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A new paradigm for a new simple chemical: butyrate & immune regulation

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Short-chain fatty acids (SCFAs) play an important role in the host system. Among SCFAs, butyrate has received particular attention for its large effect on host immunity, particularly in supplying energy to enterocytes and producing immune cells. Butyrate enters the cells through the Solute Carrier Family 5 Member 8 (SLC5A8) transporters, then works as a histone deacetylase inhibitor (HDAC) that inhibits the activation of Nuclear factor-κB (*NF-κB*), which down-regulates the expression of *IL-1β*, *IL-6*, *TNF-α*. Meanwhile, butyrate acts as a ligand to activate G protein-coupled receptors *GPR41*, *GPR43*, and *GPR109*, promoting the expression of anti-inflammatory factors. Besides, it inhibits the proinflammatory factors. Further, it can also suppress the expression of chemokines and reduce inflammation to maintain host homeostasis. This paper reviews the research progress highlighting the potential function of butyrate as a factor impacting intestinal health, obesity and brain disorders.

1. Introduction

Bacteria inhabit the human intestine, amounting to almost 10^{14} CFUs in total in the hindgut.[1] Similar estimates have also been reported in other omnivores, such as pigs. In the past, they were called intestinal commensals, which meant that only the bacteria benefitted from the co-existence. However, this conclusion has been overturned in recent years, highlighting the symbiotic nature of the relationship. Cohabitation in the intestines is beneficial to both bacteria and the host. As for bacteria, the advantage is that the host provides a habitat as well as nutrients for colonization. For the host, bacteria provide benefits in many ways. Since the intestinal tract represents the largest immune system in the body, the bacteria do not only maintain the intestinal immune tolerance but also prevent the emergence of an excessive immune response, such as allergies and systemic lupus erythematosus. Intestinal epi-

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thelial cells act as mediators between the bacteria and their hosts. Bacteria in the gut produce a variety of metabolites that act as messengers between bacteria and their hosts. These metabolites include short-chain fatty acids (SCFAs), indoles, second bile acids, and lactates.

SCFAs (acetate, propionate, and butyrate) are the main end-products of the bacterial fermentation of nondigestible dietary fibers in the large intestine.[2,3] Among the SCFAs secreted by the gut bacteria in the large intestine, acetate is the most abundant, followed by propionate and butyrate.[4] In the intestine, the concentration of SCFA gradually increases from the proximal colon to the distal colon. Recently, butyrate has received particular attention because of its extensive benefits, including acting as an energy source for gut epithelial cells and a key mediator for anti-inflammatory and antitumorigenic activities.[5,6]

G protein-coupled receptors (GPRs) are receptor-like proteins involved in signal transduction; they cause changes in the cell state by binding to chemicals in the cellular environment and activating a series of signaling pathways within the cell. Notable among the butyrate targets are *GPR41* (Free Fatty Acids Receptor 2, *FFAR3*), *GPR43* (*FFAR2*) and *GPR109* (also known as Hydroxy-carboxylic acid receptor 2). These receptors are located in the lumen-facing apical membrane of colonic epithelial cells and are activated directly by intestinal SCFA.[7] Butyrate modulates the biological responses of the host gastrointestinal tract by binding to several specific GPCRs and acting as an HDAC inhibitor, which is the most widely studied function of butyrate.[8]

2. Formation and metabolism of butyrate

The main products of microbial fermentation in the large intestine can vary significantly in their relative concentrations and production rates depending on the diet and site of production,[9] with typical ratios in feces around 3 : 1 : 1 (acetate : propionate : butyrate). Butyrate has a particularly important role as the preferred energy source for the colonic epithelium.[10] It is produced from dietary fibers through bacterial fermentation *via* two metabolic pathways. In the first pathway, two molecules of acetyl coenzyme A (CoA) yield acetoacetyl-CoA, which is then converted to butyryl-CoA. Thereafter, butyryl-CoA may yield butyrate *via* butyrate kinase. In the other pathway the CoA moiety of butyryl-CoA is transferred to an external acetate *via* butyryl-CoA: acetate CoA transferase, leading to the formation of butyrate and acetyl-CoA[11] (Fig. 2).

Monocarboxylate transporter-1 (MCT1) is an H+/monocarboxylate exchange system with a stoichiometry of 1 : 1.[12] Therefore, it is electrically neutral in the transport process and can effectively recognize short-chain fatty acids, including butyrate. The recognition of butyrate as a substrate by MCT1 forms the basis of the connection between this transporter and the intestinal tract. Moreover, SLC5A8, also known as sodium-coupled monocarboxylate transporter 1 (SMCT1), is a Na+-coupled cotransporter of various short-chain fatty acids.[13] The co-transported substrates include lactate, pyruvate, acetate, propionate, and butyrate. Moreover, the substrate specificity of SLC5A8 is very similar to that of the previously known monocarboxylate transporters,[14] except that SLC5A8 is coupled to Na+, whereas MCTs are coupled to H+. Additionally, the central deacetylase inhibition action of butyrate is also mediated by SMCT. Thus, butyrate gains entry into cells to exert its regulatory function (Fig. 1).

3 The receptors of butyrate and their activation mechanism

SCFA-sensing GPCRs include *GPR41*, *GPR43*, and *GPR109*,[16,17] which are presented on the surface of intestinal epithelial

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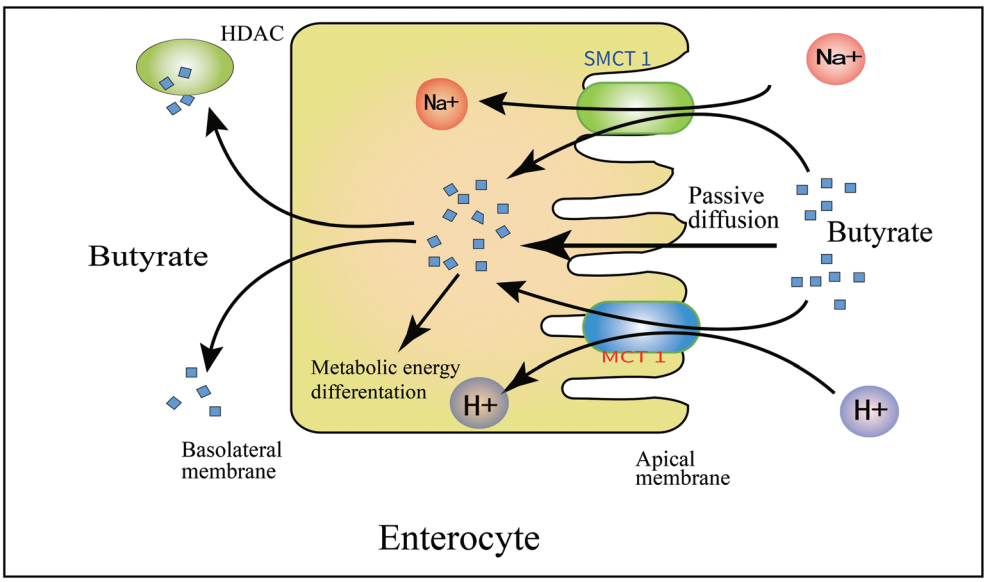

Fig. 1 Absorption of butyrate in the large intestine. Butyrate is absorbed by colonocytes, mainly *via* MCTs or SMCTs.[15]

cells, adipocytes, and immune cells. These three receptors protect against colonic inflammation and colon carcinogenesis.[18] Compared with *GPR109*, the *GPR41* and *GPR43* are more similar in that they can be efficiently activated by acetate, propionate, butyrate and other SCFAs (Fig. 2). It has been revealed that SCFAs regulate GPR41-mediated levels of genes involved in immune cell recruitment and epithelial immune barrier and thereby mediate innate immunity in epithelial cells (ECs).[19]

Li *et al.* (2018) reported that IL-6 production in the human umbilical cells could be significantly decreased by pre-treatment with butyrate for 24 h in the LPS-stimulated (12 h) group.[20] The dietary addition of flaxseed fiber increased the production of intestinal butyrate and promoted the expression of *GPR41* in hormone-producing intestinal endocrine cells, thereby reducing inflammation and improving host metabolism.[21]

The phosphorylation cascade of the mitogen-activated protein kinase (*MAPK*) is an important signaling pathway, including extracellular signal-regulating kinase ERK, stress-activated protein kinase JNK, and protein kinase p38. Research shows that SCFA can promote epithelial protection and repair from colitis development *via* the GPR41/43-MAPK pathway. These two receptors (*GPR41*/*GPR43*) are linked to IP3 formation, intracellular Ca^{2+} release, *ERK1/2* activation, and inhibition of cAMP accumulation. Le Poul reported that the SCFA-induced recombination of *GPR 41* and *GPR43* in Chinese hamster ovary cells (CHO) led to the activation of *ERK1/2*,[22] while Yonezawa T *et al.* (2007)[109] reported that the SCFA-induced activation of *p38MAPK* in human breast cancer cells by *GPR43* showed a time-dependent increase. Furthermore, other studies have shown that the *GPR41*-activated *ERK1/2* and *P38 MAPK* signaling pathways in epithelial cells induce the production of chemokines and cytokines,[23] such as down-regulating the expression of *IL-1* and *TNF-α*, up-regulating the expression of the tight junction proteins Occludin and *ZO-1*, as well as reducing the expression of *CCL20*, *CXCL2*, *CXCL3*, *CXCL5*, *CXCL8*, and *CXCL14*. This result verifies that the expression of *GPR41* mRNA positively correlates with *CCL20*, *CXCL2*, *CXCL3*, *CXCL8*, *CXCL14*, and *ZO-1*.[24]

GPR43 is mainly expressed in leukocyte populations, particularly neutrophils. There are other pathways besides inflammasome activation due to which GPR43 signaling may affect cell functions, including b-arrestin2 signaling and an alternative pathway activated by many metabolite-sensing GPCRs, including *GPR43*. For glucose metabolism, butyrate increases *PYY* and *GLP-1* expression in the colon *via* GPR41 and GPR43. *GLP-1* increases insulin and decreases glucagon production in the pancreas, and *PYY* increases glucose uptake in the muscle and adipose tissues. Butyrate increases FA oxidation in the muscle for lipid metabolism and decreases lipolysis *via* the GPR43 pathway in white adipose tissues. Meanwhile, butyrate decreases hepatic gluconeogenesis. In addition, butyrate is converted to FAs, cholesterol, and ketone bodies in the liver,[11] and the *GPR43* agonist enhances the production of *AMP*, *RegIIIg*, and β-defensins in both murine and human IECs.

Unlike *GPR41* and *GPR43*, *GPR109A* (encoded by Niacr1) can only be activated by butyrate and nicotinate, which are present in the lumen-facing apical membrane of colonic IECs.[25] In the colonic lumen, butyrate serves as an endogenous agonist for *GPR109A*.[7] Furthermore, it works through *GPR109A* in macrophages and dendritic cells to indirectly induce IL-10 þ T cells and FoxP3 þ T cells,[26] thus acting as an anti-inflammatory and anticancer agent in the colon.[7] At the same time, it connects the gut microbiome and metabolism to host physiology. Besides, *GPR109a* is a Gi/Go protein-coupled signaling receptor inhibited by the pertussis toxin (*PTX*); the activation of *GPR109a* signaling by the inflammasome pathway

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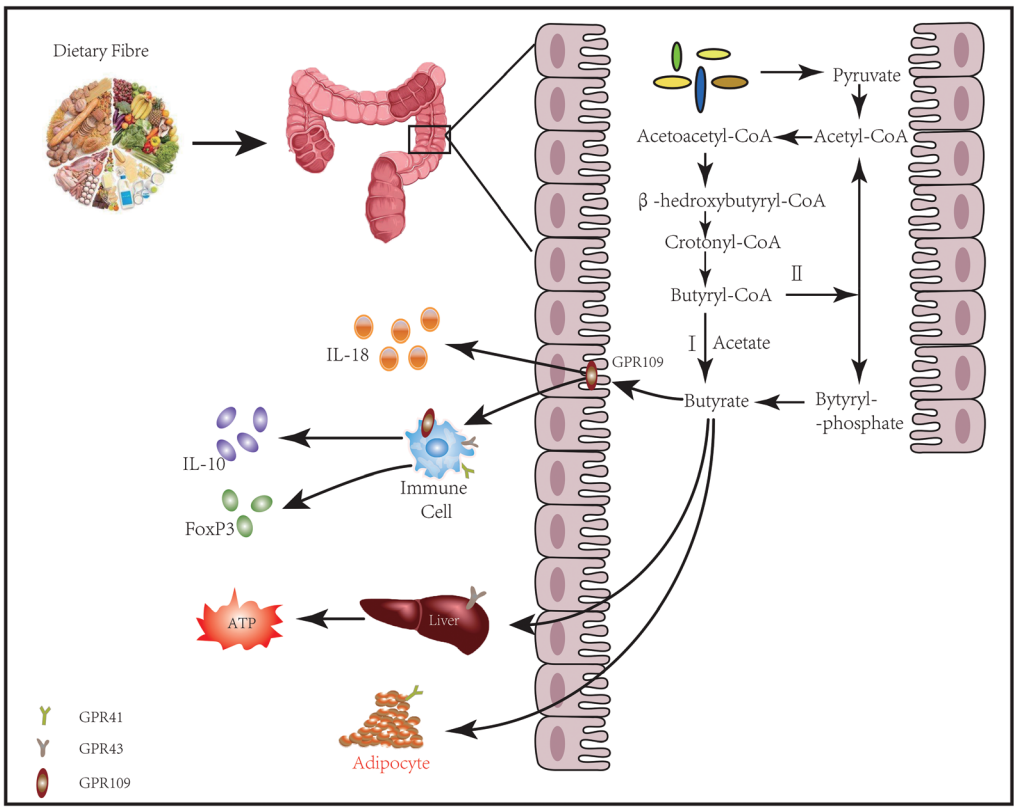

Fig. 2 Butyrate is produced in the intestine by the microbial fermentation of dietary fiber in the hindgut. Two butyrate-producing pathways and three receptor pathways of butyrate are shown above.

in colonic macrophages and dendritic cells results in the differentiation of regulatory T cells and IL-10-producing T cells.[27] Larsson *et al.*(2012) showed that the colon of conventionally housed mice expressed a higher amount of *IL-18* mRNA than germ-free mice;[28] when the sterile mice were supplemented with butyrate, the expression of *IL-18* significantly increased.[27] In 2014, in order to verify this conclusion, Nagendra Singh *et al.* (2014) tested *IL-18* expression in the colonic epithelium of WT and Niacr^{1-} mice that were administrated butyrate for one day and showed that both butyrate and niacin induced the expression of *IL-18* in the colonic epithelium of WT mice but failed to do so in Niacr1-/mice.[7] The expression of *IL-18* in colonic epithelium protects the colon against inflammation and carcinogenesis in animal models.[29] The secretion of *IL-18* also increases in intestinal epithelial cells *via* butyrate-stimulated *GPR109A* signaling. Thus, *GPR109A* seems to be essential for the butyrate-mediated induction of *IL-18* in the colonic epithelium.

4. Immunomodulatory properties of butyrate

4.1 Dendritic cells

Dendritic cells (DCs) are the most potent antigen-presenting cells (APC), which are essential in antigen recognition and presentation, as well as adaptive immune system initiation.[30] Research has shown that microbial SCFAs affect the development and homeostasis of dendritic cells in the intestinal tract of mammals. DCs secrete a variety of cytokines or membrane molecules that promote diverse T cell differentiation. For instance, butyrate effectively inhibits the production of *IL-12* in immature DCs. It has been known that *IL-12* activates the differentiation of CD4+ cells into Th1 cells by promoting the production of *IFN*.[31,32]

4.1.1 Immature DCs. Immature DCs have a strong ability for antigen uptake but express low levels of the costimulatory molecules. iDCs have low expression levels of MHC, CD40, CD80, CD86, and B7, which are co-stimulatory signaling molecules found on the surface of antigen-presenting cells and contribute to the antigen-specific activation of T cells. Butyrate inhibits the expression of *B7*, *CD40*, *CD54*, *CD86*, and MHC II molecules, thereby induces immune tolerance and interferes with the DC maturation process.[32,33] Furthermore, the expression of proinflammatory factors (LPS, TNF-α, GM-CSF, IL-4) in DCs activated by LPS is also prevented by butyrate intervention.

Regarding the regulation of the iDC pathway, it has also been reported that butyric acid inhibits the proliferation of activated CD4+ T cells, interferes with the T cell growth cycle, promotes T cell apoptosis, and causes immune tolerance by increasing the expression of indoleamine 2,3-dioxygenase

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(IDO) in iDC and creating a local low tryptophan environment. It is a rate-limiting enzyme of tryptophan catabolism. Besides, butyrate can promote the differentiation of TH0 cells to TH2 in iDCs by downregulating the suppressor of cytokine signaling (*SOCS*), a negative regulatory protein in the JAK-STAT signaling pathway.[33] The latter then secretes *IL-10* to act on iDCs, leading to a magnifying effect and thus inhibiting immune response.[34]

4.1.2 Mature DCs. Compared with iDCs, the antigen delivery ability of mDCs is enhanced. Butyrate induces the production/release of chemokines in neutrophils, DCs, and endothelial cells to regulate the recruitment of leukocytes.[35] Meanwhile, butyrate exerts a solid immunomodulatory effect on LPS-stimulated DCs by down-regulating LPS-induced *IL-6*, *IL-12B*, and protein expression in mDCs.[32,36] In addition, it has been found that SCFAs affect the secretion of both *IL-12p70* and *IL-23*; butyrate strongly inhibits their secretion in mDCs. After treatment with sodium butyrate, the secretion of *IFN-γ* is significantly reduced and the expression of co-stimulatory molecules and antigen-presentation molecules, such as *CD40*, *CD86*, and HLA class II, on mature dendritic cells is enhanced in a dose-dependent way.[37]

In conclusion, although a large body of evidence has suggested the effect of butyrate on immune tolerance in iDCs and antigen uptake in mDCs, there are mechanisms of action that remain unknown. Therefore, additional investigations are required to clearly understand the mechanism underlying the effects of butyrate on iDCs and DCs.

4.2 T cells

Butyrate makes the mDCs migrate from the periphery to the lymph nodes, activates the CD4+ and CD8+ T cells and aids T cell differentiation to promote adaptive immune responses.[38] Since the potentiation of the extra-thymic differentiation of Treg cells is dependent on the intronic enhancer *CNS1*, the Treg cell numbers are boosted upon the provision of butyrate.[39] According to N. Arpaia, butyrate does not diminish the *TGF-β* dependence of Foxp3 induction in CNS1-sufficient CD4+ T cells. Further, he has suggested that butyrate promotes the extra-thymic differentiation of Treg cells.[40] Haghikia *et al.* (2015) showed that butyrate down-regulates the LPS-induced cytokine release of *IL-12p70* and *IL-23*;[41] these cytokines polarize naïve CD4+ T cells towards *Th1* and *Th17*, thus invoking pro-inflammatory properties and inducing the differentiation of naive T cells into Treg cells. Moreover, CD4+ T cells from WT splenic DCs treated with butyrate have been shown to promote the secretion of *IL-10*.[32] The latest research on intestinal epithelial cells has indicated that butyrate induces the production of *TGF-β* in primary IECs (cell line MSIE). When MSIE cells were cultured with butyrate, enhancement was observed in the expression of *Foxp3* under Treg conditions and *IL-10* production in *Th1*, *Th17*, and Treg cells, which verifies the previous studies. It has further been suggested that butyrate promotes the production of *TGF-β* parts by its HDAC-inhibiting activity.[42]

4.3 Mast cells

Mast cells are a kind of granulocytes located in the respiratory tract, gastrointestinal tract and other tissues that are in contact with the outside environment. Moreover, it has a non-negligible role in innate immune response.[43,44] In recent years, some studies have suggested that mouse mast cells treated with butyrate inhibit the degranulation of *TNF-α* and phosphorylation of *JNK*.[45] Meanwhile, it also inhibits the activation of mast cells and reduces the contents of histamine, trypsin, *TNF-α* and *IL-6*.[46] Wang *et al.* (2018) also made similar findings in pigs. The principle is that, when inflammation occurs, mast cells are recruited to the inflamed part, leading to mast cell degranulation and the release of inflammatory transmitters, such as histamine, protease, and proteoglycan, in a short time.[47] Subsequently, *TNF-α*, *IL-6* and other regulators in the downstream pathways are expressed. Besides this, neurotransmitter substance P or complement anaphylatoxins (independent of IgE) can also induce mast cell activation through another mast cell-activating pathway that involves the *MAPK* signaling pathway.[47]

Recent evidence from studies in humans and mice demonstrate that butyrate inhibits both IgE- and non-IgE-mediated mast cell degranulation in a concentration-dependent manner. However, these effects are independent of the *GPR41*, *GPR43*, and *PPAR* receptors but are associated with the inhibition of histone deacetylases. Using transcription techniques and epigenomic analysis, Folkerts J *et al.* (2020) concluded that butyrate redistributes global histone acetylation in human mast cells, including a significant reduction in the acetylation of the Bruton's tyrosine kinase (BTK), spleen tyrosine kinase (SYK), and Linker for Activation of T cells (LAT) promoter regions. Moreover, butyrate also induces the subsequent transcriptional silencing of the *FcεRI* signaling genes, which then suppress the degranulation of mast cells to avoid hypersensitization.[48]

4.4 B cells

When focusing on B cells, we found that butyrate interferes with the differentiation of B cells, producing plasma cells instead of regulatory B cells (Breg),[49,50] and then, Breg inhibits the immune pathology by promoting the production of *IL-10*, *IL-35* and transforming growth factor beta 1 (*TGFβ1*). Besides, sodium butyrate treatment activates the P300-STAT3 pathway in B cells, and activated STAT3 (*P-STAT3*), which is highly compatible with the *IL-10* gene promoter, upregulates *IL-10* expression.[49] Recent research has revealed the effect of butyrate in suppressing arthritis in a Breg-dependent manner. Butyrate increases the level of 5-hydroxyindole-3-acetic acid (5-HIAA), which is an aryl hydrocarbon receptor (*AhR*) activator. Butyrate could also inhibit the germinal center (GC) B cells and plasmablast differentiation *via* AhR-activated pathway.[51]

Therefore, butyrate regulates the capacity of B cells to produce *IL-10* and induces pTregs.[52] These results demonstrate that microbially derived metabolites can control the balance between regulatory and mature B cell subsets.

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4.5 Epithelial cells

Butyrate provides energy to enteric epithelial cells and enhances epithelial cell differentiation and proliferation,[53] while it reduces the apoptosis of normal cells. Low-dose sodium butyrate can significantly increase the mitotic index of the jejunal epithelial cells of calves,[54] a cell breeding index, and increase the cell renewal capacity. Moreover, butyrate is a survival factor for normal cells, besides acting as a colon-cancer prevention factor. It is also a potent inducer of apoptosis in cancer cells;[55] it inhibits histone deacetylases and the associated signaling pathways in cultured cancer cells to promote cancer cell apoptosis. Some investigators have used primary non-transfer cultures to establish cell lines and evaluated the effects of butyrate on the proliferation and activation status of different cell types associated with IBD.

5. Butyrate as an inhibitor of histone deacetylase

Apart from working on the cells, these microbial metabolites also seem to influence HDACs. It has been described that SCFAs, especially butyrate, may inhibit histone deacetylation through SLC5A8.[56] Butyrate has previously been shown to conduce the dissociation of DNA and the histone octamer, causing the relaxation of the nucleosome structure so that various synergistic transcription factors can specifically bind to DNA-binding sites and active gene transcription.[57]

Based on this, butyrate is considered increasingly relevant in clinical practice. Research on the anticancer properties of butyrate has shown that it inhibits cell proliferation and induces cell differentiation and apoptosis by inhibiting HDACs.[11] In addition, butyrate has anti-inflammatory effects on the host.[58] For example, butyrate downregulates IL-1 and VCAM-1 expression in lamina propria macrophages.[20] Martin-Gallausiaux C *et al.* (2018) showed that the inhibitory effect of butyrate on histone deacetylase could activate the AP-1 signaling pathway in intestinal epithelial cells, thus regulating the release of inflammatory factors.[59] Besides, in a diabetic model in juvenile rats, butyrate has been shown to protect β-cells from death and improve glucose homeostasis by HDAC inhibition,[60] which is associated with the expression of *p*-ERK-1/2, *p*-IRS-1 and FOXO1.[61] The above results have also been verified by mice and vitro experiments.[62] In addition to acting as an antitumor and glucose metabolism regulator, butyrate partly takes on an anti-inflammatory role by inhibiting the HDAC.[58] For instance, butyrate plays a crucial role in the down-regulation of proinflammatory effectors (nitric oxide, IL-6, and IL-12[63]) and regulates cytokine expression in T cells.[11]

In summary, butyrate directly influences the HDAC *via* SLC5A8 and hence inhibits cancer cell proliferation, improves glucose homeostasis and regulates proinflammatory cytokines.

6. Effect of butyrate on the nod-like receptors 3 (NLRP3) inflammasome

Inflammasomes are multiprotein complexes that orchestrate proinflammatory cytokine secretion and cell death in immune cells.[64–66] It can be activated by microbial products, environmental factors, as well as endogenous molecules. In cultured ECs (EOMA cells), butyrate was found to significantly decrease the formation and activation of *NLRP3* inflammasomes induced by 7-ketocholesterol (*7-Ket*) or cholesterol crystals (*CHC*).[67] Activated NLRP3 inflammasome would disrupt the epithelial barrier function by down-regulating the expression of *ZO-1*, *TER*, *Occludin*, *Claudin-1*, and *E-cadherin* in cholangiocytes[68] and remarkably elevate the levels of reactive oxygen species (*ROX*) and *Claudin-2.*[69] The activation of *NLRP-3* impairs the barrier function of Caco-2 by down-regulating the expression of tight junction proteins, up-regulating *Claudin-2* expression, and then destroying the morphology.

Autophagy is closely related to the epithelial tight junction barrier, as well as the activation of inflammasomes.[70] *NLRP-3* activated by LPS significantly increases the ratio of *LC3-II*, *LC3-I*, *Beclin1*, and *Atg5*, which activates autophagy. Interestingly, autophagy destroys the intestinal barrier function and also activates *NLRP3* inflammasome.[68] SCFA plays an inhibitory role between *NLRP-3* and autophagy.[69] However, the findings of Chiu HW *et al.* (2016) do not support the previous inferences.[70] This research reported that inducing autophagy suppressed the activation of *NLRP3* inflammasomes in rats; however, autophagy inhibition contributed to *NLRP3* inflammasome activation in macrophages. SCFAs act as energy sources that protect the intestinal barrier but also act as HDAC inhibitors that suppress *NLRP3* inflammasomes. Another research indicated that butyrate promotes NLRP3 inflammasomes *in vitro.*[71]

As discussed above, research on butyrate in other animal models also supports its ability to inhibit *NLRP-3* inflammasomes. For instance, Wang *et al.* (2015) indicated that sodium butyrate (NaB) could significantly inhibit *NLRP-3* inflammasome activation and improve obesity-induced inflammation in mice.[64] When mice with vascular inflammation, atherosclerosis, or *NLRP-3*-mediated diseases like Muckle–Wells syndrome, were fed with butyrate, it could inhibit the inflammatory effect, significantly decrease cytokines, such as *IL-18* and *IL-1*, and alleviate the symptoms of the model.[72] Besides, similar improvement was also found in the models of the familial cold auto-inflammatory syndrome, urate crystal-induced peritonitis, and the DSS-induced colitis mice.[46]

Overall, butyrate can influence the immune response by inhibiting the activation of *NLRP3*.

7. Effect of butyrate on the nuclear factor kappa B (*NF-κB*) inflammatory pathway

PPAR-γ (peroxisome proliferator-activated receptors gamma) is a ligand-activated transcription factor that belongs to the nuclear receptor superfamily 8.[73] Butyrate is also a known activator of the peroxisome proliferator-activated receptors and thus has broad anti-inflammatory effects in many cell

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types.[74,75] In intestinal epithelial cells, butyrate concentrations of 0.01–1 mM induce the activation of *PPARγ* and promote epithelial barrier integrity.[76] Besides, butyrate can also inhibit *NF-κB* signaling *via* activating *PPARγ*. As reported in the literature, effective activation of *PPARγ* may be restricted to the intestinal epithelium and liver.[73]

It is known that *NF-κB* is one of the most crucial regulators of pro-inflammatory genes, such as *IL-1β*, *IL-6*, *IL-8* and *TNF-α*, and also the mediator of *COX-2* and *iNOS* expression.[77] Several studies have shown that butyrate blocks the activation of the *NF-κB* signaling pathway in various inflammation models. An inflammation model of human primary nucleus pulposus cells showed that the expression of *NF-κB* was down-regulated at the protein level after treatment with butyrate.[78] Similar conclusions were reached with bovine macrophages and Kupffer cells. Sodium butyrate attenuates LPS-induced inflammatory responses *via* blocking the *NF-κB* signaling pathways,[79,80] which reduce the expression of *IL-1β*, *IL-6*, and *TNF-α*, as well as monocyte chemoattractant protein 1 (*MCP-1*).[78] *IKB* is an inhibitory factor of *NF-κB* activation and is phosphorylated by its upstream kinases and undergoes subsequent degradation, which are essential steps for the activation of *NF-κB*.[81] Butyrate prevents *IKK* (*IKB Kinase*) and *NF-κB* from phosphorylation.[82] Interestingly, this process does not rely on the activation of *PPAR-γ*.[83]

Therefore, it is a complicated process for butyrate to regulate the host through *NF-κB*.

8. Immune function of butyrate in inflammatory bowel disease (IBD)

Inflammatory bowel diseases (IBD), which encompass Crohn's disease (CD) and ulcerative colitis (UC), are chronic and disabling inflammatory gastrointestinal disorders. The typical characteristic of IBD is a reduction in tight junctions and an increase in tissue permeability, which is also associated with inflammatory cytokines. Numerous studies show that dietary fiber supplementation is a modulator of intestinal barrier function and inflammation. Among the metabolites, SCFAs take on a major role; especially, butyrate is an important immunomodulatory molecule in the intestine.

Animal experiments and clinical studies have shown that butyrate plays an important role in repairing the intestinal mucosa and treating UC. Mice with acute colitis induced by DSS show decreased butyrate resorption in the colon, which destructs the integrity of the mucosa.[84] In the early stage of DSS-induced colitis, increased mucosal permeability is accompanied by diminished cell survival and histological changes. However, the increase in mucosal permeability can be reversed by butyrate. Clinical trials have shown that *Clostridium butyricum*, a butyrate-producing bacterium, has a good therapeutic role in IBD. Butyrate significantly increases the synthesis of mucin and thus has a good curative effect. Distal UC patients treated with sodium butyrate tablets for 6–8 weeks were found to have inhibited nuclear translocation of *NF-κB* and thereby reduced activation of *NF-κB* and *IL-1β*. These were reflected by the improvement of endoscopic and histologic scores.[85,86] Hijova E *et al.* (2017) indicated that DSS-induced colitis mice fed with a diet rich in dietary fiber, which increased the amount of SCFAs, had significantly decreased expression of inflammatory cytokines (*IL-6* and *IL-8*).[87] Further, this enhanced the expression of intestinal tight-junction proteins and the intestinal mucosal barrier function. The following year, research on a rat model of UC induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) showed that rats treated with 0.5 mM kg^{-1} butyrate showed increased production of *IL-10* and *IL-12*, meanwhile, the level of *IL-17* decreased by an *NF-κB* dependent mechanism in the intestine.[88,89] Other similar experiments verified the fact that butyrate can relieve mucosal lesions and reduce intestinal permeability by inhibiting the activation of immune cells, further relieving inflammatory injury.[90,91]

Besides, butyrate enema is considered another effective and specific therapy for proctosigmoiditis and colitis with obstinate ulcers. This highlights the importance of butyrate in the homeostasis of the colonic mucosa and supports its use in treating human diversion colitis.[92] However, Luceri *et al.* (2016)[93] obtained contradictory experimental results claiming that butyrate enema had no effect on the expression of intestinal mucosal mucin 2 (*MUC2*) and trifoliate peptide factor 3 (*TFF3*) and that the treatment of UC was ineffective. Possible reasons for this discrepancy include the method of butyrate treatment, dosage, and the state of the disease. Therefore, additional studies are required to elucidate the role of butyrate in IBD further.

9. Effects of butyrate on diet-induced obesity (DIO)

Multiple studies have shown that butyrate increases energy expenditure to counteract High Fat Diet (HFD)-induced obesity, and no significant alterations in food intake or physical activities were necessary. One mechanism by which butyrate impacts fat metabolism is *via* activating thermogenesis and dissipating chemical energy by heat through uncoupling protein 1 (*UCP1*) to regulate body energy expenditure.[94] Lipids, the primary energy substrates of thermogenesis, are decreased by butyrate by increasing fatty acid oxidation. Research in the brown adipose tissue (BAT) and white adipose tissue (WAT) shows that supplementation with butyrate could increase thermogenesis in BAT and WAT significantly.[95,96] The mechanism of action of butyrate in fat burning is related to the stimulation of lipolysis and enhanced mitochondrial function in the brown fat tissue. Fatty acid β-oxidation and skeletal muscle mitochondrial function were significantly increased after a short-term oral SB treatment in a HF diet-induced inflammatory mouse model.[51] In *ex vivo* cultured adipocytes, butyrate directly increased the expression of *LSD1*, *UCP1*, *MCT1* and the catabolic enzyme acyl-CoA medium-chain synthetase 3 (ACSM3). The inhibition of *MCT1* blocked the effects of buty-

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rate in adipocytes. Furthermore, butyrate-mediated prevention of DIO through increased thermogenesis was attenuated in lysine-specific demethylase knockout mice (*LSD1* aKO mice).[97]

In summary, butyrate improves energy metabolism *via* enhancing fat oxidation by activating BAT. Therefore, BAT and WAT seem to be potential therapeutic targets for combating diet-induced obesity and metabolic disease.

10. Butyrate and brain disorders

In neurological disorders, butyrate is potentially important for its role in anti-inflammatory processes,[98] promoting blood-tissue barrier integrity,[99] and neuromodulation.[100]

A new field of application of butyrate is in brain disorders; butyrate might have beneficial effects in treating Parkinson's disease (PD) injury. PD is a progressive neurodegenerative disorder associated with the destruction of dopamine neurons in the substantia nigra (SN) and the formation of Lewy bodies in basal ganglia. Preclinical evidence suggests that butyrate is specifically beneficial to PD in many aspects[101] as it interacts with *GPR41*. Research on the 6-hydroxydopamine-induced rat model of PD showed that sodium butyrate improved locomotor deficits, reduced premature mortality, and attenuated social deficits in an autism mouse model.[102] In addition, evidence shows that sodium butyrate could up-regulate *DJ-1* expression, a protein known as a neuron protector against excessive dopamine in oxidative stress.[103] Promoting increased butyrate levels is an approach to treating PD with implications in systemic disturbances. However, controversy regarding the commonly accepted anti-inflammatory and neuroprotective action of SCFAs was brought to light in a study using a mouse model of PD.[104,105]

Butyrate is potentially significant for it has long-term beneficial effects on ischemic injury.[106] Ischemic stroke (IS) is a prominent example of such effects. A clinical trial on ischemic brain in rats showed that treatment with sodium butyrate could stimulate cell proliferation, migration and differentiation *via* the upregulation of the brain-derived neurotrophic factor (BDNF).[107] In addition, it could restore the antibiotic-induced impairment of neuronal proliferation[108] and boost widespread neurogenesis after an ischemic insult.[107]

To sum up, in neurological disorders, butyrate promotes blood-tissue barrier integrity and neuroprotective function. Therefore, it represents a promising therapeutic approach for brain disorders.

11. Conclusion

Microbially sourced butyrate plays a crucial role in intestinal immune suppression and inflammatory response. In general, butyrate is transported into cells by SMCTs or MCTs and works as a histone acetylase inhibitor, thus reducing the intestinal mucosal levels of pro-inflammatory cytokines and suppressing the activation of the NF-KB and JAK/STAT pathways. Meanwhile, butyrate can also promote the expression of the anti-inflammatory factors IL-10 and IL-18 by binding to the GPR receptor. It promotes innate immunity, reduces epithelial permeability, and avoids antigen translocation, thereby improving intestinal health and maintaining intestinal homeostasis. In recent years, butyrate has also gained attention in diseases beyond IBD, suggesting that further research on the mechanisms of butyrate function in the brain-intestinal axis, brain-lung axis, and entero-hepatic axis is needed for a more comprehensive understanding of the benefits of butyrate.

Author contributions

Guoqi Dang: conceptualization, software, writing – original draft, writing – review & editing. Weida Wu: conceptualization, writing – review & editing, methodology. Hongfu Zhang: conceptualization, supervision, funding acquisition. Nadia Everaert: writing – review & editing, supervision.

Conflicts of interest

There are no conflicts to declare.

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