

Review Article

The role of butyrate in peripheral regulatory T cell and tolerance induction

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Abstract: Environmental cues have been proven as a pivotal participant in the shaping of immune system via metabolic control, epigenetic reprogramming and direct interaction on some key signaling pathways. Regulatory T cells (Tregs), the major negative component among immune cells, are also a considerably dynamic, plastic and heterogeneous cell population. As such, Tregs are sensitive to environmental factors, which can determine the induction of their subsets such as tissue-specific Tregs, diet-induced Tregs and microbiota-induced Tregs. Recently, short-chain fatty acids (SCFA), mainly produced by the gut microbiota, gained much attention regarding its interrelationship with the immune system. Butyrate has been shown to be a powerful and versatile SCFA, part of its beneficial effect is exerted through the induction of different types of peripheral Tregs. Here, we provide a brief overview for the role of butyrate in the induction of peripheral Tregs in disease conditions.

Keywords: Butyrate, microbiota, epigenetics, regulatory T cells

The immune system has been noted containing two complementary classes of regulatory T cells (Tregs), the thymus-derived natural Treg (nTreg) and the peripheral Treg (pTreg) [1]. In general, nTregs play an important role in the establishment and maintenance of self-tolerance, while pTregs are essential to sustain the peripheral immune homeostasis. Therefore, disturbance of either nTreg or pTreg would cause severe pathological disorders. For this reason, adoptive transfer of Tregs has long been regarded as a hopeful therapeutic approach for treatment of immune related diseases. However, this strategy faces up with problems such as off-cell effects, Treg stability and safety issues, which may impede its implication as a long-term reliable therapeutic choice [2]. Alternatively, large amount of efforts have been concentrated on the *de novo* generation and induction of pTregs such as induction of antigen-specific pTregs using MHC-peptide coated nanoparticles [3]. pTregs have a widespread organ distribution and are easily affected by the unique organ micro-environment, thereby manifesting different types imprinted

with distinct cell surface marker expression and/or functional characteristics [4, 5].

Previous studies demonstrated that intestine, a digestive organ, also serves as a major part of mucosal immune system, which contains a large repertoire of pTregs. Importantly, both diet and microbiota can induce gut Tregs to mediate tolerance toward food antigen and commensal bacterial flora [6]. The metabolic products, short-chain fatty acids (SCFAs) resulted from diet or bacterial fermentation, have been recognized to be the major contributors for induction of pTregs [7]. Butyrate, a well-documented SCFA is of particularly important. It has been indicated to possess the ability either to induce the differentiation or to promote the functionality of Tregs. Especially, consumption of diet containing *Brassica. rapa. L* could increase the percentage of butyrate-producing bacteria associated with higher butyrate content and IL-10 level in the spleen [8], and direct administration of butyrate through oral gavage regulates Th17/Treg balance to prevent 2,4,6-trinitrobenzene sulfonic (TNBS) acid-

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induced colitis in rat [9]. Studies in isolated cells also characterized its potency for induction of stable and suppressive Tregs in human subjects [10, 11]. In this mini review, we intend to provide an overview for the role of butyrate in the induction of Tregs for the maintenance of immune homeostasis in disease conditions. We also discuss the related molecular mechanisms and the potential for its derivatives in clinical applications.

Dietary fibers and microbiota

Generally, our diet is composed of a variety of macronutrients including carbohydrates, proteins, fats and fibers. There is compelling evidence that humans and other vertebrates have coevolved a reliance with gut bacteria during evolution for fiber digestion along with the production of metabolites and certain vitamins [12]. Therefore, our diet can shape gut bacterial ecology and diversity, and alterations in nutritional components (e.g., fibers) correlate with the development of human diseases [13, 14]. Indeed, consumption of diet containing high levels of fibers is associated with a lower risk death from a range of conditions [15]. Particularly, butyrate is one of the beneficial molecules emanated from bacterial metabolism of dietary fibers.

The G protein-coupled receptor (GPCR)-mediated butyrate effect

SCFA receptors belong to a family of G-protein-coupled receptor including GPR41 (FFAR3), GPR43 (FFAR2) and GPR109a (HCAR2) [16]. They serve as an important molecular link between metabolism and immune system [17]. Although much progress has been made concerning the pathological relevance of SCFA receptors in energy metabolism and disorders like type 2 diabetes and obesity, their role in immune regulation, however, has recently begun to be recognized. For example, mice deficient in *GPR43* manifest exacerbated or unresolved inflammation in models of colitis, arthritis and asthma [18], while GPR41 is required for helminth-induced anti-inflammatory cytokine secretion and regulatory T cell suppressive activity in the lung [19].

SCFA receptors have a broad expression on multiple immune cell types, with myeloid lineage showing the highest expression levels

[20]. Therefore, it is likely that those immune cells with GPCR expression contribute to butyrate-mediated protective effect observed in autoimmune models. It is believed that GPCR is indirectly engaged in butyrate function *via* dendritic cells. Oral intake of specific multi-fiber mix elevates Treg cell percentage in the mesenteric lymph nodes (MLN), which correlates with increased tolerogenic lamina propria derived CD103⁺RALDH⁺DC in the MLN, indicating a role of DC in the diet-induced Tregs [23]. GPR109a, known as niacin receptor, is also the receptor for butyrate. Butyrate-GPR109a signaling endows colonic and splenic DC with the ability to secrete retinoic acid, thereby instructing naïve T cells into Treg cells [24]. In support of this notion, acetate and propionate have been proven to prompt accumulation of nTregs in the intestine milieu through GPR43, either boosting the proliferation of nTregs or up-regulating GPR15 expression [21]. In contrast, butyrate appears to exert its immunoregulatory effect in GPR41 or GPR43-independent manner, and it mainly influences the *de novo* generation of peripheral induced pTregs, rather than thymus-derived nTregs [22].

The impact of butyrate on epigenetic regulation

Butyrate has long been recognized as a potent HDAC inhibitor. It has been well known that protein acetylation is a reversible and dynamic epigenetic modification process, which is counter-regulated by histone acetyltransferases (HAT) and histone deacetylases (HDACs). HDACs comprise a single NAD-dependent sirtuin family of class III (SIRT1-7) and three classes of Zn-dependent enzymes: class I includes HDAC1, 2, 3, and 8; class II includes HDAC4, 5, 7 and 9 (subclass IIa), and HDAC6, and 10 (subclass IIb); as well as the sole class IV HDAC11 [25]. Butyrate inhibits the activity of most HDACs except for class III SIRTs and class IIb HDAC6 and 10. The mechanism may involve the elevation of acetyl-CoA level and/or direct contact, although the binding sites remain elusive, and whether this inhibition is competitive or noncompetitive is yet to be determined [26].

Aberrant histone/protein acetylation is a causative factor in the disturbance of cell events. Therefore, Treg differentiation and function are

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also closely-related to acetylation. There is compelling evidence that Treg cells have significantly higher HDAC activity than that of CD4⁺CD25⁻ T cells. Particularly, of the ten class I and class II HDAC proteins, HDAC9 is a notable regulator for Foxp3 expression and, as a result, depletion of HDAC9 promotes Foxp3 acetylation as well as Treg suppressive function [27]. In line with this observation, pan-HDACi, but not class I-specific HDACi, were found to enhance Treg function and reduce colitis in mice. Of note, HSP70 serves as a molecular chaperon to mediate the effect of HDAC9 inhibitor on Foxp3 expression [28]. Other than HDAC9, HDAC6 [29] and SIRT1 [30] are also found capable of regulating Foxp3 expression, albeit they are non-targets of butyrate. Conversely, members of class IIa exhibit negative impact on Treg function. For example, loss of HDAC5 attenuates Treg function and generation through an unknown mechanism [31]. Collectively, the isoform-selective effects of HDAC inhibition on Treg function are yet to be fully elucidated.

Interestingly, a recent study revealed that butyrate could increase H3 acetylation in the Foxp3 promoter and the conserved noncoding sequence 1/3 region (CNS1/3), by which it preferentially promotes the generation of induced NRP1-Helios⁺CD103⁺ pTregs and, most importantly, this process seems to be irrelevant to the over-expression of critical transcriptional factors such as c-Rel, NFAT, Smad3 or Stat5 [32]. It is noteworthy that butyrate-mediated HDAC inhibition is competent of acting through dendritic cells and epithelial cells. For example, butyrate could impair functional differentiation of human monocyte-derived dendritic cells by virtue of HDAC inhibition [33], and further studies revealed that RelB could be a target responsible for the production of such Treg-supportive DC population [22]. Similarly, butyrate was found to stimulate epithelial production of retinoic acid via inhibition of epithelial HDAC [34], which forms another layer of action in favor of Treg generation.

Butyrate downstream signaling

Aside from GPCR and histone/protein acetylation, several signaling pathways are also suggested to be prominent in T cell anergy and tolerance induction. Studies in germ-free mice demonstrated that some gut-indigenous ba-

cteria are pretty important in T cell education and immune adaptation. For example, segmented filamentous bacteria (SFB) specifically deviate the generation of Th17 cells, while clostridia and bacteroids fragilis are professional to navigate T cells towards Treg fate, which largely relies on the TLR-MyD88 pathway [35, 36]. However, although butyrate is a major product originated from so-called Treg-inducing bacteria, it appears to exert its function independent of TLR-MyD88 pathway [32], indicating an additive and auxiliary role of butyrate to sustain the Treg-promoting milieu since they are initially educated.

As for other signaling pathways involved, some of them clearly emerge as a secondary effect resulted from HDAC inhibition. For example, SCFA could suppress P38 and JNK1 signaling through up-regulation of Lipin2 [37], which then activates mTOR-S6K pathway via S6K acetylation [38], and restore acetylation of tumor suppressor gene p21 to induce T cell anergy through cell cycle inhibition [39]. Studies in p21 revealed the existence of butyrate response element in the promoter region. After butyrate administration, HDAC is inhibited, which results in the higher activity of SP1/SP3 and the transcription of p21 [26]. However, not all pathways have straight forward relationships with HDAC mediated gene expression in T cells. Butyrate has also been shown to activate GSK3 β and PKA, as well as promote cAMP accumulation in T cells, by which it inhibits NFAT transcriptional activity and IL2 production without affecting calcineurin, MAPK, AP1 and NF- κ B pathways [40]. Similarly, Granzyme-B also contains butyrate response elements in the promoter region, which renders it sensitive to butyrate treatment [41]. Taken together, the co-factors interacting with HDAC or the existence of other mechanisms and pathways downstream of butyrate signaling still require further investigation.

Butyrate and its derivatives in the treatment of immune disorders

As a natural product, butyrate is quite convenient to be incorporated into the everyday diet. It can also be easily modified to generate derivatives to overcome the defects of short half-time decay and inadequate pharmacological efficacy. Chemical structure of butyrate and its derivatives are summarized in **Figure 1**.

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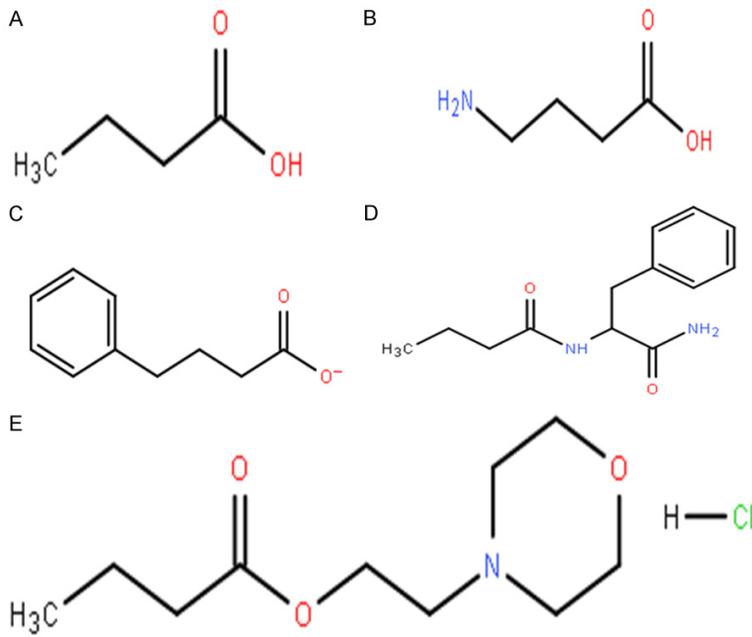


Figure 1. Chemical structure of butyrate and its derivatives. (A) butyric acid; (B) γ -Amino butyric acid (GABA); (C) 4-phenylbutyrate (PBA); (D) N-(1-carbamoyl-2-phenylethyl) butyramide (FBA), and (E) 2-(4-morpholinyl) ethyl butyrate hydrochloride (MEB).

Apart from the well-defined role of butyrate played in inflammatory bowel disease (IBD) treatment [42-44], it has recently been identified as a regulator in the skin, where butyrate invokes induction of Tregs and shows the potential to mitigate inflammatory skin reactions [45]. In type 1 diabetes (T1D) model NOD mice, administration of butyrate-containing or butyrate-yielding diet boosts Treg function and decrease serum concentration of diabetogenic cytokines, thereby providing a high degree of protection against spontaneous T1D [46].

Similar as butyrate, its derivatives also exhibit high potential for disease therapy in clinical settings. Orally administration of butyrate-releasing derivative, N-(1-carbamoyl-2-phenylethyl) butyramide (FBA), reduces colitis symptoms and colon damage with improved taste and smell [47]. Similarly, phenylbutyrate displays favorable effects on many human diseases [48]. Moreover, both GABA, and γ -Aminobutyric acid act as a major inhibitory neurotransmitter and possess high potency to inhibit autoimmune T cell response in T1D mouse models [49]. In particular, 2-(4-morpholinyl) ethyl butyrate hydrochloride, the mostly studied butyrate derivative, is capable of inducing antigen-specific T cell anergy and, by which it

prevents graft-versus-host disease [50-52]. Collectively, butyrate and its derivatives are promising agents for future clinical applications. The effect of butyrate and its derivatives on immune related diseases are summarized in **Table 1**.

Conclusion and perspectives

In the present mini review, we summarized the function of butyrate on T cell based tolerance induction, particularly for its role in peripheral induced Treg generation. Lines of evidence show that butyrate could work directly on Treg cells, leading to hyperacetylation of Foxp3 promoter and CNS region. It is noteworthy that it does not have a perceptible impact on thymus derived nTregs, which differs

butyrate from other SCFA such as acetate and propionate. The reason for this discrepancy may attribute to the low binding affinity of butyrate to GPR43 which is expressed on nTreg cells. As for the conventional T cells, butyrate and its derivatives prevent T cell activation and expansion by means of cell cycle blockage and IL-2 deprivation. Furthermore, butyrate could also act indirectly through DC and epithelial cells to secrete factors in support of Treg induction.

Despite we emphasize the immune regulatory role of butyrate played herein, the possible pro-inflammatory effect should not be out of concern. Studies also argue against the effect of SCFA including butyrate in promoting the function of effector T cells, especially within the renal system [38, 53]. This kind of "butyrate paradox" remains obscure, and microenvironment was accused for the discrepancy. For example, under steady state butyrate promotes Treg induction, while under inflammatory settings, butyrate may concurrently promote Th1/Th17 functionality and IL-23 secretion from activated DC [54], and other factors as well as cell types presented in the microenvironment could be decisive to the final outcome of butyrate's functional duality.

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Table 1. Butyrate and its derivatives in the treatment of immune related disease models

Butyrate or its derivatives	Disease entity	Modes of action	Publications
Butyrate, butyrate-yielding diet	IBD	Peripheral Treg induction	[41-45]
	Skin inflammation T1D		
FBA	DSS-induced colitis	Reduce neutrophil recruitment and maintain barrier integrity	[46]
PBA	Neuro-and lung inflammation	NF- κ B signaling suppression, alleviation of oxidative and ER stress	[47]
GABA	T1D	Inhibit diabetogenic T cell cycle progression	[48]
MEB	Transplantation	Allogenic T cell inactivation	[49-51]

FBA, N-(1-carbamoyl-2-phenylethyl) butyramide; PBA, 4-phenylbutyrate; GABA, γ -Amino butyric acid; MEB, 2-(4-morpholinyl)ethyl butyrate hydrochloride; IBD, inflammatory bowel disease; T1D, type 1 diabetes.

Mechanistically, HDAC inhibition is regarded as the main function of butyrate for a long period of time, which is also repeatedly proved to be dominant in various of cell types and disease models. However, there is no reason to exclude other possibilities. First, identification of “butyrate response element” which under the control of butyrate affects gene expression without disturbance for the activity of known important HDACs [41], suggesting the existence of acetylation on DNA-binding proteins. More recently, butyrylation and endogenous beta-hydroxybutyration have been recognized as novel histone acyl-modifications [55], which are important in metabolic regulation and inflammasome activation [56, 57]. Therefore, further studies would be necessary to address whether non-histone protein modification, or other types of histone modification are engaged in the immune system and contribute to the tolerance-inducing effect of butyrate.

In summary, butyrate serves as a bridge to link diet, microbiota, metabolic process and epigenetics to the host immune adaptation. Dietary change, fasting, exercise, and caloric restriction are long-term life style adjustment, while the beneficial effect on human health is closely related to butyrate metabolism. Given the fact of selective action on activated T cells and induction of peripheral Tregs, butyrate derivatives could be more effective immune regulators as compared to the traditional immunosuppressants.

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Disclosure of conflict of interest

None.

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