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Review

REVIEW

Gut microbiota metabolite regulation of host defenses at mucosal surfaces: implication in precision medicine

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Abstract

The gut microbiota has a well-established role in the regulation of host homeostasis. Multiple factors control the composition and function of the microbiota. The westernization of diet, a shift away from nutrient-dense foods toward diets high in saturated fats, has been implicated in the rise of chronic inflammatory diseases such as inflammatory bowel disease (IBD). Diet is critical in the development and maintenance of a healthy microbiome, where dietary fiber (found in the highest amounts in fruits, vegetables, and legumes) is metabolized by the microbiome. In turn, the bacterial metabolites of dietary fiber, short chain fatty acids (SCFAs), regulate gut homeostasis. SCFAs engage G-protein coupled receptors (GPRs) and act as histone deacetylase inhibitors (HDACi) to module epithelial and immune cell functions in the intestines, where they generally promote an anti-inflammatory state. This review highlights the functions of SCFAs and their roles in the pathogenesis of IBD to provide insights into their potential therapeutic application for the treatment of IBD for the purposes of precision medicine.

Key words: microbiota; metabolite; host defense; short chain fatty acids

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD), collectively known as inflammatory bowel disease (IBD), have emerged as a significant health challenge in the twenty-first century. IBD affects millions of people in the United States, Europe, and Asia, with increasing incidence and prevalence worldwide.[1] The role of microbiota in the pathogenesis of IBD is well established; however, the components of the microbiota that are responsible for these effects remain largely unknown. Studies have identified a crucial role for gut microbiota metabolites in modulating intestinal homeostasis and immunity, with dietary fibers and their bacterial fermentation products, short chain fatty acids (SCFAs), playing an essential part.[2,3] Of particular interest are the SCFAs acetate, propionate, and butyrate, which collectively account for >95% of the SCFA population.[4] Their importance to intestinal health cannot be overstated, as SCFAs have been linked to protection against IBD, allergic asthma, and diabetes.[5–8] In this review, we will highlight the role of SCFAs in barrier protection and in the pathogenesis of IBD.

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Formation of SCFAs

The intestines harbor trillions of bacteria that have developed both a mutualistic and symbiotic relationship with their host. The intestinal microbiome plays pertinent roles in maintaining homeostasis, and alterations in the microbiome are associated with chronic inflammatory conditions including IBD, diabetes, obesity, and allergic asthma.[6,8,9] Among various microbiota metabolites important in regulation of host physiology and health, SCFAs (including acetate, propionate, and butyrate) are derived from the bacterial fermentation of dietary fibers, such as inulin, which escape absorption in the small intestines and enter into the colon[4] (Fig. 1). Acetate is the most abundantly produced SCFA, followed by propionate and butyrate in a 3:1:1 molar ratio, respectively.[9] SCFA formation is dictated by both the type of bacteria and type of dietary fiber present in the colon. For example, most bacteria produce acetate which can be derived from acetyl-CoA or alternatively via formate, hydrogen, and carbon dioxide, via the Wood-Ljungdahl pathway.[10] Although many bacteria can produce acetate, propionate production occurs most commonly via the succinate pathway, which requires hexoses and pentoses found in the dominant phylum of *Bacteroidetes*.[10,11] Propionate can also be produced by species such as *Veilonella* using lactate through the acrylate pathway or through the propanediol pathway found in *Roseburia* and *Ruminococcususes*, which use fucose and rhamnose.[10,11] Conversely, butyrate is primarily produced through condensation of a thiol group of coenzyme A with the carboxy group of acetyl-CoA, resulting in butyryl-CoA, which can then ultimately be converted to butyrate.[10,12] There are many butyrate producing species, with the phylum *Firmicutes* being the primary producer in the human colon.[13]

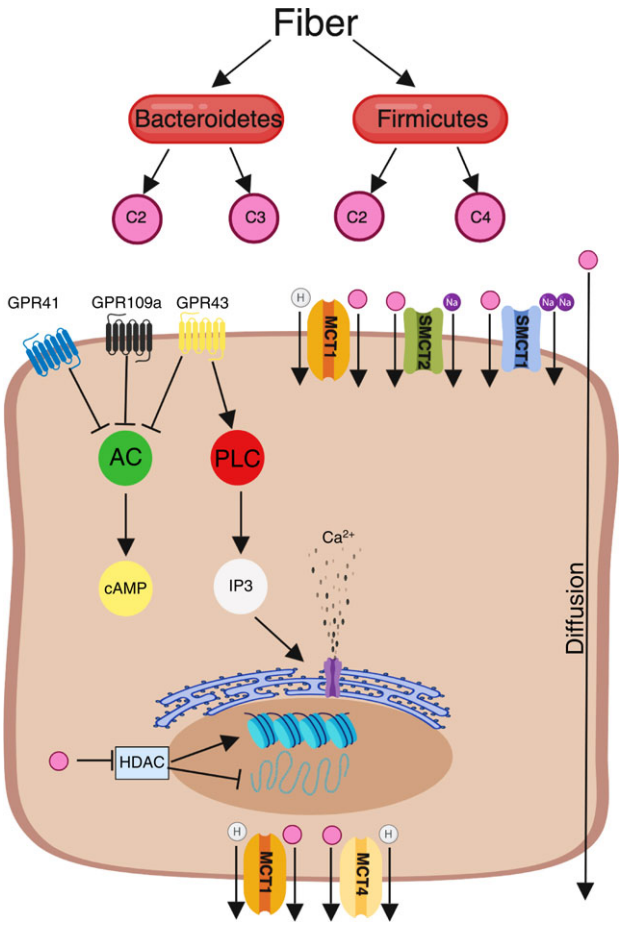

Figure 1. Formation, transport, and mechanisms of action of short chain fatty acids (SCFAs). C2, acetate; C3, propionate; C4, butyrate; HDAC, histone deacetylase; AC, adenylate cyclase; cAMP, cyclic AMP; PLC, phospholipase C; IP3, inositol triphosphate; MCT, monocarboxylate transporter; SMCT, sodium coupled monocarboxylate transporter.

Transport of SCFAs

SCFAs, particularly butyrate, provide colonic cells with 80% of their daily energy supply and thus appreciable quantities are not found in the portal vein.[14] This is in contrast to acetate and propionate, which are primarily taken up by colonocytes and transported into the portal vein for metabolism in peripheral tissues such as muscle.[4] SCFA absorption occurs by three mechanisms: passive diffusion, electroneutral, or electrogenic uptake[15] (Fig. 1). The charge of a SCFA determines whether its uptake occurs via passive diffusion or a carrier mechanism. For example, passive diffusion of SCFAs is primarily seen when SCFAs are in the protonated form; this is a major mechanism of SCFA transport at physiological pH.[16] In contrast, SCFAs in anion form are dependent on carrier-mediated uptake, which can occur through four primary transporters. Monocarboxylate transporter 1 (MCT1) and MCT4 are electroneutral transporters, which rely on hydrogen[16] in contrast to sodium coupled monocarboxylate transport 1 (SMCT1) and SMCT2, which rely on sodium and are electrogenic and electroneutral transporters, respectively.[16]

SCFA mechanisms of action

The effects of SCFAs in the intestines and elsewhere are derived from their ability to stimulate three G-protein coupled receptors (GPRs), GPR41, GPR43, and GPR109a, as well as their ability to act as histone deacetylase inhibitors (HDACi) (Fig. 1). GPR41 is coupled to the pertussis toxin-sensitive $G_{i/o}$ family, which regulates cyclic antimicrobial peptide (cAMP) production. GPR41 has its highest affinity for propionate > butyrate > >acetate.[17] GPR41 is expressed in many cells and tissues, but is found in appreciable levels in peripheral blood monocytes (PBMC), dendritic cells (DC), and polymorphonuclear neutrophils (PMN), as well as in the spleen, lymph nodes, bone marrow, lung, small intestine, and adipose tissue.[17] Conversely, GPR43 expression is more restricted, as it is located mainly in the intestines and specific immune populations such as PMN, PBMC,

monocytes, and lymphocytes.[17] GPR43 has a dual coupling to both pertussis toxin-sensitive $G_{i/o}$ as well as to the pertussis toxin-insensitive G_q. GPR43 primarily signals through $G_{i/o}$, except in the intestine, where GPR43 via its G_q coupling promotes glucagon-like peptide 1 (GLP-1) secretion.[17–20] GPR43 has affinity for all SCFAs with propionate > acetate ≥ butyrate.[17,18] Unlike GPR41 or GPR43, GPR109a engages only butyrate, while also being the endogenous receptor for niacin.[21,22] GPR109a, similar to GPR41, is coupled to the pertussis toxin-sensitive $G_{i/o}$.[21] GPR109a is expressed in the intestines, macrophages, monocytes, PMNs, DC, adipocytes, and Langerhans cells.[10,23,24] Lastly, SCFAs can act as potent HDACi with butyrate > propionate > >acetate.[25] HDACi play a role in gene modulation, protein stability, and pathway activation. With regards to gene modulation, histone acetylation allows for enhanced access for transcriptional machinery to gene promoters by relaxing the chromatin structure. Thus, histone acetyltransferases (HATs) via acetylation allow for more open and accessible chromatin, whereas HDACs remove acetylation, leading to closed chromatin and gene repression. Additionally, through their HDACi action, SCFAs also play a role in modulating protein stability and activation via acetylation, such as via modulation of p53 activity.[26]

SCFA regulation of mucus production

SCFAs are able to stimulate mucus production, which is vital for creating a barrier between the external environment and the underlying gut epithelial layer. The impact of SCFAs on mucus production was demonstrated by Finnie *et al.*,[27] who showed that butyrate increased colonic mucous glycoprotein (mucin) when incubated with epithelial biopsy specimens from colonic resection samples. SCFA regulation of mucin (MUC) gene expression was shown by Hatayama *et al.*,[28] who found that butyrate stimulated expression of MUC2, the primary mucin which comprises the colonic mucous layer, in the human goblet-like colon cells LS174T. This induction of MUC was dependent on mitogen-activated protein kinase (MEK) signaling, as the MEK inhibitor U0126 completely abrogated butyrate's effect on MUC2 protein expression. Later, this finding was extended by Burger-van Paassen *et al.*,[29] who found that butyrate, acetate, and propionate stimulated MUC2 via binding of the butyrate-responsive region by AP1. The difference in findings in the regulation of MUC2 expression at the RNA and protein level suggests a role for butyrate as both a transcriptional and translational regulator, most likely by acting via HDACi and through GPR41 or GPR43. This is further supported by the findings that propionate, which has high affinity for GPR41 and GPR43, stimulated greater MUC2 expression than butyrate at every concentration except 1mM. Further exploration of MUC2 regulation is of importance as MUC2 KO mice spontaneously develop colitis.[30,31] Beyond colitis, mucin serves an important role in protection from pathogens, as demonstrated by Jung *et al.*,[32] who showed that butyrate increases MUC 3, 4, and 12 expression while also increasing *lactobacillus* adherence and decreasing *Escherichia coli* adherence *in vitro*. Thus, the role of SCFAs in modulating mucin synthesis serves as an important mechanism by which the host can allow for the colonization of beneficial bacteria, which may outcompete pathogenic bacteria and prevent inflammation and infection. Thus, a deeper understanding of the role of mucin could lead to development of probiotics that would allow for alteration of the microbiome through colonization and expansion while also protecting from gastrointestinal infection and inflammation, which could be potentially used in precision medicine to prevent and treat gastrointestinal infection and inflammation.

SCFA regulation of antimicrobial peptides

In addition to promoting mucus production, SCFAs stimulate antimicrobial peptides (AMPs), which are critical for innate defenses against pathogens and serve as a first line of defense for the underlying epithelial layer. In this regard, Hase *et al.*[33] demonstrated that the human cathelicidin LL-37 was expressed constitutively in the colon, specifically in cells at the surface and in the upper crypts. This effect was independent of the commensal bacteria, as human fetal colon transplanted onto the backs of severe combined immunodeficiency (SCID) mice under sterile conditions demonstrated similar LL-37 expression as human colon *in vivo*. Additionally, butyrate increased levels of LL-37 in Caco-2 and HT-29 cells. The mechanism underlying the stimulation of LL-37 by butyrate was uncovered by Schauber *et al.*,[34] who showed that LL-37 expression was dependent on butyrate activation of MEK in the human colon cancer cell line SW620. The potential implications of LL-37 in host protection were unraveled by Raqib *et al.*,[35] who demonstrated that butyrate upregulated the expression of CAP-18, the rabbit homologue to LL-37, and that this upregulation was critical for protection against *shigella* infection, as pretreatment of rabbits with butyrate prior to *shigella* infection led to decreased severity of infection. This is an important finding because it suggests that prevention and treatment of gastrointestinal bacterial infections could be done through dietary intervention. However, the contribution of AMPs in the protection against specific pathogens like *shigella* must be further examined as SCFAs stimulate mucus production, and dietary deficiencies in fiber have been shown to increase mucus-degrading bacteria and susceptibility to pathogens.[36]

Aside from cathelicidin, Zeng *et al.*[37] found that in IPEC-J2 cells (a porcine-derived colon cell line) acetate, propionate, butyrate, as well as phenyl derivatives of butyrate, increased β-defensin 2 and β-defensin 3 expression. This finding was further elucidated by Xiong *et al.*,[38] who found that butyrate could stimulate the *in vivo*

expression of β-defensin 2 and β-defensin 3 in the colon and ileum of pigs, which ultimately led to protection against severe infection when pigs were challenged with *E. coli*. This effect was found to be through HDACi, as treatment of 3D4/2 cells (immortalized porcine alveolar macrophages) led to increased expression of several AMPS including β-defensin 2 and β-defensin 3. Thus, this finding suggests an important role of macrophages in AMP production in response to SCFAs, while also confirming the work of Raqib *et al.*,[35] demonstrating the potential feasibility of diet modification in the protection of gastrointestinal infection in precision medicine. Lastly, our group recently uncovered that SCFAs via GPR43 regulated the expression of REGIIIγ and β-defensin 1, 3, and 4.[39] This was dependent on SCFA induction of STAT3 and mTOR activation, as both inhibition of STAT3 and mTOR chemically or with siRNA knockdown abrogated the effects of SCFAs on AMP production.

SCFAs regulation of the epithelial layer

SCFAs regulate the daily turnover of the epithelial lining and regulate stem cell proliferation. In recent years, reports on the effects of SCFAs, specifically butyrate, on the epithelium have been conflicting. This conflicting data gave rise to the butyrate paradox, which describes differential responses of cells to butyrate when treated *in vitro* and *in vivo*.[40] This paradox was elegantly unraveled by Donohoe *et al.*,[41] who showed that cell metabolism, that is the Warburg effect, dictated the impact of butyrate on epithelial cells. This report demonstrated that tumor cells do not preferentially metabolize butyrate, leading to the intracellular accumulation of butyrate which blocks proliferation and promotes differentiation and apoptosis. However, in normal colonocytes or in tumor cells in which the Warburg effect is blocked, butyrate metabolism could promote the proliferation of colonocytes by acting as a carbon donor for acetyl-CoA and histone acetylation. This model proposes that lower doses of butyrate at the bottom of the crypt drive HAT and proliferation, whereas high doses at the top of the crypt lead to HDACi, apoptosis, and sloughing of cells into the lumen. This model was further verified by Kaiko *et al.*,[42] who showed that butyrate inhibited proliferation in cryptless animals and around areas of ulceration where the stem cell compartment would be exposed to the high luminal butyrate concentration. Thus, this study suggests that crypts, as well as colonocytes, are critical in metabolizing butyrate and creating a butyrate gradient, which permits HAT activity at the base of the crypt. Additionally, the findings of both these articles support the long-term health effects of a high fiber diet in protecting against the development of colorectal cancer.[43]

SCFA regulation of tight junctions

Tight junctions (TJs) are complex protein-protein associations between individual cells that maintain the epithelium's selective permeability. Several studies have focused on both indirect effects of SCFAs on TJs via modulation of cytokines, as well as the direct effects of SCFAs on epithelial cell TJs. In terms of cytokines, Heller *et al.*[44] showed that treatment of HT-29 cells with IL-13, a highly upregulated cytokine in UC patients, increases cell permeability, while also promoting the expression of the pore forming claudin-2. More recently, Wang *et al.*[45] showed that IL-10 KO mice have decreased zona occludin 1 (ZO1) and occludin expression and that mixed feedings of IL-10 KO mice with a diet supplemented with acetate, propionate, and butyrate could increase occludin and ZO1 expression. However, whether this effect occurred through direct actions of SCFAs on the epithelium, or through modulation of effectors such as TNFα was not investigated. This is important, as IL-10 and SCFAs are important modulators of several inflammatory cytokines such as IFNγ and TNFα, which have well-characterized roles in modulating TJ permeability.[46] Additionally, Zheng *et al.*[47] found that in the human colon cancer cell lines T84 and Caco-2, butyrate upregulated IL-10RA via a STAT3- and HDACi-dependent pathway, which led to an increase in transepithelial electrical resistance (TEER). However, KO of IL-10RA in T84 abrogated the effects of butyrate on TEER, which appeared to be facilitated by the ability of IL-10RA to downregulate the pore forming claudin-2. Furthermore, Chen *et al.*[48] recently found that butyrate protected mice from increased epithelial permeability in a GPR109a-dependent manner in a model of Trinitrobenzenesulfonic acid (TNBS) colitis. This effect was dependent on GPR109a suppression of LPS-induced phosphorylation of AKT in macrophages and was demonstrated using a transwell system where RAW246.7 macrophages were co-cultured with Caco-2 cells and pretreated with LPS in the presence or absence of butyrate. Thus, this finding exemplifies the important role macrophages play in modulating epithelial integrity through proinflammatory regulation.

SCFAs also have direct effects on epithelial cells in modulating TJ formation. For example, Feng *et al.*[49] found that butyrate increased claudin-3, occludin, and ZO1 expression in a GPR109a-dependent manner in piglets and Caco-2 cells. The effect on claudin-3 was abrogated with GPR109a knockdown (KD) in Caco-2 cells. Additionally, Cheng *et al.*[50] found that NLR family CARD domain-containing 3 (NLRC3) KO mice have increased epithelial permeability. Treatment with butyrate increased NLRC3 expression and overexpression of NLRC3 increased TEER, implicating a role for butyrate in NLR3 induction of TJs, possibly through upregulation of ZO1.

Finally, metabolism is an important driver of TJ formation. In this regard, Zhang *et al.*[51] showed that in kidney cells, activation of 5' AMP-activated protein kinase (AMPK) led to increased endogenous Ca^{2+} levels, which drove TJ formation. Additionally, Kelly *et al.*[52] showed that hypoxia inducible factor 1 (HIF-1α) expression is critical for SCFA regulation of intercellular permeability. Interestingly, AMPK activation has been shown to

stabilize HIF-1α and prevent the switch to glycolysis, the Warburg effect, implicating an important role for butyrate in modulating glycolysis.[53] Finally, Peng *et al.* demonstrated that butyrate, a known activator of AMPK, modulates TJ formation through regulation of AMPK.[54] Thus, it appears that by regulating energy status via AMPK in several tissues, butyrate may have a universal role in driving TJ formation.

SCFAs and immune regulation

The immune cells that reside intraepithelially and in the lamina propria of the intestines play a vital role in regulation of host homeostasis to microbiota, with accumulating evidence suggesting that SCFAs are the key regulator of this process (Fig. 2).

SCFA regulation of neutrophils

SCFAs have been shown to regulate neutrophil functions. In this regard, Vinolo *et al.*[55] demonstrated the differential effects of SCFAs on neutrophil killing. This was examined via the isolation of rat peritoneal neutrophils, in which butyrate inhibited the phagocytosis and killing of *C. albicans*, while also decreasing reactive oxygen species (ROS) production in neutrophils. This is in contrast to propionate, which had no effect on phagocytosis, killing, or ROS production; similarly, acetate only moderately increased ROS production. Vinolo *et al.*[56] later uncovered that butyrate and propionate treatment of neutrophils diminished TNFα, cytokine-induced neutrophil chemoattractant-2 (CINC-2αβ), and nitric oxide (NO) production in LPS-treated neutrophils. This downregulation of inflammatory cytokines was found to be HDACi-dependent and cyclooxygenase (COX) independent. These data by Vinolo *et al.* point toward a major role of HDACi in modulating neutrophil function, given the potency of butyrate compared to other SCFAs. More interestingly, it implicates butyrate as a key player in priming neutrophils in the gut, possibly to protect against invading pathogens. With these data, it would be of great interest to further examine the role of systemic butyrate, possibly through the use of tributyrin, the rapidly absorbed prodrug form of butyric acid.[57]

SCFA modulation of chemotaxis was uncovered by Sina *et al.*,[58] who examined chemotaxis of neutrophils under acute and chronic inflammation in wild-type (WT) and GPR43 KO mice. In the study, it was shown that GPR43 KO mice had decreased neutrophil influx into the colon upon both acute and chronic inflammation. Using transwell assays, they found that SCFAs activate neutrophil migration, and that this migration was abrogated with GPR43 KO. However, under non-inflammatory conditions, GPR43 KO neutrophils *in vivo* did not demonstrate any alterations in chemotaxis. Most interesting though, is that GPR43 KO aggravated acute DSS colitis, but was protective in chronic colitis. This begs the question as to the differential regulation

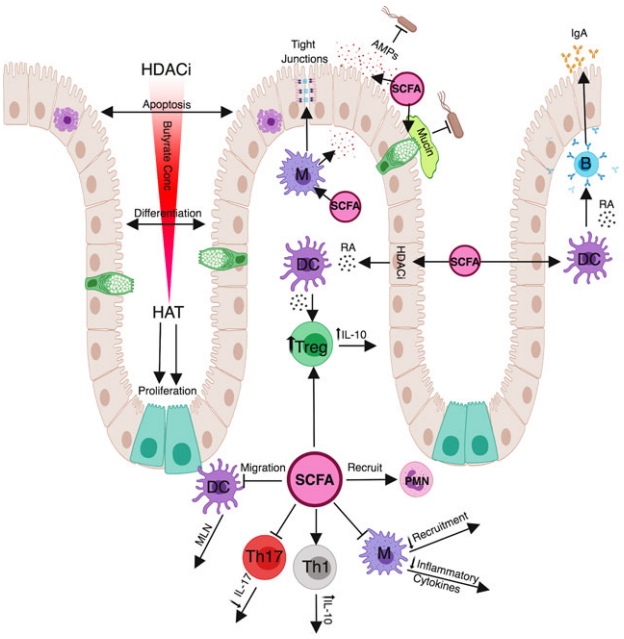

Figure 2. Short chain fatty acid (SCFA) modulation of barrier defenses. HDACI, histone deacetylase inhibitors; HAT, histone acetyltransferases; M, macrophages; AMP, antimicrobial peptides; MUC, mucin; DC, dendritic cells; RA, retinoic acid; B, B lymphocytes; Tregs, T regulatory lymphocytes, MLN, mesenteric lymph nodes; Th, T lymphocytes; PMN, neutrophils.

of GPR43 and its importance under non-inflammatory versus inflammatory conditions, and in acute versus chronic inflammation. Given that neutrophilic infiltrate is a hallmark of ulcerative colitis, it would be of interest to investigate whether a GPR43 antagonist is beneficial in modulating chronic colitis and colitis-associated cancer for the purpose of precision medicine.

The work from Vieira *et al.*[59] further demonstrated the role of SCFAs and GPR43 in neutrophil chemotaxis. Using a mouse model of gout where monosodium urate (MSU) crystals were injected into the capsule of the knee, treatment of mice with acetate led to increased neutrophil influx and elevated IL-1β. However, in GPR43 KO, the effects of acetate were abrogated, which led to decreased PMN influx and IL-1β. Later work by Vieira *et al.*[60] showed that although neutrophils and IL-1β were elevated within 6 hours of MSU deposition, treatment with SCFAs led to quicker resolution of inflammation. Thus, this finding of GPR43-dependent resolution of neutrophil inflammation in the acute setting supports the work by Sina *et al.*,[58] who showed that GPR43 KO mice are more susceptible to severe inflammation and death in the acute DSS model.

Aside from GPR43, Chen *et al.*[61] also found that dimethyl fumarate (DMF) and its metabolite monomethyl fumarate (MMF) decreased neutrophil chemotaxis into the spinal column in a model of experimental autoimmune encephalomyelitis (EAE). This effect was dependent on GPR109a expression on neutrophils, as

GPR109a KO abrogated the effects of DMF, and appears to be modulated by decreased neutrophil adhesion to endothelial cells. Thus, it appears that SCFAs via GPR43 and GPR109a are key regulators in neutrophil chemotaxis and implicate the potential systemic use of SCFAs to treat inflammatory conditions.

SCFA regulation of T lymphocytes

SCFAs modulate the differentiation of Th1, Th17, and T regulatory (Treg) cells, as well as their function. The role of SCFAs in Treg induction was demonstrated by Arpaia *et al.*,[62] who showed that butyrate could drive CNS1-dependent differentiation of extrathymic Tregs. This was further confirmed by Furusawa *et al.*,[63] who showed that luminal concentrations of SCFAs correlated with the number of Tregs present in the colon. Recently, Haghikia *et al.*[64] demonstrated that SCFAs as compared to long-chain fatty acids, were protective in the preventative setting, but not the treatment setting, in experimental EAE. This mechanism occurred via SCFA induction of Tregs, which was demonstrated by adoptive transfer of Tregs from propionate-treated or non-treated mice into recipient mice with simultaneous induction of EAE. Additionally, Schwarz *et al.* showed that butyrate induction of Tregs was protective against contact hypersensitivity reactions in the skin, similar to their role in colitis and EAE.[65] These data support that SCFAs may be an important environmental factor that could dictate the onset of inflammatory diseases; however, the ability of SCFAs to modulate inflammation after disease onset is less convincing. The lack of SCFA protection post-inflammation onset may result from their differential effects on other T cell populations as well as their concentration. For example, Sałkowska *et al.*[66] found in human Jurkat T cells that butyrate decreased RORγt expression in naïve CD4 T cells under Th17 polarizing conditions, but promoted RORγt and IL-17A expression if butyrate was added to differentiated Th17 cells. Furthermore, Park *et al.*[67] found that administration of super physiological doses of SCFAs led to the development of T cell-mediated ureteritis, which progressed to kidney hydronephrosis. These data offer interesting perspectives on the role of SCFAs on inflammation as they demonstrate that SCFAs may not be a beneficial treatment for acute inflammation, and that dosing of SCFAs could be critical in determining their therapeutic potential. Additionally, Asarat *et al.*[68] found that PBMCs co-cultured with T cells in the presence of LPS and SCFAs decreased Th17 differentiation, while increasing Treg differentiation and decreasing IL-6 production, with butyrate being the most potent inducer of Tregs. Furthermore, Zhang *et al.*[69] demonstrated that butyrate administration increases peripheral Treg induction, while increasing IL-10 and IL-12 and decreasing IL-17 and IL-23 expression. Recently, our group showed that SCFAs induce IL-10 production in Th1 effector cells in a GPR43-dependent manner mediated by Blimp-1.[70] The importance of IL-10 production in Th1 was further verified by showing that the SCFA-treated microbiota-specific Th1 cells induced less severe colitis compared to untreated Th1 cells when transferred into RAG KO mice. However, administration of an anti-IL-10R antibody abrogated the protective effects of SCFA-treated Th1 cells. Our groups' finding was further extended by Luu *et al.*,[71] who demonstrated that another SCFA, pentanoate, effectively inhibited IL-17 production in Th17 cells and increased IL-10 production, with IL-10 induction being regulated by glucose oxidation in T cells.

SCFA regulation of macrophages

SCFAs play several roles in modulation of macrophage activation, recruitment, and antimicrobial responses. The role of SCFA in the activation of macrophages was shown by Lukasova *et al.*,[72] who demonstrated the importance of GPR109a in modulating M1 macrophage differentiation by downregulating M1 macrophage markers CD68 and arginase 2. Additionally, GPR109a activation decreased IFNγ induction of monocyte chemotactic factor 1α (MCP-1α) as well as macrophage recruitment following peritoneal MCP-1α injection. This anti-inflammatory effect of SCFA receptors was extended by Nakajima *et al.*,[73] who showed that WT mice are thinner and have higher insulin sensitivity than GPR43 KO mice. To demonstrate this, it was shown that M2 macrophages isolated from WT, but not GPR43 KO mice had elevated levels of TNFα. In this context, elevated levels of TNFα expression by M2 macrophages are associated with adipocyte tissue remodeling and decreased fat accumulation. Furthermore, Chang *et al.*[74] demonstrated that butyrate via HDACi leads to the downregulation of LPS-induced proinflammatory release from macrophages, specifically affecting IL-6. Most recently, Schulthess *et al.*,[75] using single cell RNA-seq analysis, identified that butyrate induced an antimicrobial signature characterized by the expression of S100A8, S100A9, S10012, LYZ, and FCN1, which was driven by inhibition of HDAC3. Thus, these data implicate SCFAs as major modulators of basal levels of inflammation driven by macrophages, and also exemplify their potentially protective effect against pathogens through the promotion of antimicrobial responses at epithelial surfaces.

SCFA regulation of dendritic cells

SCFA regulation of DCs is critical in the induction of tolerance. In this regard, a report by Tan *et al.*[76] demonstrated the importance of GPR43 and GPR109a in development of tolerance to food antigens. Here, the lack of GPR43 or GPR109a in mice fed a high-fiber diet led to a reduction of $CD103^+$ DCs and ALDH1A2 expression [the retinaldehyde dehydrogenase-2 (RALDH2) enzyme is encoded by ALDH1A2]. RALDH2 is responsible for vitamin A metabolism to retinoic acid (RA), which is critical for the induction of Tregs by $CD103^+$ DCs.[77,78] Supporting

this evidence, it was shown that GPR43 KO and GPR109a KO mice had impaired Treg responses in the mesenteric lymph nodes (MLN), increased serum IgE, and heightened clinical anaphylaxis scores when challenged with antigen. A later report by Goverse *et al.*[79] showed that SCFAs and a high-fiber diet were able to induce vitamin A metabolism in epithelial cells and CD103$^+$ DCs and this was correlated with increased Foxp3 expression in T cells. The ability of SCFAs to induce vitamin A metabolism via ALDH1A expression in intestinal epithelial cells (IEC) was dependent on HDAC1 inhibition as demonstrated by increased expression of ALDH1A1 when IEC were treated with MS344, an HDACi targeting HDAC1. With these data, it would be of interest to examine the selective inhibition of HDAC1 in the prevention and treatment of colitis, as Treg induction has been shown to be important for protection against colitis.[63]

Recently, our group demonstrated that DCs play an important role in the induction of IgA production in the gut in response to SCFAs.[80] Here, we showed that GPR43 KO mice had decreased levels of IgA compared to WT mice and that feeding WT mice but not GPR43 KO mice with acetate led to induction of intestinal IgA. This effect of acetate was shown to be independent of T cells, as TCRβδ KO mice, which have B cells but lack T cells, also demonstrated an elevated IgA response. *In vitro*, it was shown that acetate induced RA signaling in DCs, which drove increased IgA production from B cells.

SCFAs and inflammatory bowel disease

Harig *et al.*,[81] who successfully treated a small cohort of patients with diversion colitis via rectal irrigation, first showed the relevance of SCFAs as a potential therapeutic. This finding was later extended by Scheppach *et al.*,[82] who were able to successfully treat patients with ulcerative colitis with a regiment of butyrate. The basis for using SCFAs as a treatment is exemplified by the findings of Treem *et al.*,[83] who showed that children with UC and CD have decreased fecal SCFAs, and Frank *et al.*,[84] who uncovered that patients with IBD often have a decrease in *Firmicutes* and *Bacteroidetes*, which are noted for their production of butyrate and propionate. However, despite these findings, the role of SCFAs for the treatment of colitis remains controversial. For example, Furusawa *et al.*[63] in a preventative model of colitis, showed that the treatment of mice with butyrate post transfer of CD4$^+$CD45RB^{hi} T cells prevented the onset of colitis. Additionally, Maslowski *et al.*[85] showed that acetate could reduce the severity of acute and chronic colitis in a GPR43-dependent manner, which was abrogated in GPR43 KO mice. GPR43 KO mice were more susceptible to both acute and chronic DSS colitis, with neutrophils also showing enhanced migration into the peritoneum following injection of heat-inactivated *Staphylococcus aureus*. The findings of Maslowski *et al.*[85] differ from those of Sina *et al.*,[58] who showed that GPR43 KO mice had less severe colitis in the chronic DSS model. However, because of the differences in DSS protocols, it is difficult to perform a direct comparison. Thus, further evaluation across several models of colitis should be explored, to provide stronger evidence for the use of SCFAs as a potential therapeutic in IBD patients. Consistent with the role of SCFAs in colitis prevention, Singh *et al.*[23] demonstrated the importance of GPR109a in colitis development, with GPR109a KO mice developing lethal colitis in the acute model, while also having increased risk of colorectal cancer development in the azoxymethane (AOM) DSS model. The findings by Singh *et al.*[23] in the AOM/DSS model support the work performed by Kaiko *et al.*,[42] who proposed that butyrate might play a critical role in the prevention of cancer development by preventing the proliferation of stem cells while exposed to higher luminal concentrations of butyrate. Additionally, while SCFAs may play an important role in the prevention of inflammation, Chang *et al.*[74] demonstrated that in a treatment model where butyrate supplementation began the day prior to DSS colitis onset rather than 5-7 days prior, butyrate was no better than control in terms of colitis severity in the acute DSS colitis model.[74] The reason for SCFAs' effect in prevention rather than treatment of colitis may be offered by the findings from Kaiko *et al.*,[42] who found that butyrate inhibition of stem cell expansion led to increased ulcer size in the acute model of DSS colitis. Thus, the beneficial effects of butyrate on inflammation may be partially counteracted by this delay in repair to ulcerated tissue. To circumvent this issue, in future it may be beneficial to begin investigating compounds that target individual GPRs or HDACs in IBD.

Concluding remarks

Given the importance of SCFAs in barrier protection and regulation of inflammation, dietary supplementation of SCFAs or modulation of diet to increase dietary fiber intake is an attractive option for potentially reversing the increase we see today in chronic inflammatory diseases. This could be beneficial in the preventative setting, where SCFAs have been linked to lower risk of chronic inflammatory diseases and colorectal cancer.[8,43,86] However, although SCFAs have a clear role in the regulation of host immunity, it is unclear whether SCFAs represent a feasible treatment following the onset of chronic inflammatory conditions. This is further supported by the conflicting clinical data which, to date, have failed to show conclusive evidence for the use of SCFAs in the acute setting. Nevertheless, further work is needed in this area for the purposes of precision medicine if we hope to one day treat IBD patients with chemical agonists or antagonists of GPRs or HDACs.

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Conflict of interest statement

None declared.

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