Mechanisms of mucosal healing: treating inflammatory bowel disease without immunosuppression?

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Abstract | Almost all currently available treatments for inflammatory bowel disease (IBD) act by inhibiting inflammation, often blocking specific inflammatory molecules. However, given the infectious and neoplastic disease burden associated with chronic immunosuppressive therapy, the goal of attaining mucosal healing without immunosuppression is attractive. The absence of treatments that directly promote mucosal healing and regeneration in IBD could be linked to the lack of understanding of the underlying pathways. The range of potential strategies to achieve mucosal healing is diverse. However, the targeting of regenerative mechanisms has not yet been achieved for IBD. Stem cells provide hope as a regenerative treatment and are used in limited clinical situations. Growth factors are available for the treatment of short bowel syndrome but have not yet been applied in IBD. The therapeutic application of organoid culture and stem cell therapy to generate new intestinal tissue could provide a novel mechanism to restore barrier function in IBD. Furthermore, blocking key effectors of barrier dysfunction (such as MLCK or damage-associated molecular pattern molecules) has shown promise in experimental IBD. Here, we review the diversity of molecular targets available to directly promote mucosal healing, experimental models to identify new potential pathways and some of the anticipated potential therapies for IBD.

Inflammatory bowel disease (IBD) causes chronic intestinal inflammation resulting in relapsing and remitting symptoms, including abdominal pain, diarrhoea, anaemia and weight loss[1,2]. The clinical course of IBD is highly variable, both between individuals and during the life of a given individual[1,2]. Periods of a clinically inactive disease can be disrupted by acute flares, leading to the need for medication, hospitalization and, sometimes, bowel surgery[1,2]. The mainstay of medical therapy in IBD centres is the suppression of the immune system. When this strategy fails to suppress inflammation adequately, patients are often subjected to intestinal resection to remove the affected gut[3,4]. In turn, surgery can be associated with complications, such as an anastomotic leak, sepsis, bleeding, intraperitoneal adhesions and short bowel syndrome, which lead to loss of function within the digestive system[5,6].

The pathogenesis of IBD is driven by an abnormal and prolonged T cell-mediated immune response directed towards the commensal gut microbiota that occurs in genetically susceptible individuals[7]. The known IBD risk genes are related to various immune functions, including innate immune functions such as physical barrier and autophagy[7,8]. The current models implicate multiple factors in IBD pathogenesis, including multi-layered mucosal injuries such as histological and cellular-level changes that ultimately lead to macroscopic erosions and ulcers[9,10].

Inflammatory responses at the intestinal barrier need to be tightly controlled; defective inflammation might result in tissue destruction by harmful agents, such as pathogens, whereas uncontrolled inflammation might result in host pathologies such as IBD. Besides, inflammation has a critical role in the regeneration of injured tissues. Whether a defective intestinal barrier in patients with IBD occurs owing to impaired inflammation that then leads to altered mucosal healing is not entirely clear. The relapsing–remitting course of IBD entails repeated inflammatory insults to the intestine, which must undergo a healing process to return to normal function and attain remission. Most available therapies inhibit this immune response[11,12], and few treatments attempt to harness the regenerative response. However, as it has been postulated that "tumours are wounds that do not heal"[13], promoting mucosal healing might increase the risk of oncogenic transformation. Thus, identifying pathways that disentangle tissue regeneration and tumorigenesis is a major goal in the field. In this Review, we

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https://doi.org/10.1038/s41575-022-00604-y

Key points

- Inflammatory bowel disease (IBD) has emerged as a global disease with no available cure.
- Most drugs to treat IBD are immunosuppressive, leading to increased risk of infections and cancer.
- Inter-individual variation in response to drugs means a wider range of therapeutic strategies is needed.
- Promoting mucosal healing is a promising therapeutic strategy in IBD.
- Experimental models have been instrumental to identify novel mechanisms promoting mucosal healing.
- Drugs promoting regeneration have been identified, but the examination of tumorigenesis in this setting is urgently needed.

discuss mechanisms of mucosal healing and how these might be defective in patients with IBD. Furthermore, we discuss the potential to exploit the healing process as a therapeutic alternative to treat IBD.

Defining mucosal healing

Disruption of the intestinal barrier — that is, wounding — leading to the translocation of microorganisms and other antigens into the bowel wall and to consequent uncontrolled immune activation is a key feature of IBD[9,14]. The structural basis of a healed mucosa is an intact barrier that limits bacterial translocation and consequent immune activation[14]. Thus, a functional definition of mucosal healing can be described as the re-establishment of this barrier function[9,15]. Mucosal healing can also be defined endoscopically: during the 1990s, improvement in endoscopic appearance as defined by white light endoscopy became a recommended treatment goal in clinical practice[16]. In both clinical practice and clinical trials, mucosal healing is usually defined endoscopically as the disappearance of ulcers in Crohn's disease, the absence of friability, blood, erosions and ulcers in ulcerative colitis, or as the total absence of inflammatory and ulcerative lesions for both forms of IBD. Considerable disparity still exists in the literature[17,18], although consensus has been sought in defining treatment targets[19,20]. However, it has been shown that macroscopic mucosal healing predicts sustained remission and resection-free survival[15]. Mucosal healing can also be defined histologically, going a step further than definitions by endoscopic appearance, by defining mucosal healing as the absence of inflammation on histology, which is associated with greater durability of remission[21–23]. Mucosal healing can even be defined radiologically as a lack of residual bowel wall inflammation detected by cross-sectional imaging, termed transmural healing, which was associated with lower rates of hospital admission, escalation of therapy and surgery than with mucosal healing defined only by endoscopy[24].

Increasingly over the past 20 years[19], complete mucosal healing has been identified as an important therapeutic goal in IBD[19,20,25]. In the IBSEN study, mucosal healing at the 1-year follow-up visit after diagnosis was associated with a reduced risk of future colectomy in patients with ulcerative colitis ($n = 354$) and less inflammation after 5 years, and with decreased future steroid treatment in patients with Crohn's disease ($n = 141$)[26]. In 46 patients with Crohn's disease, endoscopically defined mucosal healing after 2 years of treatment was shown to be the only factor associated with steroid-free remission at 3 and 4 years in a study of azathioprine and infliximab versus corticosteroids[27]. A systematic review of 10 studies in Crohn's disease published in 2016 found a higher odds ratio that was statistically significant for clinical remission in patients attaining mucosal healing[28]. Mucosal healing in the small intestine seems to be of similar importance; indeed, a study showed that patients with Crohn's disease ($n = 61$) in clinical remission who had a Lewis score of 350 or higher on video capsule endoscopy were at higher risk of clinical relapse compared with patients with a Lewis score of less than 350 (REF.[29]).

The currently available treatments used to induce mucosal healing almost universally act through the inhibition of immune activity, often by blocking specific inflammatory molecules. Immunosuppression is associated with infectious and neoplastic adverse effects and dealing with these consequences forms a substantial part of the experience of patients with IBD and the work of clinicians caring for them[30–34]. Therapeutic strategies to directly promote mucosal healing are lacking, but the concept of delivering mucosal healing without immunosuppression is an attractive one. Although dozens of pathways promoting mucosal healing have been identified, most, if not all, lead to over-proliferation of the intestinal epithelium and consequent tumour growth. For example, work in animal models has suggested that IL-22 and the STED7–Hippo–YAP axis, which promote intestinal regeneration, may also lead to increased intestinal tumorigenesis[35–37]. Thus, there is a lack of understanding of the targets that might promote mucosal healing without the risk of tumorigenesis. Furthermore, there are currently no clinically useful biomarkers that can define the stage of healing that the patient has reached or predict and confirm mucosal healing and regeneration. Such biomarkers would facilitate more individualized treatment strategies.

Evidence of efficacy in inducing mucosal healing varies for standard IBD treatment strategies[14]. Interestingly, glucocorticoids, which have a well-established positive effect on symptoms in acutely active IBD, might even interfere with endoscopic mucosal healing in Crohn's disease[14,38]. For example, in the IBSEN cohort, treatment with corticosteroids was a negative predictor of mucosal healing, although the underlying mechanisms are not clear[26]. For the immunomodulators, there is evidence in several studies in humans with IBD that azathioprine can both induce and maintain mucosal healing in both forms of IBD[14,38]. Methotrexate has similar effects in Crohn's disease and there is some evidence that calcineurin inhibitors can induce mucosal healing in ulcerative colitis[14,38]. Anti-tumour necrosis factor (TNF) antibody drugs (such as infliximab and adalimumab), the anti-α4β7 integrin antibody drug vedolizumab, and ustekinumab, which blocks cytokines IL-12 and IL-23, have all been shown to be effective in the induction and maintenance of clinical remission and mucosal healing in IBD[14,38]. However, all these treatment strategies are associated with an increased risk of infection.

One study used a cohort of patients identified through primary care registries and found that patients with IBD had an increased risk of infections (including upper respiratory tract infections, acute bronchitis, skin infections, *Clostridiodes difficile*, *Salmonella*, *Shigella* and *Campylobacter* infections, and herpes zoster). This risk was greater for patients with IBD treated with immunosuppressant drugs[39]. Immunosuppressant drugs also confer an increased risk of opportunistic infections, such as candidiasis, cryptosporidiosis, actinomycosis and progressive multifocal leukoencephalopathy, as well as reactivation of latent infections such as cytomegalovirus, tuberculosis and hepatitis[40]. In addition, anti-TNF therapy has also been associated with increased risk of malignancies, including haematological malignancies (lymphomas and leukaemias), colorectal, anal, gastric, pancreatic and hepatic carcinomas, and non-melanoma skin cancers[32,41,42]. However, by contrast, a study of 3,119 patients with IBD in whom 122 malignancies were identified has indicated that the use of biologic drugs and 5-aminosalicylates might mediate an anticancer effect (possibly through a reduction in chronic inflammation)[33]. Interestingly, these authors did not demonstrate the same influence on cancer rates for immunomodulators and the data regarding the anticancer effect of 5-aminosalicylates are conflicting[43–46]. Existing drugs are known to target some aspects of tissue healing, such as autophagy and induction of M2-type wound-healing macrophages, though they primarily act by inhibiting immune functions[47,48]. However, attaining mucosal healing in paediatric Crohn's disease without immunosuppression has been possible using exclusive enteral nutrition (EEN)[14,38,49]. Evidence from animal models suggests that supplementation with specific amino acids and polyamines and short-chain fatty acids could enhance mucosal healing[50], although there is a paucity of human data in this area. There is also growing evidence that nutrient status and metabolism can affect the function of the immune system, providing the metabolic substrates and signals promoting switching of T cells from quiescent to active and proliferative states[51]. However, the mechanisms by which dietary components abrogate or promote mucosal healing are not clear.

As is frequently the case in IBD, much inter-individual variation will likely complicate efforts to achieve mucosal healing. This aspect is indicated most obviously through differences between Crohn's disease and ulcerative colitis in which distinct macroscopic appearances of ulcerations are classically described[52,53]. In addition, the inflammatory infiltrate is confined to the mucosa in ulcerative colitis whereas it is transmural in Crohn's disease, with the latter also exhibiting a propensity to discontinuous inflammation in contrast to ulcerative colitis[53,54].

Intestinal epithelial cell development

The cells and molecular mechanisms that underpin the process of intestinal epithelial cell development must be well understood to harness the regenerative power of mucosal healing. The single layer of intestinal epithelial cells (IECs) that separates the external environment from the underlying tissue compartment provides a barrier function crucial for the regulation of uncontrolled translocation of potentially damaging luminal products, eventually avoiding aberrant immune cell activation against harmless compounds[55]. The IEC layer comprises absorptive enterocytes, enteroendocrine cells, goblet cells, microfold cells (M cells), tuft cells and Paneth cells, which operate in coordination with the underlying immune cells, stroma cells and enteric nervous system[56,57]. These cells are arranged in a complex and spatially defined relationship in the intestinal crypts and, in the small intestine, in the villi. Replenishment of all these cells types is fulfilled by intestinal stem cells (ISCs), which lie at the base of the crypt in a functionally defined part called the stem cell niche[58]. The structure of the crypt seems to have evolved to protect the stem cell niche from soluble metabolites present in the intestinal lumen such as microbiota-derived metabolites with the potential to inhibit stem cell proliferation[59]. Upon epithelial damage, stem cells generate new cells that differentiate into the various cell types required as they migrate towards the surface epithelium or villus. The spatial and functional architecture of the crypt–villus axis through which the train of differentiating cells flows is maintained through tightly controlled gradients of signalling molecules such as Wnt and R-spondins (involved in ISC maintenance), Notch (involved in fate decisions), and bone morphogenetic protein (BMP), which limits crypt numbers[60] (FIG. 1). One important difference between small intestine and colon crypts is the absence of classic Paneth cells in the latter[61]. Consequently, stem cell niche factors are produced by different cell compartments depending on location[62]; WNT3, epidermal growth factor (EGF) and Notch signals are mostly produced by Paneth cells in the small ISC niche, whereas in the colon stem cell niche, mesenchymal cells are the main producers of such factors (FIG. 1). Using elegant genetic mouse models, in which REG4$^+$ deep crypt secretory (DCS) cells can be depleted in vivo, Sasaki et al. demonstrated that REG4$^+$ DCS cells are necessary and sufficient to support colonic stem cell function and organoid growth in vitro[61]; therefore, it is believed that REG4$^+$ DCS cells act as Paneth cells in the colon (FIG. 1). These geographical differences in the mechanism of epithelial homeostasis have important implications for the identification of the key mechanisms of epithelial restitution and regeneration that will therefore vary between individuals and, critically, might therefore vary between ulcerative colitis and Crohn's disease. Furthermore, the appearance of Paneth cells in the colon (Paneth cell metaplasia) is considered a feature of chronicity, particularly in ulcerative colitis[53,63], indicating that, in the chronic disease state, mechanisms of restitution might deviate from those seen in acute inflammation.

Effects of inflammation

Intestinal epithelial barrier. In addition to the physical barrier composed of epithelial cells, the production of proteins, tight junctions and intracellular mechanisms to deal with invading pathogens is critical to maintaining homeostasis and must be regenerated in the process of mucosal healing (FIG. 2). Mucins are glycoproteins produced and secreted by goblet cells and are considered the

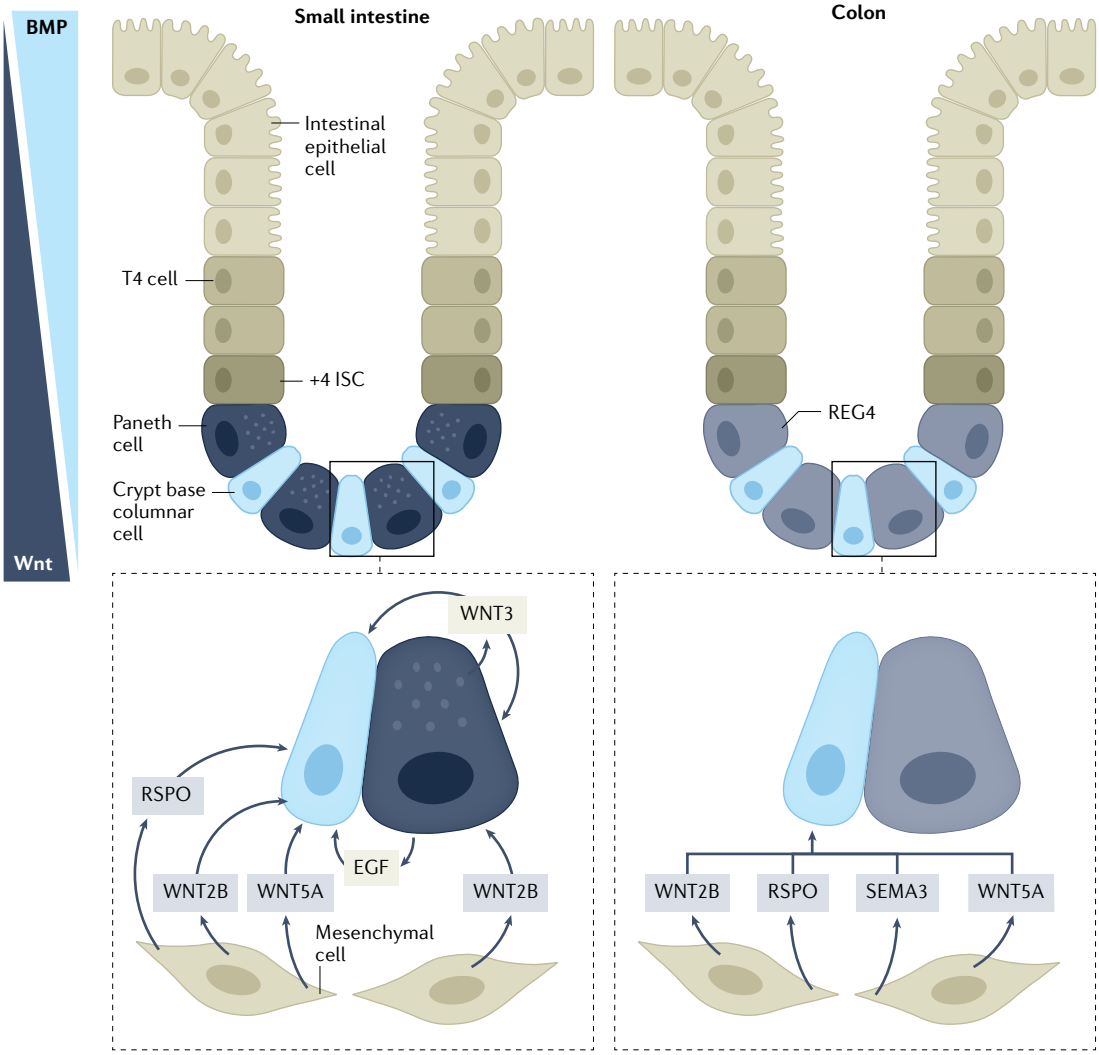

Fig. 1 | **The stem cell niche in the small intestine and colon.** Schematic diagram showing stem cell-containing crypts in the small intestine and colon. The intestinal crypts show the major epithelial cell types found and focus on the interaction between intestinal stem cells (ISCs) and their neighbouring cells within their niche. In the small intestine, stem cells are located between Paneth cells (blue cells) and at or near the position 4 (+4 ISC) within the crypt. The magnification shows interactive cells and signalling pathways that promote ISC-mediated tissue regeneration. Cell depicted in grey ($REG4^+$) within the colon crypt is an unknown cell type. On the left side, the reciprocal Wnt and bone morphogenetic protein (BMP) gradients that define the crypt–luminal axis are represented. EGF, epidermal growth factor.

major component of the mucus layer, which separates the commensal bacteria from the epithelium[64]. Microbial control is a substantial part of the functional barrier; Paneth cells are located at the bottom of the intestinal crypt in the stem cell niche and are the major source of antimicrobial peptides (AMPs)[65]. Among AMPs, Paneth cells produce and secrete defensins, cathelicidins (for example, LL-37), C-type lectins, ribonucleases (for example, RNAses) and S100 proteins[65]. The most studied C-type lectins are the regenerating islet-derived protein (REG) family, such as REGβ and REGγ, which are critical in limiting the contact between the gut microbiota and the intestinal epithelium, creating a physical barrier[66]. All these different functional barriers (for example, mucus production, AMPs and innate and adaptive responses) contribute to the physical and molecular integrity of the intestinal epithelium itself. In areas of frank erosion, there is a massive increase in intestinal permeability as the intestinal epithelium is absent but, even in areas with no erosions, intestinal permeability can be increased in patients with IBD[67], and alteration in barrier function in remission has been linked to increased paracellular permeability[68]. Barrier integrity between these transforming and migrating different cell types is maintained through a complex protein network termed tight junctions. These proteins are crucial to linking adjacent cells and sealing the space between them and have been implicated in genome-wide association studies as risk alleles for IBD[69]. Genome-wide association studies have also identified IBD risk genes that point to other epithelial functions as mechanisms leading to chronic intestinal inflammation, including genes involved in the interaction between the microbiota and the mucus layer (for example, *FUT2*) and the disruption of key intracellular processes such as bacterial handling (for example, *NOD2*) and autophagy (for example, *ATG16L1*, *GPR65*),

among others (FIG. 2). Thus, the intestinal epithelium is not static but highly dynamic and renews every 5–7 days[70], allowing continued protection of immune cells from the rich luminal microbiota.

The evidence from animal models for the role of the barrier in IBD pathogenesis suggests that the role of intestinal permeability might be complex. In mice, a transient breach of the barrier (for example, with ethanol) seemed to induce a regulatory response that protected against subsequent injury to mucosal integrity with trinitrobenzene sulfonic acid (TNBS)[71]. Moreover, a genetic model of increased permeability with knockout for a tight junction protein, junctional adhesion molecule A (JAM1), demonstrated increased colonic permeability but was not associated with increased susceptibility to dextran sodium sulfate (DSS) colitis[72]. Tight junctions, composed of claudins and occludins, have a critical role in controlling intestinal permeability, allowing the transport of water and other solutes (FIG. 2). Mice engineered to overexpress claudin 2 (a tight junction protein highly upregulated in IBD[73]) specifically in IECs resulted in a substantial increase in intestinal permeability but not in spontaneous intestinal inflammation[74]. Despite increased colonic permeability, these mice were protected from acute and chronic DSS-induced colitis[74]. By contrast, in a mouse knockout model of claudin 7 (another component of the tight junction), there was a loss of epithelial integrity, which in this model was associated with profound intestinal inflammation[75]. Myosin light chain kinase (MLCK) controls tight junctions and elevated levels are associated with loss of barrier function leading to IBD[76]. Importantly, Divertin, a small molecule, blocks the effect of MLCK1 in disrupting tight junctions and consequently reverts barrier dysfunctions, which eventually limits the development of experimental IBD[76]. Furthermore, mice with reduced activation of RhoA in epithelial cells demonstrated cytoskeleton rearrangement and aberrant cell shedding, ultimately leading to loss of epithelial integrity and subsequent inflammation[77]. Thus, increased permeability of the intestinal barrier contributes to the progression of intestinal inflammation.

Ultimately, the increased intestinal permeability that can be measured in patients with macroscopic inflammation might differ from the increased intestinal permeability that plays a part in IBD pathogenesis. Moreover, there might be key differences in the mechanisms and role of barrier impairment between ulcerative colitis and Crohn's disease. Altered gut permeability is implicated in the pathogenesis of ulcerative colitis and Crohn's disease through the link with alleles coding for elements of the gut barrier such as *CDH1* (REF.[78]), *MAGI2* (REF.[79]), *PTGER4*, *HNF4A*, *MUC1* and *MUC4* (REF.[80]). However, a human study published in 2020 examining preclinical markers of IBD onset found that serum biomarkers, including antimicrobial antibodies (thought to indicate

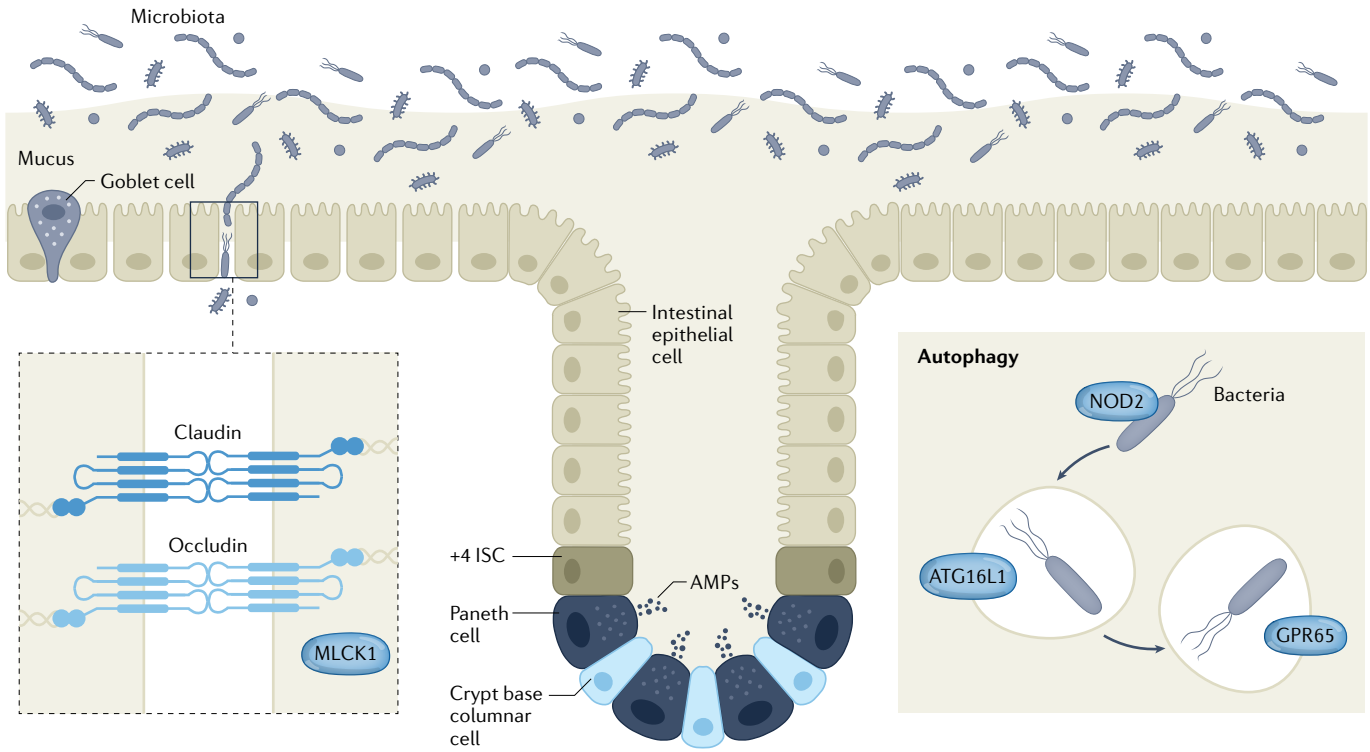

Fig. 2 | **The intestinal barrier.** Schematic diagram showing the single layer of intestinal epithelial cells and some mechanisms that prevent translocation of the gut bacteria into the lamina propria. Stem cells are located between Paneth cells (blue cells) and at or near the position 4 (+4 intestinal stem cell (ISC)) within the crypt. Goblet cells are located in the intestinal epithelium and secrete mucins to form the mucus layer. Paneth cells at the bottom of the intestinal crypt produce and secrete antimicrobial peptides (AMPs), creating a sterile environment. Tight junctions are multiprotein junctional complexes that prevent the translocation of bacteria into the lamina propria. Epithelial and immune cells underlying the intestinal epithelium might eliminate translocated bacteria through autophagy. Defects in any of these mechanisms have been associated with the initiation and progression of inflammatory bowel disease. MLCK1, myosin light chain kinase 1.

reduced barrier integrity), were linked with Crohn's disease but not with ulcerative colitis[81].

The intestinal mucosa. At the histological level, a flare of IBD might be considered a series of sterile microscopic mucosal injuries combined with inflammatory activity and consequent effort by the immune system to heal the damage and regenerate the injured tissue. The production and secretion of the non-inflammatory IgA contribute to reinforcing the physical barrier by targeting and neutralizing bacteria[82]. Furthermore, IgA can interrupt the shuttling of microorganisms and toxins inside IECs[82]. Antigen sampling and induction of adaptive immune responses are also a part of barrier function. Dendritic cells acquire luminal antigens through specialized epithelial cells called M cells[83], goblet cells[84] or CX_3CR1^+ macrophages[85], and then migrate to the draining lymph node where they generate antigen-specific T cell responses. By contrast, CX_3CR1^+ macrophages can directly capture luminal antigens by extending dendrites between epithelial cells[86]. As they extend dendrites, CX_3CR1^+ macrophages preserve barrier integrity by expressing tight junction proteins required to seal the intercellular surfaces of IECs[87].

The innate immune system provides an important layer of barrier function. For example, neutrophils are recruited at the site of barrier compromise, where they increase the assembly of the nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase complex to generate an antimicrobial oxidative burst and eventually reduce translocating pathogens[88]. In addition, neutrophils contain intracellular granules containing AMPs, proteases, metal chelators and other antimicrobial agents released upon contact with the pathogen[89]. Moreover, neutrophils produce and release neutrophil extracellular traps, which comprise DNA and chromatin in combination with toxic molecules from intracellular granules and can physically trap microorganisms and destroy them[90]. Finally, Molloy et al. demonstrated that, during infection in mice, neutrophils translocated to the intestinal lumen, generating organized intraluminal structures (similar to a cast) that encapsulated bacteria, limiting their contact with the epithelium[91].

Intraepithelial lymphocytes are embedded within the intestinal epithelium and their proximity to luminal antigens position them at the front line of defence against invading pathogens[92]. Innate lymphoid cells (ILCs) are lymphocytes lacking T cell receptors (TCRs), with reactivity against a range of antigens[93] and are subdivided into ILC1, ILC2 and ILC3, which have distinct functions in the establishment of intestinal homeostasis and repair upon injury[94]. In particular, ILC3s have been implicated as a major inducer of intestinal regeneration in mouse[95,96]. Thus, highly regulated crosstalk between the microbiota, intestinal epithelium and immune cells is compulsory for the proper function of the intestinal mucosal barrier.

Epithelial restitution: stem cell niche

Mucosal repair relies on IEC regeneration in a highly coordinated process that begins just minutes after injury. The initiation of wound healing involves a first step in which epithelial cells surrounding the injury lose their columnar polarization and then migrate to the lesion[97]. Although independent of proliferation, this initial process is regulated by cytokines, such as transforming growth factor-α (TGFα), IL-1β, EGF and IFNγ, as seen in in vitro experiments[98]. Trefoil peptides and galectins 2 and 4 can also promote epithelial regeneration in a cytokine-independent manner in vitro[99]. Furthermore, the chemokine receptors CXCR4 and CCR6 with their ligands, the chemokines CXCL12 and CCL20, respectively, modulate IEC migration to the lesion in the absence of cell proliferation[100,101]. This process is followed by IEC proliferation and differentiation, which provide the cellular pool needed to reconstitute the intestinal epithelial barrier. Cell proliferation is initiated at the stem cell niche, composed of ISCs and Paneth cells and located at the bottom of the intestinal crypt. Developmental genetic programmes involving, among others, Hedgehog, Noggin, Notch and Wnt pathways control, in different ways, the development and turnover of ISCs[60,102,103]. Cytokines, such as IL-6 and IL-22 as well as Toll-like receptor (TLR) ligands, induce the activation of the transcription factor signal transducer and activator of transcription 3 (STAT3), which induces survival and cell proliferation[104,105]. The activation of STAT3 seems to be crucial as seen in mouse models in which STAT3 inactivation results in intestinal wound healing failure[106]. Similarly, STAT5-deficient mice show impaired intestinal wound healing in a mechanism that involves activation of epithelial MLCK followed by tight junction disfunction[107]. Furthermore, deficiency of STAT5 results in impaired crypt regeneration upon irradiation-induced damage in mice, whereas STAT5 overexpression increased ISC proliferation with consequently accelerated crypt regeneration upon injury[108]. Importantly, ectopic overexpression of STAT5 is protective in DSS-induced colitis in mice[108]. STAT5 is a promising therapeutic target for induction of tissue repair in patients with IBD; however, it remains to be investigated if STAT5-induced ISC over-proliferation could lead to tumorigenesis. In addition, an IL-22 IgG4 Fc fusion protein (UTTR1147A) is currently under investigation as treatment in active ulcerative colitis and Crohn's disease with a mechanism of action presumed to be epithelial restitution via STAT3 pathways (ClinicalTrials.gov: NCT03558152, NCT03650413)[109,110].

The role of STATs in epithelial restitution is also important given the increasing clinical application of Janus kinase (JAK) inhibitors in the treatment of ulcerative colitis. These drugs block the interaction between JAKs and their associated receptors, which in turn interferes with the dimerization and phosphorylation of STAT molecules, precluding their migration into the nucleus and ultimately preventing DNA binding and targeted gene induction[111]. The efficacy of tofacitinib, which has broad effects, but predominantly inhibits JAK1 and JAK3, questions the importance of STAT-mediated epithelial regeneration in healing within ulcerative colitis. In an animal model, the process of intestinal wound healing was prolonged in the presence of high concentrations of tofacitinib[112]. In the original OCTAVE studies, tofacitinib demonstrated around 40% remission rates at

52 weeks, clearly implying that this strategy was ineffective for all patients[113]. Moreover, JAK inhibitors have yet to show convincing efficacy in Crohn's disease[114], implying that inhibition of this pathway might not be advantageous in all forms of intestinal inflammation. Disentangling the (often overlapping) roles of JAK and STAT molecules could identify patients for whom preservation of the regenerative potential of STAT signalling would be advantageous.

Mechanisms of mucosal healing

Cellular and molecular mechanisms. Immune cell crosstalk within the intestinal epithelial niche has been proposed as a key mechanism to induce intestinal tissue regeneration. In particular, immune cells act as a source of cytokines and metabolites important for ISC proliferation and IEC survival. Cytokines and soluble mediators, such as prostaglandin E_2 (PGE_2) and Wnt ligands, produced by either macrophages or lymphocytes have been shown to promote IEC proliferation[115–117]. Apoptotic clearance by macrophages has also been shown to be critical for mucosal healing as seen in experimental models of intestinal damage and repair[118]. Mechanistically, the engulfment of apoptotic cells through C-type lectin receptors induces macrophages to express anti-inflammatory (for example, IL-6, IL-11) as well as tissue-healing factors (for example, RETNLA, CHI3L3 and ARG1)[118]. Thus, macrophages limit inflammation that can otherwise lead to collateral cell death and a delay in the repair process upon intestinal injury. Macrophages seem to be critical in inducing intestinal immune homeostasis in repair as seen in mouse models in which either IL-10 or IL-10Rα was depleted specifically in macrophages while remaining intact in other cells[119,120]. Studies of tissue regeneration after cardiac injury in mice revealed that regenerating islet-derived 3β (REG3β), commonly known as an AMP, can induce the recruitment of macrophages to the site of injury and eventually promote tissue repair[119]. Although REG3β is consistently induced in several experimental models of intestinal inflammation[121,122], whether this process results in increased macrophage recruitment and induction of tissue repair is unknown.

ISC-driven regeneration is highly influenced by BMPs and signalling via Wnt, which respectively attenuate and promote cell replication. The cellular sources of Wnt proteins are mainly stroma cells, located close to the bottom of the crypts[123]. To further promote ISC proliferation, stromal cells also produce BMP inhibitors such as *Noggin* and *Gremlin1* (reviewed elsewehere[123]). In line with the critical role of stromal cells supporting ISC function, single-cell RNA sequencing (RNA-seq) analysis of the stromal cell compartment between patients with IBD and healthy individuals (5 individuals per group) or between inflamed versus non-inflamed tissues from patients with IBD (paired biopsy samples from 11 individuals) revealed a dramatic dysregulation of the inflamed stromal cell compartment in patients with IBD[124,125]. Such dysregulation was characterized by an increased activated phenotype dominated by the expression of IL-6 and LIGHT, which in turn negatively regulated the transcript levels of the stem cell markers LGR5, OLFM4 and AXIN2 in organoid cultures[124]. Although these data suggest that activated stromal cells from patients with IBD impair ISC-induced epithelial regeneration, inflamed tissues from these patients had higher LGR5 transcript levels than paired non-inflamed tissues, highlighting the need to further investigate the role of stromal cells in promoting tissue regeneration during inflammation.

New methodologies enable the investigation of cellular and molecular components of biological processes in an unbiased way. Such experiments are beginning to yield data that brings a better understanding of the process of mucosal healing. Among novel methodologies and experimental models (FIG. 3), RNA-seq analyses at the bulk, single-cell and spatial level have shed light on new cell types and mechanisms promoting tissue remodelling and regeneration[122,126].

Microbial and nutrient-derived metabolites. Emerging evidence points towards the microbiota as a critical factor in inducing tissue repair after intestinal damage. For instance, germ-free animals exhibit decreased turnover and regenerative capacity of IECs upon injury[127], suggesting that the absence of microorganisms or their products adversely affects intestinal epithelial homeostasis. Patients with IBD show alterations of the gut microbiota characterized by a reduction of diversity compared with healthy individuals[128]. Reduction in bacterial genera with anti-inflammatory functions, such as *Lactobacillus*, *Bifidobacterium* and *Faecalibacterium*, is associated with enhanced epithelium damage in patients with IBD[128–130]. Thus, the use of probiotics or faecal microbiota transplantation, aimed to restore the microbiota composition, is a promising therapeutic strategy to promote remission in patients with IBD. However, the use of faecal microbiota transplantation for this purpose is yet inconclusive[131], likely owing to our lack of understanding and standardization of the donor microbiota composition. Antibiotics have also been extensively studied in the treatment of IBD, but most trials have failed to show any beneficial effect[132]. Indeed, failure to show the beneficial effects of antibiotics to induce remission in IBD supports the importance of microbial diversity.

Certain commensal species, such as *Lactobacillus rhamnosus*, stimulate the expression of *N*-formyl peptide receptor 1 (FPR1) by epithelial cells located at the proximity of wounds inflicted in murine colons[133]. FPRs are critical regulators of intestinal homeostasis, in particular inducing proliferation and migration of epithelial cells[133]. This process involves the production of reactive oxygen species that trigger the phosphorylation of adhesion kinase (FAK) and extracellular signal-regulated kinase mitogen-activated protein kinase. Flagellated bacteria, which might translocate when the intestinal epithelium is injured, can activate TLR5 on mouse intestinal dendritic cells through the flagellum protein flagellin[134,135]. Upon activation, dendritic cells produce large amounts of IL-23 that in turn act on ILC3s to induce their production of IL-22, which acts on epithelial cells to induce the production and secretion of REG3β and REG3γ[134]. AMPs have been proposed to suppress inflammation and induce tissue recovery[136]. Moreover, IL-22 can

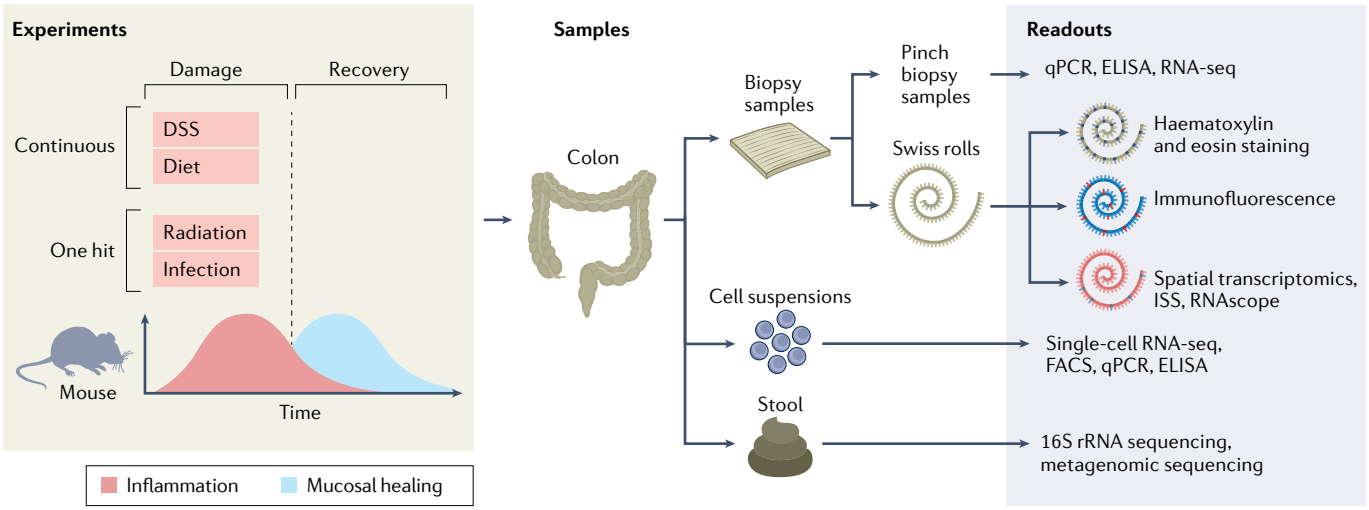

Fig. 3 | **Experimental models to study mucosal healing.** Experimental models and readouts to investigate intestinal mucosal healing are depicted in this figure. Intestinal damage can be induced by the continuous administration of dextran sodium sulfate (DSS) in the drinking water or exposure to diets such as a high-fat diet. Alternatively, one-hit irradiation or infection result in intestinal damage. Following the removal of the damaging agents, the recovery phase takes place and colonic (or small bowel) tissue can be dissected for analysis of the regenerative process. Biopsy samples, swiss rolls, cell suspensions and stool samples can be analysed for specific and complementary readouts, for example, by quantitative PCR (qPCR), enzyme-linked immunosorbent assay (ELISA; biopsy samples), immunohistochemistry, immunofluorescence, spatial transcriptomics, in situ sequencing (ISS), RNAscope (swiss rolls), single-cell RNA sequencing (RNA-seq), flow cytometry (fluorescence-activated cell sorting (FACS); cell suspension) and 16S rRNA sequencing (stool samples).

act directly on mouse and human ISCs, triggering STAT3 activation and, consequently, proliferation and increasing small intestine organoid formation in vitro[37]. Importantly, in experimental models of graft versus host disease, treatment with IL-22 results in reduced mortality, which is associated with increased intestinal epithelial regeneration[37]. Microbiota-derived tryptophan catabolites can also regulate the ILC3 capacity to produce IL-22 by activating the aryl hydrocarbon receptor (AhR)[137]. Hence, bacterial flagellin and tryptophan metabolites might trigger a cascade of events that ultimately result in increased intestinal epithelium regeneration.

In addition, anaerobic bacterial species, such as *Clostridium* clusters IV and IXa, *Bacteroides thetaiotaomicron*, and *Faecalibacterium prausnitzii*, produce short-chain fatty acids (SCFAs), which have been shown to enhance intestinal barrier integrity[138], suggesting a positive role in tissue repair. However, the microbiota-derived SCFA butyrate suppressed ISC proliferation in mice[59], whereas other SCFAs had no effects. Thus, SCFAs might have a dual role in the intestinal epithelium by reinforcing barrier integrity but inhibiting ISC proliferation. Interestingly, butyrate-metabolizing species were decreased in 127 patients with ulcerative colitis compared with 87 age-matched healthy individuals[139]. Whether this process results in decreased butyrate and the consequent increase in ISC proliferation as a regenerative process remains to be elucidated. Another microbiota-derived metabolite with regenerative properties is deoxycholate, which is a secondary bile acid that binds and activates the farnesoid X receptor (FXR)[140]. Mechanistically, deoxycholate promotes intestinal crypt regeneration by inhibiting PGE_2 production, which has been shown to limit ISC proliferation[140]. Furthermore, microorganism-derived lactate is a potent inducer of colonic proliferation in mouse models[141,142]; thus, bacterial metabolites are critical in regulating intestinal homeostasis, and modulation of the gut microbiota might be a promising strategy to increase intestinal regeneration.

Novel and potential future therapies

Exclusive and partial enteral nutrition. EEN, which involves the exclusive use of a liquid diet for a determined period, is used in many countries as standard therapy to induce mucosal healing in children, with complete transmural healing on small bowel imaging in one-third of patients[49]. However, the mechanism by which EEN promotes mucosal healing is not known. In experimental colitis and in vitro studies, EEN has been associated with inhibition of NF-κB via the p38–MSK1 pathway and reduced levels of pro-inflammatory (for example, IL-6, TNF) cytokines in colonic tissues[143,144]. However, a small study of EEN in 12 children with new-onset Crohn's disease did not demonstrate a consistent change in mucosal cytokine profiles after EEN treatment[145]. The effect of EEN is likely, at least in part, achieved through effects on the microbiota: alterations in gut microbiota in association with mucosal healing with EEN compared with mucosal healing with steroids has been reported[146]. However, the available literature regarding the microbiological changes during EEN is inconsistent[147]. Interestingly, several studies have demonstrated a lack of gut microbiota diversity during EEN[148], a finding which is at odds with the current dogma that a high microbial diversity is generally associated with gut health, but is not unexpected owing to EEN containing a paucity of substrates required for gut bacterial growth. A study of faecal metabolomics in

46 children with Crohn's disease who underwent treatment with EEN demonstrated clear differences from 11 healthy children but no relationship to the use of EEN[149]. Alternative non-microbiological mechanisms might be responsible for the anti-inflammatory effect of EEN, for example, reductions in specific amino acids[150]. Although mucosal healing has not been directly studied in EEN and partial enteral nutrition, it can be speculated that part of the mechanism might operate through continued delivery of nutrients to enterocytes with the simultaneous exclusion of food antigens that might interfere with mucosal healing. Delineating the mechanisms by which EEN and partial enteral nutrition could directly promote mucosal healing would be advantageous in better understanding its application in clinical practice. Furthermore, higher-quality studies of its use in adults could provide a non-immunosuppressive therapeutic alternative to a wider range of patients. However, for most patients, adherence to EEN is challenging and it is therefore implemented mainly in children as induction treatment. As such, there are no data regarding the long-term use of EEN. However, the beneficial effects of this treatment seem to extend beyond the period of dietary restriction, with some studies indicating a lower rate of relapse when remission is induced with EEN than with corticosteroids[151].

MSC transplantation. Autologous and allogeneic mesenchymal stem cells (MSCs) have been investigated as non-immunosuppressive therapy for IBD as both an injection of MSCs to treat Crohn's disease fistulae[152–156] and as an intravenous infusion of MSCs for luminal colitis[157–160]. In 2021, locally injectable allogeneic MSC therapy was effective for therapy-refractory ulcerative proctitis in phase II trials[161,162]. Mouse-derived MSCs have been shown to have anti-inflammatory properties and can inhibit T cell proliferation[163], and ex vivo human adipose tissue-derived MSCs reduced T cell cytotoxicity and stimulated the production of regulatory T cells[164]. However, MSCs can also have tissue-regenerative effects: local administration of autologous and allogeneic MSCs has been shown to induce wound healing and downregulate local immune responses in several studies of human fistulizing perianal Crohn's disease[165]. MSCs can differentiate into epithelial cells, myofibroblasts and fibroblasts that contribute to gut regeneration[165]. Furthermore, MSCs promote regeneration by producing several pro-angiogenic factors and altering immune cell functions such as promoting the conversion of M1 macrophages to the M2 type[165]. However, systemic administration has shown less promising results, with some patients benefiting from the therapy yet others developing a worsened disease course[165]. The exact mechanism by which the observed therapeutic benefit with MSCs occurs is unknown[165]. Controversy exists about the location and persistence of MSCs after infusion, which is likely important as there might be an alteration in the properties of MSCs depending on their environment[165]. To overcome some of the technical challenges in administering MSCs, including their initial accumulation in the lungs due to their large size relative to lung capillaries, several studies have explored the use of bioactive factors of MSCs, including MSC-conditioned medium, extracellular vesicles, exosomes and MSC cell surface proteins, in various IBD models with some success[166]. Harnessing the capacity of MSCs to regenerate damaged mucosa might expand the potential of this therapy[167].

Organoid culture engraftment. ISCs have the remarkable capacity to generate a 3D mini-gut in vitro that closely resembles the complex architecture of the intestinal epithelium observed in vivo[168,169]. These stem cell-driven and self-renewing mini-guts are called organoids and are comprised of stem cells, proliferative cells and differentiated IECs[170]. Organoid culture has proved a valuable tool for in vitro research into IBD, and their full potential is only beginning to be realized. Several groups have demonstrated the possibility of engrafting organoid-derived gut stem cells into damaged colon, resulting in tissue regeneration[171–173]. It has been shown that, after rectal transplantation of organoid culture material into the *Rag2*$^{-/-}$ DSS-colitis model, donor cells were able to regenerate intestinal crypt structures and re-establish the intestinal epithelial barrier function[170,171]. There are many challenges to establishing such a treatment, including the endoscopic technique required to deliver the organoids, the optimal clinical-grade culture system and the persistence of pathogens in the organoid culture. In addition, whether organoid culture in IBD tissue will be as successful as with non-IBD tissue-based organoids and whether autologous transplantation (immunologically simpler) would have advantages over allogenic transplantation (with the advantage of transplanting tissue lacking the genetic tendency to IBD) also need to be determined. Furthermore, the stability of the karyotype in culture will need to be defined to assess the risk of tumorigenesis. In addition, dislodgement of the grafted cells due to faecal flow in patients with active IBD and diarrhoea might also abrogate successful engraftment. Nonetheless, generating new tissue in culture that might then be grafted onto areas denuded of mucosa presents an attractive future concept.

Intestinal growth factors. Teduglutide is a more stable analogue of glucagon-like peptide 2 (GLP2), an enhancer of small bowel epithelial cell proliferation, used to treat patients with short bowel syndrome (SBS)[174,175]. In animal models, teduglutide stimulates intestinal blood flow[176], causes crypt proliferation that results in increased crypt length, villus height and mitotic index[174,175], decreases apoptosis of enterocytes[177,178], stimulates colonic growth[179], causes decreased intestinal permeability[180], and is associated with enhanced intestinal epithelial barrier function[181] as well as with enhanced intestinal fat absorption in vivo[182]. Thus, teduglutide could, theoretically, also be used in IBD to enhance mucosal regeneration after inflammatory injury. Teduglutide and GLP2 have been studied in a variety of IBD animal models, including the DSS[183–192], TNBS[187,190,191], human leukocyte antigen-B27 (HLA-B27)[193,194], TNF–actinomycin D-induced mouse[195], IL-10 knockout[196] and radiation-induced murine colitis[197] models, and demonstrated positive effects. However, the only randomized controlled trial of teduglutide in IBD, which examined its effect in

71 patients with active Crohn's disease failed to show a statistically significant difference from placebo although a positive trend was observed[198]. For the most part, patients with IBD were excluded from the original trials of teduglutide in SBS because of concerns regarding the risk of disease exacerbation with this drug[199]. However, several case studies have now reported the successful use of teduglutide combined with biologic agents in patients with SBS secondary to Crohn's disease, suggesting that exacerbation of Crohn's disease might not be a risk[199,200]. Furthermore, there are case reports of the use of teduglutide in patients with Crohn's disease and malabsorption who did not meet the criteria for a diagnosis of SBS but who were able to stop parenteral nutrition with its use, including a patient in whom fistula closure also occurred during teduglutide treatment[201].

Other intestinal growth factors that enhance epithelial repair and wound healing through angiogenesis, cellular proliferation and differentiation have been explored as treatment options for IBD. For Crohn's disease, these include subcutaneous growth hormone, which was tested in 37 adults with active Crohn's disease with positive effects on clinical disease activity scores[202]; epidermal growth factor enemas in ulcerative colitis (24 patients randomized 1:1 to receive epidermal growth factor or placebo with positive effects on clinical remission scores)[203]; two open-label studies examining granulocyte colony-stimulating factor (filgrastim) in active Crohn's disease (the first with 12 weeks of treatment in 5 patients with endoscopically active Crohn's ileitis with positive effects on mucosal healing and the second in 20 patients with clinically active Crohn's disease where positive effects were shown on clinical disease activity score)[204,205]; and granulocyte–monocyte colony-stimulating factor (sargramostim) in active Crohn's disease[206,207], where a Cochrane review reported on 3 studies including 537 patients and found no evidence of superiority over placebo in achieving clinical remission, although the quality of the evidence was judged to be low. In ulcerative colitis, a randomized controlled trial of subcutaneous keratinocyte growth factor (repifermin) in 88 patients with active disease showed no difference in rats in clinical remission[208]. Intestinal growth factors, such as keratinocyte growth factor and epidermal growth factor, have also been explored as a treatment option for IBD[209,210]. Further exploration of intestinal growth factors, particularly in studies in which mucosal healing is directly assessed, could provide tools to attain and maintain mucosal healing and therefore preserve gut function.

Myosin light chain kinase. MLCK is a protein kinase that phosphorylates the regulatory light chain of myosin II[211]. These enzymes are an important part of the mechanism of muscle contraction whereby the phosphorylation of MLC enables binding to the actin filament[212,213]. This mechanism is the main pathway for the regulation of smooth muscle contraction, including in the gut[214]. In addition, MLCK has a role in maintaining the gut barrier through effects on the tight junction. Activated MLCK catalyses the phosphorylation of MLC, which in turn results in the contraction of peri-junctional actinomyosin filaments, a mechanism for tight junction opening[215,216]. Moreover, it has been shown in CaCo cell lines that MLC phosphorylation via MLCK increased tight junction permeability and breakdown of the tight junction structural proteins ZO-1 and occludin[215]. Abnormal elevation and activity of MLCK have been observed in human IBD (associated with histological evidence of disease activity graded as inactive, mild, moderate or severe according to standard criteria[217]). Abnormal elevation of MLCK has also been described in animal models of colitis[218,219]. Increases in tight junction permeability through IL-1β-mediated increases in MLCK expression has been demonstrated to affect brain microvascular endothelial cell permeability in mice[220].

Promotion of regulatory T cells. Low-dose IL-2 therapy is known to selectively activate regulatory T cells[221], making it a promising therapy for IBD and many autoinflammatory diseases. Indeed, many IL-2 agonists and muteins are under evaluation for drug use. One example is Efavaleukin Alfa, for which phase II trials for ulcerative colitis are ongoing[222]. The exploitation of IL-2 as a target initially in the field of oncology and now in autoimmunity has been limited by the short half-life[223] and adverse effect profile (including pulmonary vascular leakage and induction of T cell anergy) of the original compounds. However, advances in bioengineering might yet enable harnessing of the pro-regulatory effects[224,225].

Extracellular matrix. The extracellular matrix (ECM) acts as a central regulator of gut regeneration and healing. Indeed, many studies have suggested that this highly structured part of the gut wall has an important role in the pathogenesis of IBD[226]. A study published in 2019 showed that oral DSS challenge to *Drosophila* causes primary damage to the basement membrane in the gut and subsequent removal leads to regeneration of the matrix[227]. These results suggest that ECM damage and regeneration are important parts of bowel wall regeneration, probably interacting with cell regeneration. Matrix metalloproteinases are important for the remodelling of ECM components[228], with enhanced expression of metalloproteases mediating matrix remodelling in patients with IBD[229,230]. Thus, medical approaches affecting the ECM, and its regeneration after injury, might be potential therapies in IBD. The use of stem cells, engraftment of cultured cells, the application of intestinal growth factors, enhancing barrier function and promotion of regulatory immune responses targeting the ECM are just a few of the wide range of potential treatment targets identified in the process of mucosal healing (FIG. 4). Future treatments should be developed to further harness the healing mechanisms contained within the human gut.

Risks

There might be risks associated with enhancing regeneration and mucosal healing; excessive stimulation of regenerative pathways could enhance neoplastic potential. In the carcinogenicity studies of teduglutide, mainly conducted in rats, benign neoplasms were observed,

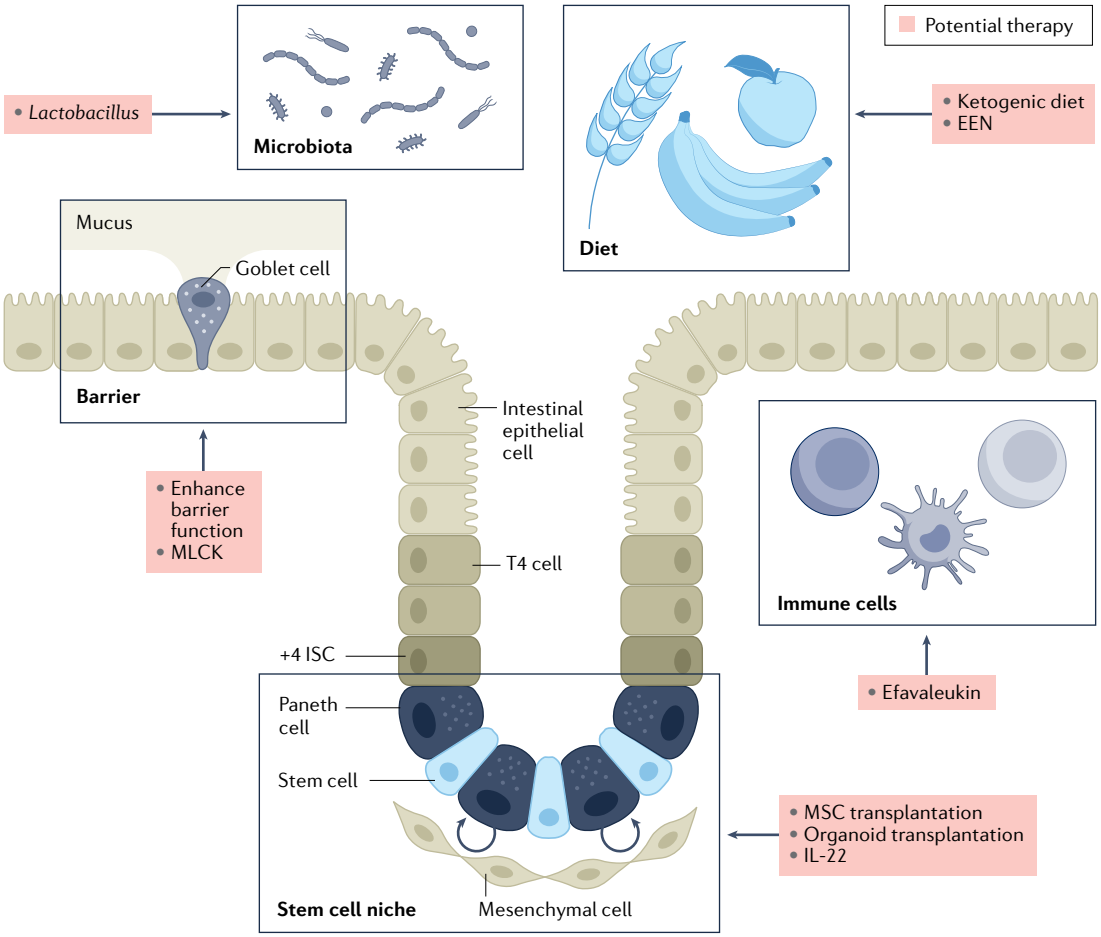

Fig. 4 | **Novel and potential future therapies targeting mucosal healing.** Intestinal mucosal healing requires the coordinated action of cellular and molecular components. These components, such as the microbiota and stem cell niche, could be exploited to promote mucosal healing. Coloured text and boxes represent potential therapies and the respective cellular and/or molecular targets to promote mucosal healing. EEN, exclusive enteral nutrition; ISC, intestinal stem cells; +4 ISC, intestinal stem cells at position 4; MLCK, myosin light chain kinase; MSC, mesenchymal stem cell.

albeit only with plasma concentrations 10–155-fold higher than those observed in humans with daily administration of teduglutide[174]. Thus, there is hope that adverse effects of tissue regeneration and healing may occur at doses higher than those required to achieve a therapeutic effect.

Another risk of directly promoting mucosal healing is that regenerative and fibrosis pathways overlap. Thus, the promotion of fibrosis could be a major barrier to the therapeutic targeting of mucosal healing. Mongersen is an experimental IBD drug that restores TGFβ1 function in the gut[231]. TGFβ1 is known to be anti-inflammatory and profibrogenic; hence, possible fibrotic complications were closely monitored in clinical trials[231]. However, such effects were not observed in phase I clinical trials in humans[231,232]. Similarly, a phase III trial in 701 patients with active Crohn's disease did not demonstrate a better therapeutic effect than placebo; although exacerbation of Crohn's disease was reported as an adverse outcome in some patients, a specific effect causing fibrotic complications was not reported[233]. In a TNBS-mediated colitis-driven intestinal fibrosis mouse model, mongersen reduced the degree of intestinal inflammation and fibrosis[231]. Indeed, it can be speculated that effective support of mucosal healing that quickly restores barrier function and therefore prevents further immune activation is likely to reduce fibrosis compared with prolonged inefficient repair along with clinically or subclinically active inflammation. In a TNBS colitis model, oral administration of a SMAD7-specific antisense oligonucleotide was not associated with intestinal fibrosis; rather, collagen deposition and fibrosis were both reduced[234]. Solutions to the problem of inducing fibrosis through targeting mucosal healing might be achieved through local targeting of treatments or specific dosing.

Conclusions

The treatment of IBD has relied almost exclusively on immunosuppression. Many of these drugs, particularly steroids and immunomodulators, have broad immunosuppressive effects leading to infectious and neoplastic adverse events. The advent of more specific immunosuppressive drugs, such as vedolizumab, broadens therapeutic options even in patients at higher risk. Nevertheless, rebuilding the mucosa without immunosuppression

is a valuable goal in future IBD therapy. Technological advances now provide tools that permit transplantation and engraftment of cells and tissue capable of regenerating damaged mucosa. Harnessing the power of growth factors and non-cellular components of the gut mucosa will enable the promotion of in vivo mucosal restitution. Delineating the nutritional requirements of a regenerating mucosa and optimizing the delivery of that nutrition could facilitate mucosal healing and potentially complement other treatments.

Published online 19 April 2022

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Acknowledgements
The authors thank members of the Villablanca lab for helpful comments. E.J.V. was supported by grants from the Swedish Research Council, VR grants K2015-68X-22765-01-6 and 2018-02533, Formas grant no. FR-2016/0005, Cancerfonden (19 0395 Pj), and the Wallenberg Academy Fellow program (2019.0315). K.S. has received support from The Swedish Medical Association (Svenska läkarsällskapet). C.R.H.H. gratefully acknowledges support from Julins Foundation, Bengt Ihre Fellowship, The Swedish Medical Association (Svenska Läkarsällskapet), the Calder Foundation, an ECCO project grant, Gastrofonden, MagTarm fund, Professor Nanna Svartz Fund and a clinical post-doc from Region Stockholm.

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
E.J.V. has received research grants from F. Hoffmann-La Roche. C.R.H.H. has received speaker fees from Takeda, Ferring, AbbVie, and Janssen and consultancy fees from Pfizer. She has acted as local principal investigator for clinical trials for Janssen and GlaxoSmithKline. She has received project grants from Takeda and Tillotts. K.S. declares no competing interests.

Peer review information
Nature Reviews Gastroenterology & Hepatology thanks Silvio Danese and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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