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Pro- and anti-inflammatory effects of short chain fatty acids on immune and endothelial cells

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ABSTRACT

In the gastro-intestinal tract, short chain fatty acids (SCFAs) have protective effects on epithelial cells. However, their effects on inflammatory cytokine production by endothelial and immune cells and the recruitment of immune cells and their trans-migration across the endothelial layer remain controversial. Both cell types are associated with the initiation and development of inflammatory diseases, such as atherosclerosis and sepsis. SCFAs modulate immune and inflammatory responses via activation of free fatty acid (FFA) receptors type 2 and 3 (FFA2 and FFA3 receptors), G protein-coupled receptor 109A (GPR109A) and inhibition of histone deacetylases (HDACs). This review will focus on the effects of SCFAs on lipopolysaccharide (LPS)- or tumor necrosis factor-alpha (TNFα)-induced inflammatory response on endothelial and immune cells function, and an overview is presented on the underlying mechanisms of the effects of SCFAs on both immune and endothelial cells, including HDACs, FFA2 and FFA3 receptors and GPR109A regulation of nuclear factor-kappa B (NF-κB) activation and mitogen-activated protein kinase (MAPK) signaling pathways.

1. Introduction

The vasculature is the main “organ” for blood supply to our vital organs and mainly consists of three layers. The luminal side of blood vessels is endothelial layer which has multiple roles in keeping the human body in homeostasis by maintaining a stable anti-inflammatory, anti-coagulant and anti-adhesive status and by controlling exchanges between circulating blood components and cells (Rubanyi, 1993). Endothelial cells also cooperate with immune cells in the regulation of local and systemic inflammation. Both endothelial and immune cells can be activated by lipopolysaccharide (LPS) and tumor necrosis factor-alpha (TNFα) and lead to endothelial and immune cells dysfunction (Dauphinee and Karsan, 2006; Madge and Pober, 2001). Endothelial dysfunction-induced by endogenous and external stimuli will effectively induce a systemic state of inflammation and other immune responses by increasing the expression and production of (pro-) inflammatory mediators, adhesion molecules and excessive immune cell adhesion and migration (Hadi et al., 2005; Iantorno et al., 2014). Immune cell adhesion to endothelial cells is mediated by adhesion molecules expressed on immune cells and endothelial cells, e.g. P-, E- and L-selectins, β_1- and β_2-intergrins, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and chemokines, e.g. monocyte chemo-attractant protein-1 (MCP-1) (Henricks and Nijkamp, 1998; Mestas and Ley, 2008). The recruitment of monocytes to the endothelial layer facilitates the transmigration to the sites of the lesion, where monocytes differentiate into macrophages which become foam cells after lipid uptake, leading to the development of atherosclerosis (Randolph, 2009). Moreover, the transmigrated immune cells aggravate the inflammatory responses by producing more cytokines, thereby creating a continuous cycle between endothelial cells and immune cells (Fig. 1). Excessive cytokine production and immune cell adhesion to the sites of lesion are two important contributors to the development of inflammatory disorders, including atherosclerosis (Ross, 1999) and sepsis (Casey et al., 1993; Paulus et al., 2011). Endogenous cytokines produced by endothelial and/or immune cells enhance the inflammatory response and initiate tissue damage. The levels of TNFα, interleukin-6 (IL-6) and LPS are regarded as diagnostic markers in inflammatory diseases (Czepiel et al., 2014; Palladino et al.,

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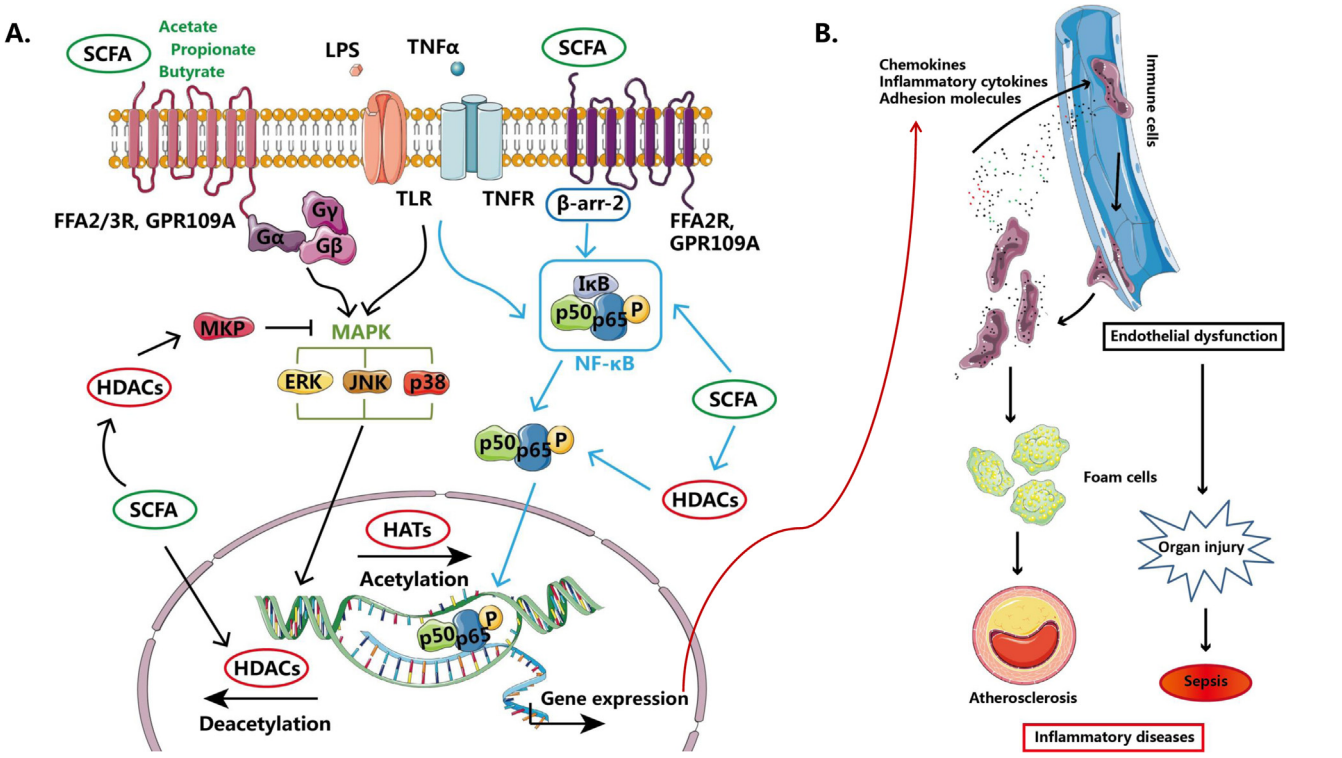

Fig. 1. Overview on the effects of SCFAs on LPS- or TNFα-induced endothelial dysfunction and immune cell activation. A: LPS or TNFα binds to its receptor and activates MAPK and NF-κB signaling pathways to modulate gene expression including inflammatory cytokines, chemokines and adhesion molecules which are important factors in the development of atherosclerosis and sepsis. SCFAs, mainly acetate, propionate and butyrate, can regulate MAPK signaling pathway and activation of NF-κB via activation of FFA2 and FFA3 receptors and GPR109A or inhibition of HDACs activity. B: LPS or TNFα-dependent production of inflammatory cytokines, chemokines and adhesion molecules induce immune cell adhesion and recruitment to the inflammation lesion. The recruited immune cells produce excessive cytokines and attract more immune cells which forms a vicious circle which leads to foam cell formation and the development of atherosclerosis. Besides, endothelial dysfunction and activated immune cells are also important in the progression of organ injury and sepsis.

2003). However, the therapeutic potential of neutralizing antibodies for these cytokines failed to diminish the outcome of inflammatory diseases (Casey et al., 1993; Glauser, 1996; Palladino et al., 2003). Therefore, new efficient drugs to prevent, combat and cure inflammatory diseases are needed.

Epidemiological evidence indicates that increased consumption of dietary fibers improves cardiovascular function and reduces systemic inflammation, atherosclerosis as well as immune disorders (Anderson et al., 2009; Esposito and Giugliano, 2006), while low fiber diets are associated with increased inflammatory disorders (Morrison and Preston, 2016). For example, after two weeks addition of supplementary soluble fiber in the diet, the levels of circulating pro-inflammatory mediators such as TNFα, IL-6 and IL-8 were reduced (Macfarlane et al., 2013). Dietary fibers affect host physiology by the production of metabolites, such as short chain fatty acids (SCFAs) (Li et al., 2014; Threapleton et al., 2013; Vinolo et al., 2011c). SCFAs, mainly acetate, propionate and butyrate, are fermentation metabolites of carbohydrates produced by the intestinal microbiome. The total SCFA concentration in the lumen of the colon decreases progressively from the proximal to the distal end from 70 to 140 mmol/l to 20–70 mmol/l respectively (Topping and Clifton, 2001) with the ratio of acetate, propionate and butyrate in the colon being 60:25:15 (Tazoe et al., 2008). SCFAs are absorbed by the colonic epithelial cells, pass the portal vein, but mostly are then metabolized by the hepatocytes in the liver. A small fraction of SCFAs can pass the liver and result in a low but measurable concentration in the systemic circulation. Moreover, intravenous administration of SCFAs (Fukumori et al., 2011), sodium butyrate (Katoh and Tsuda, 1987) or oral administration of tri-butyrin (a prodrug of butyrate) (Miyoshi et al., 2011), increases the systemic SCFA concentrations.

Although there is a clear correlation between high fiber consumption and changes the host immune status, the underlying mechanisms are largely unknown. SCFAs form a link between dietary intake and improved health outcomes in Western societies. However, there is insufficient evidence for appropriate clinical or public health interventions with clearly defined outcomes using SCFA formulations in intervention or treatment of cardiovascular diseases. This review aims to offer an integrated view of recent achievements in understanding the effects of SCFAs on LPS- or TNFα-induced endothelial and immune cell dysfunction, in particular, aspects of inflammatory cytokine production and migration and recruitment of immune cells, as well as the main mechanisms involved in the inflammation modulatory effects of SCFAs.

2. SCFAs as agonists of free fatty acid (FFA) receptors type 2 and 3, G-protein coupled receptor 109A (GPR109A) and inhibitor of histone deacetylases (HDACs)

SCFAs appear to have a regulatory function in cardiovascular disorders. The downstream effects of SCFAs are mainly ascribed to two pathways: (1) activation of receptors, including FFA2 and FFA3 receptors, and GPR109A, and (2) inhibition of HDACs (Tan et al., 2014).

2.1. SCFAs as agonists of FFA2 and FFA3 receptors and GPR109A

The FFA receptors are G protein-coupled receptors, which are dose-dependently activated by free fatty acids and are involved in various physiological and pathophysiological processes (Brown et al., 2003; Hara et al., 2013). FFA2 and FFA3 receptors and GPR109A are the main receptors for SCFAs. FFA2 and FFA3 receptors are differentially expressed on cells and regulate diverse cellular functions. The FFA2

receptor is mainly expressed on immune cells, including neutrophils, eosinophils, dendritic cells and monocytes, indicating a broad role in inflammatory and immune responses (Kim et al., 2014). The FFA3 receptor is mainly expressed on pancreas, spleen and adipose tissue and has been implicated in obesity and other metabolic diseases. However, FFA3 receptors are also expressed on immune cells, but at lower levels compared to FFA2 receptors (Kim et al., 2014). The FFA2 receptor is likely the main receptor contributing to the effect of SCFAs on inflammatory and immune disorders, but the effects via FFA3 receptors on metabolic disorders have also been widely recognized (Ulven, 2012). SCFAs can activate FFA2 and FFA3 receptors and their potencies on activation of FFA2 and FFA3 receptors are different. The optimum chain length for the activation of the FFA2 receptor spans two to three carbon atoms (acetate and propionate) and for the activation of the FFA3 receptor ranges from three to five atoms (propionate and butyrate), both with half maximal effective concentration (EC_{50}) of around 0.5 mM (Brown et al., 2003; Le Poul et al., 2003). Therefore, the potency rank orders of SCFAs for the FFA2 receptor is acetate ~ propionate > butyrate, and for the FFA3 receptor is propionate ~ butyrate > acetate (Ulven, 2012).

SCFAs activate FFA2 and FFA3 receptors and trigger different downstream signaling cascades. The activated FFA3 receptor is coupled to $G_{\alpha i}$ and inhibits adenylyl cyclase, and therefore decreases the levels of cyclic AMP (cAMP). Activation of the FFA2 receptor is linked with both $G_{\alpha i}$ and $G_{\alpha q}$ and decreases cAMP levels and increases cytoplasmic calcium concentrations (Brown et al., 2003). The FFA2 receptor also engages an alternative signaling pathway mediated by β-arrestins-2, producing anti-inflammatory effects by inhibition of NF-κB. For example, the pro-inflammatory cytokines IL-6 and IL-1β were downregulated by activation of FFA2 receptors and knocking-out β-arrestin-2 recovered their expression (Gao et al., 2004; Lee et al., 2013). There are, as far as we know, no reports linking FFA3 receptors to β-arrestins (Lee et al., 2013).

GPR109A is classified as an orphan G-protein coupled receptor and known as hydroxyl-carboxylic acid 2 (HCA_2) receptor. GPR109A is expressed primarily on adipocytes and also expressed on immune cells including neutrophils and macrophages (Chai et al., 2013). But there is no information indicating its presence on endothelial cells. Interest in GPR109A is growing since its discovery as the receptor for niacin a decade ago, along with deorphanisation as the receptor for endogenous ligand 3-hydroxy-butyrate. SCFAs, mainly butyrate, show property to activate GPR109A. Activation of GPR109A is also coupled to the inhibitory G protein G_i/G_o (Martin et al., 2009) and activation of GPR109A can recruit β-arrestins from the cytosolic compartment to the cell membrane (Chai et al., 2013). GPR109A is also linked to the regulation of vascular inflammation in atherosclerosis (Chai et al., 2013).

Taken together, signaling through FFA receptors could result in different outcomes in different cell types depending on receptor expressions and binding of different subunits of FFA receptors or β-arrestins. For example, butyrate inhibited reactive oxygen species production in neutrophils in a pertussis toxin (PTX)-sensitive manner, while acetate increased reactive oxygen species production in macrophages in a PTX-insensitive manner (Vinolo et al., 2009a). These findings stress the opposing signaling via FFA2-$G\alpha_{q/11}$ and FFA3- $G\alpha_{i/o}$.

2.2. SCFAs as HDAC inhibitors

Most of the HDACs are ubiquitously expressed in immune, endothelial and vascular smooth muscle cells. HDACs and histone acetyltransferases (HATs) modulate acetylation of histone protein which is involved in epigenetic DNA modification (Zhou et al., 2011). HDACs are an evolutionary conserved family of proteins that include four classes: I (HDAC1–3 and 8), II (HDAC4–7 and HDAC9–10), III sirtuins (SIRT1–7) and IV (HDAC11) and are present in the cytoplasm or nucleus of cells (Didonna and Opal, 2015; Gray and Ekstrom, 2001). Inhibition of HDACs activity causes an increase in acetylation in histone proteins and decreases positive charge on histones. The decreased positive charge reduces binding of histones to the negatively charged DNA, leading to an open structure of DNA/chromatin, which facilitates the binding of transcription factors, such as signal transducer and activator of transcription3 (STAT3), NF-κB and forkhead box P3 (FOXP3), thereby initiates gene transcription (Fig. 1A). Although, inhibition of HDACs theoretically results in an increase in gene transcription, HDAC inhibitors can either inhibit or facilitate specific gene expression, depending on the promoter and chromatin status (Reichert et al., 2012). HDAC inhibitors were originally developed as anti-cancer agents (Ropero and Esteller, 2007). Now we know that HDACs are also involved in the regulation of inflammatory gene expression, vascular integrity and the development of cardiovascular diseases, including atherosclerosis and sepsis (Ciarlo et al., 2013; Ordovas and Smith, 2010; Rafehi et al.; Xu et al., 2014).

HDAC inhibitors can be structurally classified into at least four classes: hydroxamates, cyclic peptides, aliphatic acids and benzamides (Kim and Bae, 2011). Trichostatin (TSA) and suberoylanilide hydroxamic acid belong to hydroxamates class and are very potent inhibitors with an efficacy at a nanomolar to low micromoloar range. SCFAs belong to aliphatic acids class with effective inhibitors of HDAC enzymes in the millimolar range (Licciardi et al., 2011). These HDAC inhibitors are generally known as broad-spectrum inhibitors. SCFAs, mainly butyrate and propionate, inhibit class I and class IIa HDACs, and downregulate SIRT1 expression (Schilderink et al., 2013; Yu et al., 2014). Butyrate and propionate are non-competitive inhibitors of HDACs and specifically inhibit the activity of HDAC1 and HDAC3 (Vinolo et al., 2011b). Among the SCFAs, butyrate is the most potent inhibitor of HDACs with approximately 80% inhibitory efficiency, while the inhibitory efficiency of propionate is approximately 60% (Kasubuchi et al., 2015). Most, but not all of the studies indicate that acetate has no HDAC inhibitory activity. For instance, acetate enhanced IL-6, IL-8 and TNFα production in LPS-exposed macrophages accompanied with increased acetylation of pro-inflammatory gene histones (Kendrick et al., 2010). HDAC activity may be inhibited by SCFAs after entering the cell via passive diffusion or sodium-coupled monocarboxylate transporters ((SMCT-1)/Slc5a8 and via FFA receptor activation (Sun et al., 2017). However, the roles of FFA receptors in inhibiting HDACs are controversial. For example, inhibition of HDACs by activation of FFA3 receptors in Chinese hamster (*Cricetulus griseus*) ovary cell lines suppressed histone acetylation (Wu et al., 2012). SCFA-induced inhibition of HDACs in colon tissue was largely FFA2 receptor dependent (Smith et al., 2013) while acetate may influence the inflammatory process by regulating epigenetic modification in a FFA receptor-independent manner (Andrade-Oliveira et al., 2015). Furthermore, butyrate and propionate inhibited HDAC activity, independent of FFA2 and FFA3 receptors (Aoyama et al., 2010). It is not completely clear whether this effect is direct or indirect, and further studies are necessary to confirm any causal relationship between FFA receptor activation and HDAC inhibition.

3. The roles of SCFAs in the regulation of inflammation in immune cells

The immune system protects the host against pathogens by secreting inflammatory cytokines and mediating the clearance of pathogens. However, excessive production of cytokines will lead to systemic inflammation and pathological diseases (Swirski and Nahrendorf, 2013). SCFAs modulate inflammation by regulating immune cell cytokine production. For example, butyrate and propionate decrease LPS-induced TNFα and nitric oxide synthase (NOS) expression in monocytes (Vinolo et al., 2011b). These effects are mediated by activation of FFA2 and FFA3 receptors and GPR109A or inhibition of HDACs.

3.1. FFA2 and FFA3 receptors and GPR109A mediate the pro- and anti-inflammatory effects of SCFAs in immune cells

FFA2 and FFA3 receptors expression is upregulated by LPS stimulation in monocytes and macrophages and this indicates a potential role of these receptors during systemic inflammation (Ang et al., 2016). However, it is not clear which of the two FFA receptors is more important or whether they cooperate to induce anti-inflammatory or pro-inflammatory effects, because controversial results are reported depending on ligand, cell type, organ and disease.

SCFAs reduced IL-8 production in the airways during airway inflammation by activation of FFA2 and FFA3 receptors on neutrophils and macrophages (Halnes et al., 2017). Acetate also inhibited LPS-induced TNFα secretion from mice and human mononuclear cells by activating FFA receptor pathways (Masui et al., 2013). Administration of propionate to allergic mice reduced inflammatory mediators, such as IL-4, IL-5 and IL-17A in the lungs, through an FFA3 receptor-dependent manner (Trompette et al., 2014). In macrophages, butyrate had anti-inflammatory effects (decreasing inducible NOS (iNOS), TNFα, MCP-1 and IL-6 production) by activation of FFA3 receptors (Ohira et al., 2013). These effects were incompletely blocked by PTX since PTX blocked the response to FFA3 receptor activation, but not to FFA2 receptor activation, indicating that the anti-inflammatory effects of butyrate are associated with FFA3 receptor and other non-FFA receptor pathways. These observations indicate that FFA2 and FFA3 receptors act as anti-inflammatory receptors and FFA2 and FFA3 receptor agonists could offer new opportunities for the treatment of inflammatory diseases.

In contrast, pro-inflammatory roles of activated FFA2 or FFA3 receptors are also reported and associated with the activation of MAPK, phosphoinositide 3-kinase (PI3K) or rapamycin (mTOR) signaling pathways (Seljeset and Siehler, 2012; Thorburn et al., 2014). Activation of FFA2 and FFA3 receptors by acetate increased the production of cytokines (IL-6, CXCL1 and CXCL2) via activation of the extracellular signal-regulated kinases 1/2 (ERK1/2) and p38MAPK signaling pathways. Deletion of FFA2 or FFA3 receptors in mice diminished IL-6 production and delayed the expression of interferon gamma (INFγ) and chemokines (Kim et al., 2013), thereby protecting these mice against inflammatory tissue destruction. These studies indicate the pro-inflammatory effects of activation of FFA2 and FFA3 receptors and antagonists of FFA2 and FFA3 receptors may have protective effects in inflammatory diseases.

GPR109A expression is upregulated by cytokines such as IFN-γ in macrophages (Schaub et al., 2001), pointing to a role for GPR109A in immunity and inflammation. GPR109A activation and its direct anti-inflammatory potential in the vasculature had also emerged. Activation of GPR109A inhibits TLR4-induced expression and secretion of TNFα, IL-6 and MCP-1and reduces progression of atherosclerosis (Digby et al., 2012). Among the SCFAs, only butyrate binds to GPR109A with low affinity and activation of GPR109A by butyrate exerts anti-inflammatory effects in colonic inflammation (Chai et al., 2013; Singh et al., 2014). However, there is a lack of information about GPR109A-mediated effects of butyrate on immune cells in the regulation of cardiovascular function. Since GPR109A shows anti-inflammatory effects, it is worthwhile to investigate GPR109A-mediated effects of SCFAs on immune cells in cardiovascular disorders, such as atherosclerosis.

Due to the opposite effects of activation of FFA2 and FFA3 receptors and unravelled roles of GPR109A, it remains unclear whether the use of an agonist or an antagonist of FFA2 and FFA3 receptors would be preferred in clinical settings. In a study in humans, it was demonstrated that GLPG0974, an FFA2 receptor antagonist, did not meet clinical endpoints due to the induction of mild-to-moderate ulcerative colitis in spite of a reduction in neutrophil activation and infiltration (Suckow and Briscoe, 2017). In general, acetate and propionate stimulate while butyrate inhibits immune cell function (Bolognini et al., 2016). This might explain the conflicting data when mixtures of SCFAs are used, and may be further complicated when non-receptor mediated anti-inflammatory effects are involved. FFA receptors may provide a link between diet, gut microbiota and host immune homeostasis, and highlight the importance of FFAs in the regulation of inflammatory and immunological processes.

3.2. HDACs mediate the pro- and anti-inflammatory effects of SCFAs in immune cells

Butyrate and propionate showed anti-inflammatory activities by inhibition of HDACs in macrophages and dendritic cells. Butyrate and propionate decreased LPS-induced TNFα production in mononuclear cells via inhibiting NF-κB activation, and the effects of butyrate and propionate were similar to the HDAC inhibitor TSA (Usami et al., 2008). In another study, treatment with butyrate and propionate suppressed TNFα production and NF-κB activity, and promoted the production of anti-inflammatory cytokine IL-10 in LPS-activated mononuclear cells and neutrophils by inhibition of HDACs (Aoyama et al., 2010; Chang et al., 2014; Vinolo et al., 2011c). However, these roles of HDACs in modulating the inflammatory response were demonstrated by non-specific HDAC inhibitors. Specific HDAC inhibitors should be used to investigate the specific role of each HDAC subtype.

HDAC3-deficient macrophages were unable to activate almost half of the inflammatory gene expression program when stimulated with LPS. Especially, the IFNβ-dependent branch of the LPS response was almost completely abrogated because of the reduced basal and LPS-inducible IFNβ expression. These data indicate a central role for HDAC3 in inflammation and may have relevance for the use of selective HDAC inhibitors as anti-inflammatory agents (Chen et al., 2012).

HDAC5 belongs to class II and is regulated by phosphorylation of serine residues at the N-terminus of the enzyme. Class II HDACs can shuttle between the nucleus and cytoplasm. The capacity to translocate enables the interaction with cytoplasmic non-histone proteins such as NF-κB (Poralla et al., 2015). Knock-down of HDAC5 significantly reduces the LPS-induced production of TNFα and MCP-1 in murine and human macrophage cell lines, and over-expression of HDAC5 significantly elevated the production of these cytokines as well as anti-inflammatory IL-10. These effects were accompanied by increased NF-κB activity (Poralla et al., 2015). Therefore, HDAC5 has a regulatory function in the pro-inflammatory response of macrophages.

HDAC6 is involved in the regulation of inflammatory and immune responses, specifically at the level of the antigen-presenting cells / T cell immune synapse, regulatory T cell function and macrophage responses (Halili et al., 2010). HDAC6, as a transcriptional activator, is required for the production of IL-10 by macrophages and inhibition of HDAC6 disrupted the anti-inflammatory STAT3/IL10 axis in macrophages (Cheng et al., 2014b). However, the primarily nuclear protein HDAC11 represses IL-10 gene expression in macrophages (Cheng et al., 2014a). Two different HDACs are recruited to the same gene promoter to dictate divergent transcriptional responses.

Taken together, these data indicate that the HDAC subtypes do have inflammation modulatory properties on immune cells. Hitherto, due to their broad-spectrum inhibition of HDACs, it is not completely clear which specific HDAC mediates the effects of SCFAs on stimuli-induced immune cell dysfunction. This might be the reason for the pleiotropic effects of SCFAs. Therefore, specific HDAC knockdowns in different cell types and animals are necessary for investigating the roles of the different HDACs and SCFAs in cardiovascular function and disease in which (chronic) inflammation plays a pivotal role.

4. The roles of SCFAs in regulation of inflammation in endothelial cells

Data on the roles of FFA2 and FFA3 receptors and HDACs in the effects of SCFAs on endothelial cells is scarce despite of the fact that SCFAs (acetate, propionate and butyrate) attenuate TNFα- or LPS-

induced endothelial activation by inhibiting the production of pro-inflammatory cytokines (IL-6 and IL-8) (Li et al., 2018) and FFA2 and FFA3 receptors as well as HDACs are expressed on/in endothelial cells (Lee et al., 2012; Pluznick, 2014). Voltolini *et al.* studied the involvement of FFA2 and FFA3 receptors in the effects of SCFAs on regulation of inflammatory cytokine production in endothelial cells and found that the LPS-induced mRNA expression of FFA2 receptors and inflammatory genes, such as IL-6 and IL-8 was attenuated by sodium propionate (Voltolini et al., 2012).

HDAC inhibitors stimulate anti-inflammatory signaling pathways in the endothelium, pointing to a therapeutic potential of HDAC inhibitors in the treatment of inflammatory diseases. SCFAs, especially butyrate as a HDAC inhibitor, protect against vascular inflammation and atherosclerosis, thereby modulating endothelial function, pro-inflammatory cytokine production and oxidative stress (Davie, 2003; Hoffman et al., 2016). Administration of two structurally unrelated HDAC inhibitors, TSA and sodium butyrate, alleviated sepsis-induced lung injury accompanied with LPS-induced IL-6 and cyclooxygenase-2 (COX-2) expression in human intestinal endothelial cells (Ogawa et al., 2003). Based on limited information, FFA2 and FFA3 receptors and HDACs are involved in the anti-inflammatory effects of SCFAs. However, the roles of SCFAs in regulation of endothelial dysfunction and cardiovascular diseases and the exact roles of FFA2 and FFA3 receptors and HDACs in the effects of SCFAs are still an open area for fundamental research.

5. FFA2 and FFA3 receptors and HDACs mediate the effects of SCFAs on the recruitment of immune cells to endothelial cells

SCFAs not only modulate the production of pro-inflammatory cytokines but also affect migration and recruitment of immune cells to endothelial cells, which is also an important step in the development of inflammatory diseases including atherosclerosis and sepsis (Fig. 1B) (Aguilar et al., 2014; Ince et al., 2016; Zapolska-Downar and Naruszewicz, 2009). These effects are mediated by modulation of adhesion molecules expression on immune and endothelial cells by activation of FFA2 and FFA3 receptors or inhibition of HDACs.

5.1. FFA2 and FFA3 receptors mediate the effects of SCFAs on the recruitment of immune cells and adhesion molecule expression in immune cells

The data on FFA2 and FFA3 receptors mediated effects of SCFAs on the recruitment of immune cells are still controversial. SCFAs induce migration and recruitment of neutrophils to inflammatory sites by activation of FFA2 receptors following activation of MAPK signaling pathways (Sun et al., 2017; Vinolo et al., 2011a, 2009b). On the contrary, some data indicated that SCFAs inhibit the recruitment of immune cells. Butyrate suppressed INFγ-induced ICAM-1 and lymphocyte function-associated antigen-3 (LFA-3) expression on monocytes (Bohmig et al., 1997). Propionate and butyrate decreased neutrophil migration and L-selectin expression, whose effects were diminished in $FFA2R^{-/-}$ mice (Sina et al., 2009). These discrepancies might be due to the different cell types, the activation status of cells used and the different adhesion molecules investigated.

5.2. HDACs mediate the effects of SCFAs on adhesion molecule expression in endothelial cells

Differential effects of SCFAs on expression of ICAM-1 and VCAM-1 on endothelial cells were found and HDACs might be involved in the effects of SCFA. Incubation of human umbilical vein endothelial cells (HUVECs) with butyrate or propionate increased ICAM-1 expression, but not VCAM-1 (Menzel et al., 2004; Miller et al., 2005). This was supported by another study in which butyrate increased ICAM-1 expression by inhibition of histone acetylation (Miller et al., 2005; Ogawa et al., 2003). These data suggest that SCFAs facilitate adhesion molecules expression and the recruitment of immune cells. However, pre-incubation of TNFα-stimulated HUVECs with butyrate or propionate significantly decreased VCAM-1 but not ICAM-1 expression and reduced the adhesion of monocytes and lymphocytes to HUVECs (Li et al., 2018; Menzel et al., 2004; Zapolska-Downar and Naruszewicz, 2009; Zapolska-Downar et al., 2004). Butyrate inhibited VCAM-1 in oxLDL-induced EA.hy926 cells (HUVEC-derived cell line) via inhibition of NF-κB. This led to a reduced migration and adhesion of monocytes to the lesion area, indicating that butyrate may have a role in the prevention and treatment of atherosclerosis (Aguilar et al., 2014). Due to the diverse effects of SCFAs, different subtypes of HDACs might be involved in the regulation of adhesion molecules expression.

HDAC3 is highly expressed in endothelial cells and is involved in regulating vascular function. However, the role of HDAC3 differs in the regulation of different adhesion molecules. Inflammation-induced down-regulation of HDAC3 is associated with an NF-κB-dependent increase in ICAM-1 expression, indicating that HDAC3 inhibits ICAM-1 expression (Li and Sarna, 2012; Paz-Priel et al., 2011). In contrast, HDAC3 knockdown or inhibition represses TNFα-induced monocyte adhesion via inhibition of VCAM-1 expression in HUVEC (Inoue et al., 2006) indicating that HDAC3 facilitates VCAM-1 expression in endothelial cells.

HDAC4 is also involved in the regulation of vascular function by affecting endothelial cells. Kruppel-like factor-2 (KLF-2) is a novel transcriptional regulator of endothelial pro-inflammatory activation that inhibits the expression of pro-adhesive factors, such as VCAM-1, and immune cell adhesion to the endothelial monolayer. KLF-2 expression was significantly reduced by TNF-α via activation of NF-κB and HDAC4 (Kumar et al., 2005). Therefore, HDAC4 modulates VCAM-1 expression and recruitment of immune cells by regulation of KLF-2.

It is clear that SCFAs modulate the migration and recruitment of inflammatory cells to the endothelium by regulating the expression of adhesion molecules. However, their diverse effects on adhesion molecules expression might be due to their broad spectrum effects on activation of FFA2 and FFA3 receptors and inhibition of different HDACs.

6. Mechanisms involved in the effects of SCFAs on regulation of inflammation and recruitment of immune cells

Generally, FFA receptors are phosphorylated and then transduce a signal leading to the internalization and desensitization of FFA receptors. Activation of FFA receptors down-regulates NF-kB downstream genes expression and regulates several intracellular pathways including MAPKs (ERK, c-Jun N-terminal kinase (JNK) and p38MAPK) (Huang et al., 2017). Other studies also show that HDACs can modulate NF-κB activation and MAPK signaling pathway (Jeong et al., 2014; Roger et al., 2011).

6.1. NF-κB activation

NF-κB mediates the transcription of multiple pro-inflammatory genes and is pivotal in immune and inflammatory responses. Using promoter deletion mutagenesis and reporter gene analysis, it was demonstrated that NF-κB is crucial for LPS- and cytokine-activated promoter activity of over 200 genes involved in the development of inflammatory diseases. These genes include cytokines (TNFα, TNFβ, IL-1β, IL-2, IL-3, IL-5, IL-8, IL-12, IL-18), chemokines (IL-8, MIP-1α, MIP-2, MCP-1), adhesion molecules (ICAM-1, VCAM-1, E-selection, P-selectin) and enzymes (iNOS, COX-2) (Baeuerle and Baichwal, 1997; Liu and Malik, 2006; Pahl, 1999). Hence, inhibition of NF-κB activation can inhibit expression of multiple pro-inflammatory genes, reduce tissue neutrophil influx and prevent endothelial leakage. The order of potency for the suppression of NF-κB activity is butyrate > propionate > acetate (Tedelind et al., 2007), which is similar to the order for the inhibition of HDACs (Miyoshi et al., 2008).

Traditionally, NF-κB activity is regulated by signal-induced IκB

degradation leading to NF-κB activation. However, NF-κB transcriptional activity can also be modulated by acetylation and deacetylation of proteins in the NF-κB pathway and by accessibility of NF-κB target genes. For example, CBP/p300, upstream of NF-κB, interacts with HATs to induce gene expression (Zhong et al., 2002) and subunits of NF-κB (p65 and p50) interact with HDACs to repress transcription (Ashburner et al., 2001). The acetylation status of p65 modulates its binding to IκBα. Acetylated p65 interacts weakly with IκBα, whereas deacetylated p65 by HDAC3 enhances p65 binding to IκBα, which in turn, can result in the export of NF-κB complexes from the nucleus back to the cytoplasm (Chen et al., 2001). Moreover, the association of p65 with HDAC1 and HDAC2 inhibits the expression of NF-κB-regulated genes at both basal and induced levels (Fig. 1A) (Ashburner et al., 2001). HDAC1 directly associates with the Rel homology domain of p65 which modulates NF-κB activation or repression. HDAC2 does not interact with NF-κB directly, but can regulate NF-κB activity via its association with HDAC1. Nuclear NF-κB in unstimulated cells mainly consists of p50 homodimers coupling with HDAC1 bound to DNA and repressing NF-κB dependent gene expression, such as IL-6, IL-8, iNOS and TGFβ. Following activation, p50/p65 heterodimers, containing phosphorylated p65, translocate into the nucleus and displace DNA-bound p50/HDAC1 (Zhong et al., 2002). These mechanisms ensure that only NF-κB activates transcription in activated cells.

Butyrate and propionate are known as HDAC inhibitors and also shown to modulate NF-κB activity. For example, butyrate upregulated IL-10 production and repressed the production of pro-inflammatory molecules IL-12, TNFα, IL-1β, NO by inhibiting NF-κB activity (Ni et al., 2010; Saemann et al., 2000; Usami et al., 2008). Moreover, propionate inhibited the production of NO by macrophages, an effect associated with inhibition of NF-κB activation (Usami et al., 2008). However, there is still no direct evidence proving that the effect of butyrate or propionate on inhibition of NF-κB activity is mediated by inhibition of HDACs.

6.2. MAPK signaling pathways

MAPK signal transduction pathways including ERK, JNK and p38MAPK are involved in regulation of multiple cell functions. The ERK signaling pathway is a major regulator of cell proliferation, while JNK and p38MAPK are associated with inflammation processes. MAPK signal transduction pathways can be interfered by HDACs. Acetylation status of mitogen-activated protein kinase phosphatase-1 (MKP-1) enhances its interaction with MAPK substrates, dephosphorylates ERK, JNK and p38 MAPK, and negatively regulates MAPK signaling. Simultaneous inhibition of HDAC1–3 increased MKP-1 acetylation and decreased LPS-induced phosphorylation of p38MAPK in macrophages but not in MKP-1 null macrophages. Finally, inhibition of HDAC1- 3 decreased LPS-induced expression of TNFα, IL-1β, iNOS and nitrite synthesis (Jeong et al., 2014). However, the effects of HDACs might also be independent of the MAPK signaling pathways. TSA (HDAC inhibitor) treatment strongly inhibited TNFα and IL-6 production in a time- and dose-dependent manner in macrophages. However, TSA did not inhibit ERK1/2 or p38 phosphorylation or NF-κB, c-jun or IRF7 nuclear translocation in these macrophages. Instead, TSA strongly increased Mi-2β, a transcriptional repressor recruited to the IL-6 promoter in macrophages exposed to LPS. TSA also reduced the binding of Mi-2β to the Tnf promoter (Roger et al., 2011).

Moreover, FFA receptors can be linked to different signaling cascades downstream of MAPK. FFA2 and FFA3 receptor activation induces phosphorylation of ERK1/2 and FFA2 receptor activation induces phosphorylation of p38MAPK, while FFA receptors can weakly activate JNK (Seljeset and Siehler, 2012). It has been shown that propionate-induced FFA3 receptor activation is followed by ERK1/2 activation, whereas treatment with acetate, the FFA2 receptor agonist, resulted in weaker activation. There is a clear link between FFA2/FFA3 receptors/HDACs and NF-κB/MAPK and between SCFAs and FFA2/FFA3 receptors/HDACs, however it is not clear if activation of FFA2 and FFA3 receptors or inhibition of HDACs by SCFAs regulates NF-κB or MAPK signaling pathway mediating the effects of SCFAs on immune and endothelial cells.

7. Conclusions and future perspectives

SCFAs might play an essential role in regulation of inflammation and contextually result in either protective or causative effects, by stimulating or dampening production of inflammatory cytokines, as well as inhibiting or facilitating migration and recruitment of immune cells, which are likely mediated by a combination with cell surface receptors (FFA2 and FFA3 receptors and GPR109A) or inhibiting intracellular enzyme activity (HDACs) (Fig. 1A). Although, the effects of SCFAs on regulation of endothelial and immune cell (dys)function are complex, SCFAs still show promising therapeutic potential in the treatment of inflammatory and cardiovascular diseases. There are some key research questions that remain to be investigated because the latest clinical trials investigating the effects of receptor agonists or HDAC inhibitors in inflammatory disorders failed (Suckow and Briscoe, 2017). First, more information on FFA2 and FFA3 receptors and HDACs mediated effects of SCFAs in endothelial cells in the development of inflammatory diseases is needed. Second, most studies used non-specific receptor agonists and antagonists or HDAC inhibitors which might contribute to the variable effects on regulation of inflammation. Therefore, specific receptor agonists and antagonists and specific HDAC inhibitors are required. Besides, the downstream molecular processes involved in the activation of receptors and/or inhibition of HDACs will not only elucidate the underlying mechanisms, but also offer the explanation for diverse effects of SCFAs. Third, the pleiotropic roles of different SCFAs indicate that the effects of acetate, propionate and butyrate treatment alone or in combination should be investigated.

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