Short-chain fatty acids: linking diet, microbiome and immunity

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Abstract

The short-chain fatty acids (SCFAs) butyrate, propionate and acetate are microbial metabolites and their availability in the gut and other organs is determined by environmental factors, such as diet and use of antibiotics, that shape the diversity and metabolism of microbiota. SCFAs regulate epithelial barrier function as well as mucosal and systemic immunity via evolutionary conserved processes that involve G-protein-coupled receptor signalling or histone deacetylase activity. Indicatively, the anti-inflammatory role of butyrate is mediated through direct effects on the differentiation of intestinal epithelial cells, phagocytes, B cells and plasma cells, and regulatory and effector T cells. Intestinally-derived SCFAs also directly and indirectly affect immunity at extra-intestinal sites, such as the liver, the lungs, the reproductive tract and the brain, and have been implicated in a range of disorders, including infections, intestinal inflammation, autoimmunity, food allergies, asthma, and responses to cancer therapies. An ecological understanding of microbial communities and their interrelated metabolic states, as well as the engineering of butyrogenic bacteria may support SCFA-focused interventions for the prevention and treatment of immune mediated diseases.

[H1] Introduction

Mutualistic relationships between multicellular organisms and the microbiome have occurred throughout evolution[1]. This is illustrated by the adaptation of metabolism and immune responses at mucosal surfaces to the presence of a large number and diversity of bacteria, viruses and fungi. This adaptation allows multicellular organisms to establish non-inflammatory homeostasis with the microbiota. The host and microbiome are functionally connected through diet, metabolism and pathogen defence mechanisms such that together they can be regarded as a holobiont [G][1]. Changes in the environment and in lifestyle that have an impact on homeostatic holobiotic interactions can consequently affect susceptibility to infections and immune-mediated disorders. Understanding such mechanisms is particularly important given the rapid increase in incidence of immune-mediated inflammatory diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis, type 1 diabetes, asthma and obesity worldwide[2]. Environmental factors that contribute to this trend include a Western-style diet (high in fat, protein and salt intake but low in plant fibre consumption) and the increasing use of broad-spectrum antibiotics that directly influence the commensal microbiota[3, 4]. Indeed, changes in the microbiota at mucosal surfaces as a consequence of a Western lifestyle have been associated with immune-mediated inflammatory disorders[5].

In particular, microbiota-derived metabolites such as short-chain fatty acids (SCFAs) [G] — which include acetic acid (acetate), propionic acid (propionate) and butyric acid (butyrate) (**Box 1**) — have marked effects on mucosal and systemic immune responses, and low levels of SCFAs in the intestine have been directly associated with susceptibility to inflammatory and allergic diseases[5] (**Supplementary Table 1**). SCFAs are predominantly metabolised by enterocytes and hepatocytes, for which they provide an energy source[6]. Among all SCFAs, butyrate is the best understood modulator of the immune system with beneficial effects observed across many species, including insects[7], birds[8], mice[9] and humans[10]. Butyrate can negatively impact the fitness of non-pathogenic microbes underscoring the potential far-reaching and direct influence of SCFAs in shaping the gut microbiome[11].

In this Review, we discuss the factors that affect SCFA levels and the molecular mechanisms by which SCFAs link diet, the microbiota and the host immune system, thereby determining the balance between homeostasis and immune-mediated disorders (**Fig. 1**). This article focuses mainly on intestinal derived SCFAs, and particularly butyrate, and how they regulate mucosal barrier integrity, mucosal immunity, and susceptibility to intestinal immune disorders. In addition, extra-intestinal homeostasis and pathologies, including those of the reproductive tract, liver, brain and lung are discussed. Dissecting the emerging molecular insights into the functions of SCFAs is crucial for understanding the links between environmental risk factors and immune-mediated diseases, and might lead to new treatment options for immune mediated disorders and cancer immunotherapies.

[H1] Factors affecting SCFA levels

The production of SCFAs in the intestine by specific subsets of anaerobic bacteria (**Box 1**) is modulated by a range of environmental and host factors.

[H2] Environmental factors

Many environmental factors affect the composition of the intestinal microbiota and thus the production of SCFAs at mucosal sites. These include gestational age at birth[12], delivery mode[13, 14], feeding mode (breastfeeding or formula)[15], diet[16], use of antibiotics[9, 17], infections, and stress[18] (**Fig. 1**). Microbiota profiles are shaped early in life, as the gastrointestinal tract of the newborn becomes colonised by the maternal microbiota during delivery and through maternal contact and breastfeeding[19]. The colonisation of the neonatal gut relies on the maternal vaginal microbiota, primarily the butyrogenic bacteria *Lactobacillus* after vaginal deliveries, whereas the skin microbiota, in particular *Staphylococcus spp*. contribute to colonisation after caesarean sections[13, 20]. Breastfeeding is another key factor in determining microbiota composition in the neonate, as bacteria are vertically transferred from mother to infant[21, 22]. The butyrogenic bacteria *Bifidobacteria* and *Lactobacillus* are found at higher levels in the intestines of breastfed infants as compared to formula-fed infants[15]. Abnormal development of the intestinal microbiota in neonates has been associated with inflammatory diseases in childhood and adulthood[23]. During the first two years of life, the

93 microbiota undergoes marked changes in composition until more stable communities
94 develop; gut metabolite profiles change in parallel, and low acetate and high succinate
95 levels at birth are followed by high acetate and formate levels at around the first year of life,
96 and high propionate and butyrate levels when approaching two years[24] (**Fig. 1**). The gut
97 microbiota is relatively stable during adulthood, but ageing has been linked with a reduction
98 in the abundance of bacterial species that support SCFA production, such as *Bifidobacteria*
99 spp.[25],and an associated decline in fecal SCFA levels[26].
100
101 Dietary fibres provide appropriate substrates for the bacterial production of SCFAs[16]. A fibre-
102 rich Mediterranean diet, characterised by regular consumption of fruit, vegetables, cereals,
103 legumes and a high intake of olive oil and seafood, alongside restricted consumption of red
104 meat and confectionery, has been associated with a direct increase in intestinal SCFA levels,
105 which may partly account for prevention of IBD[27]. In addition to promoting microbiota
106 diversity and SCFA production, a high-fibre diet reduces the levels of inflammatory markers,
107 such as JAK/STAT and MAP kinase signalling in monocytes, B cells and T cells[28]. By contrast, a
108 low-fibre diet leads to decreased SCFAs, with a compromised colonic mucus barrier function
109 and enhanced susceptibility to the pathobiont *Citrobacter rodentium* in mice[29]. A diet with a
110 high intake of processed foods, red meat, saturated fats, and simple carbohydrates (as
111 those found in white sugar and white flour), and a low intake of fruits and vegetables,
112 provides only limited substrates for SCFA production. As such, a Western diet has been
113 associated with low levels of faecal and serum SCFAs and reduced microbial fermentation[30].
114 Recent evidence has also linked high salt intake, which is a characteristic of a Western diet,
115 to a reduction in SCFA levels (butyrate in particular) and to intestinal inflammation in mice[31].
116 Use of antibiotics — which cause dysbiosis in humans[17] and directly reduce levels of SCFAs in
117 the intestine in mice[9] — has been associated with the rapid increase in prevalence of
118 chronic immune-mediated disorders[4]. There is strong epidemiological evidence linking
119 antibiotic use in early life to the development of inflammatory diseases such as asthma[32, 33]
120 and IBD[34] later in life. Microbiota composition shortly after birth shapes allergic airway
121 responses in mice [35], and the disruptive effect of perinatal antibiotics on microbiota and host
122 immunity confers susceptibility to asthma[36].
123

Viral infections can also affect the abundance and diversity of the microbiota and, thereby, SCFA biosynthesis. For example, SARS-CoV-2 infection causes a depletion of butyrogenic bacteria, such as *Faecalibacterium prausnitzii*, and an enrichment of opportunistic pathogens including *Clostridium heathewatu* and *Streptococcus* spp. in the gut[37, 38]. SARS-CoV-2-infected individuals with severe illness had substantially low faecal SCFA levels, which remained reduced for more than 30 days after disease resolution, suggesting that SARS-CoV-2 infection induce long-lasting effects on the gut microbiota and SCFA synthesis[39].

Finally, social stress impacts on the intestinal microbiota limiting levels of acetate and butyrate, whilst increasing infection susceptibility and immunopathology in mice infected with *Citrobacter rodentium* [18].

[H2] Host genetic factors.

Little is known about how host genetic factors might affect SCFA metabolism. Biological sex impacts gut microbiota, partly via the direct effects of the sex hormones testosterone and oestrogen on microbiota composition and diversity[40]. Furthermore, the levels of SCFAs in the intestine of antibiotic-treated mice are decreased to a greater extent in female mice than in male mice, suggesting complex host–environment interactions in determining SCFA metabolism[41].

A recent microbiome-wide association study suggests that host genetics can influence the gut microbiome, promoting increased microbial production of butyrate and correlating with enhanced insulin response in type 2 diabetes[42]. Additional evidence that host genetics can affect the ecological niche of butyrogenic bacteria comes from rare Mendelian disorders. Defects in the genes that encode for guanylate cyclase 2C (*GUCY2C*), the sodium/hydrogen exchanger 3 (*SLC9A3*) or the chloride anion exchanger (*SLC26A3*) cause congenital diarrhoea and susceptibility to spontaneous intestinal inflammation[43, 44, 45]. Congenital diarrhoea is characterised by high concentrations of sodium in the stool, increases in Enterobacteriaceae, and a reduction of bacterial strains that support the growth of butyrogenic bacteria (such as *Bifidobacteria*) and of butyrogenic bacteria themselves (such as *Faecalibacterium prausnitzii*) before the onset of intestinal inflammation[46]. This suggests

that a diet high in salt and defective sodium uptake owing to genetic defects may have similar effects on the composition of the microbiota and the associated SCFA metabolism.

[H1] SCFA-induced cellular signalling

The SCFAs acetate, propionate and butyrate have been shown to signal through surface-expressed free fatty acid receptors or through the G-protein-coupled receptors (GPCRs) GPR41, GPR43 and GPR109a, which are expressed on epithelial cells, adipose tissue and immune cells such as neutrophils, dendritic cells (DCs), macrophages and T cells[47] (**Fig. 2**). Activation of these GPCRs suppresses cAMP-dependent signalling pathways and concurrently activates alternative signalling pathways, such as mTOR downstream of GPR41, 43 and GPR109s[48]. The differential expression of these GPCRs across tissues and their variable affinities for individual SCFAs underlie the diverse functions of SCFAs[47]. GPR41 is present in a wide range of tissues, whereas GPR43 is mainly expressed in lymphatic tissues and various immune cells. GPR41 and GPR43 both bind acetate, propionate and butyrate, whereas GPR109a is activated mainly by butyrate. Activation of GPR43 by SCFAs can have multiple effects depending on the cell type. For example, it can induce activation of the NLRP3 inflammasome and interleukin 18 (IL-18) secretion by colonic epithelial cells, promote the recruitment of neutrophils to inflammatory sites, and enhance the differentiation and suppressive function of $FOXP3^+$ regulatory T (T_{reg}) cells[47].

Butyrate and propionate exert their effects also through the inhibition of histone deacetylase (HDAC) **[G]** activity (**Fig. 2**). When SCFAs enter cells either by passive diffusion or via the transmembrane carriers sodium-coupled monocarboxylate transporter 1 (SMCT1; encoded by *SLC5A8*) and lactate transporter monocarboxylate transporter 1 (MCT1; encoded by *SLC16A1*), they can bind directly to intracellular HDACs and inhibit their activity. In addition, at low concentrations, butyrate and propionate can enhance histone acetylation through their metabolism to acetyl-CoA, which is an acetyl donor to histone acetyltransferases such as p300[49]. Butyrate can also contribute an acetyl group to for histone acetylation[50]. By increasing histone acetylation and increasing the amount of open chromatin, butyrate impacts gene expression in intestinal epithelial cells, macrophages, DCs and lymphocytes, particularly T_{reg} cells[51, 52, 53]. Changes in various pathways, including those

associated with the cell cycle, cell differentiation, antibacterial responses, inflammation, fatty acid metabolism and oxidative stress have been observed in studies examining in the effects of butyrate exposure on immune and non-immune cells[52, 54, 55].

Butyrate regulates transcription also via histone acylation, including propionylation, butyrylation and crotonylation[49, 56, 57]. Histone butyrylation, but not histone acetylation or propionylation, restricts the chromatin binding of transcription factors containing bromodomains. Butyrate promotes histone crotonylation by inhibiting the decrotonylase activity of HDAC2[57], as well as through its conversion into butyryl-CoA and crotonyl-CoA, which serves as a precursor for histone crotonylation by p300–CBP[58].

Emerging evidence indicates that the HDAC-inhibitory activity of butyrate also modifies lysine acetylation of non-histone proteins such as the NF-κB subunit p65[59]. Butyrate enhances acetylation of p65 through inhibition of HDAC3 and HDAC6, resulting in differential recruitment of NF-κB to proinflammatory gene promoters both *in vitro* and *in vivo*[59]. Furthermore, SCFAs, in particular butyrate, have recently emerged as activators of intracellular receptors that control immune responses, such as peroxisome proliferator-activated receptor-ɣ (PPARɣ)[60] and aryl hydrocarbon receptor (AhR)[61], in various cell types.

Together, the diverse molecular targets of SCFAs illustrate how varying gradients of different SCFAs in different tissues can target immune and non-immune cells to mediate a wide range of homeostatic functions at the mucosal barriers, regulate immunity in the intestine and at distal organs, and, potentially, protect from immune-mediated diseases.

[H1] SCFA functions at mucosal barriers

SCFAs regulate mucosal immunity via both immune and non-immune cell targets (**Fig. 3**). They help to maintain the epithelial barrier function at mucosal surfaces, they influence the antimicrobial and inflammatory functions of monocytes and macrophages, and they have anti-inflammatory and tolerogenic effects on lymphocytes.

[H2] Barrier integrity and function

SCFAs act on epithelial stem cells, epithelial columnar cells and enteroendocrine cells through GPCRs, but also through their inhibitory effect on HDACs. They directly augment barrier function in the healthy intestine by promoting the production of antimicrobial factors, regulating epithelial cell turnover, and maintaining epithelial barrier integrity. For example, in vitro studies have shown that SCFAs stimulate sub-epithelial myofibroblasts to produce prostaglandin, which then triggers mucin expression by epithelial cells[62]. Butyrate directly induced mucin expression in human polarized goblet cell lines[63], as well as in perfused rat colon[64]. In addition, SCFAs could stimulate the production of antimicrobial peptides **[G]** such as the cathelicidin LL37 in human epithelial cell lines[65]. GPR43 activation in response to butyrate promoted the production of β-defensins and RegIIIγ in human and murine colonic cell lines, by stimulating mTOR and STAT3 phosphorylation[48].

Butyrate has a crucial role in regulating the intestinal stem cell niche and the turnover of epithelial cell progenitors. Specifically, butyrate inhibits proliferation of intestinal stem and progenitor cells and delays wound repair via the transcription factor Forkhead Box O3 (FOXO3)[66]. Furthermore, butyrate-dependent inhibition of HDAC8 has been shown to prevent intestinal epithelial cell death during intestinal inflammation via the repression of hexokinase 2 expression and subsequent inhibition of mitochondrial respiration[67]. These functions are likely to be tightly regulated, as during homeostasis columnar colonocytes metabolise butyrate as an energy source and potentially prevent butyrate from reaching proliferating epithelial stem and progenitor cells within the crypt.

In addition to supporting epithelial cell turnover, SCFAs regulate barrier integrity via the formation of tight junctions. Butyrate increased expression of the tight junction proteins, including expression of claudin 3 and claudin 4 in an epithelial cell line[68], and of claudin-1 and occludin in a mouse model[69]. Moreover, SCFAs have been shown to act via inhibiting autophagy and NLRP3 activation to reverse the decreased transepithelial electrical resistance, the increased paracellular permeability and the morphological disruption of tight junction proteins that are commonly observed upon stimulation with lipopolysaccharide (LPS)[70]. Butyrate enhances intestinal epithelial O_2 consumption, thereby stabilising expression of hypoxia-inducible factor (HIF)[71], a transcription factor that promotes intestinal

barrier repair via the induced expression of intestinal trefoil factors, mucin and tight junction proteins[72].

Together, these findings suggest that SCFAs, and in particular butyrate, have mainly homeostatic and anti-inflammatory effects on the intestinal epithelium. Human epithelial colonic organoids are rendered less responsive to butyrate by the pro-inflammatory cytokine tumour necrosis factor (TNF), which reduces the expression of butyrate receptors and transporters[73]. These data have important implications for the therapeutic administration of butyrate during active inflammation in IBD, as they suggest a context specific regulation of its anti-inflammatory and barrier protective impact on barrier immunity, as despite its anti-inflammatory, barrier-protecting effect in homeostasis butyrate might not be able to suppress inflammatory responses during TNF-associated hyperinflammation.

[H2] Myeloid cells

SCFAs act directly on DCs to enhance their immune regulatory properties. Butyrate and propionate inhibit LPS-induced maturation of human monocyte-derived DCs *in vitro*, reducing the production of IL-12 and limiting $CD8^+$ T cell activation[74].

Butyrate can regulate colonic macrophage function in vivo. The inhibitory effect of butyrate on the production of pro-inflammatory cytokines, such as IL-6 and IL-12, or other pro-inflammatory mediators, such as nitric oxide, by LPS-stimulated macrophages might contribute to the hyporesponsiveness of intestinal macrophages to bacterial stimulation[51]. In addition, butyrate has been shown to promote oxidative phosphorylation (OXPHOS)[9] and reduce glycolysis[52], thereby coordinating a metabolic rewiring in macrophages. In the context of allergic airway inflammation, butyrate and propionate suppressed the activation of both mouse- and human-derived alveolar macrophages and decreased airway inflammation in an asthma model, partly through GPR43 activation and HDAC inhibition[75].

In parallel to its anti-inflammatory properties, butyrate increases the production of antimicrobial peptides, including calprotectin and cathelicidin, by macrophages through HDAC3 inhibition, thereby increasing macrophage antimicrobial activity against

enteropathogens *in vivo*[52]. Similarly, in a mouse model of mastitis induced by *Staphylococcus aureus*, butyrate promoted HDAC3 inhibition thereby activating an antimicrobial programme in macrophages to protect against pathogens[76]. Although the above studies indicate a function of SCFAs in macrophage activation, it can be speculated that butyrate predominantly regulates macrophages to mitigate inflammation and tissue injury either via the direct suppression of inflammatory programmes or via the elimination of tissue damage inducing pathogens. The specific impact of SCFAs depends on homeostatic versus pathogenic context and the cellular types involved.

Butyrate imprints epigenetic programmes in macrophages that are likely to have strong translational implications for inflammation and tumour immunology. Indeed, the functions of butyrate cannot simply labelled as anti-inflammatory or immunosuppressive since mice deficient in the butyrate receptor Gpr109a, which is expressed on DCs and macrophages in the intestine, were more susceptible to developing colon cancer[77] again highlighting an context specific role of butyrate.

SCFAs regulate the chemotactic recruitment and antibacterial responses of neutrophils through both HDAC inhibition[78] and GPR43 signalling[79]. In the context of IBD, butyrate suppressed migration and neutrophil extracellular trap (NET) formation in neutrophils derived from patients with ulcerative colitis and Crohn's disease[80]. Other HDAC inhibitors could mimic the suppression of neutrophil responses. These findings were confirmed in a mouse model of colitis, in which butyrate alleviated intestinal inflammation via inhibition of NET release and the downregulation of proinflammatory cytokines, chemokines and calprotectin[80].

[H2] T cells

SCFAs can regulate T cell differentiation and function through inhibition of HDACs and GPCR signalling. Butyrate induces intestinal T_{reg} cell differentiation by enhancing histone H3 acetylation in the *Foxp3* promoter and other conserved noncoding sequence regions of the *FoxP3* locus; as a result, it ameliorates the development of colitis induced by adoptive transfer of T cells[53]. Propionate, but not acetate, has also demonstrated some HDAC inhibitory activity that enhanced extrathymic T_{reg} cell generation in the periphery[81]. In

addition to SCFAs inducing T_{reg} cell differentiation via HDAC inhibition, acetate, propionate and butyrate, when administered together, induced the differentiation of colonic T_{reg} cells and protected mice against colitis in a Gpr43-dependent manner[82]. Butyrate also accelerates fatty acid oxidation via its conversion to butyl-CoA and the downstream activation of carnitine palmitoyltransferase 1A. This upregulation of fatty acid oxidation was shown to enhance T_{reg} cell differentiation in vitro[83].

In addition to directly targeting T cells to induce T_{reg} cell differentiation, butyrate has been shown to modulate the expression T_{reg} cell-inducing molecules in epithelial cells or DCs. Indicatively, it can stimulate epithelial cells to produce retinoic acid, a metabolite of dietary vitamin A that is crucial for the generation of gut specific T_{reg} cells by intestinal DCs[84]. The effects of SCFAs on epithelial cell and DC expression of vitamin A-converting enzymes may at least in part account for the increased numbers of intestinal T_{reg} cells[85]. Of note, another regulatory function of retinoic acid in the intestine is its ability to induce antibody class switching to IgA, which is in keeping with the increased levels of intestinal IgA induced by SCFA administration[85, 86]. Similarly, butyrate acts on DCs in the intestine via the butyrate receptor Gpr109a[77] and the butyrate transporter Slc5a8[87] to support DC-induced differentiation of T_{reg} cells; Slc5a8-dependent uptake of butyrate induces DCs to express *IDO1* and the retinoic acid-converting enzyme *Aldh1A2*[87].

SCFAs can impact the differentiation of naive T cells to effector T cell subsets such as T helper 1 (T_H1) cells and T_H17 cells. Butyrate favours T_H1 cell differentiation by upregulating T-bet expression and downregulating the expression of Rorγt and other T_H17 cell-associated transcription factors[88].

In addition to regulating T cell differentiation, SCFAs influence cytokine expression in T cells. Butyrate-dependent secretion of IL-10 by T_H1 and T_H17 cells reduced the severity of colitis in a mouse model of adoptively-transferred gut microbiota-specific T cells [88]. Butyrate has been shown to increase IL-10 expression in differentiated T_H1 cells from both healthy individuals and patients with IBD through GPR43-mediated upregulation of the transcription factor BLIMP1[89]. In T_{reg} cells, butyrate induces IL-10 via the increased acetylation of p70 S6 kinase and the downstream phosphorylation of the ribosomal protein S6, which increases

mTOR activity as required for IL-10 production[90]. Moreover, SCFAs — particularly butyrate — stimulate IL-22 production by $CD4^+$ T cells and innate lymphoid cells (ILCs) via upregulating expression of AhR and HIF, which both maintain intestinal homeostasis[91]. Butyrate-dependent IL-22 production protected mice from intestinal inflammation in a model of *Citrobacter rodentium* infection. Finally, propionate directly suppresses IL-17 production by γδ T cells through an HDAC-dependent mechanism and dampens IL-17 secretion in human γδ T cells derived from patients with IBD[92].

SCFAs promote the survival, differentiation, cytotoxic activity, and memory function of $CD8^+$ T cells by modifying cellular metabolism[93,94]. The activation of $CD8^+$ T cells involves increased mitochondrial mass, and SCFAs serve as substrates for fatty acid oxidation through their metabolism to acetyl-CoA, which fuels the TCA cycle and OXPHOS. Butyrate supports preferential use of OXPHOS through sustained glutamine utilisation and fatty acid catabolism in $CD8^+$ T cells. Acetate can restore the hyporesponsive effector functions of glucose-restricted $CD8^+$ T cells by increasing IFNy production and histone acetylation[95]. In addition, butyrate has been shown to enhance the antitumour activities of cytotoxic T lymphocytes and chimeric antigen receptor (CAR) T cells, through mTOR activation and HDAC inhibition, resulting in metabolic and epigenetic reprogramming that promotes the IL-12 signalling pathway in the tumour microenvironment[96].

[H2] B cells

The gut microbiota and SCFAs promote B cell differentiation and intestinal IgA responses. SCFAs favour the differentiation of B cells into plasma cells and stimulate class switching to IgA by replenishing the acetyl-CoA pool, thereby promoting mitochondrial energy production and glycolysis to support antibody production in both mouse and human B cells[97]. SCFAs induce histone hyperacetylation on genes encoding activation-induced cytidine deaminase (*Aicda*) and immunoglobulin heavy chain, which are involved in plasma cell differentiation and immunoglobulin class-switch recombination, independently of GPCR activation[97]. SCFAs also enhance the generation of $CXCR5^+$ T follicular helper cells **[G]** *in vitro* and *in vivo*, which support B cell differentiation[97].

At physiological range, butyrate and propionate inhibit class-switch recombination, somatic hypermutation and plasma cell differentiation in C57BL/J6 mice through a B cell-intrinsic, dose-dependent, HDAC-inhibitory activity[98]. Butyrate and propionate-induced miRNAs downregulate expression of *Aicda* and *Blimp1* in mouse and human B cells, impairing intestinal and systemic antibody responses[98]. At low SCFA doses, the miRNA-mediated inhibition of *Aicda* and *Prdm1* expression is insufficient to override the enhancement of class-switch recombination. Furthermore, butyrate promotes the suppressive capacity of regulatory B cells by amplifying AhR activation, leading to a decrease in the severity of arthritis in experimental models[99].

[H2] Innate lymphoid cells

Innate lymphoid cells (ILCs) contribute to host protective immunity and tissue regeneration. Butyrate stimulates the activation of the PI3K–AKT and mTOR signaling pathways in a GPR43-dependent manner, and thereby promotes the proliferation of intestinal ILC1s and ILC3s, while simultaneously suppressing the expansion of intestinal ILC2s[100]. Propionate[101] increases the number of colonic IL-22-producing ILC3s *in vivo* through Gpr43 signalling, whereas butyrate[91] stimulates the production of IL-22 by RORγt$^+$ ILC3s via GPR41 signalling and HDAC inhibition. Similarly, butyrate inhibits the proliferation and cytokine response of ILC2s in the context of airway inflammation, protecting against ILC2-driven airway hyperreactivity, potentially through its HDAC-inhibitory activity[102]. Among ILCs, ILC2 cells exhibit the highest expression of SCFA transporters, which contribute to an increased intracellular SCFA reservoir and the subsequent HDAC inhibition[100]. In summary, SCFAs regulate the plasticity of colonic ILCs, maintaining gut epithelial barrier integrity, and preventing against tissue injury, infection and immunopathology.

Overall, the influence of SCFAs on the mucosal barrier and immune cells function underscores their role in orchestrating finely tuned immune responses within the microenvironment of the gut and extra-intestinal tissue sites, and highlights how a focus on SCFAs might help understand and treat intestinal and extra-intestinal immune disorders.

[H1] SCFAs in intestinal immune disorders

Several immune disorders have been associated with changes in abundance of butyrogenic bacteria and levels of SCFAs (**Supplementary Table 1**). However, the strength of evidence linking the effects of SCFAs to human immunopathology is variable. Layers of evidence include: comparative metagenomic data on the abundance of bacterial species or groups in health and disease; measures of SCFA concentrations in stool or body fluids; abundance of butyrogenic bacteria or cross-feeding **[G]** communities as measured by bacterial culture; assessment of functional effects of SCFAs on primary cells or cell lines in vitro; assessment of functional effects of SCFAs, butyrogenic substrates or butyrogenic bacteria in model organisms; and clinical trials in humans (ranging from pure observations to randomised controlled trials). For many disorders, current evidence suggests that causal associations with SCFAs are plausible and evidence of the therapeutic effectiveness of targeting SCFA-associated mechanisms is just emerging. Here, we discuss the link between SCFAs and inflammatory bowel disease or intestinal inflammation, whereas the potential role for SCFAs in extra-intestinal infections, in pregnancy, autoimmune diseases and allergies are discussed in the following section and in Box **2**.

[H2] Inflammatory bowel disease

IBD comprises Crohn's disease and ulcerative colitis and is characterized by a dysregulated inflammatory response in the intestine towards components of the gut microbiota[103]. This immunopathology involves defective epithelial barrier function, defective bacterial sensing by epithelial cells and innate immune cells, and activation of commensal-specific inflammatory T cells that produce pathogenic cytokines. Perturbations in the gut microbiota and dysbiosis are a characteristic feature of IBD, with both low abundance of butyrogenic bacteria such as *Faecalibacteria*, *Roseburia hominus* and *Bifidobacteria*, and reduced levels of butyrate[104, 105, 106, 107]. A recent meta-analysis of SCFAs in patients with IBD found decreased levels of butyrate and acetate, although inconsistent results were reported across different studies[108]. TNF treatment reduces butyrate consumption in intestinal epithelium organoids from patients with IBD, and this suggests that inflammation renders the epithelium less responsive to butyrate[73]. Decreased expression of the butyrate transporter MCT1 in colonocytes of patients with Crohn's disease indicates a potential reduction in their capacity to uptake butyrate[73]. Thus, intestinal inflammation may reduce both the levels of

butyrogenic bacteria and utilisation of butyrate. Recent evidence indicates that healthy individuals with a high genetic risk for IBD have decreased *Roseburia* spp. in the gut microbiota[109]. Crucially, these data indicate that IBD-associated dysbiosis is not just a consequence of intestinal inflammation but may in fact contribute to disease initiation. Mouse studies show that the anti-inflammatory effects of *Faecalibacteria prausnitzii* in experimental IBD are mediated by butyrate directly. To this end, butyrate produced by *F. prausnitzii* downregulated the pro-inflammatory IL-6–STAT3–IL-17 signalling pathway via HDAC1 inhibition and transcriptional repression, whilst promoting expression of *FoxP3* and therefore the maintenance of T_{reg} cells[110]. Together, these data indicate that dysregulation of butyrate levels in the intestine may be an important susceptibility factor in IBD.

Epidemiological evidence suggests that disruption of the gut microbiota by antibiotic use early in life is associated with increased risk of IBD[34]. This association is particularly strong when antibiotics are given in the first year of life, indicating that there may be a critical period during which antibiotic use causes strong and long-term microbial disruption with long-term consequences for the immune system. Most recently, prenatal use of antibiotics in the third trimester of pregnancy has been associated with very early onset IBD in children[111], suggesting that effects on the maternal microbiota before birth may influence the infant's future risk of intestinal inflammation via transgenerational microbiome transfer. In mice, antigen-specific tolerance to gut bacteria is induced during a specific pre-weaning interval, involving goblet cell-associated antigen passages that enable exposure of intestinal immune cells to microbial antigens and T_{reg} cell differentiation[112]. Although it is not clear whether SCFAs are required during these processes, a study in mice showed that the intestinal microbiota induced a robust immune response at weaning that was supported in part by SCFAs and involved the generation of RORγt^{+} T_{reg} cells. Inhibition of this weaning reaction led to increased susceptibility to colitis, allergic inflammation and cancer, later in life[113]. These studies illustrate the importance of maintaining a stable intestinal microbiota at specific times during development, in particular around weaning, with crucial implications for susceptibility to disease later in life. Further evidence that the effects of antibiotics may be related to the disruption of SCFA generation comes from studies in mice showing that SCFA levels are markedly reduced during antibiotic use and that repopulation of the microbiota after antibiotic treatment causes intestinal macrophages to become overactive,

driving long-lived pro-inflammatory T cell responses. Supplementation of antibiotics with SCFAs, particularly butyrate, prevented macrophage dysfunction and abolished the pro-inflammatory T cell response in this model[9].

Although a causal role of defective SCFA metabolism in IBD is plausible, there is only a limited number of studies that show therapeutic efficacy of SCFAs administered directly to humans with IBD[10]. SCFA enemas are effective in reducing symptoms in subsets of patients with ulcerative colitis[114]. Butyrate enemas also reduce disease activity index in these patients, although a subsequent trial indicates only minor effects on colonic inflammatory parameters [115]. A pilot trial investigated the efficacy of encapsulated butyrate as an adjuvant to conventional therapy for maintaining remission in patients with Crohn's disease and ulcerative colitis[116]. Butyrate adjuvant therapy led to a decrease in faecal levels of the intestinal inflammation marker calprotectin, stimulated the growth of butyrogenic bacteria, and enhanced the quality of life for patients with ulcerative colitis (although no impact on disease activity scores was observed). At this point, it is important to caution that SCFAs alone are unlikely to be a universal solution for conditions associated with microbial dysbiosis. Despite ample evidence suggesting a protective role for SCFAs such as butyrate in the pathogenesis of IBD, not all nutritional strategies that are known to be effective in Crohn's disease are associated with increased butyrate or SCFA levels. For example, the first-line therapy for paediatric Crohn's disease is exclusive enteral nutrition, an approach that leads to reduced microbial diversity and lower butyrate levels[117]. It remains to be determined whether strategies that selectively support butyrogenic bacteria would enhance therapeutic efficacy overall[118].

[H2] Intestinal infections

Microbiota prevent invasion of mucosal surfaces by bacterial or viral pathogens. For example, commensal *Bifidobacteria* spp. can protect against infection with enteropathogenic *Escherichia coli*[119] and from necrotizing enterocolitis[120]. Protection against enteropathogenic *E. coli*, might be partly owing to the generation of acetate by *Bifidobacteria*, as acetate promotes epithelial barrier defences and prevents bacterial translocation[119]. However, the ability of *Bifidobacteria* to support the growth of butyrogenic bacteria[121] may also account for the antimicrobial effects of these commensals. SCFAs

diminish the duration and severity of intestinal infections with bacterial pathogens, including *Shigella* spp.[122], *Salmonella enterica* serovar Typhimurium[123] and enteropathogenic *E. coli*[119], as they promote epithelial defences, mediate colonisation resistance **[G]** , and inhibit bacterial virulence and the formation of biofilms. SCFAs, especially butyrate, reduced the fitness of *Clostridium difficile* in a model of antibiotic-induced *Clostridium difficile* infection, and can directly suppress *Clostridium difficile growth* in a concentration-dependent manner[124, 125]. In line with this, lower faecal butyrate levels are observed in human subjects with *Clostridium difficile* infection compared to those without[125]. In addition, butyrate imparts antimicrobial activity to macrophages in a manner dependent on HDAC3 inhibition and prevents the dissemination of intestinal *Citrobacter rodentium* infection in mice[52].

Environmental enteric dysfunction is a global health problem characterized by defective small intestinal function in children who lack access to clean water and good sanitation. It is associated with stunted growth and increased mortality owing to overgrowth of *E. coli*, *Shigella* spp. and *Campylobacter* spp. together with reduced numbers of butyrogenic *Clostridia*[126]. Low levels of bacterial metabolites including 2-hydroxyisobutyrate have been found in the faeces of undernourished, hospitalized children in Zambia with morphological evidence of enteropathy[127]. A symbiotic preparation containing *Lactobacillus plantarum* and fructo-oligosaccharide as a source of fibre for the generation of SCFAs has been shown to prevent sepsis and death of at-risk infants in rural India[128].

Together, these findings underline the importance of SCFAs in preventing infectious disease of the intestine and indicate the potential for preventing diseases by increasing the levels of SCFAs such as butyrate. The mechanisms by which SCFAs exert anti-inflammatory, tolerogenic effects in response to commensal microorganisms yet also prevent infections are likely to involve, at least in part, their antimicrobial properties[52]. The beneficial effects of butyrogenic bacteria in preventing dissemination of intestinal infections has been hypothesised to result from the adherence of these commensals to intestinal mucosal cells that blocks translocation of pathogenic bacteria from the intestinal lumen into the bloodstream[128].

[H1] Intestinal SCFAs at extra-intestinal sites

The colonic lumen is the richest source of SCFAs in the body, with excessive SCFAs transported to the liver and a fraction released into the peripheral blood. At extra-intestinal sites, the tissue concentrations of SCFAs vary (**Box 3**). Intestinally-derived SCFAs have immunomodulatory effects at extra-intestinal sites such as the reproductive tract, liver, brain and lung. The systemic immunomodulatory effects of SCFAs are mainly mediated by their effects on precursor cells in the bone marrow.

[H2] The gut–reproductive tract axis

SCFAs produced by the vaginal microbiota have homeostatic and immunomodulatory effects in the female reproductive system (**Box 3**)[129]. Intraperitoneal injection of butyrate attenuates LPS-induced endometritis in mice by suppressing LPS-induced inflammatory responses[130]. The progression of endometriomas in mice and the growth of human endometrioma cells can be inhibited by butyrate-mediated inhibition of HDAC1 and upregulation of the tumour suppressor RAP1GAP[131]. SCFAs in the uterus also have profound consequences for pregnancy. Dietary administration of butyrate to pregnant rats significantly improved litter size and embryo implantation, and conferred an increase in progesterone levels[132], and feeding female mice a high-fibre diet during pregnancy increased the frequencies of thymic and peripheral T_{reg} cells with higher levels of expression of the autoimmune regulator *Aire*[133]. SCFAs from the colonic lumen of pregnant mice reach developing embryos through the maternal liver and bloodstream to shape embryonic energy metabolism, generating resistance to obesity and metabolic syndrome in offspring [134].

[H2] The gut–liver axis

Intestinal SCFAs can reach the liver via the hepatic portal vein and account for an estimated 30% of the hepatic energy supply. Butyrate has anti-inflammatory effects on Kupffer cells **[G]** in the liver; *in vivo* administration of butyrate to rats via portal vein transfusion enhanced production of the immunosuppressive arachidonic acid metabolite prostaglandin E2 (PGE2) by Kupffer cells, and *in vitro* butyrate supplementation to Kupffer cells increased PGE2 production while suppressing TNF secretion[135]. Dietary supplementation with butyrate for six weeks prevents liver injury and inflammation in mice with non-alcoholic-fatty liver

disease (NAFL), as indicated by decreased expression of pro-inflammatory cytokine genes (*Il1b and Tnf*) and activation of Kupffer cells in the liver[136]. In rat models of NAFL, butyrate inhibited NF-κB-mediated inflammation, which contributes to disease amelioration[137]. Mechanistically, butyrate upregulates PPARα expression in the liver by increasing H3K9 acetylation on its promoter to facilitate binding of the canonical NF-κB subunit p65.

Early-life antibiotic treatment markedly reduces butyrate levels in the liver of mice, which leads to impaired IL-18 signalling and, in turn, suppresses the mitochondrial function and maturation of liver-resident natural killer (NK) cells[138]. Dietary butyrate or supplementation with butyrogenic bacteria could stimulate IL-18 production in Kupffer cells and hepatocytes in a GPR109a-dependent manner to restore the maturation of liver-resident NK cells in mice exposed to antibiotics early in life[138].

[H2] The gut–brain axis

SCFAs are key mediators of the microbiota–gut–brain crosstalk, and are involved in the modulation of microglia **[G]** function, production of neurotrophic factors, and maintenance of the blood–brain barrier integrity, as well as in neurogenesis[139]. SCFAs can cross the blood–brain barrier to control microglia structure and maturity[140]. Acetate, specifically, is metabolised by microglia to acetyl-coenzyme A, fuelling metabolic pathways, such as the TCA cycle and the production of citrate and ATP, ultimately supporting the maturation of microglia during steady state[141]. Interestingly, germ-free mice have increased permeability of the blood–brain barrier, and this can be reversed by colonisation of the intestine with butyrogenic bacteria, which induce an increased expression of occludin[142]. Sodium butyrate can trigger AKT activation of microglia, both in vitro and in vivo, concomitantly with the inhibition of IL-1β and TNF production downstream of LPS stimulation, and this effect is mediated through HDAC inhibition[143]. The hypothesis that some circulating SCFAs affect the central nervous system is supported by findings in a mouse model of multiple sclerosis: oral administration of non-absorbing antibiotics (which have only local, non-systemic, effects on the gut microbiota) increased demyelination of nerves, and this was markedly ameliorated by oral butyrate (but not acetate or propionate)[144]. In this setting, butyrate inhibited HDAC activity in oligodendrocytes, thus restricting demyelination and enhancing remyelination. The neuroprotective roles of SCFAs in humans have been recognised in several neurological disorders, including multiple sclerosis, stroke and Parkinson disease[145]. Patients with

Parkinson disease demonstrate reduced levels of butyrate and other SCFAs in the intestine[146]. It is currently unclear how alterations in SCFA levels link to symptoms and disease progression, although reduced SCFA levels and biomarkers of mild intestinal inflammation (faecal calprotectin, IL-8, IL1-β) positively correlated with earlier onset of disease[147].

[H2] The gut–lung axis

In addition to the limited local supply of SCFAs in the lung from the lung microbiota, intestinal SCFAs that enter the bloodstream can modulate pulmonary immune responses through their effects on myeloid cell precursors in the bone marrow that seed the lung (**Fig. 4**). Increased circulating levels of propionate resulting from a high-fibre diet in mice were shown to generate anti-inflammatory DCs that seed the lung to limit allergic T_H2 cell responses[148]. Accordingly, treatment of mice with the antibiotic vancomycin, which depletes butyrate-producing bacteria, exacerbated allergic airway disease, whereas supplementation with butyrate prevented any allergic reactions associated with vancomycin[149]. These data support human epidemiological studies linking antibiotic use in early life with the propensity to develop asthma in adulthood[32, 33]. Early-life disruption of the microbiota and restoration of SCFAs seems to be key to altering allergic responses in the adult lung[150], and this might be partly owing to the capacity of SCFAs to cause long-term imprinting of immunity via epigenetic alterations[151]. However, it remains unclear whether SCFA treatments can prevent allergy in humans. Lower levels of SCFAs are detected in the faeces of allergic children as compared to non-allergic children[152]. Children fed a diet rich in yoghurt, fish, fruit and vegetables were found to have increased levels of faecal butyrate, and children with the highest levels of intestinal butyrate were less likely to develop asthma[153]. Children at 3 months of age with a family history or risk factors for atopy were found to lack the microbial pathways that support butyrate fermentation[154]. Furthermore, in a 6-year follow-up study, children who were raised in a farm-related environment and had a low incidence of asthma, atopic sensitization and food allergy, were characterized by high faecal butyrate levels [155]. These studies and the immune pathways that may be involved in regulating allergic responses in the lung by SCFAs have been summarised elsewhere[151]. The suppression of the production of type 2 cytokines by human lung ILC2s is one of the key mechanisms via which butyrate alleviated ILC2-driven airway hyperreactivity in vitro[102].

Intestinal SCFAs also impact respiratory infections, mainly via their effects on myeloid cell precursors in the bone marrow. Both propionate and butyrate boost $CD8^+$ T cell responses in the lung to protect against influenza infection in mice[93]. Mechanistically, increased SCFA levels generated in the intestine enhance the generation of $Ly6C^-$ monocytes in the bone marrow. In the lung, $Ly6C^-$ monocytes differentiate into macrophages with reduced expression of the neutrophil-attracting chemokine CXCL1. Thus, neutrophil recruitment to the airways is blunted during infection, limiting tissue pathology. In parallel, $CD8^+$ T cell effector function is supported via the metabolic rewiring by SCFAs[93]. A role for intestinal-derived acetate has been described in protection against secondary bacterial infections in the lung following influenza virus infection, with acetate enhancing the bactericidal activity of alveolar macrophages to reduce bacterial loads and lung pathology, thereby increasing survival of mice co-infected with influenza A virus and *Streptococcus pneumoniae*[156]. These data may partly explain the propensity of antibiotics to worsen influenza symptoms in mice[157].

Humans with higher levels of butyrate-producing bacteria in their intestine are less likely to develop viral infections of the lower respiratory tract[158], and various clinical trials show the beneficial effects of probiotic bacteria **[G]** that support SCFA production in the prevention and reduced severity of respiratory tract infections[159]. It is noteworthy that alterations in the gut microbiota of patients with COVID-19 commonly include reduced bacterial diversity and a reduction of butyrate-producing and -supporting bacteria, including *Bifidobacteria*, *Lactobacilli*, *Roseburia* and *Faecalibacterium praunitzii*, and both changes correlate with disease severity[37, 38]. Manipulation of the intestinal metabolite environment holds promise for reducing symptoms in hospitalised patients with COVID-19 based on preliminary evidence that a high-dose butyrate-supporting probiotic mixture administered three times a day reduced gastrointestinal symptoms and the risk of developing respiratory failure in these patients[160]. However, further studies with greater numbers of patients will determine the efficacy of probiotic treatment following infection with SARS-CoV-2.

[H1] The therapeutic potential of SCFAs

Low levels of SCFAs have been implicated in susceptibility to infection as well as in inflammatory, autoimmune, and allergic diseases (**Box 2**). Manipulation of SCFA levels holds promise for both preventative strategies and immunomodulatory therapies that induce or maintain remission in inflammatory diseases. There are various pharmacological approaches to increase SCFA levels in the gut. The most developed of these are butyrate administration to increase its levels in the gut lumen and modulation of the butyrate metabolism using prebiotics **[G]** and probiotics. Other approaches such as specific diets, faecal microbiota transplantation **[G]** and administration of genetically modified bacteria **[G]** have also reached preclinical or clinical trial stages.

Oral[116] or rectal administration of butyrate by enema[161] or suppositories has been used to increase local levels of butyrate, with enema application leading to increases in both intraluminal and portal vein levels of butyrate[161]. This procedure did not alter butyrate levels in peripheral blood due to a first-pass effect **[G]** in the liver. Targeted delivery of butyrate to the lower intestinal tract using polymeric micelles effectively restored intestinal homeostasis and suppressed immune activation in mouse models of colitis and peanut allergy[162].

The selective use of prebiotics and probiotic bacteria and bacterial communities can optimise niches for butyrogenic bacteria[163]. A large number of preclinical studies have focused on the roles of *Faecalibacterium prausnitzii*, *Roseburia hominus* and *Clostridium* spp. in modulating intestinal inflammation (reviewed in [164]). Recent technological advances that allow the metabolism and interactions of individual bacterial species to be predicted using metagenomic and metabolomic profiling are likely to improve such approaches in the future[165]. It is important to derive ecological principles involving both natural and genetically modified strains of bacteria in probiotic preparations that will enable the selective establishment of butyrogenic communities[166]. Genetically modified probiotics, such as *E. coli* strains engineered to produce increased butyrate levels, have demonstrated efficacy in reducing disease activity and intestinal damage in colitis models[167]. However, the relative effectiveness of genetically modified probiotics compared to that of natural probiotics remains unclear and clinical trials in this regard are currently limited[168].

Faecal microbiota transplants aim to replace a dysbiotic microbiota with a 'healthy' one and have shown promising results in *C. difficile*-induced pseudomembranous colitis[169, 170], resulting in Food and Drug Administration (FDA) approval. However, their efficacy in other disorders is less clear. In ulcerative colitis, the efficiency of faecal microbiota transplants depended on the presence of butyrogenic bacteria and the butyrate production capacity in the donor microbiome[171]. Increased colonisation with *Clostridium* cluster XIVa and reduced *Bacteroidetes* were found in the subgroup of patients who responded to the therapy. Conversely, low levels of *Clostridium* clusters IV and XIVa correlated with a poorly sustained response to faecal transplant, and this could be improved by transplantation of microbiota enriched in *Clostridium* clusters IV and XIVa[171]. Further studies are required to define the donor microbiome components that predict efficient colonisation and matching with the host microbiome that are required to achieve optimal SCFA production and sustained therapy response. It will also be important to optimise methods that favour colonisation by butyrogenic communities and to understand how to combine faecal microbiota transplants with nutritional maintenance and use of prebiotics and probiotics.

An increase in intestinal and systemic SCFAs, including butyrate, can also be achieved using diets rich in plant fibres that enhance the growth of SCFA-generating bacteria[172] or rich in fermentable prebiotics such as resistant starch **[G]**[173]. Use of diet to modulate SCFA levels has particular promise during pregnancy, where it is often unethical to test the effects of pharmaceutical compounds or other treatments. The therapeutic benefits of an adapted diet in pregnancy may be substantial, with recent evidence in mice suggesting that maternal gut-derived SCFAs have direct beneficial effects on offspring by promoting T_{reg} cell differentiation[133]. Moreover, a high-fibre diet (or oral SCFA administration) during pregnancy, was shown to reverse maternal obesity-induced gut microbiota changes and cognitive and social deficits in the offspring of obese mice[174]. Two clinical trials (NCT04520594 and NCT04522271) are underway to evaluate the effectiveness of resistant starch as an adjuvant therapy that enhances butyrate-producing bacteria in paediatric IBD [175].

Animal models show that modulating host microbiota in early life has profound effects on allergic airway responses[35], in keeping with the strong epidemiological evidence linking

antibiotic use in early life with onset of asthma in humans [32, 33]. This suggests a critical window of opportunity in early life for replacement of SCFAs as a potential way to prevent asthma and atopy. Targeting *Bifidobacteria* might be a therapeutic option in this case, as they have evolved in the intestinal niche to express genes for utilization of human milk oligosaccharides (HMOs) from breastmilk, to generate SCFAs and thereby prevent systemic inflammation and immune dysregulation[176]. Indeed, levels of intestinal *Bifidobacteria* are reduced in children with asthma[177], and administration of *Bifidobacteria* improved symptoms and quality of life in children with allergic rhinitis and asthma[178]. Prevention of allergy and atopy by supporting *Bifidobacteria* or a high fibre diet during pregnancy has strong potential to counteract the transgenerational impact of microbial dysbiosis that can be caused by antibiotics during pregnancy or during early life postpartum[179].

Finally, SCFA levels have been associated with the efficacy of various cancer therapies, including chemotherapy and immunotherapy[180]. The immunomodulatory properties of SCFA, particularly butyrate, might apply on tumour-specific cytotoxic T lymphocytes and CAR T cells[96]. Butyrate has been shown to boost the antitumor immune response of cytotoxic $CD8^+$ T cells through IL-12 signalling both in vitro and in vivo[181]. Patients with gastrointestinal cancer that responded to oxiplatin exhibited elevated plasma butyrate levels compared to non-responders. Similarly, patients with solid tumours that responded to immunotherapy (nivolumab or pembrolizumab) displayed increased faecal concentrations of SCFAs (acetic, propionic, butyric, valeric acid) and high levels of isovaleric acid in the blood, which correlated with longer progression-free survival[182]. This suggests that manipulating SCFA levelss in combination with cancer therapies maybe a useful therapeutic strategy.

It is as yet unclear whether the health benefits of the above SCFA-focused therapies are solely related to the presence of butyrate and other SCFAs or microbial metabolites. With increasing understanding of the biochemical mechanisms underlying the biological effects of SCFAs, there is potential to harness these more selectively using HDAC inhibitors and GPCR ligands.

[H1] Conclusion

SCFAs are bacteria-derived metabolic products at the interface between mucosal microbial communities and the host. An increasing prevalence of immune-mediated disorders have been associated with dysbiosis and a reduction in bacteria that produce SCFAs, particularly butyrate. Modulating butyrate levels enables the manipulation of inflammatory responses in model systems, and targeting SCFA metabolism has the potential to prevent disease and to maintain remission in immune-mediated disorders.

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Author contributions

The authors contributed equally to all aspects of the article.

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Peer review information

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Figure 1 | Environmental factors drive microbial metabolism and diversity with effects on SCFA production and inflammation.

a. Environmental and host factors including birth mode, breast feeding, diet, genetics, gender, age, use of antibiotics and inflammation modify the composition and metabolic capacity of intestinal microbial communities. Many of these factors have been shown to affect butyrogenic bacterial communities, such as *Faecalibacterium pranusnitzii*, *Anaerostipes*, *Roseburia spp.*, *and Eubacterium spp*, and the levels of butyrate levels in the intestine, as well as production of other short-chain fatty acids (SCFAs) including acetate and propionate. An ecosystem of bacteria drives fermentation of nutrients including plant fibres and amino acids into SCFAs. Cross-feeding between butyrate-producing and non-producing strains contributes to the production of butyrate. Fermentation by-products from *Bifidobacteria* (acetate, formate, lactate and succinate)[183] feed butyrate producing bacteria

including, *Anaerostipes caccae* and *Roseburia intestinalis*. *Blautia* and *Lactobacilli* are common producers of acetate and lactate, respectively, which can cross-feed butyrate producers, such as *Anaerostipes* and *Eubacterium spp*[184]. b. Dynamics of metabolites including SCFAs in the stool during the first two years of life in humans. Alterations in gut metabolite profile shifts from low acetate and high succinate levels in the early phase to high acetate levels in the middle phase, and ultimately reaching high propionate and butyrate levels at two years of life[24].

Figure 2 | Effects of SCFAs on GPCR-mediated signalling and HDACs.

The microbiota-derived short-chain fatty acids (SCFAs) acetate, propionate and butyrate signal through the G protein-coupled receptors (GPCRs) GPR43, GPR41 and GPR109A, with effects on a range of cell types, including epithelial stem cells, columnar epithelial cells, innate immune cells (dendritic cells (DCs), monocytes and macrophages) and lymphocytes (B cells and differentiated plasma cells, naïve T cells, regulatory T (T_{reg}) cells, and T helper 1(T_H1), T_H2, T_H17 effector cells. Butyrate also has histone deacetylase inhibitory activity. The differential distribution of SCFAs and differential expression of SCFA receptors and HDACs on the various cell types explain the context-specific effects of SCFAs in homeostasis and disease.

Figure 3 | The role of butyrate in intestinal mucosal immunity.

Butyrate regulates the intestinal mucosal barrier integrity and function through a variety of mechanisms. In the intestinal lumen, butyrate reaches concentrations of between 10 and 20mM and is a key energy source for intestinal epithelial cells. In columnar epithelial cells, butyrate signals via G protein-coupled receptors (GPR41, GPR43 or GPR109A) and histone deacetylase (HDACs) inhibition to activate the NLRP3 inflammasome and induce the downstream expression of IL-18, antimicrobial peptides and the tight junction proteins Claudin and Occludin. In addition, butyrate controls epithelial stem cell turnover and apoptosis by increasing promoter activity for the transcription factor FOXO3. In the lamina propria, butyrate levels range between 1 and 5mM and regulate the homing and differentiation of immune cells, modify the balance between pro- and anti-inflammatory

cytokine production, and induce the antimicrobial activity and phagocytic activity of macrophages. Butyrate inhibits the transcription of pro-inflammatory cytokines such as interleukin-12 (IL-12) and tumor necrosis factor (TNF) in monocytes and macrophages, increases the production of antimicrobial peptides, calprotectin (S100A8/S100A9), and modifies cellular metabolism, thereby inducing autophagy. In addition, butyrate controls the plasticity of the innate lymphoid cell subset ILC3 and IL-22 production. Butyrate can also shape adaptive immunity. It affects B cell- and plasma cell differentiation and intestinal IgA production, it supports FOXP3 expression and IL-10 secretion during the differentiation of regulatory T (T_{reg}) cells, and increases the ratio of T helper 1 (T_H1) to T helper 17 (T_H17) cells via the upregulation of T-bet expression and downregulation of RORγt. MCT1, lactate transporter monocarboxylate transporter 1. SMCT1, Sodium-coupled monocarboxylate transporter 1. mTOR, Mammalian target of rapamycin. HIF, Hypoxia-Inducible Factor. AhR, Aryl hydrocarbon receptor. FAO, Fatty acid oxidation. OXPHOS, Oxidative phosphorylation. TCA, Tricarboxylic acid cycle.

Figure 4 | The role of butyrate in lung mucosal immunity.

At the lung epithelial lining, there is little local production of butyrate by the lung microbiota, but intestinally-derived butyrate indirectly affects lung immunity and susceptibility to respiratory infections via it effects on the bone marrow precursor cells and blood-derived cells that enter the lung mucosa. The levels of intestinally-derived butyrate range between 1 and 10µM in the bone marrow and the blood. Butyrate downregulates dendritic cell ~~act~~activation and ~~ie~~migration~~-~~ by reducing the expression of costimulatory molecules CD80 and CD86~~and activation~~, thereby limiting allergic T_H2 responses. In addition, butyrate modulates the differentiation of naïve T cells into T helper 9 (T_H9) cells and regulatory T (T_{reg}) cells, for example via the upregulation of the transcription factor FOXP3. Butyrate also suppresses B cell and plasma cell differentiation by downregulating the expression of the transcription factors AICDA and BLIMP1; is associated with reduced mast cell degranulation in response to IgE, as a result of the downregulation of tyrosine protein kinases BTK, SYK, and LAT; decreases ILC2 proliferation and cytokine production following the downregulation of the transcription factor GATA3; and promotes eosinophil recruitment by inhibiting its adhesion to blood vessel endothelium and ~~,~~ ~~which affect~~ chemotactic responses and direct enhance apoptosis in the lungs. MCT1, lactate transporter

monocarboxylate transporter 1; SMCT1, Sodium-coupled monocarboxylate transporter 1; IL-5, interleukin 5; BCL-XL, BCL-2 like 1; MCL-1, induced myeloid leukemia cell differentiation protein 1.

Box 1 | The bacterial origin of short-chain fatty acids

Fatty acids with 1–6 carbons are classified as short-chain fatty acids (SCFAs). They are by-products of bacterial fermentation, produced predominantly in the colon by members of the gut microbiota that use undigested dietary fibre as an energy source. Acetic acid (acetate), propionic acid (propionate) and butyric acid (butyrate) are found at high levels in the human and mouse colon at a ratio of approximately 60% acetate to 20% propionate and 20% butyrate[185]. SCFAs are most concentrated in the intestine and exhibit a gradient within the body. Butyrate is primarily absorbed by the gut epithelium, whereas propionate and acetate are primarily absorbed in the liver and periphery, respectively.

Specific subsets of anaerobic bacteria, in particular members of the *Clostridium*, *Enbacterium* and *Butyrivibrio* genera, produce SCFAs at high levels in the intestine[186]. Clostridial clusters IV and XIVa are highly efficient butyrate producers, with the dominant species of the clostridial cluster IV in the human colon being *Faecalibacterium pranusnitzii*, and with *Roseburia spp.*, *Anaerostipes and Eubacterium spp.* being important butyrate producing members of clostridial cluster XIVa [186]. However, some of the butyrogenic effects of dietary fibre are the result of cross-feeding between butyrate-producing and non-butyrate-producing strains[187] (see also **Fig. 1**), which is promoted by anoxic conditions in the colon[188]. For example, butyrogenic bacteria, such as *Anaerostipes caccae* and *Roseburia intestinalis*, can use the fermentation by-products from the initial breakdown of dietary carbohydrates by other non-butyrogenic bacteria — such as acetate, succinate and lactate (from *Bifidobacteria*[183])— to produce butyrate. Multiple factors impact butyrate production, including species richness and key molecular factors such as hydrogen sulfide, environmental pH and resource competition[189]. Interspecies interactions in bacterial communities with high species richness illustrate that it is essential to consider bacterial communities rather than individual bacterial species when exploring the factors that

contribute to changes in SCFA levels[190]. Interestingly, clusters of bacterial taxa associated with increased availability of oxygen (and therefore limiting the anoxic conditions that promote bacterial cross-feeding) are altered in humans with a poor prognosis or therapeutic unresponsiveness in inflammatory bowel disease[105].

Box 2 | SCFAs in autoimmune disease and allergies

[bH1] Multiple sclerosis

Levels of butyrate as well as of butyrogenic bacteria are low in stool of patients with multiple sclerosis (reviewed in [191]). Oral administration of butyrate ameliorated disease in the mouse models of cuprizone-induced multiple sclerosis[144] and experimental autoimmune encephalomyelitis[192].

[bH1] Type 1 Diabetes

Reduced levels of butyrate-producing bacteria are found in patients with type 1 diabetes[193, 194] and administration of butyrate and acetate protected mice against diabetes[195]. Moreover, butyrate could alleviate antibiotic-driven type 1 diabetes in female offspring of nonobese diabetic mice[196]. Several mechanisms seem to account for the protective effects of butyrate against diabetes, including a reduction in the frequency of autoreactive T cells[195, 197]. Individual SCFAs may act in distinct ways: acetate limits the expansion of autoreactive T cells in lymphoid tissues through a reduction in B cell numbers together with reduced expression of MHC class I, MHC class II, CD86 and IgA, whereas butyrate increases the number of functional T_{reg} cells[197]. Butyrate supplementation improved glucose metabolism and insulin sensitivity in mice transplanted with a type 1 diabetes-associated gut microbiota[194]. However, oral supplementation of butyrate does not have a significant impact on innate immunity or residual beta cell function in individuals with long-standing type 1 diabetes[198]. Metagenomic studies combined with metabolomics show that the abundance of *Eubacterium rectale*, *Bacteroides pectinophilus* and *Roseburia intestinalis*, and levels of the butyrate-producing 4-aminobutanoate degradation V pathway, correlate with butyrate levels and increased insulin responsiveness[42].

[bH1] Graft-versus-host disease

SCFAs have been shown to protect mice from T cell-mediated damage in acute graft-versus-host disease (GVHD) by inducing GPR43-dependent ERK phosphorylation and NLRP3 inflammasome activation in the host[199]. However, the role of butyrate in GVHD is complex. Butyrate is reduced in the intestine of mice with GVHD, and manipulation of the microbiota to enhance butyrate-producing *Clostridium* strains prevents acute GVHD[200]. Recipients of allogeneic hematopoietic stem cell transplantation (HSCT), who had been administered anaerobe-targeting antibiotics, had reduced abundances of butyrogenic bacteria and bacterial genes for butyrate biosynthesis, together with higher incidence of and mortality from acute gut/liver GVHD[201]. Reduced butyrate levels are strongly associated with acute GVHD in patients after allogeneic HSCT [201], and with pediatric HSCT recipients who later develop chronic GVHD[202]. In HSCT recipients, donor fecal microbiota transplantation that results in an increase of butyrate producers contributes to the amelioration of intestinal GVHD[203]. An increased abundance of butyrogenic bacteria is associated with reduced severity of GVHD[204]. However, although butyrate seems to help to prevent GVHD, it might impede recovery in the intestine once GVHD-associated colitis has started. Increased abundance of butyrogenic bacteria in the human intestine correlated with an increased risk of developing steroid-refractory GVHD[205]. Mechanistically, direct exposure of colonic stem cells to butyrate following epithelial architecture loss during colitis impairs colonic mucosal recovery from GVHD.

[bH1] Allergies

Food, respiratory and cutaneous allergies in children are associated with reduced levels of butyrogenic bacteria[206]. High levels of butyrate and propionate in faeces of young children correlate with a decreased susceptibility to food allergy[153], and, vice versa, early-life reduction in faecal butyrate levels has been associated with food allergy later in life (reviewed in [207]). It is thought that the presence of butyrate in human milk may regulate key tolerogenic mechanisms involved in protecting against the development of food allergy[208]. Supplementing infant formula with *Lactobacillus rhamnosus GG* expands butyrogenic bacteria, boosting tolerance to cow's milk in infants, and protecting against asthma, eczema, urticaria and rhinoconjunctivitis in children with cow's milk allergy[209]. These protective effects of butyrate have been confirmed in mouse models of food allergy[162]. High fibre diet

in mice protects against food allergy and increased levels of intestinal butyrate and acetate, alongside increased retinoic acid activity in $CD103^+$ dendritic cells[210].

Children with atopic dermatitis[211], infants with atopic eczema[212, 213, 214] or infants with a predisposition to develop allergic sensitisation later in childhood[154] have gut microbiome signatures associated with reduced ability to derive SCFAs, particularly butyrate. Butyrate protected against skin inflammation that resembles allergen-induced atopic dermatitis and against systemic allergen sensitization in a mouse model, by promoting skin barrier function[181, 215]. Butyrate promotes epidermal keratinocyte differentiation and the production of key structural proteins and lipids, by fuelling fatty acid oxidation (directly and indirectly) and altering mitochondrial metabolism and lipid synthesis. Butyrate also suppresses the expression of IL-33, which is an alarmin implicated in the pathogenesis of atopic dermatitis and reduces *Staphylococcus aureus*-induced skin inflammation in a mouse model of atopic dermatitis[216]. These findings emphasise the significance of SCFAs in the skin as signalling molecules protecting against inflammatory skin disease.

Box 3 | Production of SCFAs at extra-intestinal sites

In addition to their production by the gut microbiota and their systemic dissemination, short-chain fatty acids (SCFAs) are also produced locally by the microbiota at the skin and the female genital tract. The immunomodulatory effects of SCFAs are mediated by different mechanisms depending on the tissue and might have opposing effects.

[bH1] Skin SCFAs

Whereas intestinal butyrate inhibits HDAC3 in macrophages to blunt their response to Toll-like receptor (TLR) [51], SCFAs produced in the skin, mainly propionate and valerate, inhibit HDAC8 and HDAC9 in keratinocytes, thereby rendering them tolerant against TLR ligands and promoting pro-inflammatory cytokine production[217]. HDAC8 and HDAC9 inhibition enhances MAP kinase signalling in keratinocytes and promotes cytokine responses, including release of IFNβ, ultimately leading to the maturation of dendritic cells (DCs) and subsequent T cell proliferation[218]. These data demonstrate that local SCFAs have tissue-

specific mechanisms of epigenetic control of immune tolerance. However, it is important to consider SCFAs individually, as butyrate can have anti-inflammatory effects also in the skin, where it reduces contact hypersensitivity responses and leads to expansion of T_{reg} cell populations[219].

[bH1] Reproductive tract SCFAs

The upper reproductive tract of female mammals isn't sterile, but is primarily colonised by *Prevotella* spp., *Lactobacillus iners* and *Lactobacillus crispatus*, although this microbiota is highly diverse and scarce compared with the vaginal microbiota[220]. Conversely, the lower reproductive tract is colonised predominantly by commensal *Lactobacillus* spp., which prevent genital tract infections by producing large amounts of lactic acid that lower vaginal pH[221]. During bacterial vaginosis, several types of anaerobic bacteria including *Gardnerella vaginalis* dominate the vaginal microbiota[222], shifting metabolites towards lower lactic acid levels and synthesis of high levels of butyrate, propionate and acetate[223]. In healthy women with a *Lactobacillus*-dominated vaginal microbiota and a physiological level of SCFAs, lactic acid exerts anti-inflammatory effects on cervicovaginal epithelial cells. However, during bacterial vaginosis, increased levels of vaginal SCFAs reduce the anti-inflammatory effects of lactic acid, resulting in a pro-inflammatory environment, compromise barrier integrity and ultimately contribute to the development of a dysbiotic vaginal environment[224].

Glossary

Holobiont. Ecological unit of a host and other species including commensal bacteria and viruses residing in the intestine or skin.

Short chain fatty acids (SCFAs). Comprise fatty acids with a carbon backbone of 1 to 6 carbons, formate (C1), acetate (C2), propionate (C3) and butyrate (C4), and valerate (C5).

Histone deacetylase (HDAC) Enzymes that mediate protein deacetylation in particular histone acetylation to modify histone function, DNA accessibility and gene transcription.

Antimicrobial peptides. Peptides that are essential for the defence of intestinal barrier surfaces against invading bacteria.

T follicular helper cells. Specialised CD4+ T cells that support B cell responses.

Cross-feeding. Exchange of metabolites among different species or strains of microorganisms as energy or metabolic source.

Colonisation resistance. Ability of the intestinal microbiota to prevent expansion of new microorganisms.

Kupffer cells. Liver resident specialised mononuclear phagocytes that line the hepatic sinusoids.

Microglia. Brain resident phagocytes that protect the cerebral microenvironment.

Faecal microbiota transplantation. Transfer of microbes from the stool of one individual to another.

Genetically modified bacteria. Bacteria that have been engineered to express specific genes in order to modify the bacterial ecosystem, to produce metabolites or proteins, and to modify host function.

Probiotic bacteria. Bacteria that provide health benefits when consumed.

Prebiotics. Substances such as carbohydrates that stimulate the growth of probiotic bacteria.

First-pass effect. Reduction of a metabolite due to metabolism at a specific location in the body which leads to a reduction

Resistant starch. Starch that is resistant to digestion in the small intestine.

TOC blurb

Short chain fatty acids (SCFAs) are produced by certain microbiota and regulate mucosal barrier integrity and immune cell functions. This Review summarizes latest insights into how SCFA levels might determine inflammatory and allergic disease outcomes by controlling the crosstalk between diet, the microbiome, and immunity.