

Resolution of Inflammation and Gut Repair in IBD: Translational Steps Towards Complete Mucosal Healing

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Despite significant recent therapeutic advances, complete mucosal healing remains a difficult treatment target for many patients with inflammatory bowel diseases (IBD) to achieve. Our review focuses on the translational concept of promoting resolution of inflammation and repair as a necessary adjunctive step to reach this goal. We explore the roles of inflammatory cell apoptosis and efferocytosis to promote resolution, the new knowledge of gut monocyte-macrophage populations and their secreted prorepair mediators, and the processes of gut epithelial repair and regeneration to bridge this gap. We discuss the need and rationale for this vision and the tangible steps toward integrating proresolution therapies in IBD.

Key Words: inflammatory bowel disease, Crohn, ulcerative colitis, mucosal healing, inflammation

BACKGROUND

Inflammation is a protective host response to “danger”.¹⁻³ Once the threat or insult, usually infection or trauma, is neutralized, a coordinated and active process of resolution and repair begins to restore tissue integrity and function.^{4,5} Ulcerative colitis (UC) and Crohn disease (CD) are characterized by immune-mediated nonresolving chronic inflammation of the gastrointestinal tract, which if untreated will progress toward the natural complications of uncontrolled inflammation, namely the development of fibrosis (strictures in CD; “hose-pipe” colon in UC) or organ damage and subsequent organ failure (abscess and fistula formation in CD; toxic megacolon in UC). Many factors (host genetics, the complex gut tissue environment, microbial dysbiosis, impaired gut barrier function, and dysregulated innate/adaptive immune system) drive

the pathogenic immune response and underlie the emphatic failure to resolve gut inflammation in IBD.⁶⁻⁸

From general anti-inflammatory and immunosuppressive therapies using 5-aminosalicylates, corticosteroids, and thiopurines in the pre-2000s, we have moved toward biologic therapies that enable specific targeting of proinflammatory mediators such as tumor necrosis factor (TNF) and interleukin (IL)-12/23p40 and toward integrins to reduce inflammatory cell migration. More recently, a new family of small molecules to block Janus kinase signaling, which regulates multiple inflammatory pathways,^{9,10} have also become available in clinics. These therapies have undoubtedly been successful, and many more new drugs are on the horizon.¹¹

Despite these promising advances, there is a consistent “therapeutic ceiling”¹²⁻¹⁷ to the ability of such approaches to bring about complete mucosal healing—the total resolution and healing of ulcerations and a full return to healthy gut mucosa. Complete mucosal healing is the most coveted treatment target with the best long-term implication in prognosis.¹⁸ It is particularly noteworthy that this is only achieved in about 50% of patients with moderate to severe IBD, despite intensive medical therapy.^{13,19,20} The CALM study in CD, for example, showed that even with early aggressive medical therapy using anti-TNF and azathioprine and guided by biomarkers (fecal calprotectin [S100a8/9] and C-reactive protein), endoscopic mucosal healing was seen in less than 50% of patients after 48 weeks of treatment.¹³ In the conventionally managed group from the same study, this rate was even lower at 30%. Three recent keynote clinical trials in UC from 2017 to 2019, the VARSITY,¹⁴ UNIFI,²¹ and OCTAVE²² studies, showed endoscopic mucosal improvements of 39.7% vs 27.7% (vedolizumab vs adalimumab), 51.1% vs 28.6% (ustekinumab vs placebo), and 54.7% vs 13.1% (tofacitinib vs placebo), respectively, after almost a year of continuous treatment. These studies highlight

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the present-day situation and the limitations of available medical treatments.

Because the key driving factors that initiate and perpetuate IBD mucosal inflammation are not fully known, the dominant drug development model so far has been based on the principle of long-term continuous inhibition of the abnormal immune response in IBD.²³ Whether such an approach, by further intensification (eg, combining different biologics) or stratification (eg, using biomarkers to select treatment), can provide the game-changing improvement is questionable. In this context, our review focuses on the therapeutic concept of promoting inflammation resolution as the extra factor required to overcome the therapeutic ceiling phenomenon. In essence, we posit a simple dual-approach model where **complete mucosal healing = anti-inflammatory + proresolution therapy**. We discuss this with a particular focus on key immune cells, such as monocytes, macrophages, and neutrophils, and the importance of dismantling the inflammatory mucosal environment as the prerequisite to mucosal healing. Despite a developing mature field in the resolution of inflammation, there are no present treatments that are wholly focused on this resolution in IBD, or indeed in any other major chronic inflammatory conditions. Here we discuss the tangible routes toward translation in IBD.

INFLAMMATION: FROM INITIATION TO RESOLUTION

In general, the immediate inflammatory response is mediated by receptors of the innate immune system, such as toll-like receptors (TLRs) and nucleotide-binding oligomerization-domain protein–like receptors.²⁴ In such settings, tissue-resident immune cells contribute by producing inflammatory mediators,

including cytokines such as chemokines, eicosanoids, and products of proteolytic cascades. The main effect of these mediators is to elicit an inflammatory environment: plasma proteins and leukocytes (mainly neutrophils) that are normally restricted to the blood vessels gain access. The activated endothelium allows recruitment and selective extravasation of neutrophils into the tissues. Here they become activated and exert their effector functions by releasing the contents of their granules, potent proteases, and oxidants that are damaging in an indiscriminate manner^{25,26} (Fig. 1).

A successful acute inflammatory response results in the elimination of the harmful stimuli (eg, infectious organisms such as bacteria, fungi, parasites, and viruses) followed by a resolution and repair phase, which is thought to be mediated mainly by tissue-resident and recruited macrophages.²⁷ Neutrophils undergo apoptosis, a process that promotes several proresolution pathways, particularly when paired with their uptake via phagocytosis by macrophages (also known as efferocytosis). This leads to neutrophil clearance and further release of anti-inflammatory and reparative cytokines and mediators.²⁸ Macrophages play a further role to dampen inflammation and initiate wound repair by clearing debris and producing growth factors and mediators that provide trophic support to the tissue environment²⁹ (Fig. 2).

MUCOSAL INFLAMMATION LANDSCAPE IN IBD

Although these steps from inflammation initiation to resolution are reasonably well defined in infection or tissue injury, they are poorly understood in the context of IBD—in particular, regarding the timeline of key immunological events that shape the complex IBD mucosal milieu.^{30,31} A persistent innate inflammatory

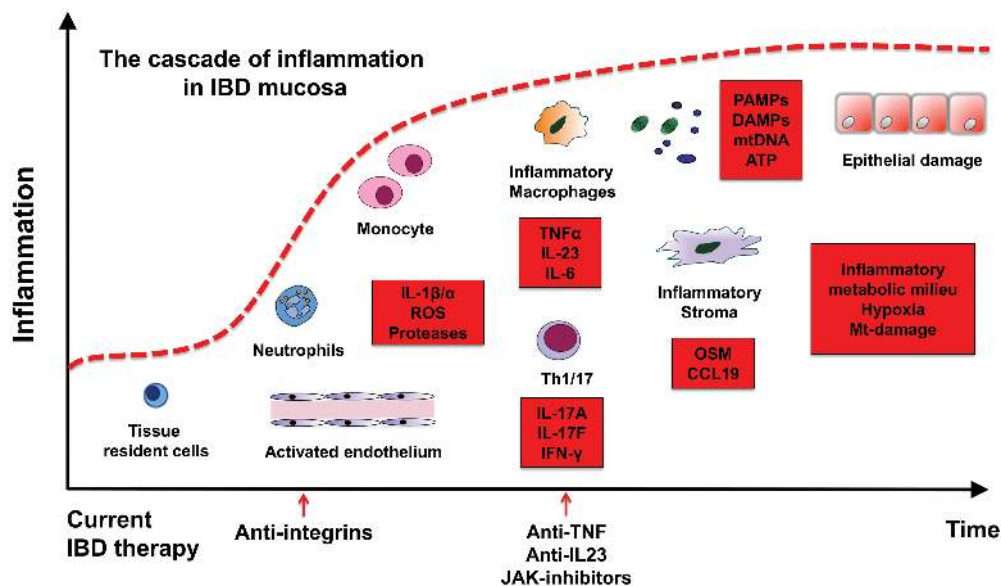


FIGURE 1. From initiation to the development of the chronic nonresolving inflammatory milieu in the IBD gut mucosa. Main biologic treatments such as anti-integrins, anti-TNF, IL-23p40, and Janus kinase inhibitors block single factors to reduce inflammation. Potentiating factors in the red box. ATP indicates adenosine triphosphate; mtDNA, mitochondrial DNA.

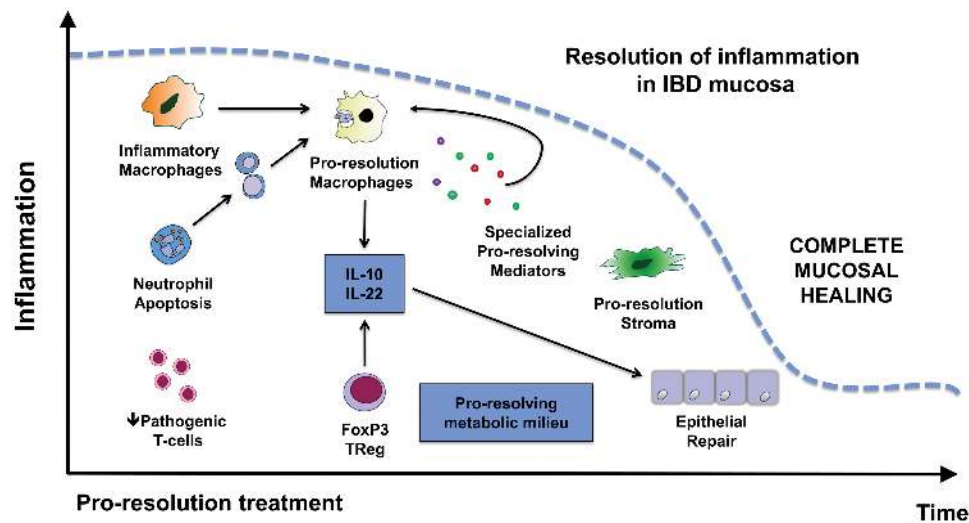


FIGURE 2. Promoting resolution of inflammation in IBD. Key events and pathways that can be targeted to promote and accelerate resolution and repair following IBD treatment.

process acquires new pathogenic characteristics. Excessive production of IL-1, IL-6, IL12/23, and TNF α result in polarizing cytokine conditions that drive distinct abnormal T-cell responses⁷ and subsequent uncontrolled tissue remodeling, such as fibrosis.³² Recent advances in single-cell technology are now providing novel insights into the IBD inflammatory landscape.³³⁻³⁶ Three recent studies have presented comprehensive high-resolution cell-type mapping of the inflamed mucosal milieu in UC and CD using a single-cell RNA-sequencing approach (scRNAseq).^{33, 35, 36} Of interest, they showed a few findings with common themes for UC and CD relevant to this review.

First, this mucosal milieu is unsurprisingly complex. By harnessing the power of single-cell analysis and rather than focusing on discrete cells or mediators, researchers have identified “modules” of proinflammatory cells that are bound by transcriptional functional programs. For instance, Martin et al³⁶ described a module comprising inflammatory macrophages, activated dendritic cells, T-cells, and stromal cells with highly correlated transcriptional profiles occupying the inflamed ileum affected by CD, which they termed the GIMAT module (IgG plasma cells, inflammatory mononuclear phagocytes, and activated T- and stromal cells). In UC, such a complex modular inflammatory network is also present. By using receptor-ligand pair analyses to construct cell-cell interaction network, Smillie and colleagues³⁵ showed that the most dominant modules in UC are also focused around inflammatory macrophages and stromal cell populations. These studies extend previous findings using more classical approaches to show the accumulation of phenotypically distinct monocytes/macrophages in both CD and UC.³⁷⁻³⁹

Second, inflammatory macrophages and stromal cells that dominate the IBD mucosal milieu have high expressions of the proinflammatory cytokines oncostatin M (OSM), IL-23, IL-6, IL-1 β , IL-1 α , and TNF.³⁶ In particular, stromal cells appear to form part of a positive feedback loop to maintain an

inflammatory environment through the expression of monocyte [chemokine (C-C motif) ligand] CCL2/CCL7 and neutrophil [chemokine (C-X-C motif) ligand] CXCL2, CXCL3, and CXCL8 chemoattractants. Earlier genomic-expression analysis showed significant enrichment of IBD-associated genetic variants to be associated with these immune processes, and in particular the function of macrophages.⁴⁰⁻⁴² These included *GPR65*, an IBD-risk gene shown to inhibit proinflammatory cytokine production in macrophages;⁴³ *GBP5*, which promotes NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome activation;⁴⁴ and *MAFB*, a transcription factor controlling macrophage differentiation and self-renewal.⁴⁵

Third, 2 scRNA studies consistently showed that enrichment of such cell types or their mediator profile (high GIMAT and OSM-OSM receptor expressions, respectively) are associated with resistance to anti-TNF treatment in UC and CD.^{33, 36} In essence, such a complex modular system underlies the treatment-resistant inflammatory gut landscape. The role for OSM was shown in an earlier study by West et al⁴⁶ showing that high stromal OSM expression predicted anti-TNF failure in UC. In an independent cohort of 441 patients, the GIMAT module was also associated with anti-TNF nonresponse. Taken together, these studies demonstrate that dysregulated macrophage and stromal cell activity are prominent features of the IBD mucosal milieu and represent a roadblock to complete mucosal healing and restoration of homeostasis.

PROMOTING RESOLUTION OF INFLAMMATION AND REPAIR AS A THERAPEUTIC CONCEPT

The challenge is to develop effective strategies that will first disrupt the self-perpetuating environment that sustains immune cell activation and initiate and then accelerate the process of inflammation resolution to achieve complete mucosal

healing (Box 1). The concept of proactive reparative immunology has been covered recently.⁴⁷ Here we align some of these ideas with IBD-specific pathogenic factors and discuss the translational opportunities that can be developed in conjunction with this goal.

BOX 1. RESOLUTION OF INFLAMMATION AS A THERAPEUTIC CONCEPT.

1. Disrupt the mucosal environment that sustains the activation of innate immune cells:
 - a. Interrupt key signaling network in inflammatory modules in the IBD mucosal milieu.
 - b. Manipulate inflammatory and/or epigenetic cues that shape the differentiation of inflammatory monocytes/macrophages—for example, metabolism, hypoxia, reactive oxygen species, short-chain fatty acids.
 - c. Block the signaling and recruitment of key inflammatory cells to the inflamed gut, such as proinflammatory monocyte-derived macrophages.
2. Initiate and accelerate the process of inflammation resolution:
 - a. Promote neutrophil apoptosis such as cyclin-dependent kinase (CDK)-inhibitor drugs.
 - b. Promote macrophage efferocytosis and its proresolution macrophage phenotype.
 - c. Directly harness proresolution macrophage products such as resolvins, protectins, and maresins.
3. Promote a state that allows deep repair and complete mucosal healing:
 - a. Encourage epithelial repair and regeneration such as IL-22.
 - b. Tackle the inflammatory signals from the stroma that mediate resistance to current biologic therapy in IBD such as OSM and CCL19.

Neutrophils in the Resolution of Inflammation

Neutrophils constitute 60% to 70% of circulating leukocytes in human blood. These short-lived “first responder” cells are recruited in abundance in IBD mucosa (particularly in UC) and are equipped with an arsenal of proteases and oxidants to execute host defense duties during the onset of inflammation.^{48, 49} Although much prevailing data are focused on the role of neutrophils in acute inflammation, they have an increasingly recognized contribution to chronic inflammation.⁵⁰ Neutrophils deposit a trail of granule proteins such as α -defensins and CXCL12 that recruit monocytes to inflammatory sites;⁵¹ cathelicidins that are present in neutrophils (LL-37 in humans; cathelicidin-related antimicrobial peptide (CRAMP) in mice) and promote the adhesion of monocytes via formylated peptide receptor 2 (FPR2)⁵²; and neutrophil alarmins such as S100a8/9, mitochondrial DNA, and high-mobility group box-1 that all enhance the inflammatory function of macrophages.⁵³⁻⁵⁵ The IBD mucosa is associated with prolonged neutrophil survival⁵⁶ and possibly proinflammatory neutrophil cell death such as (neutrophil

extracellular trap) NETosis in UC.^{57, 58} However, in CD, a defect in acute inflammation has been suggested.⁵⁹ It is purported that neutrophils fail to migrate to the inflammatory site, resulting in impaired bacterial clearance, which then sustains a chronic inflammatory response.

The mechanism of neutrophil death is important to the resolution process.⁶⁰ Neutrophil death via apoptosis, a process of programmed cell death, prevents the release of its toxic contents and is the first step to turning off inflammation. Apoptotic neutrophils are taken up by macrophages (efferocytosis), initiating a feed-forward proresolution program that is characterized by the release of tissue-repairing cytokines, such as transforming growth factor- β (TGF β) and IL-10, that counteract proinflammatory pathways.^{27, 28} Uptake of apoptotic neutrophils by macrophages also suppresses the transcription of *IL23*, which encodes for the IL-23 protein, a key IBD proinflammatory cytokine.⁶¹ This central tenet provides the platform for therapeutic intervention. Neutrophil apoptosis can be induced pharmacologically (eg, using cyclin-dependent kinase inhibitors such as R-roscovitine, tanshinone IIA, and ectoine).⁶²⁻⁶⁵ Of interest, tanshinone IIA, a Chinese medicinal herb identified from a large compound screen, was shown to potently stimulate egress of neutrophils from sites of inflammation in a zebrafish injury model.⁶⁴ Anti-oxidants, such as N-acetylcysteine, also promote apoptotic cell clearance by inhibiting Ras homolog family member A (RhoA) and reactive oxygen species production.⁶⁶ These drugs have the therapeutic potential to accelerate resolution of inflammation.⁶⁷

Whereas neutrophils drive inflammation at one end of the spectrum, they are also important in tissue repair, and specific aspects of this built-in biological response can be exploited.⁶⁸ Of the approximately 300 proteins contained within neutrophils, some have properties that are important in repair.⁶⁹ One such protein, annexin A1 (ANXA1), is released by dying neutrophils, where it interacts with FPR2 to attenuate chemokine-triggered activation of integrins, thereby reducing further inflammatory cell recruitment.⁷⁰ The ANXA1/FPR2 interaction also promotes macrophage efferocytosis.^{71, 72} Lipoxin A4, a proresolving lipid mediator released by the neutrophils, is a major “stop signal” for neutrophil migration.⁷³ Of interest, the production of lipoxin A4 is reduced in IBD,⁷⁴ and its administration is beneficial in a hapten-induced mouse colitis model.⁷⁵ Alpha-defensins are also released from neutrophils, with a functional effect of increasing the phagocytic capacity of macrophages and dampening their release of inflammatory mediators.⁷⁶ These proresolution angles coupled with approaches to inhibit neutrophil-mediated chronic inflammatory functions (eg, inhibiting the neutrophil alarmins and NETosis) offer a rich realm of targets for IBD treatment.

Inflammatory Monocyte Recruitment

In health, intestinal macrophages are relatively anti-inflammatory and hyporesponsive to microbial stimuli, an adaptation that allows them to exist in an antigen- and microbe-rich

environment. They are vital for the maintenance of intestinal homeostasis through the removal of apoptotic and senescent cells and the production of regulatory cytokines that also limit collateral damage associated with excessive inflammation.⁷⁷

⁷⁸ Like macrophages in other tissues, those in the gut wall are epigenetically shaped by local environmental cues.^{77, 79} In IBD there are marked changes to the macrophage compartment resulting from increased immigration of classical (CD14^{hi}) monocytes, leading to the accumulation of proinflammatory CD11c^{hi} monocytes/macrophages in the inflamed colon.^{37, 38, 80, 81} In CD, paired blood and gut scRNAseq analysis showed that increased gut inflammatory macrophages were associated with a depletion of circulating monocytes in patients enriched with the GIMAT module.³⁶

Targeting leukocyte—and specifically monocyte—recruitment has been widely considered in IBD.⁸² Research has reported that CCR2 is the essential chemokine receptor that mediates the entry of monocytes into the circulation and subsequent recruitment into the site of the inflamed gut.^{83, 84} Genetic ablation and antibody-mediated blockade of CCR2 is protective against mouse experimental colitis.⁸⁴⁻⁸⁶ Potential therapies could inhibit the recruitment of proinflammatory monocytes and their inflammatory products, but a few complexities are evident. For instance, it is not clear whether monocyte blockade would have a collateral effect on the maintenance of the resident macrophage pool, which also relies on CCR2-dependent monocytes, or if distinct monocyte subsets are recruited to healthy vs inflamed/repairing tissue. Moreover, the fate of monocytes in the IBD mucosa remains poorly understood, and it is plausible that although initially proinflammatory, monocytes may be conditioned by the local gut environment to become proresolving macrophages over time. In this scenario, monocyte blockade may prove counterproductive.

Anti- α 4 β 7 integrin vedolizumab, a current IBD biologic treatment, is thought to exert its effect by inhibiting the adhesion of α 4 β 7-expressing lymphocytes to gut mucosal vascular addressin cell adhesion molecule 1.^{87, 88} A recent study showed that vedolizumab also blocks gut homing for α 4 β 7-expressing monocytes.⁸⁹ Of particular interest, vedolizumab was shown to affect the recruitment of nonclassical (CD14⁺CD16⁺⁺) monocytes, which preferentially develop into wound-healing macrophages. Vedolizumab resulted in poor gut wound healing in vivo,⁸⁹ which has a potential clinical impact, such as healing following IBD surgery.⁹⁰ Moreover, a recent study examining monocyte heterogeneity in mice described the presence of prorepair monocytes during the recovery phase of dextran sulfate sodium (DSS)-induced colitis that were marked by their high expression of *Ym-1*.⁹¹ In a further study, *CCR2*-deletion in a mouse model of surgery delayed recovery from inflammation and postoperative ileus,⁹² again supporting a proresolution role for these cells. Hence, targeting monocytes may be more complicated than first thought. Cellular lineage tracing in experimental models may

provide further information regarding monocyte heterogeneity and temporal recruitment mechanism(s). Timing of blocking monocyte recruitment is potentially critical in developing this approach in the clinic.

Macrophage Efferocytosis of Apoptotic Cells

Macrophages are incredibly plastic and adapt in response to signals received from their immediate microenvironment. This biological feature provides an angle for potential intervention.⁹³ Although historically considered as either proinflammatory or anti-inflammatory, macrophages also exhibit profound healing and antifibrotic, proresolving, and tissue-regenerating characteristics.²⁹ A key event in the programming of macrophages begins following the uptake of apoptotic cells, which reduces the expression of proinflammatory cytokines and chemokines from macrophages⁹⁴ while promoting production of the immunoregulatory cytokines TGF- β and IL-10.^{27, 28} Macrophage efferocytosis also results in activation of peroxisome proliferator-activated receptor- γ and liver X-receptor, which enhance the expression of the phagocytic receptors CD36 and Mer proto-oncogene tyrosine kinase,^{95, 96} further enhancing the potential to clear dying cells. Moreover, major transcriptomic changes occur in intestinal macrophages following the uptake of apoptotic intestinal epithelial cells, including the downregulation of inflammatory genes (several of these are IBD susceptibility genes: *Lsp1*, *Mrpl20*, *S100a10/11*, and *Sept1*), pattern recognition receptors (*Clec4a*, *Clec4b1*, *Cd209a*, and *Tlr2*), and inflammatory leukotriene biosynthesis (*Alox5ap*).⁹⁷ Such data provide the framework to understand how macrophages are programmed toward a specific beneficial phenotype.

Trained Macrophage Immunity

Immunological memory is thought to be a defining feature of the adaptive immune system. However, recent work has shown that myeloid cells of the innate immune system may be able to “remember” the stimuli they encounter by undergoing functional, metabolic, and epigenetic reprogramming, which facilitates an altered response upon restimulation—a phenomenon that has become known as trained immunity.⁹⁸ This response further opens an additional translational avenue that involves suppressing or enhancing the trained proinflammatory and prorepair macrophage functions, respectively.⁹⁹ Exploring the factors that shape monocyte-macrophage function in the gut, such as the epigenetic modifications that monocytes undergo as they are recruited into the IBD gut, offers tractable targets.¹⁰⁰⁻¹⁰² These targets include manipulating the environmental cues, tissue factors, or epigenetic signals that confer their inflammatory properties. The roles of immune metabolism,¹⁰³ tissue hypoxia,¹⁰⁴ presence of extravasated pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs; bacterial lipopolysaccharide, mitochondrial

DNA, and reactive oxygen species [ROS])^{105, 106} are also tractable targets for interventions to disrupt the proinflammatory features of monocytes and their progeny. Exposure to butyrate, a short-chain fatty acid produced by gut bacteria, during monocyte-macrophage differentiation enhances monocyte antimicrobial activity via the histone-deacetylase-3 epigenetic regulation of S100a8/9.¹⁰⁷ As discussed herein, such potential translation will require further understanding of the macrophage populations that persist in the inflamed gut during the evolution of IBD.¹⁰⁸

Macrophage-Specific Therapeutics

In defining specific molecular targets for monocytes or macrophages, the next challenge is to find ways to deliver therapeutics to enable this objective. Parallel developments in oncology, where there is a need to target tumor-associated macrophages (TAMs), in a similarly complex tumor microenvironment, provide some insights into how this might work in IBD.¹⁰⁹ For example, macrophage-targeting nanoparticles (liposomes/PEGylated nanoparticles/folate receptor targeting agents) could be synthesized to deliver specific pathway inhibitors or agonists to promote the differentiation of inflammatory myeloid cells into macrophages with a proresolving phenotype.¹¹⁰⁻¹¹³ Nanoparticles are emerging as key translational moieties in targeting TAMs. For examples in cancer, immunonanomedicines target TAMs primarily by blocking specific TAM survival or affecting their signaling cascades, restricting their recruitment to tumors, and re-educating tumor-promoting TAMs to the tumoricidal phenotype.¹¹⁴ In another development, nanobiologics exploit high-density lipoprotein properties to deliver specific therapeutics.¹¹⁵ These areas remain in early stages of translation but are likely to become one of the next important steps to precision medicine in IBD.

Products of the Proresolving Macrophage as IBD Treatment

Downstream from this secreted products of the proresolving macrophage are closer to translation. Proresolving macrophages secrete resolvins, protectins, and maresins, long-chain fatty acid mediators (secreted proresolving mediators [SPMs]) shown to promote resolution of tissue injury in a wide variety of pathologies.¹¹⁶ The SPMs are a large class of signaling molecules produced by macrophages through the metabolism of ω -3 polyunsaturated fatty acids. The specific SPMs protectin; resolvin D1, D2, E1; and maresins have been shown to attenuate mouse colitis.¹¹⁷⁻¹²⁰ They act by blocking neutrophil recruitment and mediating the phagocytosis and clearance of apoptotic neutrophils by macrophages. It is noteworthy that these resolving bioactive lipids are synthesized from ω -6 and ω -3 polyunsaturated fatty acids (PUFAs). High dietary ω -6 PUFA

with proinflammatory potential is associated with increased risk of UC,¹²¹ but ω -3 PUFA has proresolution properties.¹²² However, ω -3 PUFA dietary treatment has not been shown to be effective in IBD,¹²³⁻¹²⁵ most likely reflecting the complex and context-specific nature of integrating metabolites and immunity.

Macrophages also produce a variety of growth factors, such as insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF)- α , TGF- β , and Wnt proteins that regulate epithelial and endothelial cell proliferation, myofibroblast activation, stem and tissue progenitor cell differentiation, and angiogenesis.²⁹ A cocktail of macrophage products (termed SuperMAPO) obtained through a culture of macrophages with apoptotic thymic cells has been shown to be effective in ameliorating mouse models of arthritis and IBD.¹²⁶ In a further study, Yoon et al¹²⁷ showed that instillation of apoptotic leukocytes can also induce the production of proresolving cytokines in an acute pulmonary inflammation model, although whether this cytokine production relies on local tissue macrophage activity remains unclear.

Inflammatory Stroma and Activated Endothelium

The advent of single-cell technologies has led to a much deeper understanding of the role of the intestinal stroma in intestinal inflammation. Indeed, UC is characterized by major changes in the composition and inflammatory status of the stromal compartment,³³ which is becoming recognized as an amplifier and driver of disease chronicity in IBD. Many potential specific targets in the IBD stroma recently, for example, endothelial atypical chemokine receptor 1 (ACKR1), stromal OSM,^{36, 46} and CCL19/CCL21.³³ These appear to be key factors that maintain the IBD milieu and resistance to anti-TNF treatment. West et al⁴⁶ showed that in a mouse model of anti-TNF-resistant IBD, mice lacking OSM developed significantly less disease than their wild-type counterparts, with reduced colonic chemokine and cytokine production accompanied by attenuated signs of inflammation. This was shown especially in the late disease course and could be recapitulated by OSM neutralization. In previous research, OSM has been targeted for rheumatoid arthritis in phase 1 and 2 clinical trials using a humanized anti-OSM monoclonal antibody, but little clinical efficacy was seen for this chronic inflammatory condition.¹²⁸ Regardless, OSM remains a good potential therapeutic target and is a proven biomarker for anti-TNF α treatment failure.

Epithelial Repair

A healthy intestinal mucosa is maintained by (1) a secreted barrier, a generous mucus layer laced with antimicrobial peptides, and (2) a physical one, comprising epithelial cells with tight-junctions and innate immune receptors such as TLR2 and TLR5 that can initiate a response when the barrier

is breached.¹²⁹ Intestinal epithelial cell regeneration and differentiation occur at the intestinal stem cells located at the crypt base driven by 4 important signaling molecules: Wnt, Notch, bone morphogenic proteins, and hedgehog.¹³⁰ Given that barrier dysfunction precedes the development of clinical IBD^{131, 132} and that incomplete mucosal healing is associated with a high risk of relapse, treatments that are focused on epithelial repair are important components of a proresolution treatment approach.¹³³

The mechanisms of epithelial repair can be divided into 3 phases. The first phase is epithelial restitution, where epithelial cells lose their columnar polarity and migrate to the site of the injury to rapidly seal the defective barrier.¹³⁴ Note that TGF- α/β , trefoil factors, and galectin-2 and -4 are vital for this process.^{135, 136} Second, epithelial cells receive signals, such as cytokines, growth factors, and bacterial products that lead to the induction of transcription factors, such as signal transducer and activator of transcription (STAT)-3 and -5 and nuclear factor-kappa B (NF- κ B) that promote epithelial homeostasis.^{133, 137} Finally, the new epithelial cells follow a well-defined differentiation process into mature intestinal epithelial cells (IECs) of either the absorptive or secretory lineage.¹³⁸

The concept of epithelial repair in IBD is not new and has been countenanced upon for more than 20 years.¹³⁹ Such therapies have included trefoil factors,¹⁴⁰ neutrophil-borne defensins and cathelicidins,¹⁴¹ ANXA-1 and its mimetic peptide Ac2-26,¹⁴² lipoxins,¹⁴³ EGF,¹⁴⁴ fish oil,¹⁴⁵ and probiotics.¹⁴⁶ Despite positive EGF clinical trial data in UC,¹⁴⁴ further clinical development has been stymied by the fear of overstimulation of epithelial proliferation and subsequent colorectal cancer development.¹⁴⁷ Recent work in an scRNAseq study focusing on the colonic IECs in UC has uncovered novel leads, such as WAP four-disulfide core domain 2 (WFDC2),³⁴ an anti-protease molecule that inhibits bacterial growth and is involved in the repositioning of goblet cells in UC. Further work from Parikh et al³⁴ showed that WAP four-disulfide core domain 2 is an important goblet cell-secreted antibacterial factor that is required to prevent colonization and invasion during epithelial barrier breakdown.

The identification of IL-22 (of the IL-10 family) as a prorepair cytokine has generated significant interest. In 2008, Sugimoto et al¹⁴⁸ showed that IL-22 could ameliorate intestinal inflammation. Further studies have shown that IL-22 activates the epithelial STAT-3 pathway to promote intestinal stem cell, antimicrobial peptide, and mucin production and the subsequent expansion and survival of the epithelial cells.^{149, 150} In addition, IL-22 has a major protective role in intestinal graft-vs-host disease.¹⁵¹ Some of the beneficial effects of anti-TNF α have been ascribed to increased IL-22 production.¹⁵² However, there is a fine balance to maintain because IL-22 is potentially a key factor in colon cancer¹⁵³ and has been shown to promote colitis in some settings.¹⁵⁴ Upregulating IL-22 function can be

achieved by using the endogenous inhibitor IL-22BP, directly via IL-22-Fc fusion protein UTR1147A or by using a ligand-based approach to activate the Aryl hydrocarbon receptor (AhR)-IL-22 pathway. A clinical trial using the traditional herbal remedy *indigo naturalis* to activate the AhR-IL-22 pathway has been shown to induce a clinical response in active UC, but this is limited by potential adverse

BOX 2. CHALLENGES IN TRANSLATING PRORESOLUTION AND REPAIR IN IBD.

1. Where to position in clinical trials in IBD?

These treatments are likely to play an adjunctive role to current treatments that inhibit the proinflammatory mucosal response (eg, anti-TNF α). Treatment duration should be short-term and focused on patients with active IBD at an early stage. Study endpoints like mucosal healing rather than clinical response are likely to be more informative.

2. Is there a dominant proresolution or repair mechanism to target?

It is unclear if there is a hierarchy of importance and whether there are likely to be UC- or CD-specific therapies. Some such as neutrophil-targeted approaches may be better for UC whereas stratification according to biological response, such IL-22 levels, may guide therapeutic decision-making.

3. Do we have tools to accurately to monitor whether complete mucosal healing has been achieved?

More comprehensive ways to assess mucosal healing (combined radiological, histologic, and endoscopic methods and biomarkers such as C-reactive protein and S100a8/9) are necessary. More specific mechanistic biomarkers may be needed depending on the type of treatment, such as cytokine-based therapies.

4. How to deliver proresolution and repair treatments in the clinical setting?

Gut mucosal drug delivery systems (such as 5-aminosalicylic acid/mesalazine) are necessary to ensure that adequate levels of proresolution/repair drugs reach the inflamed mucosa. Their effects may be different in inflamed vs normal tissue states. More cell-specific delivery methods (eg, macrophage nanobiologics) as discussed are areas for further studies.

5. What are the potential unintended consequences of proresolution and repair treatments?

Neoplasia and fibrotic complications may be factors to consider. A potential mitigation step is to ensure that these therapies are time-limited to the active and early phase of IBD (eg, at time of diagnosis) and that drugs are delivered to inflamed rather than normal tissue.

effects, such as liver and lung toxicity.¹⁵⁵ Other cytokines including TGF β and IL-28 also have roles in mediating epithelial repair. For example, IL-28 induces IEC proliferation and promotes wound healing via STAT1 signaling.¹⁵⁶ Inhibition of Smad7, a blocker of the TGF β receptor, via antisense oligonucleotides showed initially positive results in CD, but its clinical development was terminated because of lack of efficacy.^{157, 158}

The rationale for epithelial repair/regeneration treatment is strong, but its place within the IBD treatment armamentarium requires further thought. As this area continues to grow, the practical place for such an approach may require stratification involving patients with severe IBD with extensive gut tissue damage, ideally with a more specific drug delivery mechanism that avoids systemic exposure and in a time-limited fashion.

CONCLUDING REMARKS

The therapeutic ceiling of current IBD treatments indicates strongly that a combined approach targeting anti-inflammatory, proresolution, and repair processes is necessary (Fig. 3). This mature field of inflammation resolution offers many potential therapeutic targets (Table 1), yet it is pertinent to note that there are surprisingly no current bona fide proresolution or repair treatments available to patients with IBD. The tangible route to clinical translation poses several challenges (Box 2).

One key step for therapeutic progression is to generate the willingness to develop high-quality clinical trials to test the efficacy of potential proresolution/repair treatments. Such studies should be adjunctive in nature: for example, can they

improve complete mucosal healing in conjunction with current biologic therapy? Some prior considerations are necessary. First, current trial endpoints of response that are heavily based on clinical activity (eg, the CD Activity Index and the Mayo Score for CD and UC, respectively) may not reflect the true efficacy of proresolution/repair treatments. Here, objective readouts of mucosal and histological inflammation and healing may be more instructive. Second, useful molecular tools or systems to track mucosal healing in a dynamic manner in IBD are lacking. Measurements of S100a8/9 are increasingly adopted in IBD clinical trials but are less useful in CD. Third, novel delivery systems with specificity for the inflamed gut only—or better, to the implicated cell type—are fundamental. This specificity would reduce concern over potential proresolution/repair treatment effects on normal tissue, particularly the risk of neoplasia.

Although the direction of translation is clear, many of the concepts of resolution and repair are derived from acute inflammatory or injury models and conditions. These findings may not be generalizable to an immune-mediated condition like IBD where the gut mucosa is in close apposition with the complex luminal environment. New studies, such as the important scRNAseq studies, continue to reveal further complexities in both UC and CD, raising the prospect of disease-specific proresolution treatment. It is clear that there is some ground to cover with regard to understanding IBD-specific inflammation resolution; nevertheless, there is enormous potential to develop new simple treatments that harness the resolution and repair process. In conjunction with current available treatments, there is a real possibility to break the current therapeutic ceiling, to finally facilitate universal complete mucosal healing for all patients with IBD.

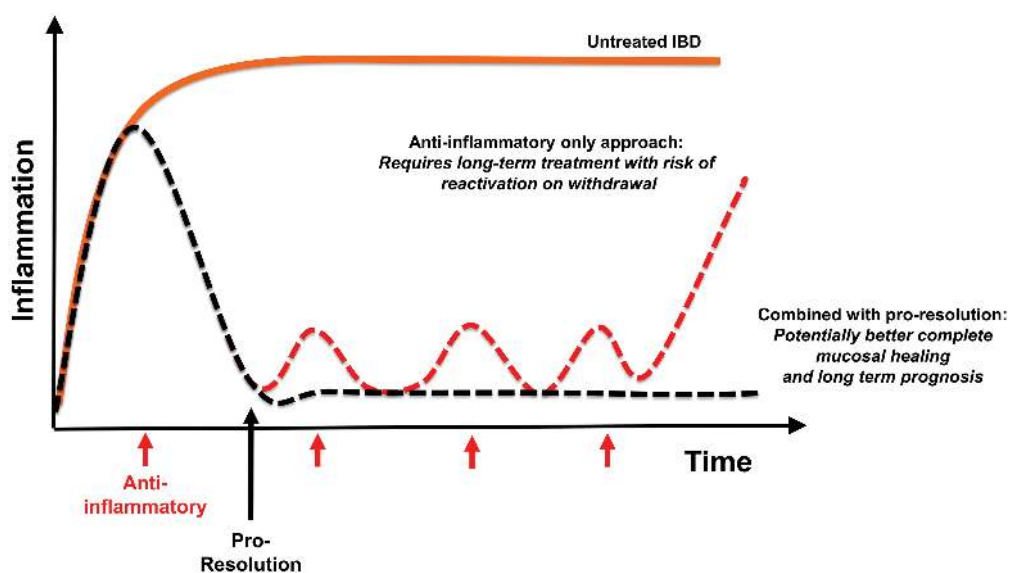


FIGURE 3. Therapeutic positioning of proresolution/repair approaches in IBD. Combined anti-inflammatory and proresolution therapy potential to significantly improve beyond current rates of complete mucosal healing in IBD.

TABLE 1. Proresolution and Repair Targets in IBD

IBD Inflammation Resolution and Repair Targets*				
Target Category/Method	Specific Target	Condition/Model	Species Studied (in vivo unless stated)	Reference
Targeting immune cell recruitment				
Block or ↓production of monocyte and neutrophil chemo-attractants	Theoretical (CCL2/CCL7) (CXCL2, CXCL3, and CXCL8)	Targets identified but not evaluated in IBD	n/a	36
↓Proinflammatory monocyte-derived macrophages	CCR2-blocking	DSS colitis	Mouse	84-86
Resolvins, protectins, and maresins	See use of proresolving macrophage products below.			
	Lipoxin A4	Hapten-induced colitis	Mouse	75
Annexin A1	Annexin A1	Not evaluated in IBD	n/a	72
↓Inflammatory signals from the stroma and endothelium	Endothelial AKTB1, stromal OSM CCL19/CCL21	Targets identified in scRNA in people with IBD	Human	33, 36, 46
	Stromal cytokine OSM genetic deletion or blockade	IBD mouse model; <i>Helicobacter hepaticus</i> infection and systemic IL-10 receptor blockade	Mouse, wild-type, and <i>OSM</i> ^{-/-}	46
	OSM targeting with anti-OSM monoclonal antibody	Patients with rheumatoid arthritis; not evaluated in IBD	Human	128
Clearance of inflammatory cells				
↑Granulocyte/neutrophil death	CDKI	Several inflammatory mouse models (not IBD)	Mouse	62, 63
	N-acetylcysteine, inhibiting RHOA, and reactive oxygen species production	Xenobiotic-induced lung inflammation; not evaluated in IBD	Human in vitro; rat in vivo	65
↑Granulocyte/neutrophil egress	CDKI	Tail fin resection	Zebrafish	64
↑Efferocytosis and other effete inflammatory cells	Apoptotic neutrophil cell therapy	Acute pulmonary inflammation model; not evaluated in IBD	Mouse	127
	Apoptotic neutrophil products, annexin A1	Not evaluated in IBD	Human in vitro	71
	Apoptotic neutrophil products, α-defensins	Thioglycollate model of peritonitis; not evaluated in IBD	Human in vitro; mouse	76
Promoting proresolving immune cells				
↑Monocytes; wound healing macrophages	Increasing adhesion of α4β7-expressing monocytes; nonclassical (CD14 ⁺ CD16 ⁺⁺) monocytes	Findings from vedolizumab-treated IBD patients; blocking in mice with intestinal surgery	Human; mouse	89, 90
	Increasing prorepair monocytes	Theoretical; prorepair cells identified in DSS-induced colitis	Mouse	91
	Increasing CCR2-dependent monocyte-derived macrophages†	Blocking delayed recovery from postoperative ileus in mouse	Mouse	92
Modulating macrophage function to proresolving				
↑Efferocytosis	CDKI	Pneumonia model; not evaluated in IBD	Mouse	62

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TABLE 1. Continued

IBD Inflammation Resolution and Repair Targets*				
Target Category/Method	Specific Target	Condition/Model	Species Studied (in vivo unless stated)	Reference
Change immune metabolism	Block succinate dehydrogenase	Not evaluated in IBD	n/a	103
	Butyrate	Not evaluated in IBD	Mouse in vitro; In vitro monocyte-macrophage differentiation	107
Macrophage-targeting nanoparticles	Liposomes/PEGylated nanoparticles/folate receptor targeting agents	Evidence from targeting TAMs; not evaluated in IBD	n/a	110
↓Macrophage inflammatory signature	Macrophage uptake of apoptotic intestinal epithelial cells	DSS colitis	Mouse	97
Exploiting pro-resolving macrophage products				
Delivery of resolvins, protectins, and maresins (SPMs)	Resolvin E1	DSS colitis	Mouse	117
	Resolvin D1 and Resolvin D2	DSS colitis	Mouse	118
	Maresin 1	DSS- and 2,4,6-trinitrobenzene sulfonic acid-induced colitis	Mouse	119, 159
Lipoxin A4	Lipoxin A4	Human IBD expression data; hapten-induced colitis	Human Mouse	74, 75
Macrophage (SuperMAPO) products	Products from macrophages after culture with apoptotic cells	Arthritis model; DSS colitis	Mouse	126
Accelerating epithelial cell growth/regeneration				
IL-22	IL-22 and STAT3	Human IBD expression data; DSS colitis	Human and mouse in vitro; mouse in vivo	149-151
	Activate AhR-IL-22 pathway	Multicenter, double-blind trial, human UC	Human	155
TNF α	Anti-TNF α Increased IL-22	CD patients receiving anti-TNF monoclonal antibody	Human	152
IL-28	IL-28 via STAT1 signaling	Patients with IBD; DSS colitis	Human; mouse	156

*Although presented in isolation based on published findings, the targets listed below should be considered as potentially interconnected.

[†]CCR2 deletion has both potential anti-inflammatory effects and causes delayed wound healing; the monocyte-derived macrophage department is very complex. n/a, not available.

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