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# Gut microbiome mediated macrophage alterations in the intestine of IBD patients

Writing Assignment

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## Layman summery.

In Europe 2.5-3.0 million people are diagnosed with inflammatory bowel disease, or IBD. These patients often suffer from a chronic inflamed gut with symptoms ranging from vomiting to abdominal pain. We do not know precisely what causes IBD, even though it is clear a major part of the symptoms can be explained due to a dysregulated immune system. Normally the immune system plays a very important role in watching out for pathogens invading the body. When it finds an infection, it is actively working on eliminating the infection, and whenever the source of infection is removed it goes back to a more quiet stage. In people with IBD, the immune system is either turned up even when it shouldn't be, or not turned down after the infection is gone. Although we don't have a cure for IBD yet, we know some cells of the immune system that are dysregulated in IBD patients. Of particular interest are macrophages as these cells are involved in both the immune response and the resolution of it.

Traditionally, macrophages are divided into two different subtypes, termed M1 and M2. M1 macrophages are associated with inflammatory functions and are essential to eliminate pathogens. M2 macrophages, on the other hand, have mostly anti-inflammatory functions and are involved in tissue repair and removing debris. The signals from the surrounding tissues determine whether a macrophage develops into a M1 or M2 macrophage. There are a lot of different signalling molecules and cells involved in this development choice, one of them being butyrate. This is a small molecule made by some of the bacteria of the gut during digestion. Whenever levels of butyrate are high, it in essence signals to the macrophages that everything is going fine and that no harmful substances are nearby. Therefore, macrophages are differentiating into an anti-inflammatory M2 phenotype. Additionally, as macrophages are not required at high levels whenever there is no inflammation, butyrate ensures fewer macrophages are recruited to the intestine. Whenever there are too many pathogenic signals, the effect of butyrate is overridden, and the immune system is turned up, resulting in macrophages now differentiating into an inflammatory M1 macrophage. In patients with IBD the levels of butyrate are substantially lower due to the fact that there are less butyrate-producing bacteria present within the gut. However, butyrate is not the only signal involved in the regulation of inflammation. Therefore, this review summarised the current knowledge of 1) how butyrate affects macrophage development, 2) how this is dysregulated in IBD patients and 3) whether or not butyrate is a potential drug for treating IBD symptoms.

It is clear that IBD is a complex disease and only targeting the dysregulated inflammation and one cell type of the immune system is not enough to treat IBD entirely. However, we do see a slight improvement of the quality of life and less severe signs and symptoms following butyrate treatment. Therefore, even if butyrate on its own may not cure IBD, it has a potential to be used in combination with other therapies.

Review

# Microbiome-mediated macrophage alterations in the intestine of IBD patients

*Anne Floor Holtrop*

*Intestinal dysbiosis and a dysregulated immune system has been well recognized in the aetiology of inflammatory bowel disease (IBD). However, mechanisms and pathways involved remain elusive. Accumulating evidence suggests that bacterial metabolites might greatly impact the immune regulation of the intestine. Butyrate, a short chain fatty acids (SCFA), which is metabolized by the gut bacteria from otherwise indigestible fibre-rich diets, has been shown to ameliorate IBD symptoms. Although the exact mechanisms remain partly understood, it is known that a major drop in butyrate-producing bacteria is associated with the increased pro-inflammatory environment. The increased amount of inflammatory cues facilitate macrophages to differentiate into a pro-inflammatory phenotype. Hence, enhancing butyrate levels in therapy could be a potential treatment for IBD. This review summarised the current knowledge on how butyrate affects intestinal macrophages, in terms of recruitment and phenotype polarization. With the incidence of IBD outpacing death and global gains in life expectancy, newly diagnosed IBD patients' cases are being added to the pool of prevalent individuals on a continuous basis. Therefore, broadening our knowledge about different pathways is essential for individualized treatment planning and improving the quality of life of IBD patients.*

***Keywords: SCFAs, IBD, immune cells, macrophage, polarization, dysbiosis***

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## Introduction.

Inflammatory bowel disease (IBD), comprising of both ulcerative colitis (UC) and Crohn's disease (CD), is a persistent and recurrent inflammatory disease of the gastrointestinal tract (GIT) that already affects 2.5-3.0 million people in Europe alone.[1] Inflammation has a cobblestone-like pattern, which means that inflamed patches are interrupted by healthy tissue. For UC, this is primarily restricted to the intestinal mucosa, whereas in CD can affect any section of the GIT from the mouth to the anus.[2] Conventional therapies for IBD, such as 5-aminosalicylates (5-ASA), corticosteroids, immunosuppressives, or biologics,[3] are primarily focused on inducing and sustaining remission in order to alleviate secondary complications, rather than addressing the underlying pathogenic process (as reviewed in 4). As diminished intestinal epithelial integrity and persistent inflammation are the primary indications of IBD severity, early research focused on the immunological pathways in IBD patients and how they differed from healthy individuals. However, because a dysregulated immune system alone cannot account for all of the clinical phenotypes observed in IBD, current research is moving toward the notion that immunopathogenic processes are only one part of disease aetiology and must thus be evaluated in the context of other factors (**Fig 1**),[2] including genetics, environmental stimuli and altered intestinal microbiota composition.[5]

The GIT contains the largest compartment of the immune system of any tissue within the body, as it is continuously exposed to a wide range of foreign antigens and other environmental agents. During an infection, a large number of immune cells, such as macrophages, are recruited to neutralize or destroy microorganisms. If not appropriately controlled, this can pot-

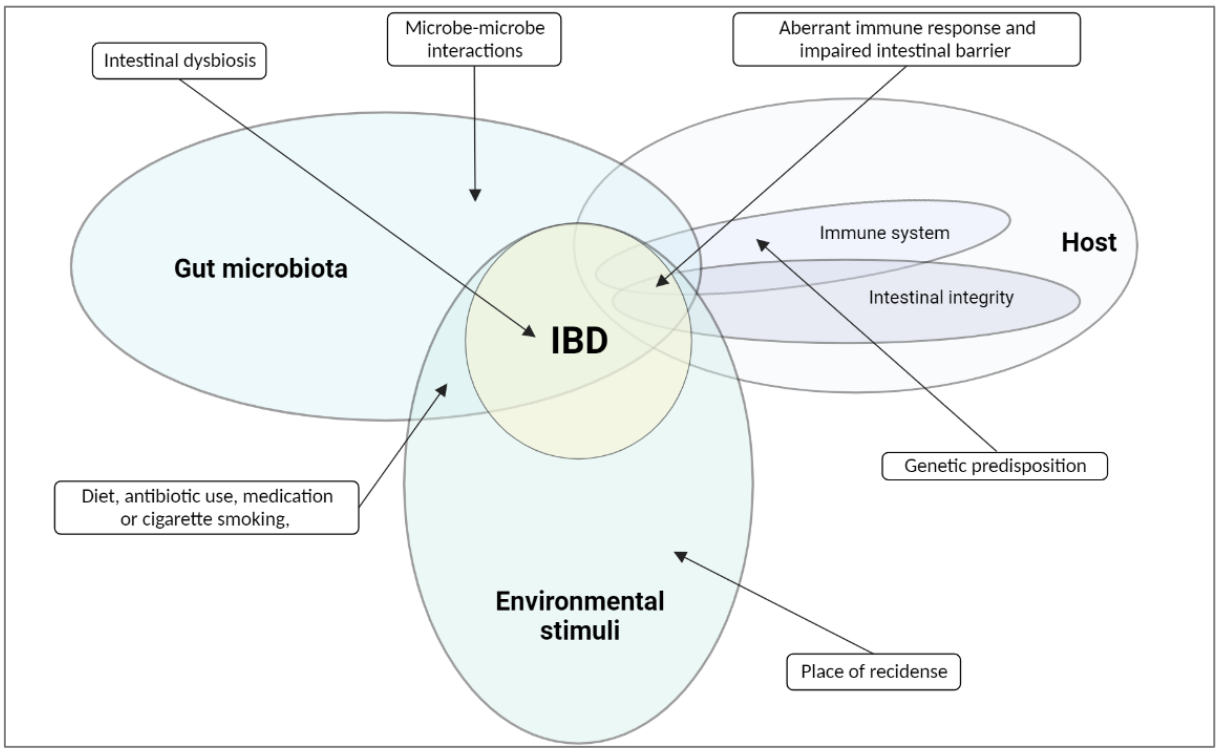


*Figure 11: A Venn diagram visualizing several interactions between the gut microbiota, host and environmental factors in the pathogenesis of inflammatory bowel disease.* The gut microbiota, the environment and the host influence and modulate each other to form a physiological balance.[21] In IBD this is disturbed, leading to e.g. dysbiosis or an aberrant immune response. Adapted from Wu *et al*. (2021).[68] Figure made with Biorender.

entially induce host cell damage. As a result, there is a trade-off between eliminating the pathogen and avoiding host tissue harm. Hence, a proper and dynamic balance of pro-and anti-inflammatory pathways exists and changes over time (**Fig 2ab**), which is distorted in IBD (**Fig 2c**).[2] Several lines of research show that this distortion is affected by both the composition and chemical signalling of the intestinal microbiota.[6] Although it remains elusive whether changes in the (relative) numbers of the intestinal microbial population or its secretome (i.e. dysbiosis) precedes inflammation or vice versa.

The microbial species associated with the gut of an IBD patients facilitates a more inflammatory environment. Part of the increased inflammatory cues in IBD are associated with a major drop in butyrate-producing bacteria.[7–10] Butyrate is a short-chain fatty acid (SCFA) produced during anaerobic fermentation of dietary fibres by the intestinal microbiome,[11] affecting a range of host immune cell types, including macrophages. Whenever butyrate-levels drop during intestinal dysbiosis the conditioning of macrophages towards a more anti-inflammatory phenotype is distorted. However, the underlying molecular mechanisms of how butyrate alters this process are only partly understood. Therefore, the purpose of this review is to summarize the current understanding of the relationship between butyrate and how alternations of its concentration impacts macrophage functionality within the intestine in the context of IBD. The first part will discuss how intestinal dysbiosis is related to the clinical symptoms as seen in IBD, zooming in on the role of butyrate. Lastly, this review offers further speculation on the mechanisms underlying the effects of butyrate on macrophage functionality, mainly in terms of motility and polarization.

## Intestinal dysbiosis exacerbates pro-inflammatory environment.

The term "microbiota" refers to the diverse collection of microorganisms, including bacteria, fungi, viruses, archaea and protozoa, found in the human GIT.[12] It is engaged in metabolizing dietary materials into bioactive food components,[13] vitamin synthesis,[14] fostering immunological maturation and metabolic homeostasis.[15] The composition of one person's microbiota is in constant flow under the influences of both internal and external variables, such as diet, ingested drugs, immune system activity and concentrations of microbial products.[16] Due to high α- and β-diversity (i.e. within one sample and between samples, respectively), there is no one microbial profile that all healthy people share.[17] Nevertheless, at the phylum level the intestine of a healthy gut is predominantly composed of the *Bacillota*

and *Bacteroidota* phyla, followed to a much lesser extent by the *Actionmycetota* and *Pseudomonadota*.[10,18] Whenever there is a loss of beneficial microbiota's local distribution, complexity, diversity or functional composition, the ratio of "pathogenic" to "protective" bacteria shifts towards the former,[19] also referred to as intestinal dysbiosis. Whether dysbiosis causes inflammation or vice versa, evidence points towards the fact that dysfunctional microbiota-immune interactions aggravate resolution of (chronic) inflammation in IBD. This was confirmed via the use of animal models in which germ-free mice displayed dramatically reduced disease severity or delayed onset of dextran sulphate sodium (DSS) induced colitis.[20] On the other hand, several of the risk factors for developing IBD or a greater severity of IBD progression are related to major microbiota-remodelling,[21] such as antibiotic and probiotic therapy,[12,22] cigarette smoking, and dietary alterations.[12]

Intestinal dysbiosis in IBD leads to a shift towards a pro-inflammatory environment with activated immune cells, which is often accompanied by defects in the epithelial barrier. Breach of this mucosal barrier may result in unlimited passage of microbes and foreign antigens into the lamina propria,[23] further facilitating the pro-inflammatory environment. Although neither UC nor CD is identified by an unique microbiota composition profile, there are multiple overlapping alterations documented in research compared to healthy individuals.[24,25] The most consistent observation has been a decrease in the variety of the *Bacillota's* "protective" commensal bacteria,[7–10] which has been linked with a reduced capability to regulate both inflammation and the corresponding resolution. This is predominantly due to decreased relative numbers of butyrate-producing bacteria of the *Bacillota's* phylum,[7–10] such as *R. hominis*, [26,27] *F. prausnitzii*,[28,29] and the *Clostridium IXa* and *IV* groups.[9] Furthermore, decreased abundance of the members of the *Bacteroidota* phylum,[9] and an increase in the *Pseudomonadota* populations have been described in several studies.[9,10] Moreover, numbers of facultative anaerobic bacteria of the more "pathogenic" *Enterobacteriaceae* family were found to be increased in the intestine of IBD patients as well.[30]

To summarise, intestinal dysbiosis disrupts the integrity of the intestinal barrier leading to translocation of foreign antigens into the lamina propria. A subsequent inflammatory response of the host immune system enhances progression of IBD symptoms. Part of the increased inflammatory cues in IBD are associated with a major drop in butyrate-producing bacteria,[7–10] which will be discussed in more detail in the next chapter.

## Butyrate levels facilitate a more anti-inflammatory environment.

Intestinal dysbiosis in IBD patients is accompanied by an imbalance in the production of microbial metabolites, such as short-chain fatty acid (e.g. butyrate), secondary bile acids and tryptophan.[31] In a healthy intestine, SCFA are produced by anaerobic fermentation of dietary fibres. These chemicals have a number of impacts on the host's metabolism and immune system. The three major SCFAs produced are acetate, propionate and butyrate, which are aliphatic carboxylic acids containing 1 carbon in the carboxylic function and respectively 1, 2 and 3 carbons in the aliphatic tail.[11] For all human colonic regions (i.e. ascending, distal, sigmoid and transverse regions), the relative molar ratios for acetate, propionate and butyrate are 60-20-20, respectively.[32,33] Both butyrate and propionate are predominantly absorbed by colonic epithelial cells and thereby provide energy for vital activities, whereas acetate has a more systemic function.

Butyrate is by far the most extensively studied SCFA, as it affects a variety of processes within the intestine. First of all, butyrate is mainly known as a fuel for colonocytes,[34] and due to its involvement in multiple pathways related to the maintenance and repair of the intestinal barrier.[35] Without butyrate, epithelial cells switch their metabolism from β-oxidation to anaerobic glycolysis, resulting in lower oxygen consumption,[32] resulting in an increased abundance of *Enterobacteriaceae*.[36] *Chang et al.* (2014) demonstrated that in the presence of these bacteria and a decline in butyrate-producing bacteria, lamina propria macrophages modify the intestinal microbial communities by removing undesired populations of bacteria via the release of proinflammatory mediators. They contended that, until the right microbial balance is restored and butyrate levels return to normal, this pro-inflammatory milieu might act as a trigger for the intestinal immune system to shift to either a more M1 phenotype in an attempt to regain homeostasis in the gut.[37] Lastly, butyrate has reported to act as a histone deacetylase (HDAC) inhibitor,[38] meaning it can modify gene expression and alter the epigenetic landscape of its target cells. Hence, it is no surprise butyrate plays a pivotal role in the regulation and modulation of the human inflammatory responses and affects a range of host immune cell typs.[39]

As a reduction of butyrate remodels the gut microbiota composition via both its impact on the oxygen levels and on the local immune responses, it is plausible

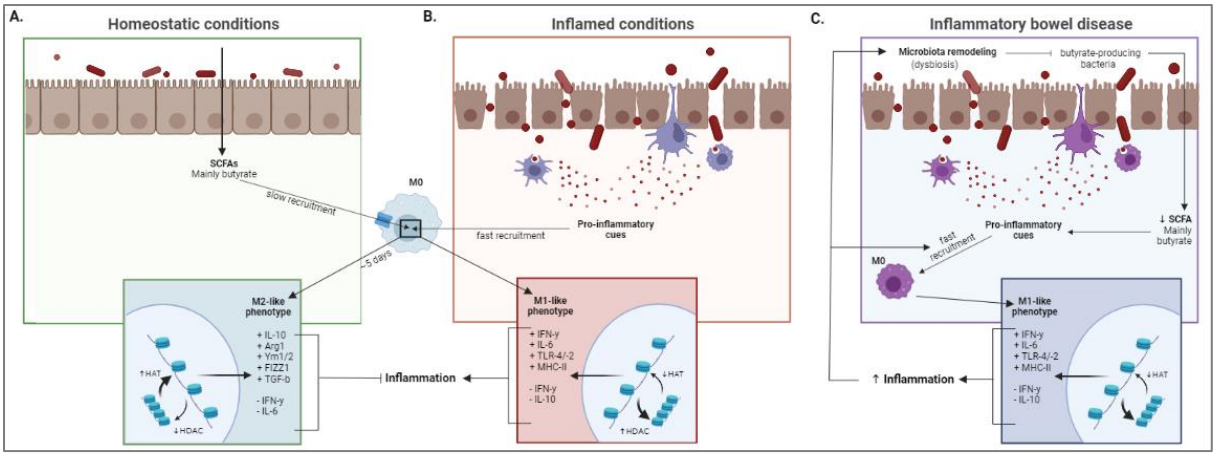


*Figure 2: simplified schematic overview of macrophage polarization and recruitment under different conditions.* **(A)** under homeostatic conditions, the gut microbiome produces SCFAs, particularly butyrate. Butyrate ensures a slow recruitment of monocyte-derived macrophages from the bloodstream and conditions the ones that do enter the intestine in around 5 days towards an M2-like phenotype. As butyrate functions as a HDAC, the epigenetic landscape changes and there is upregulation of several M2-related markers (e.g. Il-10, Arg1, Ym1/2, FIZZ1, TGF-b) and downregulation of M1-related markers (e.g. IFN-γ and IL-6). Under more proinflammatory conditions **(B)** there is a faster recruitment of M0 macrophages, which are subsequently conditioned into an M1-like phenotype. These TLR-4/-2 responding macrophages ensure a more inflammatory environment until the pro-inflammatory cues are removed. **(C)** There are increased amounts of pro-inflammatory signals in IBD, which keep the intestine inflamed, similarly as in (B). However, when inflammation develops, microbiota remodelling occurs, increasing dysbiosis. This in turn reduces the relative number of butyrate-producing bacteria, which restarts the inflammation cycle. *Abbreviations used*: SCFA, short-chain fatty acid; IL, interleukin; Arg1, arginase 1; FIZZ1, inflammatory zone protein 1; TFG-b, transforming growth factor beta; IFN, interferon; TLR, Toll-like receptor); MHC-II, major histocompatibility complex II; HAT, histone acetyltransferases; HDAC, histone deacetylases. Adapted from Deleu *et* al. (2021).[69] Created with BioRender.

that butyrate supplementation could ameliorate the course and/or progression of IBD. Indeed, there have already been several clinical trials that investigated potential benefits of either increased levels of butyrate or butyrate-producing bacteria.[40–43] First of all, a pilot trial of Hallert *et al.* (2003) demonstrated that specifically increasing faecal butyrate levels diminishes intestinal inflammation and severity of abdominal pain in patients with UC.[43] Second, in another (double-blind and placebo-controlled) pilot study the group of Facchin *et al.* (2020) found that oral butyrate-treatment was associated with a significant increase in the quality of life (QoL) for both UC and CD patients.[41] QoL improvement was mostly related with an increase in SCFA-producing bacteria in UC patients and a large rise in butyrogenic colonic bacteria in CD patients. Lastly, another trial demonstrated that butyrate-treated individuals with UC showed prolonged remission.[40]

To summarise all of the above, the clinical trials so far demonstrate that oral butyrate or dietary fibre intake reduces inflammation and clinical symptoms of IBD patients. A plausible hypothesis is that consuming butyrate or dietary fibre raises the luminal concentration of butyrate, which promotes the proliferation of "beneficial" bacteria within the intestine. Although the underlying molecular pathways of how butyrate facilitates a more anti-inflammatory environment remain incompletely understood, it is clear butyrate facilitates a more anti-inflammatory. One possible mechanism could be the way butyrate attenuates macrophage polarization and recruitment. Especially taken into account macrophages are both involved in the anti-inflammatory response in IBD remission and in active colitis. The current understanding of these pathways will be discussed in more detail in the next chapter.

## The interplay between intestinal macrophages and butyrate levels.

Macrophages are white blood cells of the innate immune system that are known to phagocytose and digest pathogens such as cancer cells, bacteria, and cellular debris. Aside from phagocytosis, they serve important functions in intestinal homeostasis and a variety of physiological processes, including metabolism, tissue healing, and tissue remodeling.[44] Depending on the stimuli within the resident tissue, macrophages can display different functional phenotypes and migration properties. Traditionally, macrophages can be classified into non-activated (M0), pro-inflammatory (M1), or anti-inflammatory (M2) subsets.[45] Nonetheless, it should be recognized that, as we have come to better understand the function of macrophages, this M1/M2 paradigm simply does not

reflect reality. Macrophage phenotypes are therefore more accurately represented by a continuum, rather than a binary system.

During IBD progression, the balance between inflammation and resolution is distorted and tilted towards chronic inflammation.[46] Indeed, a defect in either M2 polarization or the associated cytokines has been shown to increase the severity of (DSS-induced) colitis, whereas adoptive transferring of M2 macrophages or induction of M2 polarization has been shown to reduce the severity of experimental colitis.[46] As it is known that butyrate levels facilitate a more anti-inflammatory environment, this chapter will discuss the current understanding of how butyrate levels impacts both macrophage polarization and recruitment.

## Butyrate conditions macrophages towards an anti-inflammatory phenotype.

Under homeostatic conditions (e.g. healthy or during remission of IBD), newly arriving inflammatory monocytes are gradually conditioned *in situ* into the phenotype associated with resident macrophages (i.e. M2-like) in around five days (**Fig 1a**).[47,48] Tissue resident M2-like macrophages (a.k.a. alternatively activated macrophages) are characterized by, among others, the expression of interleukin 10 (Il-10), tumour necrosis factor β (TNF-β) ,[48] resistin-like-α (or FIZZ1), arginase 1 (ARG1) and chitinase 3-like 3 (or Ym1).[49] This combination safeguards a hypo-responsiveness to Toll Like Receptor (TLR-) mediated activation.[48] Within a more inflammatory environment due to recognition of invading microorganisms or foreign antigens, monocyte-like macrophages develop into an M1-like phenotype that are TLR-responsive (**Fig 1a**, right panel). M1 macrophages (a.k.a. classically activated macrophages) are important effectors in inflammatory processes, in which they produce pro-inflammatory mediators like tumour necrosis factor α (TNF- α), IL-1α, IL-β, IL-6 and nitric oxide (NO).[50,51] Hence they are avidly phagocytotic and are highly involved in antigen presentation to activate the adaptive immune system.

The M1/M2 balance shifts towards the former during the prolonged presence of inflammatory cues in the colon, as seen during active colitis in IBD patients (**Fig 1c**). Butyrate has been extensively studied for its affect on this M1/M2 balance, as higher levels have been associated with an M2-skewed macrophage population (i.e. mimicking the situation in a more healthy gut). First of all, Ji *et al.* (2016) demonstrated that butyrate can of alter the epigenetic landscape of macrophages, thereby increasing the IL-4 induced expression of M2 markers (e.g. Ag1, Fizz1 and Ym1) in bone marrow-derived macrophages (BMDMs). They provided a molecular mechanism of butyrate-mediated protection against DSS-induced colitis in which butyrate enhanced STAT6 phosphorylation partly via inhibiting *histone deacetylase inhibitor 1* (HDAC1) gene expression and increasing histone 3 lysine 9 acetylation (H3K9) acetylation.[52] Besides upregulating M2-associated markers, butyrate-treatment results in an enriched transcription profile beneficial for killing pathogens.[53] However, as mentioned before, the conditioning of M0 into M2-like macrophages takes around 5 days. Indeed, the same epigenetic changes can not be replicated by short-term (~3h) exposure, as effects were quickly lost following withdrawal.[38] Alongside epigenetic changes, butyrate induces a massive metabolic shift in intestinal macrophages from glycolysis to both oxidative phosphorylation and lipid metabolism.[53] This is evidenced by metabolomic investigations that demonstrate significant reductions in levels of glycolysis proteins such as succinate, trans-aconitate, and citrate,[54] even though the exact molecular mechanism remains elusive.

Evidence shows that Yes associated protein (YAP), of the Hippo pathway, negatively affects M2 polarization of macrophages and promotes the M1 phenotype.[55] Indeed, Zhou *et al.* (2010) showed that higher YAP expression, of the Hippo pathway, in macrophages deteriorate symptoms of IBD.[56] Even though there is no direct proof that butyrate affects the Hippo pathway in intestinal macrophages specifically, Dai *et al.*, (2019) showed that butyrate stimulates proliferation of intestinal smooth muscle (ISM) cells through this pathway *in vitro*.[57] It is therefore conceivable that they interact in intestinal macrophages in some way. Collectively, butyrate slowly alters the epigenetic landscape of the macrophage population within the intestine towards an anti-inflammatory phenotype and upregulates certain pathways associated with this phenotype.

## Butyrate levels reduces recruitment and migration of intestinal macrophages.

Macrophage mobility remains a key feature in promoting immune defence against infections and inflammation regardless of tissue of residency. Environmental cues, such as cytokine gradients and uniform fields of chemokines, influence the pace of migration, differentiation, and recruitment of macrophages to the site of infection within the periphery. Migration of macrophages into the intestine by chemotaxis and increased cellular adhesion is predominantly mediated by the C-C chemokine receptor type 2 (CCR2).[58–60] During the conditioning steps of monocyte-like macrophage into resident macrophage in the intestine, the CCR2 receptor is down regulated as it is no longer required to actively recruit M0-like macrophages into the periphery.[58]

During acute colitis in IBD, both the mRNA and protein levels of CCR2, and its ligand macrophage chemoattractant protein-1 (MCP-1, also known as CCL2) are significantly increased. Subsequently, there is substantial infiltration of monocyte-derived macrophages.[61,62] Consistent with this observation, DSS-induced colitis is attenuated in mice after either neutralization or deletion of CCL2 or CCR2.[58,59] The fact that CCR2-deficient animals do not develop experimental colitis due to faulty monocyte recruitment underlines the fact that the pathogenic function of newly recruited macrophages into the gut periphery.[48] However, the exact molecular mechanism remains elusive and warrant further investigation.

As mentioned before, butyrate is a non-competitive, reversible HDAC1 inhibitor, causing macrophage hyperacetylation.[63] Lipopolysaccharide (LPS)-augmented Src and focal adhesion kinase (FAK) transcripts are repressed as a result of epigenomic remodeling.[63] FAK has previously been linked to macrophage motility, with a deletion resulting in a broad motility deficiency impacting chemotaxis and random movement.[64] In summary, these data indicate that butyrate can impact the levels of newly recruited M0-like macrophages into the intestine via several different pathways.

## Discussion.

The pathogenesis of IBD remains incompletely understood. However, it has been more clear over the last few decades that the microbiome, immune system, genetics, and environmental variables form a highly complex interaction network that are all involved (**Fig 1**). Understanding the underlying mechanisms that lead to either the onset or progression of IBD is crucial in order to find new or additional patient therapies.[2,5] Modulation of the microbiota, particularly of butyrate-producing bacteria of the *Bacillota's* phylum, has already shown promising results in small pilot trials in both UC and CD patients.[40–43] We now know that enhanced butyrate levels in IBD patients are associated with further remodelling of the microbiota,[41] prolonged remission,[40] and improved clinical symptoms.[43] Considering macrophages are the most abundant immunological cell type within the intestine, and that is implicated in both pro- and anti-inflammatory environments; this review asked the question whether butyrate-treatment affects intestinal macrophage functionality. Indeed, several complementary studies show that, following butyrate exposure, macrophages can be conditioned over a period of time into a more M2-like phenotype (**Fig 2a**).[47,48] During these conditioning steps, the newly arrived macrophages from circulation are reprogrammed via both epigenetic remodelling and the enhancement of pathways associated with anti-inflammatory properties. At the same time, recruitment of macrophages from the blood stream is reduced via the downregulation of the CCR2/CCL2-axis. On the other hand, whenever butyrate levels are reduced or when there is a breach of the intestinal barrier, macrophages are programmed into a more pro-inflammatory environment over a shorter period of time (**Fig 2b**), which, if not regulated properly, results in many of the symptoms associated with IBD (**Fig 2c**).

From a therapeutic standpoint, targeting the M1/M2 balance as a therapy has the potential to re-establish appropriate interactions between the intestinal microbiota and the immune system which are distorted in IBD.[46] If this could be (partly) realised through maintaining adequate levels of butyrate, this could be a promising and non-invasive strategy. However, the concept of the M1/M2 paradigm within the laboratory relies on a predetermined collection of parameters, which may be considered a major oversimplification that more accurately represents the outsides of the spectrum *in vitro*, rather than the whole spectrum visible *in vivo*. As a result, future studies should concentrate on understanding the interchanging and switching between M1- and M2-like phenotypes, as well as mapping the environmental cues that are involved. Rather than only looking into the induction of M1 and M2 macrophages from M0. Moreover, it is known that a dysbiotic microbial community, once established, substantially affects both the local mucosal and systemic landscape of immune cells, thereby creating a feedback loop in which the host immune system and its microbiota cross-regulate each other. Hence, altering the butyrate-levels may affect the inflammation part of the IBD aetiology and induce microbiota remodelling, but there remains to be little to no research about whether or not the beneficial effects seen in the current trials can be sustained for a longer period of time.

It has been more clear over the last few decades that the microbiome, immune system, genetics, and environmental variables form a highly complex interaction network (**Fig 1**). As a result, therapy that focuses on only one of the numerous factors is more likely to treat a specific group of symptoms rather than the underlying cause. Additionally, it should be noted that the relationship between the gut microbiota and inflammation as seen in IBD is more dynamic than just cause and effect. Ultimately, a deeper understanding of the mechanism of how the interplay between the microbiome and the host is functioning is required to clarify causation or correlation, as timing of both processes is difficult to determine in humans.[65] Despite the fact that butyrate appears to have a significant role in the beginning and development of IBD, it is just one of the components underlying the aetiology (**Fig 1**). Therefore, it is unlikely that butyrate-treatment alone can either prevent or cure IBD. It is more likely that treating IBD symptoms requires a combination of therapies to modulate different pathways

within the intestine. For example, one double-blind and placebo-controlled study already demonstrated that combined therapy of topical 5-ASA and sodium butyrate significantly improved the disease activity than 5-ASA treatment alone.[66]

This review contributed to a better understanding of how butyrate is thought to influence macrophage polarization and migration within the gut and pointed out the idea that it could be beneficial to use butyrate-treatment in combination with other drugs to target different pathways in IBD patients. With the incidence of IBD outpacing death and global gains in life expectancy, newly diagnosed IBD patients' cases are being added to the pool of prevalent individuals on a continuous basis. As a result, the direct and indirect health-care burden of managing IBD in the worldwide population is considerable, and it is predicted to significantly expand in the coming years, putting major strain on both the individuals and the health-care system.[1,67]

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