory cytokine that plays a key role in maintenance of immunological homeostasis and inflammatory responses. Mucosal TGF-β1 has been shown to be abnormally downregulated in active CD patients and the subsequently diminished counter-regulatory TGF-β1 response to the inflammatory stimuli has been implicated in CD pathogenesis [155]. This is due to enhanced levels of SMAD7 (Mothers against

452 decapentaplegic homolog 7), an intracellular protein that binds TGF-β receptor and prevents
453 TGF-β1-associated and SMAD-associated signaling, leading to SMAD7 as a potential
454 therapeutic target for reducing intestinal inflammation [156]. Mongersen, a 21-base single-strand
455 phosphorothioate antisense oligonucleotide that hybridizes to the human SMAD7 mRNA,
456 facilitates RNase H-mediated RNA degradation. The molecule is delivered locally into the lumen
457 of the terminal ileum and ascending colon in a modified release formulation consisting of pH-
458 dependent methacrylic acid-ethyl acrylate copolymers; its ability to downregulate SMAD7 and
459 alleviate CD-like colitis was first shown in a mouse model [157]. Successful clinical benefit of
460 SMAD7 targeting by mongersen was shown in a phase II study in 166 active CD patients with a
461 significantly higher rate of clinical response than placebo [158]. The molecule is currently being
462 investigated for efficacy and safety in an ongoing phase III study in active CD patients [159].
463 IL-10 is an anti-inflammatory, immunomodulatory cytokine involved in immune homeostasis,
464 downregulating inflammatory pathway and regulating mucosal inflammation. Defects in IL-10
465 activity may contribute to the development of IBD inflammatory symptoms. Administration of
466 recombinant human IL-10 was found to be ineffective in active CD patients [160]. However,
467 targeting of the IL-10 pathway has shown some promise in mouse models when an antibody-
468 antigen complex targeted to DEC-205 (a type-I cell surface protein expressed by dendritic cells)
469 has shown to interfere with the function of Th1 cells by an enhanced secretion of IL-10. This
470 modulates the ability of $CD4^+$ $CXCR3^+$ T cells to migrate to the sites of inflammation via
471 downregulation of CXCR3 expression, preventing autoimmune mediated inflammation in the
472 small intestine and colon [161].
473 IL-4, also an anti-inflammatory and immunoregulatory cytokine, functions by inducing Th2-type
474 $CD4^+$ T cells to shift to a Th1 response and is significantly downregulated in IBD. IL-4 gene

475 therapy (plasmids carrying IL-4 cDNA) in TNBS-induced murine colitis showed promise in
476 reducing disease severity along with reducing the levels of IFN-γ, TNF-α and IL-6 mRNA [162].
477 However, further studies on the long term effects of pro-regulatory cytokine therapy are needed
478 before application to human studies.
479
480 **Stem cell mediated regeneration of damaged intestinal mucosa**
481 In recent years there have been great advances in the knowledge of stem cell therapy and a
482 growing interest in the application of autologous stem cell transplantation in autoimmune
483 diseases including IBD. However, the therapeutic potential of stem cell transplantation requires
484 in-depth investigation along with addressing the practical and ethical issues associated with it.
485 Haematopoietic stem cell (HSC) transplantation and mesenchymal stem cell (MSC)
486 transplantation have shown some promise in patients for treatment of IBD and are currently
487 being evaluated in clinical trials. HSC transplant leads to the generation of a 'new' immune
488 system free of autoimmunity involving preparatory chemotherapy that eliminates the immune
489 system and after HSC transplant, the haematopoietic stem cells generate new tolerant T-cell
490 population [163]. Ditschkowski *et al.* has reported that 10 out of 11 IBD patients (7 CD patients
491 and 4 UC patients) remained free from the disease after allogeneic HSC transplantation [164].
492 However, the largest randomized clinical trial of HSC transplantation conducted till date in
493 refractory-CD patients showed no significant clinical improvement in inducing sustained disease
494 remission in patients receiving HSC therapy compared to those receiving standard CD treatment
495 [165]. HSC therapy was also associated with significantly more adverse events compared to
496 conventional therapy along with the death of 1 patient. These findings raise serious concerns
497 over the toxicity and lack of clinical efficacy associated with HSC therapy and does not support

498 the widespread use of HSC transplantation in refractory CD patients. Further research is
499 warranted to study the risk factors associated with the significant toxicity of HSC therapy and to
500 evaluate the possible benefit of maintenance immunosuppressive therapy in patients to regain
501 responsiveness.
502 MSCs inhibit maturation of APCs, T-cell proliferation, IFN-γ production and decrease the levels
503 of proinflammatory cytokines IL-6, IL-12 and TNF-α and increase the IL-10 and TGF-β levels
504 inducing tolerance and epithelial healing [166]. Adipose-derived MSCs have two biological
505 functions that are useful for the regeneration or repair of damaged tissues, their ability to reduce
506 inflammation and differentiation potential. Following a small phase I trial confirming feasibility
507 and safety of adipose-derived MSCs for the treatment of fistulizing CD, a phase II clinical study
508 evaluating the safety and efficacy of adipose-derived MSCs in combination with fibrin glue in
509 perianal complex fistulas was conducted [167]. Local administration of adipose-derived MSCs
510 with fibrin glue was found to be significantly more effective than fibrin glue alone in the
511 induction of fistulae healing. It also improved the patient's quality of life. Allogeneic adipose-
512 derived MSCs were evaluated in a phase I/IIa study for the treatment of complex perianal
513 fistulizing CD following a local injection [168]. The therapy was found to be promising in terms
514 of the fistulae tract closure, as well as being safe treatment that could potentially overcome the
515 problems associated with surgery and systemic anti-TNFs. Allogeneic adipose-derived MSCs
516 (Cx601) are now being evaluated in a phase III clinical study at a dose of 120 million cells (5
517 million cells/ml) for complex perianal fistulizing CD treatment [169]. Cx601, at a dose of 60
518 million cells (5 million cells/ml), is also being evaluated in a phase I/IIa study for safety and
519 efficacy of the treatment to induce remission of moderate active UC [170]. Remestemcel-L
520 (Prochymal®) are MSCs prepared from bone marrow aspirates of healthy human donors. In an

open-label phase II study of remestemcel-L in moderate-to-severe CD patients, 3 out of 9 patients showed clinical response by day 14, along with a significant improvement in CD activity index and IBD questionnaire scores at day 28 compared to baseline [171]. Based on these findings, a larger phase III study was initiated, and is currently ongoing, to evaluate the efficacy for the treatment of moderate to severe CD [172]. However, there is a lack of mechanistic data analyzing the effectiveness of the agent in CD, such as the contribution of T cells/APCs inhibition and the reparative or regenerative effects in the intestinal tissue, raising concerns over the long term application of the therapy [171].

The MultiStem® product is a bone marrow derived non-hematopoietic adult adherent stem cell product based on MAPC isolation and extraction protocols. The cells express distinct cytokine profiles, due to different culture conditions, compared to MSCs leading to different phenotypes [173]. The product, believed to reduce inflammation, exert immunomodulatory effects and promote tissue repair, was evaluated for safety and efficacy in a randomized, double-blind, placebo-controlled, phase II clinical trial for the treatment of moderate to severe UC [174]. The product failed to show a significant difference in clinical efficacy compared to placebo in the primary end-points-change in endoscopic score from baseline, as measured by modified Baron score at 8 weeks and change in Mayo rectal bleeding subscore from baseline at 4 and 8 weeks.

Several IBD susceptible genes have been identified to explain the potential mechanism of stem cell transplantation being efficient in IBD treatment, which include *NOD2/CARD15* expressed in IECs, peripheral blood monocytes and macrophages [175]. Transfer of IBD has been shown in cases where a person develops UC when transplanted with stem cells from sibling with the same disease or development of CD following bone marrow transplantation from donor without CD symptoms but carrying polymorphic forms of IBD susceptible genes *NOD2* and *CARD15*.

Therefore, it is important to note that stem cells that do not carry any polymorphic forms of susceptible IBD genes are important for remission in IBD patients [2, 176]. Stem cell therapy theoretically appears to be a valuable tool for treatment of IBD but it still remains to be seen further whether it can overcome the issues of safety and show consistent efficacy in larger clinical settings.

Gut associated lymphoid tissue for targeting cytokines

Ochi *et al.* and Forster *et al.* investigated the immune modulation of inflammatory diseases by mucosal delivery (oral and nasal) of CD3-specific IgG2 monoclonal antibody (muromonab-CD3 or OKT3) to induce regulatory T-cells that are preferentially induced at mucosal surfaces, secreting IL-10 and TGF-β, inducing tolerance and avoiding the abnormal mucosal inflammatory reaction by reducing levels of inflammatory cytokines IFN-γ and TNF-α associated with IBD [177, 178]. Orally delivered CD3-specific antibody at a dose of 5 µg was taken up in the gut associated lymphoid tissue (GALT). The oral therapy was found to be superior in action as compared to intravenous delivery, with no depletion of T-cells and apoptosis observed, along with reduced side effects via the oral route. The antibody appears in the gut villi 30 mins to 3 hours after oral administration as compared to intravenous delivery in which the antibody appears in the serosal surface of the gut [177]. Oral muromonab-CD3 is currently being evaluated in a phase II study for safety and efficacy in active UC patients [179]. Visilizumab (Facet Biotech) is another investigational humanized IgG2 monoclonal antibody that is in clinical phase targeting the invariant CD3 chain of the T-cell receptor CD3 to reduce inflammation in severe UC and moderate to severe CD [180, 181]. However, visilizumab (HuM291) at an intravenous dose of 5 µg/kg was not effective in achieving response, remission

or mucosal healing in patients with severe corticosteroid-refractory UC [182]. The patients showed a trend towards a greater rate of colectomy and more prone to symptoms of cytokine release and serious cardiac and vascular disorders. These studies highlight the early stage progress and also the challenges in the translation of CD3 targeting as an effective therapy in UC. The failures in CD3 targeting also challenges our understanding of the pathophysiology of UC.

Adenosine deaminase inhibition

Brown and colleagues have shown a dual mechanism of adenosine deaminase (ADA) inhibition by pentostatin. The first mechanism was by targeting and reducing T_{eff} cell numbers and reduction of T_{eff} derived cytokines TNF-α, IFN-γ as well as macrophage-related IL-1β and IL-6. The second mechanism was the potent anti-inflammatory effect of pentostatin, independent of its lymphodepletion effect, showing reduction in IL-1β, IL-6 and TNF-α in a colitis IL-$10^{-/-}$ mouse model [183]. The study demonstrated that ADA might be a potential target for IBD treatment, however further studies investigating safety and efficacy in humans are warranted.

Oxidative/Anti-oxidative balance restoration

It has been previously shown that the macrophages oxidative burst activity is enhanced in IBD condition, leading to production of NADPH oxidase, which then mediates the production of superoxide and other reactive oxygen intermediates such as hydrogen peroxide and hypochlorous acid causing inflammatory conditions [184]. Therefore, anti-oxidants can be potential candidates for anti-inflammatory therapy in IBD by maintaining the balance between reactive oxygen species production and the anti-oxidant property. Phenylethanoid acteoside isolated from

590 *Plantago lanceolata* L. has been shown to exhibit anti-oxidative potential and ameliorates acute
591 or chronic inflammation *in-vivo* in a DSS induced colitis model, along with suppression of
592 proinflammatory cytokine release such as TNF-α, IFN-γ and GM-CSF [185]. Similarly, other
593 anti-oxidant molecules that have shown promise, mainly in UC, via modulation of oxidant/anti-
594 oxidant balance and reduction in production of iNOS, COX-2 and proinflammatory cytokines
595 such as TNF-α and IL-1βare Acacia ferruginea [186], Rhizophora apiculata [187], naringin
596 [188], amentoflavone [189], olmesartan medoxomil [190], Lacto-Wolfberry [191], and
597 resveratrol [192].
598
599 **Nutraceutical intervention**
600 Supplementation with specific amino acids such as arginine, glutamine, cysteine, threonine,
601 serine, methionine and proline has suggested that they can be useful in mucosal healing and this
602 can possibly lead to their overall beneficial effect in IBD by reducing risk of relapse [193]. Liu *et*
603 *al.* demonstrated colonic mucosal healing in DSS-induced colitis rats by administration of a
604 cocktail of supplement amino acids (threonine, methionine and monosodium glutamate) but this
605 mixture of amino acids showed no influence on the mucosal inflammatory status in the same
606 model [194]. Glutamine is an immunomodulatory essential amino acid that has been widely
607 shown to function as an anti-inflammatory agent via several proposed mechanisms, mainly
608 suppression of T cell migration [195], enhancement of heat shock proteins expression [196],
609 endoplasmic reticulum stress signaling, anti-apoptotic effects [197], and by inhibition of NF-$_{K}$B
610 and STAT signaling pathways [198], leading to reduction of proinflammatory cytokines and
611 subsequent amelioration in inflamed state. Other amino acids that have shown promise are L-
612 cysteine and L-arginine. L-cysteine was shown to significantly reduce proinflammatory

cytokines TNF-α, IL-6, IL-12p40 and IL-1β and increased the expression of caspase-8, inducing apoptosis, in DSS-induced porcine colitis model [199]. L-arginine on the other hand was shown to have anti-inflammatory activity via attenuation of GM-CSF and chemokines C-X-C motif ligand 1 and macrophage inflammatory protein α, as well as cytokines IL-1α, IL-1β, IL-6 as well as IL-17, suggesting a broad role in colitis [200]. Casein glycomacropeptide (169 amino acids) is a C-terminal part of kappa casein that is released in whey during the manufacture of cheese by the action of chymosin. Casein glycomacropeptide can modulate gut microbiota and regulate immune responses and has been considered as a promising candidate for UC treatment [201]. Experimental mice colitis model has shown anti-inflammatory effects of casein glycomacropeptide via NF-κB/p65 pathway inhibition and alleviation of weight loss and morphological/histological damage [202]. In a randomized clinical trial in active distal UC patients, casein glycomacropeptide (fraction of bovine whey protein) as a nutritional therapy was found to be safe and showed a similar disease-modifying effect to that of mesalazine [203].

Novel cholinergic anti-inflammatory pathway targeting

Gallowitsch-Puerta *et al.* recently discovered a cholinergic anti-inflammatory pathway, which is a rapid and locally acting nervous system based pathway that inhibits cytokine stimulation and response [204]. The signals are transmitted via the vagus nerve, which is the tenth cranial nerve originating in the brain stem and connects brain to the colon through an indirect pathway. The function of the vagus nerve is to maintain balance in the autonomic nervous system by controlling heart rate, blood pressure and digestion. Its main principle neurotransmitter is acetylcholine which modulates organ function by interaction with the peripheral muscarinic receptors. Signals transmitted via this vagus nerve converge with cytokine-producing cells which

express the nicotinic acetylcholine receptor alpha 7 (nAChRα7), which is an essential component of the cholinergic anti-inflammatory pathway as it inhibits the release of TNF, IL-1β, IL-6 and IL-18, but not IL-10. Borovikova *et al.* showed, in rats, that direct electrical stimulation of this afferent vagus nerve fibres exposed to endotoxins, which activate cytokine release, lead to the inhibition of proinflammatory TNF-α and cytokine release significantly, avoiding the damage to tissues and potential inflammation [205]. The α-7 agonists have shown to decrease the activation of NF-$_K$B pathway, a transcription factor which regulates the release of cytokines. This provides an opportunity to exploit this pathway to therapeutic advantage in IBD which is thought to be caused by the excessive cytokine activity leading to inflamed intestinal mucosa.

CONCLUSIONS

The complex and poorly understood aetiology and gut pathophysiology of IBD, as well as discordances in translating results from animal studies to human trials, have restricted the discovery of novel targets and the subsequent therapies from reaching the patients. A comprehensive analysis of the differences in gut pathophysiology in UC and CD patients has presented several gastrointestinal parameters that can potentially be exploited as novel targets for IBD. The exploitation of GATA-3, IP-10 proteins, and TLR inhibitors, currently being investigated in clinical stage, emphasizes on the growing shift from the conventional TNF-α based biologics approaches towards newer targeting opportunities that may represent the future of IBD treatment. Recent developments in emerging therapies such as restoration of pro-regulatory mechanisms, HSCs and MSCs transplantation and CD3 targeting has shown some potential that requires further trials and in-depth investigation. Ongoing and future explorations and a more insightful understanding of the diseased gut pathology will be an important step

659 towards the translation of ‘promising’ targets into clinically effective therapeutics that can
660 revolutionize the treatment of IBD.
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669 **References**
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Table I Current and emerging biopharmaceutical therapies in inflammatory bowel disease which are established in clinical stage

Drug	Molecular Weight (kDa)	Mechanism of action	Status/Brand name	Company	Route of administration	Indication
TNF-α inhibitors						
Infliximab	144.19	TNF-α inhibitor	Marketed/Remicade®	Janssen Biotech	iv	UC, CD
Adalimumab	144.19	TNF-α inhibitor	Marketed/Humira®	Abbott	sc	UC, CD
Certolizumab pegol	90.8	TNF-α inhibitor	Marketed/Cimzia®	UCB	sc	CD
Golimumab	147	TNF-α inhibitor	Marketed/Simponi®	Janssen Biotech/MSD	sc	UC
TNF-Kinoid	-	TNF-α inhibitor	Completed phase II	Neovacs	iv	CD
AVX-470	-	TNF-α inhibitor	Completed phase-Ib	Avaxia Biologics	oral	UC, CD
AG014	-	*L.Lactis* secreting TNF-α inhibitor	Completed phase I	ActoGeniX	oral	UC
CAM inhibitors						

Natalizumab	149	CAM α4-integrin inhibitor	Marketed/Tysabri®	Biogen	iv	CD
Vedolizumab	146.8	CAM α4β7-integrin inhibitor	Marketed/Entyvio®	Takeda	iv	UC, CD
Etrolizumab	144.1	CAM α4β7, αEβ7 - integrin inhibitor	Phase III	Genentech	sc	UC
Vatelizumab	-	α2β1-integrin inhibitor	Phase IIa	Sanofi	sc	UC
PF-00547659	-	MAdCAM-1 inhibitor	Phase II	Pfizer	sc	UC, CD
AMG 181	144	MAdCAM-1 inhibitor	Phase II	Amgen/AstraZeneca Plc.	sc	UC, CD
Alicaforsen (ISIS 2302)	6.36	ICAM-1 inhibitor	Completed phase II	Atlantic healthcare/Isis Pharmaceuticals	rectal	UC

Interleukin inhibitors						
Low dose IL-2	17.6	Selective stimulation of regulatory T cells	Phase II	ILTOO Pharma	sc	UC, CD
Daclizumab	142.6	CD25 (IL-2Rα) inhibitor	Completed phase II	Facet Biotech	iv	UC
PF04236921	-	IL-6 inhibitor	Phase II	Pfizer	sc	CD
Tralokinumab	143.87	IL-13 inhibitor	Completed phase II	AstraZeneca Plc./Medimmune	sc	UC
Dectrekumab (QAX-576)	-	IL-13 inhibitor	Completed phase II	Novartis	iv	CD
Anrukinzumab	145.4	IL-13 inhibitor	Completed Phase II	Pfizer	iv	UC
GSK1070806	-	IL-18 inhibitor	Completed phase I	GSK	iv	UC, CD
Ustekinumab	145.64	IL-12/23p40 inhibitor	Phase III	Janssen Biotech	iv, sc	CD

BI 655066	-	IL-23p19 inhibitor	Phase II	Boehringer Ingelheim	sc	CD
ATR-107 (PF05230900)	-	IL-21 inhibitor	Phase I	Pfizer	iv,sc	CD
NNC0114-0006	-	IL-21 inhibitor	Completed phase II	Novo Nordisk A/S	iv	CD
AMG 139	-	IL-23 inhibitor	Phase II	Amgen/AstraZeneca Plc.	iv, sc	CD
LY3074828	-	IL-23 inhibitor	Phase II	Eli Lilly and Company	iv	UC
Immunomodulators						
Visilizumab	150	Anti-CD3	Completed phase II	Facet Biotech	iv	UC, CD
Muronomab CD3 (OKT3)	146.09	Anti-CD3	Phase II	Brigham and Womans Hospital/Therapix Biosciences	oral	UC
NI-0401	-	Anti-CD3	Completed phase I/IIa	Novimmune	iv	CD
Abatacept	92.3	Anti-CD28/B7.1-2	Completed phase III	Bristol-Myers Squibb	iv	UC

FFP104	-	Anti-CD40	Phase I/II	FF Pharma	iv	CD
Ciclosporin (CyCol®)	1.202	T cell inhibitor	Completed phase IIa	Sigmoid Pharma	oral	UC
Ciclosporin (CyCron®)	1.202	T cell inhibitor	Completed phase I	Sigmoid Pharma	oral	CD
P28GST (glutathione S-transferase)	28	Th1 response inhibitor; Th2 and regulatory response inducer	Phase II	University Hospital, Lille	injection	CD
Type 1 T-cell	-	Antigen specific immune suppression	Completed Phase I-IIa/Ovasave®	TxCell	iv	CD
RDP-58	1.38	TNF-α, IFN-γ, IL-2 and IL-12 inhibitor	Completed phase II	Abbott	oral	UC, CD
Mongersen (GED-0301)	-	SMAD7 inhibitor	Phase III	Celgene Corporation	oral	CD

Mongersen (GED-0301)	-	SMAD7 inhibitor	Phase II	Celgene Corporation	oral	UC
Denosumab	147	Receptor activator of NF-$_K$B ligand inhibitor	Phase I/II	University of Manitoba	sc	CD
Chemokine inhibitors						
Bertilimumab	-	Eotaxin-1 inhibitor	Phase II	Immune Pharmaceuticals	iv	UC
Eldelumab (BMS-936557, MDX-1100)	146.5	IP-10 (CXCL-10) inhibitor	Completed phase II	Bristol-Myers Squibb/Medarex	iv	UC, CD
GSK3050002	-	CCL20 inhibitor	Completed phase I	GSK	iv	UC
E6011	-	Fractalkine/CX3CL1 inhibitor	Phase I/II	Eisai Co., Ltd.	iv	CD
Stem cell therapies						

Remestemcel-L (Prochymal®)	-	Adult stem cells	Phase III	Osiris Therapeutics	iv	CD
Cx601	-	Allogeneic adipose-derived MSCs	Phase III	TiGenix S.A.U	il	CD
Cx601	-	Allogeneic adipose-derived MSCs	Phase I/IIa	Instituto de Investigación Hospital Universitario La Paz	ic	UC
ALLO-ASC	-	Allogeneic adipose-derived MSCs	Phase I	Anterogen Co., Ltd.	iv	CD
PDA-001	-	Human placenta derived stem cells	Phase II	Celgene	iv	CD
PF-05285401	-	Adult adherent stem cells	Completed Phase II	Pfizer/Athersys	iv	UC
Enzyme inhibitors						
GS-5745	-	MMP9 inhibitor	Phase II	Gilead Sciences	sc	UC, CD

KHK4083	-	OX40 (CD134) inhibitor	Phase II	Kyowa Hakko Kirin Pharma Inc.	iv	UC
Enzyme agonist						
Dolcanatide	1.68	Guanylate cyclase-C agonist	Completed phase Ib	Synergy Pharmaceuticals	oral	UC
TLR agonists						
BL-7040	-	TLR-9 agonist and acetylcholinesterase inhibitor	Completed phase IIa	BioLineRx, Ltd.	oral	UC
Kappaproct (DIMS0150)	6	TLR-9 agonist	Phase III	InDex Pharmaceuticals	intrarectal	UC
Probiotics						
AG011	-	Probiotic of L.Lactis secreting IL-10	Completed phase I	ActoGeniX	oral	UC

VSL#3	-	Probiotic of 8 bacterial strains, protects epithelial barrier function	Phase IV	VSL Pharmaceuticals	oral	UC, CD
LPS inhibitor						
Bovine intestinal alkaline phosphatase	140-160	LPS inhibitor	Completed phase II	AM-Pharma	oral	UC
GATA-3 inhibitor						
SB012 (DNAzyme hgd40)	-	GATA-3 inhibitor	Phase I/II	Sterna Biologicals GmbH & Co. KG	rectal enema	UC
GLP receptor agonist						
ZP1848	-	GLP-2 agonist	Completed phase Ia	ZealandPharma	sc	CD

			and Ib			

TNF, tumor necrosis factor; IFN, interferon; CAM, cell adhesion molecule; ICAM, intercellular adhesion molecule; MAdCAM, mucosal vascular addressin cell adhesion molecule; IL, interleukin; GLP, glucagon-like peptide, sc, subcutaneous; iv, intravenous; il, intralesional; ic: intracolonic

Table II Pathophysiological changes in the gut of ulcerative colitis and Crohn's disease patients as compared to healthy volunteers.

	Healthy	Ulcerative colitis	Crohn's disease
Luminal pH			
Small Intestine	6.1-7.0[206]	6.1-8.3[207, 208]	6.0-7.4[208, 209]
Colon	5.8-7.7[206]	2.3-7.5[207, 208]	5.2-7.0[208, 209]
Transit time (hrs)			
Small Intestine	1.5-5.4[206]	5.6 ± 2.4[210],a	1.8-6.6[211] ,b
Colon	41.1-62.3[212]	9.5-39.1[212]	N.C
Bacterial flora			
Small Intestine			
Duodenum	10^2 microorganisms/gram luminal content[213]	N.C	
Jejunum	10^2 microorganisms/gram luminal content[213]	N.C	
Ileum	10^7-10^8 microorganisms/gram luminal content[213]	N.C	*Faecalibacterium prausnitzii*[214] ↓ Enterobacteriaceae[215] ↑ *E. coli*[215] ↑

			Ruminococcus gnavus[215] ↑
Colon	10^{11}-10^{12} CFU/ml[216]	Ruminococcus spp[10] ,d Eubacterium spp[10] ,d Fusobacterium spp[10] ,d Lactobacillus spp[10] ,d Proteobacteria[10] ,d Bacteroidetes[10] ,d	*E. coli*[10] ,c *Faecalibacterium prausnitziid*[10] ,c,d ↓ Bacteroidetes[10] ,c ↑ Bifidobacterium[10] ,d ↓ Firmicutes[10] ,c,d ↓ Enterobacteriaceae[10] ,c,d ↑ *Ruminococcus gnavus*[10] ,d ↑
Lipopolysaccharides (LPS) in colonic lumen	~50 µg/ml[217]	N.C[217]	Increased[217]
Intestinal alkaline phosphatase			
Colon	1.0 ± 0.1 units/mg[218]	2.8-fold decrease[219]	2.4-fold decrease[219]
Carcinoembryonic antigen-related cell adhesion molecule 6	N.C	N.C in colonic tissue[10]	Increased expression in ileal enterocytes[10]

Intestinal barrier function	N.C	Widening of barrier tight junctions leading to increased permeability[220]	2 to 3-fold increase in intestinal permeability, reduced levels of anti-microbial defensins[221-223]
Colonic mucus layer	N.C	Decreased thickness[220] Goblet cell count[33] ↓ Trefoil factor 3 (TFF3)[33] ↑ MUC2 and MUC3 glycoproteins[33] ↓	Increased thickness[224, 225] Goblet cell count[33] ↑ Trefoil factor 3 (TFF3)[33] ↑ MUC2, MUC3 and MUC4 Glycoproteins[33] ↑ α-defensins HD5, HD6[224],e β-defensins HBD1, HBD2, HBD3 and HBD4[224],f ↓
Immune cells	N.C	Mast cells[225] T-cells[12, 14] Neutrophils[12, 14] Macrophages[12, 14] ↑	Mast cells[15, 33, 224-226] Macrophages[226] Eosinophils/secreted proteins[226] ↑

		Eosinophils/secreted proteins[12, 14]	Basophils[226]
Colonic mucosal immunoglobulins (Ig)			
IgA	N.C[44]	Decreased[44]	N.C[44]
IgG	3 µg/ml[45],g	512 µg/ml[44],h	256 µg/ml[44],h
IgG1	1.8 µg/ml[45],g	479 µg/ml[44],h	121 µg/ml[44],h
IgG2	1.3 µg/ml[45],g	N.C	185 µg/ml[44],h
IgG3	0.2 µg/ml[45],g	51 µg/ml[44],h	36 µg/ml[44],h
Transferrin receptor expression	N.C	Increased expression[227]	Increased expression[227]
Neonatal Fc receptor (FcRn)	N.C	Increased expression[59]	Increased expression[59]
Cytokines	N.C	Proinflammatory cytokines[17, 102, 228-230]: IFN-γ TNF-α IL-5 IL-6	Proinflammatory cytokines[6, 63, 230, 232-234]: IFN-γ TNF-α IL-6 IL-12

		IL-12 IL-13 IL-18 IL-23 IL-27 Pro-regulatory cytokines [6, 63, 155, 230, 231]: IL-4 ↓ IL-10 ↓ TGF-β1 ↑	IL-16 IL-17 IL-18 IL-21 IL-27 Pro-regulatory cytokines [6, 63, 155, 230, 231]: IL-4 ↓ IL-10 ↓ TGF-β1 ↓
Interferon gamma-induced protein (IP-10)/CXCL-10	N.C	5.98-fold higher expression[235],i	4.76-fold higher expression[235],i
Natural killer activating receptor 2D (NKG2D)	N.C	N.C[236],i	Upregulation of $CD4^+$ T cells expressing NKG2D[236],i
GATA-3 and STAT-4 signalling proteins	N.C	Increased expression[11],i	N.C[11],i
OX40 (CD134)	No colonic lamina propria expression[85]	Increased colonic lamina propria expression[85]	Increased colonic lamina propria expression[85]
Enzymes			
Colon tissue	N.C	Guanylate cyclase-C[91] ↓	Guanylate cyclase-C[91] ↓

		Inducible nitric oxide synthases (iNOS)[237] Unidentified serine proteases[238] Trypsin[238] Neutrophil elastase[238] MMP-1, -2, -3 and -9[239]	Inducible nitric oxide synthases (iNOS)[240] MMP-1, -2, -3 and -9[239]
Faeces	N.C	Fecal proteolytic activity[241] Alpha-1-antitrypsin[241] Neutrophil elastase[241]	Fecal tryptic activity[242]
Toll-like receptors (TLRs)	Low expression of TLR-2 and TLR-4; High expression of TLR-3 and TLR-5[150],j	Increased expression of TLR-2, TLR-4 and TLR-5 by intestinal dendritic cells[10, 149] No change in TLR-3 expression[150],k	Increased expression of TLR-2, TLR-4 and CD20 cells by intestinal and colonic dendritic cells[10, 149] Lower expression of TLR-3[150],k
Heat shock proteins (HSP)	N.C	HSP 27[243] ↓ HSP70[243] ↓	HSP 27[243] ↓ HSP70[243] ↓

NC, no change; [a]measured using mesalazine microspheres; [b]measured using pH sensitive capsule; [c]mucosal sample; [d]fecal sample; [e]ileal CD; [f]colonic CD; [g]intestinal mononuclear cells; [h]colonic washings taken at endoscopy; [i]expression levels in colon tissue compared to non-IBD subjects; [j]intestinal biopsies from non-IBD subjects; [k]intestinal biopsies from UC and CD patients; IFN, interferon; TNF-α, tumor necrosis factor alpha; TGF-β1, transforming growth factor beta 1; IL, interleukin; IP-10, interferon-γ-inducible-protein-10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; MMP, Matrix metalloproteinases; STAT-4, signal transducer and activator of transcription-4; SMAD-7, Mothers against decapentaplegic homolog 7; ↑ enhanced; ↓ reduced.

Table III Cutting edge in novel anti-inflammatory molecules that function via suppression of cytokines through inhibition of signaling pathways

Molecule	**Signal pathway inhibited**	**Suppressed proinflammatory mediators**	**Reference**
Curcumin	NF-$_K$B, JAK-STAT, JNK, MAPK	IFN-γ, TNF-α	[244-248]
Rutin		IFN-γ, TNF-α, IL-1β	[249]
Vanillin	NF-$_K$B	IFN-γ, TNF-α, IL-1β, IL-6	[250]
Everolimus		IFN-γ	[251]
7-*O*-succinyl macrolactin A	NF-$_K$B, PI3-kinase/Akt/mTOR	ICAM-1, VCAM-1, TNF-α, IL-6, MCP-1, IL-8	[252]
Corilagin	NF-$_K$B	TNF-α, IL-1β, IL-6	[253]
AS1940477	p38 α MAPK	TNF-α, IL-1β, IL-6	[254]
N-[3 (aminomethyl)benzyl]acetamidine (1400W)	iNOS signaling pathway inhibition	TNF-α, IL-6	[255]
Apolipoprotein E-mimetic peptide COG112	NF-$_K$B	TNF-α, IL-1β, IL-17	[256]
Arsenic trioxide	NF-$_K$B, caspase-3	TNF-α, IL-1β, IL-12, IL-17, IL-18 and IL-23	[257]
BIHC: 3,3'-((2-butyl-5-	NF-$_K$B	TNF-α	[258]

chloro-1H-imidazol-4-yl)methylene)bis(4-hydroxy-2H-chromen-2-one)			
Semapimod	MAPK (p38 and JNK)	TNF-α, IL-1β, IL-6, MIP-1α,β, TNF-α	[259]
Telmisartan	NF-$_K$B		[260]

Table IV Promising probiotic approaches proposed to be potential novel treatment of IBD

Strain	**Mechanism of action**	**Indication**	**Reference**
*Escherichia coli Nissle*1917	Induction of anti-microbial defensins, inhibition of adhesion of pathogens	UC	[261]
Lactococcus lactis genetically modified	Secretion of anti-TNF-α antibodies	UC, CD	[262]
Lactococcus lactis (LL-Thy12) genetically modified	Secretion of IL-10	CD	[263]
Clostridium butyricum	Induction of IL-10 production	UC	[264]
Saccharomyces boulardii	Decreases secretion of TNF-α and IL-6, increases IL-8 secretion	UC, CD	[265]
Bacillus subtilis PB6	Inhibits phospholipase A2, decreased expression of IFN-γ, TNF-α, IL-1β, IL-6	UC, CD	[266]

Faecalibacterium prausnitzii	Inhibition of IL-17 and increase in IL-10 and IL-12	UC, CD	[267]
Lactococcus lactis subsp. cremoris FC	NF-$_K$B inhibition, decrease in TNF-α, IL-6, IL-8, iNOS and MIP-2	UC, CD	[268]
Lactobacillus suntoryeus (Lactic acid bacteria)	TLR-4-linked NF-$_K$B inhibition, decrease in TNF-α, IL-1β, IL-6 and COX-2	UC, CD	[269]
Lactobacillus casei Shirota	Suppression of IL-6 via NF-$_K$B signaling pathway inhibition	UC, CD	[270]