REVIEW

Mucosal healing and inflammatory bowel disease: Therapeutic implications and new targets

Megan Lynn Otte, Raju Lama Tamang, Julia Papapanagiotou, Rizwan Ahmad, Punita Dhawan, Amar B Singh

Megan Lynn Otte, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198, United States

Raju Lama Tamang, Julia Papapanagiotou, Rizwan Ahmad, Punita Dhawan, Amar B Singh, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198, United States

Corresponding author: Amar B Singh, PhD, Professor, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, 600 42nd Dewey, Omaha, NE 68118, United States. amar.singh@unmc.edu

Abstract

Mucosal healing (MH) is vital in maintaining homeostasis within the gut and protecting against injury and infections. Multiple factors and signaling pathways contribute in a dynamic and coordinated manner to maintain intestinal homeostasis and mucosal regeneration/repair. However, when intestinal homeostasis becomes chronically disturbed and an inflammatory immune response is constitutively active due to impairment of the intestinal epithelial barrier auto-immune disease results, particularly inflammatory bowel disease (IBD). Many proteins and signaling pathways become dysregulated or impaired during these pathological conditions, with the mechanisms of regulation just beginning to be understood. Consequently, there remains a relative lack of broadly effective thera-peutics that can restore MH due to the complexity of both the disease and healing processes, so tissue damage in the gastrointestinal tract of patients, even those in clinical remission, persists. With increased understanding of the molecular mecha-nisms of IBD and MH, tissue damage from autoimmune disease may in the future be ameliorated by developing therapeutics that enhance the body's own healing response. In this review, we introduce the concept of mucosal healing and its re-levance in IBD as well as discuss the mechanisms of IBD and potential strategies for altering these processes and inducing MH.

Key Words: Inflammation; Injury/repair; Mucosal healing; Mucosal barrier; Therapeutics; Colitis

Core Tip: Mucosal healing (MH) is vital in maintaining intestinal homeostasis and protecting against infection and injury. MH has emerged as an important clinical criterion in effective treatment of inflammatory bowel disease (IBD). However, there remains a relative lack of therapeutics that can restore MH due to the complexity of the disease and healing processes. Through increased understanding of the molecular mechanisms of MH, tissue damage from IBD may be ameliorated by developing novel therapeutics. Here, we introduce the concept of MH and its relevance in IBD, and discuss the mechanisms of IBD and potential strategies for altering these processes for inducing MH.

INTRODUCTION

Mucosal healing (MH) is a process of wound repair that restores the integrity of damaged epithelial barrier and homeostatic function after an injury compromises barrier integrity[1]. It is a complex process regulated by multiple cell types, through distinct mechanisms in response to highly specific stimuli within multiple signaling and cytokine pathways[1]. For simplification, the MH process is considered to have three phases: Epithelial restitution, proliferation, and differentiation and maturation[1]. Restitution consists of epithelial cells migrating into a wound within hours, followed by proliferation of epithelial cells in hours to days, and finally differentiation of intestinal stem cells into all mature intestinal cell types[1-3]. Each phase is induced and regulated by multiple cytokines, growth factors, and cell types reviewed in[1] and can be influenced by many factors that enhance or prevent wound healing as well as by the source of barrier injury. When the homeostatic process of wound healing is slowed or delayed by external or genetic factors, chronic inflammation may develop because repair of the intestinal epithelial barrier (IEB) and subsequent reduction of inflammation will not occur unless wound healing mechanisms are present[3]. Due to inflammation and chronic immune response, the consequences of impaired MH are chronicity of autoimmune diseases, including Inflammatory Bowel Disease, (IBD) and its progression to colorectal cancer.

INFLAMMATORY BOWEL DISEASE OVERVIEW AND PATHOGENESIS

Inflammatory Bowel Disease (IBD) is a term that represents autoimmune inflammatory diseases of the gut, with ulcerative colitis (UC) and Crohn's Disease (CD) being the major disease types; however, the etiology of IBD remains unclear. IBD is known to have genetic and environmental risk factors, but the mechanisms by which these factors induce IBD are not well-understood[4]. Common symptoms include abdominal pain, diarrhea, weight loss, malnutrition, and particularly in CD, nausea, vomiting, intestinal blockages, fistulae, and abscesses[4,5]. IBD is an increasingly common and often debilitating disease, affecting up to 200 individuals *per* 100000 people in the United States[4]. Onset of IBD often occurs before the age of 30, and patients experience poor quality of life along with high risk of developing colorectal cancer due to the chronic and progressive symptoms and persistent inflammatory state[1,4]. Though multiple treatment mechanisms exist, including corticosteroids, anti-inflammatory medications, monoclonal antibodies, stem cell treatments, and surgery, IBD cannot currently be cured[4]. Therefore, continued research into the mechanisms of pathogenesis and development of new pharmaceuticals and treatment methods is vital to decrease mortality and improve quality of life for IBD patients.

Autoimmune disease pathogenesis is difficult to study due to the multifactorial causes of disease and complex molecular mechanisms, as well as the predicament of patients not presenting to the clinic until late in the disease development process. Although the disease complexity and difficulty in identifying the initial molecular instigators has thus far precluded full understanding of the specific molecular mechanisms of IBD pathogenesis, the general overarching processes have been elucidated. During IBD pathogenesis, the IEB and mucosal layers become damaged and inflamed due to injury and/or infection, which can develop into a state of chronic inflammation and reduced IEB integrity. Genetic factors can also play a role in pathogenesis, particularly in CD through genes such as a variant that impairs autophagy and dysregulates the IEB and gut microbiota[6,7]. The damage to the IEB results in microbial and antigen exposure in the intestinal lumen, leading to an inflammatory cascade and disturbed homeostasis[8,9]. Multiple cytokines and immune cell types are also thought to contribute to the pathogenesis of or protection against IBD; therefore, further study of the complex interactions governing the maintenance or breakdown of gut barrier homeostasis is imperative to deeper

understanding of IBD pathogenesis and the development of effective treatments.

MUCOSAL HEALING AND PATHOLOGICAL RELEVANCE IN IBD

The main goals of IBD treatment are two-pronged: Reducing symptoms and preventing new inflammation and intestinal injury through traditional treatment methods, and recently a new, ambitious goal of inducing wound healing of existing inflammation and damage[8]. Some current treatments may contribute to both treatment goals; however, many existing clinical treatments are more targeted toward the traditional goal of preventing inflammation and damage. Even with the advent of some newer MH-focused treatments, additional avenues of further enhancing MH should be explored. Although clinical remission or preventing new inflammation is possible, patients may still have residual disease symptoms during remission due to the defective wound healing process leaving previous intestinal damage unrepaired. Additionally, up to half of IBD patients experience non-response or loss of response to standard therapeutics, leading to relapse[10]. Hence, MH induction presents an attractive goal in effective long-term treatment of IBD and prevention of relapse, and thus also prevention of progression to colitis-associated colon cancer. In this review, we will summarize the main current and prospective treatments of IBD, as described in Table 1, and their benefits and limitations towards the goal of reducing inflammation and achieving MH. More detailed analysis of the mechanisms of action, safety, and efficacy profiles of current IBD therapies and clinical trials can be found in Neurath *et al*[11]. However, in this review, we will emphasize areas that have not yet been as extensively clinically explored, including the temporal control of gut immune function, which presents novel potential for fine-tuning of the immune system in MH and restoration of gut homeostasis. We will examine three major factors contributing to the pathogenesis and tissue damage of IBD, as shown in Figure 1: Gut barrier dysfunction, gut dysbiosis, and inflammatory cytokine responses. Specifically, we will focus on the prospect of altering these factors and associated pathways summarized in Table 2 for both the reduction of inflammation and induction of MH.

CURRENT STATUS OF IBD THERAPIES AND FOCUS ON ENHANCED MUCOSAL HEALING

Traditional Therapies

Historically, there has been a disconnect between the expectation of IBD treatment promoting MH and real treatment outcomes, as many therapies for UC and CD primarily target symptom relief and reduction of chronic inflammation[12]. Corticosteroids, Methotrexate, and surgery are typically utilized to achieve these goals, but they do not promote MH as the primary therapeutic endpoint[13,14]. Because UC and CD are progressive diseases, patients may still experience intestinal damage even during periods without physical symptoms, and disease progression is typically only slowed by these treatments, not stopped[12,15]. However, achieving MH may help stop disease progression as well as decrease symptom severity. New treatment plans broaden the therapeutic focus to include inducing MH through a variety of mechanisms, such as by altering the gut microbiome and altering inflammatory and anti-inflammatory cytokines with antibodies or exogenous cytokine therapies.

Inflammation Reduction and Immune Modulation Therapies

One such mechanism for emphasizing MH includes suppressing specific parts of the patient's immune system to decrease the main contributors of chronic inflammation. When new inflammation is reduced, it may then be possible for the wound healing process to begin to keep up with the rate of tissue damage. Methods of achieving this goal include enteral nutrition (EN), partial EN (PEN), 5-aminosalicylates (5-ASA), and Azathioprine treatments, which focus on decreasing IBD-associated inflammation by suppressing the host immune system in a less global manner than corticosteroids or preventing the production of inflammatory molecules. EN is a dietary treatment for IBD patients that can either totally (with EN) or partially (with PEN) replace solid food intake with specialized formula[16]. Although the mechanism by which EN induces MH is currently unknown, microbiome changes are implicated and are hypothesized to aid in decreasing chronic inflammation[17]. While typically a pediatric treatment, a pilot study shows MH in adults following EN and PEN treatment as well[16]. Currently, the largest drawback of EN treatment is low patient compliance, especially in adults[16]. 5-ASA drugs, which are used to induce remission in early IBD, are shown to induce MH in 43.7% of patients[18]. Azathioprine is a purine analog inhibiting purine metabolism and blocking T cell activation and co-stimulation, therefore functioning by suppressing the immune system and decreasing inflammation in IBD[11,19-21]. It is shown to achieve MH independently in some cases (30.1%) alone but is more successful when used in conjunction with anti-TNF-α antibodies such as Infliximab (44%)[22]. Other immunomodulating drugs shown to induce MH when given with monoclonal antibodies include Cylosporine and Tacrolimus[23].

Table 1 Summary of inflammatory bowel disease therapeutics

Treatment type	Available therapeutics	Mucosal healing relevance/ Success
Corticosteroids	Prednisone/ Prednisolone/ Methylprednisone	Prednisone treatment for 14 d (20 mg/day) decreased mucosal inflammation indicating a possible role in developing short-term MH[139]. 29% of patients in one study displayed endoscopic remission after steroid treatment[140].
Nutritional therapy	Enteral nutrition (EN)/ Partial enternal nutrition (PEN)	EN/PEN induce MH in both adults and children[14].
Aminosalicylates (5-ASA)	Sulfasalazine/ Mesalamine/ Olsalazine/ Balsalazide	On average induce MH in 43.7% of patients[141].
Immunomodulators	Azathioprine/ 6-mercaptopurine	Azathioprine alone has achieved MH in 16.5% of cases and in 43.9% when used in combination with antibody therapies[18]. After 16 wk of mercaptopurine treatment, patients in remission showed a 47.1% rate of MH[142].
	Cyclosporine	Shown to induce MH when used in conjunction with Vendolizumab[143].
	Tacrolimus	Shown to induce MH when used in conjunction with Vendolizumab[143].
	Methotrexate	After 36 wk, methotrexate treatment had a MH rate of 47.4%[142].
Monoclonal antibody/ Biologic therapies	Adalimumab	Induced MH in 24% of patients treated[24].
	Certolizumab	Clinical response rate at weeks 2 and 12 was 29.7% and 52.8% (respectively) in CD[25].
	Infliximab	Treatment induced MH in up to 60.3% of patients in phase 2 clinical trials[23].
	Natalizumab	MH achieved by 42.3% of patients after 14.1 mo of treatment[144].
	Risankizumab-rzaa	Endoscopic response and deep remission observed in 55% and 29% of patients (respectively), indicating MH[27].
	Ustekinumab	Treatment of individuals with moderate to severe CD showed MH *via* a reduced disease score after 8 wk[19].
	Vedolizumab	Has shown to induce MH in up to 50% of UC patients and 29% of CD patients in clinical trials[26,27].

MH: Mucosal healing; EN: Enteral nutrition; PEN: Partial enteral nutrition; 5-ASA: Aminosalicylates; CD: Chron's Disease; UC: Ulcerative colitis.

Monoclonal Antibody Therapies

Monoclonal antibodies targeting inflammatory cytokines are an emerging class of IBD therapies that more directly focus on permitting or inducing MH and are an attractive method of IBD treatment. Monoclonal antibody drugs targeting multiple cytokines are approved or in trials, and function by removing inflammatory cytokines. Anti-tumor necrosis factor-alpha (TNF-α) antibody-based drugs such as Infliximab, Adalimumab, and Certolizumab are biologic therapies that have achieved significant clinical success and are now widely used as front-line treatments for IBD, with the goal of reducing inflammation and promoting MH. The target of anti-TNF-α therapies is a pro-inflammatory cytokine contributing to the chronic and severe inflammatory response observed in UC and CD, and the monoclonal antibody functions by downregulating pro-inflammatory molecules and restoring IEB integrity, allowing the body to begin to heal[8,24-26]. Infliximab is an Immunoglobulin G1 anti-TNF-α antibody that is shown to induce MH. Active phase 1 and 2 clinical trials of Infliximab in UC patients find that, respectively, 45.5% of patients and 60.3% of patients exhibit MH[27]. Adalimumab is another anti-TNF-α antibody presenting some indication of MH effects. In the double blind, randomized, placebo controlled clinical trial, 24% of patients display MH at week 52 compared to 0% in the placebo group[28]. Certolizumab pegol is an anti-TNF-α antibody fragment targeting soluble and trans-membrane TNF. Its role in promoting MH is not yet well-investigated, but it demonstrates symptom relief and remission at week 8[29]. In some patients, anti-TNF-α treatments are ineffective or lose efficacy over time. In these patients, biologics targeting other inflammatory cytokines are employed as IBD treatment options. For example, Ustekinumab is a monoclonal antibody that targets interleukin (IL)-12 and IL-23 pro-inflammatory cytokines. Ustekinumab significantly reduces SES-CD (Simplified Endoscopic Activity Score for Crohn's Disease) scores in patients over 44 wk, implying its ability to enhance MH[30]. Similarly, Risankizumab, an IL-23 antibody, induced remission in 45% of patients, with an endoscopic response rate of 29%[31]. Additionally, Natalizumab, an anti-α4-integrin antibody, induces MH in 42.3% of patients[32]. Vedolizumab, on the other hand, is an $\alpha_4\beta_7$ integrin receptor antagonist and biologic medication, with potential immunosuppressive effects localized to the colon. In a clinical trial, 50% of UC patients and 29% of CD patients display MH following long-term use[33,34].

Although inflammation reduction and monoclonal antibody treatments have greatly enhanced the quality of IBD treatment in recent years, lack or loss of response occurs with concerning frequency, and even in patients who respond well to treatments, complete resolution of the disease has not been

Table 2 Summary of molecular pathways involved in mucosal healing

Pathways/ Mechanism of action	Associated models studied	Ref.
EGFR signaling	*In vitro*, colorectal cancer mice, EGFR mutant mice	[43,116]
Hippo/YAP signaling	*In vitro*, YAP-1 transgenic mice	[36,59]
Notch signaling	Villin-Claudin-1 transgenic mice	[41,42]
Wnt/β-catenin signaling	*In vitro* and *In vivo models of injury/repair*	[44,60,61]
Vitamin D receptor (VDR) signaling	*In vitro*, VDR knockout mice	[45]
Src/focal adhesion kinase	*In vitro*, Mechanical colonic wound in mice, Nox1 and AnxA1 knockout mice, oral gavage in mice	[76-78]
Autophagy/ATG16L1	Patient biopsies; ATG16L1 T300A knock-in mice; Atg5-manipulated mice	[6,7,104]
SCFA-mediated signaling [acetate, propionate, butyrate, *etc.*]	*In vitro*, Patient biopsies, oral gavage in mice. T-cell induced colitis, trinitrobenzenesulphonic acid (TNBS) colitis	[83-85,91,93,100,101,114]
TLR-mediated signaling	DSS colitis	[109,110,112]
MyD88 mediated bacterial sensing	Mechanical colonic wound, MyD88 knockout mice	[111]
Prostaglandin-endoperoxidase synthase 2 enzyme (PGE2)	*In vitro*, mechanical colonic wound, Ptgs2 knockout mice, Ptger4 knockout mice	[111,112]
Mucin 2 signaling	*In vitro*, DSS colitis, EGFR mutant mice	[80,116]
IL-6/IL-22/IL-23/STAT3 signaling	DSS colitis, Th2-mediated colitis, cytokine deficient mice, bone marrow transplant mice, T-cell induced colitis, human and mouse intestinal organoid culture	[94,97,98,136-138]
TGF-β signaling	*In vitro*, DSS colitis, TGF-β transgenic mice	[50,130,131]
IL-10 signaling	*In vitro*, mechanical colonic wound in mice, IL-10-deficient mice	[132,133]

EGFR: Epidermal growth factor receptor; YAP: Yes-associated protein 1; ATG16L1: Autophagy related 16 like 1 protein; Atg5: Autophagy related 5; SCFA: Short chain fatty acid; TNBS: Trinitrobenzenesulphonic acid; TLR: Toll-like receptor; DSS: Dextran sodium sulfate; PGE2: Prostaglandin-endoperoxidase synthase 2 enzyme; IL: Interleukin; STAT3: Signal transducer and activator of transcription 3; TGF-β: Transforming growth factor-β.

achieved[10]. This is due to the complex nature of disease development through many aberrant proteins and signaling pathways, as well as the multi-faceted process of MH that must be approached from many angles to restore complete IEB homeostasis. Overall, while current therapies offer evidence of permitting MH in IBD, some of these therapies do not directly promote MH through enhancing the processes of restitution, proliferation, or differentiation, but rather by simply inducing immune suppression to decrease inflammation and associated injury. Subsequently, there is a critical need to better understand the processes of inflammation and MH in IBD to aid in the development of actively MH-inducing IBD therapies.

IBD TISSUE DAMAGE MECHANISMS AND IMPLICATIONS FOR MH INDUCTION

Gut Barrier Functions and Mucosal Healing

A major role of the intestinal epithelium is its function as a barrier against the luminal environment and antigens; a role that is critical in maintaining normal mammalian homeostasis[35]. Accordingly, IEB dysregulation is a major factor in gut inflammation; thus, reinforcement of IEB integrity is a key consideration when developing more effective treatments for gut inflammatory diseases, including IBD. The MH process has been shown to help reinforce barrier integrity[36]. A complex and dynamic coordination between epithelial and immune factors facilitates MH, however, the 'paradoxical' role of the barrier-integral proteins in promoting MH remains ill-understood. Here, we summarize the key components of IEB regulation to illustrate the dynamic causal relationship between MH and the proteins comprising the structural and functional units of the gut barrier.

The physical component of the IEB consists of a single layer of epithelium, with cells linked by junctional complexes along the apicobasal axis. Excellent reviews have described the types and roles of junctional complex proteins in barrier integrity, thus here we focus on mechanisms by which regulation of these proteins influences inflammation and MH[35-39]. Tight junctions, the most apical cell-cell adhesions are considered the "gate" of the IEB and consist of multiple proteins, including the Claudin family of proteins, Occludin, junctional adhesion molecule (JAM) and the zonula occludens (ZO)-proteins[40]. Studies in cell and mouse models demonstrate through genetic manipulation that tight
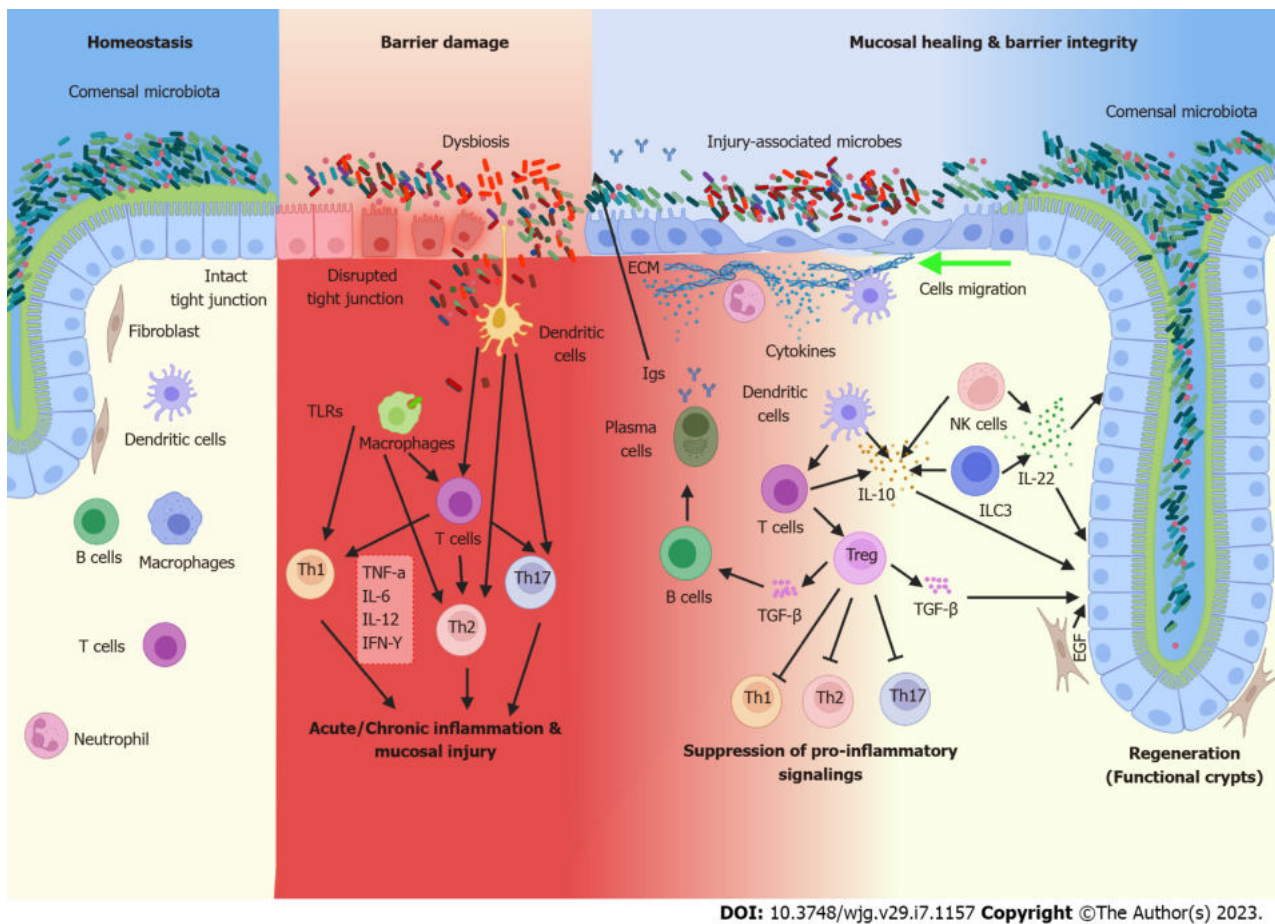
Figure 1 Pictorial depiction of inter-connection between the immune system, inflammation, and microbiota in mucosal inflammation, associated injury, and healing. Left: normal mucosal homeostasis; Middle: Inflammatory lesions damage the mucosal barrier between the gut lumen and the rest of the body. Barrier damage leads to immune cell activation, cytokine release, and feedback cycles of deteriorating inflammation driven by microbes crossing the damaged barrier; Right: Migration of circulating restitutive immune cells to the wound area, the release of repairing cytokines; crosstalk among extracellular matrix and epithelial cells for proliferation and migration; switching of microbiota and cytokines for mucosal healing and functional crypt regeneration.

junction proteins are not merely static structural entities of the IEB; they perform additional non-canonical roles of regulating epithelial cell proliferation, survival, differentiation, and migration, which are the same processes integral to MH. In this regard, ZO-1 regulates expression and nuclear localization of the transcription factor ZO-1-associated nucleic-acid-binding protein to influence cell proliferation in a density dependent manner[41,42]. Recent studies also show that the ZO-proteins modulate Hippo/Yes-associated protein 1 signaling, a critical regulator of crypt growth and MH[43]. A similar role of JAM in regulating intestinal epithelial cell (IEC) proliferation has been reported[44,45]. Occludin, on the other hand, is shown to regulate IEC apoptosis and survival[46,47]. Recent studies demonstrate that the Claudin family of proteins is integral to tight junction structure and function and plays a complex role in gut inflammation and regenerative processes. For example, Claudin-1 overexpression in the intestinal epithelium of mouse models of IBD induces significant dysregulation of Notch/Wnt signaling and severe colitis, resulting in delayed recovery from colitis-associated injury[48, 49]. Claudin-2 is unique among the Claudin proteins expressed in the intestine, as it is primarily expressed at the crypt base among proliferative undifferentiated cells and associates inversely with the differentiation state of IECs[48]. Of note, Claudin-2 is a direct target of Epidermal Growth Factor Receptor (EGFR), Wnt/β-catenin, and Vitamin D receptor signaling, all of which promote intestinal MH [50-52]. Claudin-3 on the other hand, which functions as a receptor for *Clostridium perfringens*, is sharply downregulated in the biopsies of the IBD patients, and loss of its function in the mouse gut promotes colitis-associated cancer[53-55]. We have also previously reported that Claudin-3 Loss enhances gp130/ IL-6/STAT3 (signal transducer and activator of transcription 3) signaling, which promotes colitis-associated injury/repair[50]. Interestingly, the loss of intestinal Claudin-7 expression results in spontaneous inflammation due to dysregulation of epithelial-extracellular matrix interactions[56,57]. Claudin-7 also regulates intestinal stem cell function in association with the Epithelial Cellular Adhesion Molecule protein[58,59]. Conversely, Claudin-15 Loss in the IEB results in a mega-intestine[60]. Hence, dysregulation of tight junction protein expression in the IEB results in IBD, indicating that restoration of tight junction homeostasis is a vital component of MH and a promising target for treatment

development.

Like tight junction component proteins, adherens junction proteins contribute significantly to gut inflammation and MH. Here, E-cadherin, a protein whose expression indicates an epithelial phenotype, contributes to inflammation-associated epithelial repair by regulating the epithelial-to-mesenchymal transition, a process associated with cell proliferation and migration[58,61,62]. Specifically, E-cadherin expression inhibits migration of IECs and wound healing[63]. Contrastingly, complete E-cadherin loss causes a severe inflammatory phenotype characterized by villus blunting, which is a marker of premature epithelial death, and incomplete brush border development[64]. The stabilization of E-cadherin expression is facilitated partly by binding with the cytoplasmic domain of p120-cadherin. Accordingly, p120 knockout mice displayed disrupted intestinal integrity and early death from intestinal injury[65]. Other heterodimeric adherens junction proteins, α- and β-catenin, are key players in the regulation of Hippo and Wnt signaling[66,67]. Although α- and β-catenin expression dictates IEC proliferation and differentiation during injury repair, their expression counter-acts each other. Moreover, ubiquitination of β-catenin by α-catenin aids the degradation of β-catenin, balancing the Wnt signaling pathway[68]. Taken together, these findings support a complex and dynamic interdependence between gut barrier regulation and MH, which should be considered for therapeutic potential.

Gut Dysbiosis, IBD, and Mucosal Healing

Aberrant microbiome-immune interactions can lead to improper immune activation and are potentially responsible for the clinical and endoscopic observations in IBD patients. Mechanisms of microbial involvement in IBD include production of short-chain fatty acids (SCFAs), interaction with autophagy pathways, activation of immune cells, TLR signaling, and prostaglandin pathways[69-73]. Excellent reviews have covered the details of such interactions and the dynamic association with gut inflammatory processes[71-75]. Thus, here we focus primarily on how gut microbiota may contribute to MH processes under normal and/or inflammatory conditions.

Gut dysbiosis is mediated by pathogenic microbes harboring genes encoding toxin proteins, which disrupt the IEB *via* disassembly or redistribution of tight junction proteins. For example, human epithelial cells treated with *Escherichia coli* or *Salmonella typhimurium* demonstrate downregulation of ZO-1 and Occludin proteins while by contrast, Claudin-2 is upregulated[76-78]. *Shigella flexneri* and *Campylobacter jejuni* are involved in deregulating E-cadherin, as well as activating IL-8 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thereby inducing barrier dysfunction and inflammation[79,80]. Overall, studies suggest that pathogenic gut bacteria promote chronic mucosal inflammation by dysregulating IEB integrity.

On the other hand, commensal gut bacteria seem to promote the initial stage of epithelial restitution, as studies in germ-free mice show impaired rates of epithelial cell migration, which is dependent on the formation of focal adhesions[81,82]. In this context, a commensal bacterium activates the focal adhesion kinase, thereby enhancing epithelial restitution and promoting mucosal wound repair in a redox-dependent manner[83-85]. In a mouse colonoscopy-based wound healing model, an abundance of anaerobic bacteria such as *Akkermansia spp* augmented early stages of MH[86]. The selection of mucin-producing bacteria from the mucin layer also helps close mucosal wounds[87,88]. These microbes help generate SCFAs such as acetate, propionate, and butyrate, which are considered the primary energy sources of gut colonocytes and are therefore critical supporters of IEB restoration and integrity following tissue damage[89-92]. The major producers of SCFAs include the genus *Bacteroides*, *Clostridium* clusters IV and XIVa, and *Bifidobacterium*, though they use diverse mechanisms to achieve homeostatic outcomes[89,93,94]. For example, *Bacteroides ovatus* decreases lipopolysaccharide-induced inflammation and produces indole-3-acetic acid that likely promotes IL-22 production by immune cells, yielding beneficial effects in epithelial regeneration[95,96]. SCFAs produced by fiber-fermenting commensal microbes are also linked to upregulation of Foxp3+ T regulatory (Tregs) cell development, which have a widely documented role in protection against epithelial injury and colitis [97]. Inhibition of histone deacetylases and/or activation of the latent form of transforming growth factor-β (TGF-β) to act as a potent inducer of Tregs are potential mechanisms of SCFAs[98,99]. SCFAs also mediate activation of STAT3 which plays a vital role in mucosal homeostasis[100,101]. *Clostridia*-related segmented filamentous bacteria promote IL-23 production by antigen-presenting cells, which activate type 3 innate lymphoid cells (ILCs) to initiate an IL-23R/IL-22/STAT3 loop, thereby producing serum amyloid A which promotes IL-17 production by Th17 cells[102-105]. The importance of the SCFA propionate is the augmentation of dendritic cell and macrophage hematopoiesis precursors that impact intestinal immunity to control the growth of invading mucosal pathogens[106,107]. A breach in this regulation is a central mechanism in triggering, maintaining, and exacerbating IBD. Supplementation with another important SCFA, butyrate, rescues deficiencies in mitochondrial respiration and increases autophagy in the colonocytes of germ-free mice compared to conventionally raised mice[108]. Due to the critical role in repair of gut dysbiosis, the regulation of specific gut bacteria and SCFAs may therefore possess significant potential for clinical treatment of IBD.

As introduced above, accumulating evidence suggests a causal interdependence between autophagic flux in the intestinal epithelium and gut microbiota colonization. In this regard, CD patients who are homozygous for the ATG16L1[T300A] gene variant exhibit higher abundance of Enterobacteriaceae, Bacteroidaceae and Fusobacteriaceae in the inflamed ileum compared with patients homozygous for the wild

type ATG16L1 allele[6]. Similar findings have been obtained from mice where expression of the autophagy gene Atg7 is genetically knocked out in the gut epithelium, as these mice display altered microbial composition with enrichment in *Clostridium septum*, *Eubacterium cylindroides*, and *Bacteroides fragilis* compared to wild type mice[109]. ATG16L1[T300A] variant mice also show changes in fecal microbiota composition compared to wild type mice, displaying an increase in the order Bacteroidales, which is associated with increased Th17 and Th1 cells in the colon and ileum lamina propria without the development of intestinal inflammation[7]. However, Atg5 deficient mice display reduced bacterial diversity, as observed in IBD patients, and contain a low number of the Lachnospiraceae, Ruminococcaceae, and Akkermansia families that control inflammatory responses[110]. Of note, a role of autophagy in regulating intestinal stem cell function and mucosal injury/repair has been demonstrated by several recent studies[111,112]. Taken together, these studies highlight a complex causal integration between host cell autophagy processes and intestinal microbial communities in regulating intestinal homeostasis and injury/repair.

Additionally, infiltrating immune cells such as macrophages and neutrophils responding to gut dysbiosis comprise essential components of intestinal wound healing by altering aberrant physiological parameters of the local microenvironment, such as microbe-associated molecular patterns (MAMPs) and decreased oxygen levels from the formation of reactive oxygen species[86,113-115]. Of note, Toll-like receptors (TLRs) expressed on multiple immune cell lineages induce signaling pathways upon binding by MAMPs and improve outcomes in experimental mouse colitis models *via* the promotion of wound healing[116,117]. Specific microbes in proximity to the wound bed also activate host epithelial proliferative signaling through a formyl peptide receptor pathway[83,84]. Studies employing a mechanical colonic wound method further disclose a protective role for prostaglandin E2 (PGE2) in re-establishment of the IEB through a TLR2/MyD88-dependent manner[118,119]. A follow-up study from the same group shows that in the early repair phase, a TLR2/PGE2 axis is required for barrier establishment; however, PGE2 must then decrease to allow for epithelial proliferation and regeneration [118]. In this context, Jain *et al*[120] elegantly demonstrates that temporal regulation of the bacterial metabolite PGE2/deoxycholate during colonic repair is critical for crypt regeneration[120]. The highly specific and time-dependent switching of microbial colonization and signaling pathways can therefore act to promote MH in a localized manner.

Several therapeutic approaches have been examined by administration of prebiotics or probiotics to regulate the microbiota. For example, butyrate enemas are effective in treating experimental colitis and UC patients[121,122]. Also, p40, a protein produced by *Lactobacillus rhamnosus GG* (LGG), activates host epithelial EGFR signaling and mediates wound healing[123]. Of note, the mechanism of MH promotion by LGG is *via* a positive effect on epithelial barrier maturation by upregulation of Claudin-3[124]. Recently, genetically modified probiotic bacteria–based precision delivery of human EGF also appears to be a promising intervention against mucosal inflammation through crypt-derived MH and barrier restoration[125,126]. Firmicutes, such as *Faecalibacterium prausnitzii* play an essential role in mucosal barrier homeostasis by regulating NF-κB activation and IL-8 production[125,126]. In another study, oral gavage with *Faecalibacterium prausnitzii* during dextran sodium sulfate (DSS) colitis improves outcomes compared to mice treated with DSS alone, likely due to participation of Claudin-1 and Claudin-2[125]. The probiotic mixture known as VSL #3, containing 4 strains of *Lactobacilli*, 3 strains of *Bifidobacteria*, and one strain of *Streptococcus* is effective in preventing pouchitis and in treating UC flareups[127]. This probiotic functions by partially upregulating mucin production and restoring the IEB by stimulating ZO-1 and Occludin expression while decreasing Claudin-2[127]. Taken together, a complex interdependence exists between gut microbiota and MH processes in the promotion of barrier integrity that should be fully explored for its intriguing potential in improving clinical outcomes.

Inflammatory Cytokines in IBD and Mucosal Healing

Due to dysregulation of many cytokines and growth factors in IBD and the regulatory importance of many of these same molecules in MH, we discuss the potential of altering immune signaling and inflammatory cascades in restoring proper intestinal homeostatic balance. Increasing knowledge of the coordination of these pathways will contribute to the development of more effective and targeted therapies to ameliorate disease while preserving essential immune system function.

Major cytokines and growth factors that are considered pro-inflammatory in IBD include IL-1β, interferon-gamma (IFN-γ), TNF-α, and IL-6. During an infection in the gut, IL-1β, TNF-α, and IFN-γ are shown to be produced by inflammatory monocytes, among which IL-1β and TNF-α are associated with increased IEB permeability[2]. TNF-α and IFN-γ are also produced by ILCs and serve to recruit and activate additional inflammatory cells[3]. TNF-α is particularly well-studied and often targeted in the treatment of autoimmune diseases as detailed in our discussion of monoclonal antibody therapies above. Although necessary for innate immune responses against acute pathogens and acute DSS colitis, when produced chronically by T cells TNF-α can be a major contributing factor to the loss of epithelial barrier and development of autoimmune disease[128-130]. IFN-γ has been shown to be regulated during wound healing of skin epithelium by Tregs, where lack of Tregs resulted in increased IFN-γ, accumulation of pro-inflammatory macrophages, and hindered wound healing[131]. Gut microbiota can also impact immune system activation through cytokine signaling. Kuhn *et al*.[132] demonstrates that intraepithelial lymphocytes (IELs) must interact with commensal Bacteroidales order microbes to produce IL-

6 in response to acute *C. rodentium* colitis infection, aiding in repair of the IEB *via* increased Claudin-1 expression[132]. Despite the necessary effects of pro-inflammatory cytokines for immune system response and homeostasis, each also possesses drawbacks. When the location, amount, or duration of cytokine production becomes dysregulated, chronic inflammation and disease can result. IFN-γ, TNF-α, and IL-6 are upregulated after secretion by immune cells in the chronic inflammatory state associated with IBD[3,130,132]. These cytokines are known to increase gut permeability by altering tight junction protein expression[132-134]. In particular, increased IL-6 in the IECs and lamina propria mononuclear cells increases Claudin-2, which promotes intestinal permeability and is known to be upregulated in IBD[134,135]. Interestingly, it is IELs that produce IL-6 in a protective manner during acute infection through the c-Jun N-terminal Kinase pathway, rather than the Mitogen-activated protein kinase/extracellular signal-regulated kinase pathway, suggesting both duration and cell type-specific layers of complexity in the role of IL-6[132,134]. It is likely that similar multi-layer and highly context-specific pathways exist for other inflammatory cytokines as well. Therein lies the challenge of harnessing pro-inflammatory cytokines and signaling pathways in the immune system for MH: Targeting modifications toward the decrease of detrimental chronic effects without impairing their beneficial and homeostatic functions. Continuing in the discussion of IL-6, global reduction of IL-6 expression can decrease chronic inflammation in IBD and enhance MH, but may also increase susceptibility to infection[132,136]. It would therefore be ideal to develop a method of reducing IL-6 production in IECs while leaving expression in IELs intact to avoid increasing the risk of dangerous infections in patients. The potential in fine-tuning inflammatory cytokine expression to promote MH is great, but much work remains to ensure it can be accomplished safely without severe detrimental effects to other functions of the immune system.

Conversely, several other cytokines, growth factors, and cell types function primarily in an anti-inflammatory role in IBD and intestinal homeostasis and offer additional mechanisms to enhance MH by resolving chronic inflammation. Growth factors and cytokines considered to be anti-inflammatory include TGF-β, IL-10, IL-22, and IL-17. TGF-β is a well-studied growth factor that has been demonstrated by Beck *et al*[137]. to play a role in the restitution, or IEC migration, phase of MH, evidenced by the lack of IEC migration and impaired wound healing after TGF-β inhibition in DSS colitis[8,137]. Additionally, TGF-β when produced by a macrophage secretome called SuperMApo aids in removal of apoptotic cells and resolution of inflammation in IBD models[57,138]. Importantly, since this secretome produces TGF-β from a singular cell type, rather than globally, its administration can provide the context- and location-dependent production of beneficial TGF-β while avoiding potential opposing or off-target effects. Macrophages and Th2 cells also produce IL-10, which enhances the proliferation phase of MH, maintains immune tolerance to the many antigens encountered by the IEB, and promotes barrier integrity[139,140]. Similarly, the production of IL-17 by anti-inflammatory Tregs helps with IEC proliferation and blockage of detrimental microbiota in colitis models, providing protection against IBD[141]. One of the major players in protection against IBD is IL-22, which is produced by multiple cell types and promotes MH by more than one mechanism, as reviewed in[142]. Most importantly in IBD, IL-22 is produced by ILCs, CD4+ T cells, and NK cells, demonstrating activation of both the innate and adaptive immune system[143,144]. IL-22 primarily acts on intestinal stem cells (ISCs) and IECs and functions by activating STAT3 signaling, which induces IEC proliferation and therefore MH[101,142,143,145]. Regarding ISCs, IL-22 both protects them from depletion during intestinal inflammation and induces regeneration[101,143,145]. Though some of these cytokines and growth factors can be inflammatory under certain circumstances, in the context of IBD they function in an anti-inflammatory manner and are beneficial in promoting MH. Some cytokines even demonstrate potential to be produced or administered selectively in only beneficial locations or cell types. Therefore, anti-inflammatory cytokines and growth factors present promising options for treatment of IBD through selectively reducing inflammatory signals and inducing or enhancing the MH process.

CONCLUSION

In recent years, IBD has become increasingly common in the United States and abroad. As progressive and debilitating diseases, UC and CD have long-term negative implications on health. In the past, treatments have focused on reducing the clinical manifestations of the disease, often leaving underlying disease mechanisms active in the gut[12,15]. With increased understanding of the mechanisms and complex pathogenesis of IBD, further innovation in treatment approaches must occur to improve patients' long-term outcomes. Subsequently, instead of merely hoping to reduce symptoms, doctors now desire therapies that actively aid in healing and regeneration of damaged tissue. MH has therefore emerged as the main goal for research and treatment end points to ensure long-term remission, survival, and a good quality of life for patients. Going forward, understanding the interactions regulating the breakdown and regeneration of the IEB, as well as overarching gut homeostasis processes, will be paramount to treating and curing patients with IBD. Monoclonal antibody therapies offer a promising start to revolutionizing treatment, aiming not only to reduce clinical manifestations, but also to interrupt disease activity on a cellular and molecular level. However, even newly developed antibody therapies

cannot by themselves completely resolve IBD and restore total gut homeostasis. Therefore, the three approaches to targeting the molecular machinery governing IBD of restoring the IEB, regulating the gut microbiota, and altering the cytokine signaling-mediated immune response are all being studied as potential mechanisms for achieving MH. Optimal future treatment protocols for IBD will ideally include a combination of these approaches, with the intent of restoring intestinal homeostasis by balancing expression of multiple proteins and repairing several of the many dysregulated pathways involved in IBD pathogenesis.

FOOTNOTES

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