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REVIEW

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The Influence of the Gut Microbiota on Host Physiology: In Pursuit of Mechanisms

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The results generated from the NIH funded Human Microbiome Project (HMP†) are necessarily tied to the overall mission of the agency, which is to foster scientific discoveries as a basis for protecting and improving health. The investment in the HMP phase 1 accomplished many of its goals including the preliminary characterization of the human microbiome and the identification of links between microbiome diversity and disease states. Going forward, the next step in these studies must involve the identification of the functional molecular elements that mediate the positive influence of a eubiotic microbiome on health and disease. This review will focus on recent advances describing mechanistic events in the intestine elicited by the microbiome. These include symbiotic bacteria-induced activation of redox-dependent cell signaling, the bacterial production of short chain fatty acids and ensuing cellular responses, and the secretion of bacteriocins by bacteria that have anti-microbial activities against potential pathogens.

INTRODUCTION

During the past fifteen years, our understanding of the composition and dynamics of the intestinal microbiota has become increasingly clear [1,2]. We have discovered that the microbiome consists of several hundred genera of bacteria, which may be grouped generally into the Bacteroidetes and Firmicutes taxonomic divisions [3]. The density of bacterial populations differs from $\sim 10^{2-3}$ in proximal ileum and jejunum, $\sim 10^{7-8}$ in the distal ileum, and $\sim 10^{11-12}$ colony forming units (cfu) per gram within the ascending colon [4]. Constituents of the microbiota occupy either a planktonic niche within the fecal stream, are adherent to the gut mucosa, or are associated mucous layer [5]. The continuing dynamic dialog between host cells and the microbiota are well studied across a variety of metazoans, and have unveiled commonalities of interaction across diverse phyla [6].

The intestinal microbiome thrives in a nutrient rich and thermostable environment and provides the host with metabolic nutrition, the facilitation of energy extraction, the competitive exclusion of pathogenic microorganisms and many other beneficial functions [7]. The gut resident microbes are crucial for normal immune development and homeostasis, as well as regulatory effects on epithelial growth, differentiation and cytoprotection, thus exemplifying a balanced symbiotic relationship between the host and its resident bacterial flora [8,9]. However, aberrations ("dysbiosis") in the intestinal microbial population has been shown to be also associated with weaknesses in gut barrier function and in innate and systemic immune dysregulation, although the extent to which the altered microbiome diversity is causal, or occurs as a result of disease remains an open question? [10]. By extension, the "hygiene hypothesis" conceives that the increased incidence of inflammatory bowel diseases (IBDs) and metabolic disorders, may be, at least in part, a result of a poverty of early exposure to, or the antibiotic destruction of the normal microbiota [11,12]. Furthermore, correlations between a dysbiotic gut microbiome have established links with a wide variety of effects on the host, from neoplasia [13] to psychiatric conditions [14,15]. As a result, increased research efforts have focused on approaches that supplement the gut microbiota

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†Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; DUSP3, dual specificity phosphatase 3; FA, fatty acid; FFAR3, free fatty acid receptor 3; GLP-1, glucagon-like peptide-1; GPCRs, G-protein-coupled receptors; HMP, human microbiome project; IBDs, inflammatory bowel diseases; ING, intestinal gluconeogenesis; MAPKP, mitogen activated protein kinase phosphatase; MCT, monocarboxylate transporter; Nox, NADPH oxidase; Nrf2, NF-E2-Related Factor 2; POMC, proopiomelanocortin; PPARγ, peroxisome proliferator-activated receptor γ; PTEN, lipid phosphatase; PTPs, protein tyrosine phosphatases; PYY, peptide YY; ROS, reactive oxygen species; SCFA, short-chain fatty acid.

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with live bacteria that are known to elicit positive influences on the host. This approach, termed 'probiotics', has described incidences where beneficial bacteria suppress inflammation, strengthen gut epithelial barrier function, promote epithelial restitutional responses, and offer potential interventional therapy for disorders of the gastrointestinal tract and beyond [16,17].

Many proposals have been put forward to define probiotic organisms. One in particular, following expert consultation and working group outputs on probiotics at the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), proposed a definition of "live microorganisms, which when consumed in adequate amounts, confer a health benefit on the host" [18]. Indeed, there is ever increasing literature based on laboratory and clinical investigations validating the use of probiotic bacteria as pharmacotherapeutic interventions in human health and disease [19,20]. In addition, there is an ever growing awareness of the need to characterize the molecular mechanisms by which probiotics elicit their beneficial effects on the host. Several molecular mechanisms defining the action of a eubiotic microbiota, and of probiotics on the host have been postulated, and here, three such mechanisms will be discussed, namely the (1) lactobacilli-induced and redox-dependent modulation of cell signaling pathways in the gut epithelium, (2) the production of short chain fatty acids (SCFAs) by lactic acid bacteria that are absorbed by enterocytes and mechanistically modulate physiological processes, and (3) the production of antimicrobial substances by commensal bacteria or probiotics that act on other (potentially pathogenic) bacteria.

HOST CELL AND MICROBE CROSS-TALK VIA REDOX SIGNALING

The microbiota occupying the intestinal lumen can influence many physiological processes. Up until the turn of the millennium, most studies of bacteria in the intestine involved characterization of pathogenic prokaryotic organisms, many of which induce inflammatory signaling networks in the host tissue [21-23]. However recently, more focus has been directed towards studying how non-pathogenic commensal bacteria can influence physiological and homeostatic pathways in the host, and in particular the molecular mechanisms of host cell and microbe cross-talk [24,25]. Here, discoveries reported during the past few years demonstrating that certain taxa of enteric commensals can stimulate cellular signaling via the generation of reactive oxygen species (ROS) in the gut epithelia will be discussed.

The first identification of deliberate ROS production within host cells was the observation that professional phagocytes such as neutrophils are able to generate ROS following bacterial contact [26]. Here, oligopeptides produced by prokaryotes which have a bacterial-specific N-formyl group (such as N-formyl methionyl-leucyl-phenylalanine (fMLF)) are sensed by formyl peptide receptors (FPRs) situated on the surface of neutrophils. The sensing of fMLF by FPR then initiates a signaling cascade that eventuates in the catalyzed generation of ROS by NADPH oxidase 2 (Nox2) [27]. NADPH oxidase (Nox) enzymes are also expressed in non-phagocytic cells, with Nox1 and Duox2 expressed in the intestine where they are involved in ROS generation in cells following bacterial contact with enterocytes [28-30]. Furthermore, orthologs of the Nox enzymes are conserved across multicellular life, where their function in generating ROS to control cellular proliferation and differentiation is well-documented. These include the control of the development of *Drosophila* haematopoietic progenitors [31], the control of the transition from proliferation to differentiation in the plant root [32], the control of regeneration of an amputated *Xenopus* tadpole tail [33], and the regulation of mouse spermatogonial stem cell self-renewal [34].

A recent finding showed that lactobacilli similarly induced ROS generation in intestinal epithelial cells via the catalytic action of Nox enzymes, with downstream effects including cell proliferation in the intestinal stem cell niche of *Drosophila* or murine intestines [35]. In this study, pure strains of bacteria isolated from the fly gut lumen were gnotobiotically fed to germ-free larvae. Of those tested, only *Lactobacillus plantarum* induced the dNox-dependent generation of cellular ROS, and ROS-dependent epithelial cell proliferation at time points up to four hours after ingestion. This observation was recapitulated in mammalian systems where strains of lactobacilli (especially the probiotic *Lactobacillus rhamnosus* GG strain) potently induced the generation of physiological levels of ROS in cultured cells. In addition, using an epithelial cell-specific Nox1-deficient (B6.Nox1$^{\Delta IEC}$) mouse, ingestion of *L. rhamnosus* GG was shown to induce Nox1-dependent ROS generation and cell proliferation in the murine intestine. Together, data from the *Drosophila* and mouse models show a conserved mechanism by which probiotic lactobacilli enhance epithelial development and homeostasis [35]. In a contemporary study, it was reported that FPR1-mediated sensing of fMLF by the enterocytes activates redox signaling cascades that promote restitution of an injured mucosa [36]. This study showed that *L. rhamnosus* GG, or purified preparations of fMLF could stimulate FPR1, and potentiate the generation of Nox1-dependent ROS leading to cell proliferation and migration within colonic wounds [37]. These discoveries establish a function for FPR1 in perceiving the commensal enteric microbiota which actively facilitated mucosal wound restitution following injury.

As mentioned, non-radical ROS generated by Nox enzymes function as regulators of many cell signaling pathways [38]. The cellular consequences of generated ROS are dependent on the subcellular sites and duration of generation [39-41]. ROS are short-lived molecules with very small radii over which they exert their reactive in-

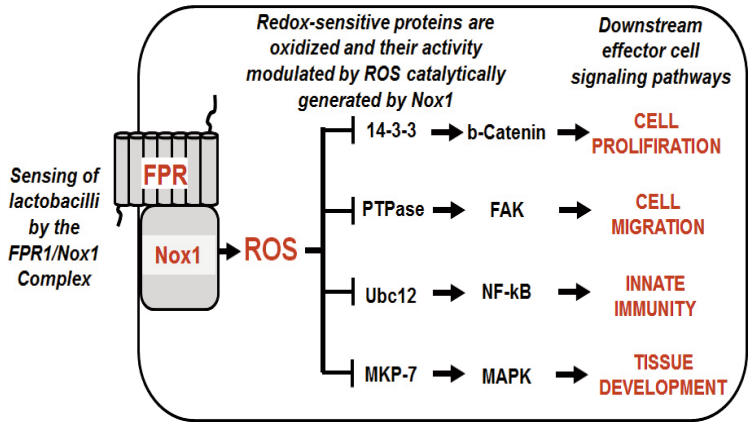

Figure 1. Supplementation of a normal diet with probiotics influences a plethora of physiological processes via the modulation of the cellular redox state. Bacteria in release formylated peptides, which are detected by sentinel formyl peptide receptors (FPRs) situated at the apical edge of colonocytes. Ligand binding induces the enzymatic generation of ROS by NADPH oxidases 1 (Nox1) that effectively transduce a signal following microbial product attachment via generated ROS. The ROS generated oxidizes sensor proteins, such as MKP-7, PTPase, UBC12, and 14-3-3 thereby activating downstream physiological process controlled by these proteins including tissue development, cell migration, inflammation, and cell proliferation respectively. Future studies will focus on the specific nature of factors released by probiotic bacteria that stimulate ROS generation in enterocytes. In addition, Mass spectrometry analysis of tissues will reveal further ROS-sensitive proteins that are target molecules of lactobacilli-induced ROS generation within enterocytes.

fluence. Indeed, some sentinel receptors physically associate with Nox to limit the ROS-mediated reactive influences to the immediate vicinity of target effector proteins. The molecular mechanism by which ROS control cell signaling pathways is by the oxidation of reactive cysteine residues within proteins [42-44]. These proteins have a graded perception of cellular ROS levels, which thus acts to transduce this information to proteins via the reversible oxidation of cysteine residues. In particular, cysteines within proteins that have a very low-pK_a exist as thiolate anions (Cys-S^-) and are easily oxidized by ROS [45]. Examples of proteins harboring regulatory redox-sensitive thiolates that have been shown to be sensitive to lactobacilli-induced ROS generation include the lipid phosphatase (PTEN) [37], MAPKPs such as DUSP3 [46,47], low-molecular weight (LMW)-PTP [48], protein tyrosine phosphatases (PTPs) [49], and enzymes involved in sumoylation and neddylation reactions [50]. As mentioned, each of these proteins has been shown to respond to increasing levels of ROS generated in cells in response to contact with lactobacilli, together outlining a molecular mechanism by which probiotics transduce their message into gene regulatory events and exerting their influence on host physiology (Figure 1).

CYTOPROTECTION BY PROBIOTIC BACTERIAL-ACTIVATION OF KEAP1/NRF2/ARE SIGNALING

Another well-characterized cell signaling circuitry that is sensitive to cellular ROS generation is the Keap1/Nrf2/ARE signaling module. Nrf2 (NF-E2-Related Factor 2) and its antagonist Keap1 (Kelch-like ECH-Associated Protein 1) are central components that induce cytoprotective responses to xenobiotics within the host [51]. The pathway is evolutionarily conserved across metazoan model systems including *Caenorhabditis elegans* [52], *D. melanogaster* [53], zebrafish [54], and mouse [55]. The activity of Nrf2 in the cytoplasm is regulated by the physical binding action of its inhibitor, Keap1 [56]. Under uninduced conditions, Keap1 binds to Nrf2, promoting Nrf2 fate towards Cullin-dependent E3 ubiquitin ligase proteosomal degradation. Electrophilic stress in the cytoplasm leads to the oxidation of cysteines within Keap1 resulting in a change in Keap1 conformation, and a release of Nrf2. Nrf2 then passes into the nucleus where it binds to an antioxidant response element (ARE) promoter sequence resulting in activating the expression of a battery of cytoprotective factors [57]. Examination of the relationship between bacterial-dependent ROS generation and Nrf2 pathway activity revealed that lactobacilli-induced, and Nox1 mediated generation of ROS activated Nrf2-dependent cytoprotective genes, and mediated organismal cytoprotection against oxidative stress in *Drosophila*, and against radiological insult in mice [58]. Thus, the Nrf2/Keap1/ARE signaling pathway represents another signaling mechanism by which the host senses and responds to microbial stimuli and activates cytoprotection and cell proliferation (Figure 2).

Since it has now been established that lactobacilli can induce the activation of Nrf2 signaling, this opens the possibility of identifying a mechanism by which probiotics influence other disease states that are regulated by Nrf2. As mentioned, the Nrf2 pathway has been extensively

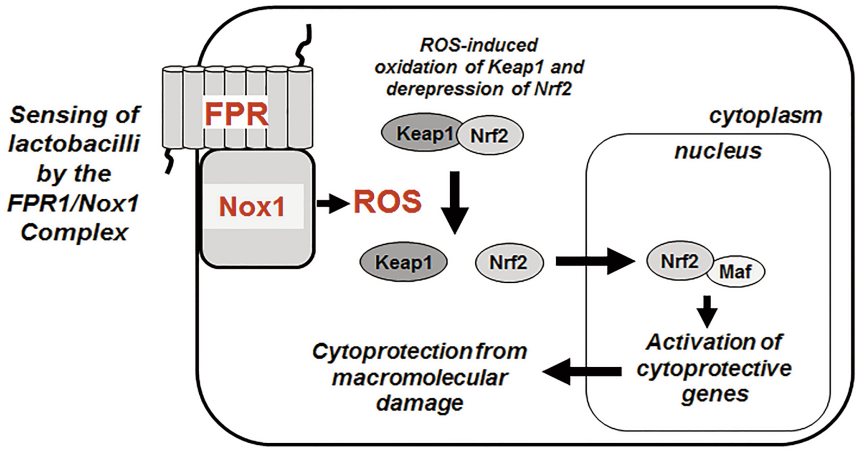

Figure 2. During homeostatic conditions, the Keap1 proteins binds with the nuclear factor Nrf2, inhibiting its translocation to the nucleus and the induction of the transcription of Nrf2-dependent reading frames. Lactobacilli-induced ROS generation causes the oxidation of cysteine residues within Keap1, which results in conformation change of Keap1 and the release of its inhibitory acuity on Nrf2. Nrf2 is then free to translocate into the nucleus where it binds to a DNA site known as an anti-oxidant response promoter elements. Nrf2 binding to these sites eventuates in the expression of a battery of phase II detoxifying genes that function in cytoprotection. The gene products from this regulon function to protect proteins in the cytosol from excessive ROS induced oxidation, thereby promoting cell survival, especially in circumstances of simultaneous exogenous oxidative insults. Future studies will focus on identifying the potencies of candidate probiotic bacteria for their capacities to stimulate cytoprotective Nrf2 pathway signaling.

studied in relation to cytoprotection against xenobiotic stresses inducing basal regulon of several hundred genes [59]. In addition, investigations into Nrf2 pathway function revealed that it also regulates cellular processes other than cytoprotection, including redox homeostasis in the aging heart [60], neurodegenerative diseases [61], cancer cell growth and chemoresistance [62-64], oxidative stress and inflammatory pathways [65], and diabetes [66]. Physiologically, ROS are generated during epithelial tissue inflammation, chiefly as a result of respiratory burst by monocytes at the site of injury. Here, Nrf2-responsive genes protect stem cell populations and facilitate restitutive cellular proliferation [67]. Together, each of the above are examples of cellular processes that are potentially modulated by probiotic stimulation of Nrf2 pathway.

In summary, ROS are enzymatically generated in epithelial cells after contact with lactobacilli. These ROS then function as signaling messengers due to their ability to transiently oxidize thiol groups within redox sensitive proteins. These biochemical alterations then regulate a network of effector proteins that are critical regulatory steps in innate immunity, cellular motility, and cell proliferation and differentiation pathways. Thus, ROS generation by lactobacilli (and other lactic acid bacteria) is a mechanistic description for the established effects of the microbiota on gut physiology that have to date only been phenomenologically reported.

THE PRODUCTION OF SHORT CHAIN FATTY ACIDS BY THE MICROBIOME AND THEIR ABSORPTION INTO THE HOST TISSUE

It is now firmly established that specific subsets of bacteria directly influence metazoan physiology through their metabolic activities [3]. Importantly, recent advances have revealed the nature of the molecular interactions between microbe-derived gut metabolites and host signaling pathways. Here, a number of studies describing the molecular mechanisms by which gut microbiome-generated short chain fatty acids (SCFAs) influence physiological processes within the host will be discussed.

SCFAs are products of the fermentation of indigestible foods by constituents of the gut microbiome. The main source of substrates driving the fermentation within the gut are complex carbohydrates such as starch or dietary fiber [68,69]. More than 95 percent of the SCFAs produced by bacteria in the gut are acetate, propionate, and butyrate, with fractions of caproate, formate and valerate constituting the other 5 percent. Most SCFA production occurs in the colon, where the three most abundant SCFAs may reach levels of 100 mmol/kg, and often existing at a relative ratio of 3:1:1 acetate to propionate to butyrate [70,71]. Amounts of SCFAs are particularly high in diets rich in foodstuffs that contain β-glucan or α-galactosides, with gut transit time of food also a contributing variable to amounts of SCFAs produced [72].

Colonic absorption of SCFAs is highly efficient with less than 10 percent of all the SCFAs expelled in the fecal stream. SCFAs are absorbed via the (1) hydrogen-coupled monocarboxylate transporter 1 (MCT 1), MCT 2 and MCT 4 [73], by (2) dynamic exchange with bicarbonate, as well as by (3) non-ionic diffusion of protonated SCFAs at the apical tips of colonocytes [74,75]. SCFAs absorbed into colon are transported into the hepatic portal vein and liver where they may be further metabolized before entering circulation. By contrast, SCFAs absorbed in the rectum can bypass the liver and directly enter systemic

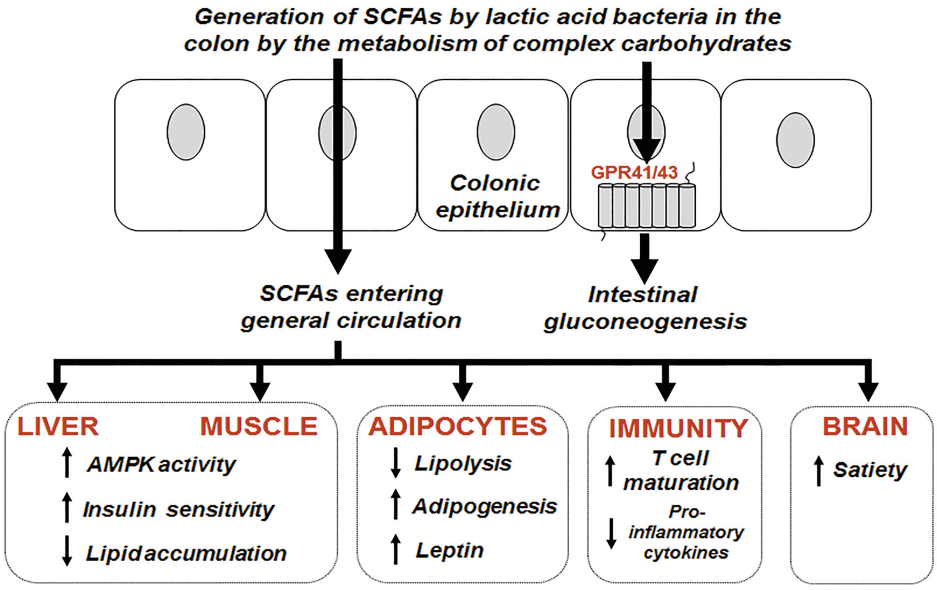

Figure 3. The fermentation of complex carbohydrates mainly in the colon by lactic acid bacteria produces short chain fatty acids (SCFAs), with about 95 percent of the species produced being acetate, propionate and butyrate. Propionate and butyrate mainly influence their effects on physiology in the colon and liver, with smaller amounts entering general circulation. In the colon, SCFA bind to the G-couples receptors GPR41 and GPR43, eventuating in and increase in the production of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) which control glucose metabolism in tissues, including intestinal tissue. High levels of acetate entering general circulation triggers reactions in adipose tissue, liver and muscle, brain, and influences on immunity. The molecular mechanisms of how SCFAs induce their responses is currently the subject of intense research focus. Investigations are being undertaken to deduce the extent to which SCFAs act directly on tissue remote from the intestine, or whether they act indirectly by inducing the expression of signaling factors produced in gut tissue which then circulate systemically.

circulation. Thus, systemic SCFA levels in individuals depend on dietary habits, the rates of SCFAs synthesis by the microbiome, and the efficiency of colonic absorption. Indeed, in clinical analysis, marked increases in acetate and propionate concentrations were detected in the serum postprandial to starch supplementation [76]. In colonocytes, liver, and skeletal muscle, SCFAs are sensed by G-protein coupled receptors (GPCR). These include GPR41 which is primarily activated by propionate, and GPR43 which is activated by all three SCFAs [77]. In addition, GPR109a which responds only to butyrate, has been shown to be expressed in colonocytes, adipose tissue, and immune cells [78]. Sensing of SCFAs within colonocytes, especially of butyrate levels, is required for optimal physiological functioning, as will now be discussed in the following paragraphs.

HOST FACTORS THAT MEDIATE SCFA INFLUENCE ON METABOLISM

The diversity of the gut microbiota and the abundance of SCFA-producing bacteria have been associated with energy harvesting and body weight. Specifically, several studies have implicated SCFAs as modulators of organismal body weight and gluconeogenesis when supplemented with specific controlled feeding regimes. For example, mice on a high fat acetate supplemented diet had reduced levels of body fat compared to non-acetate controls; an effect thought to be due to acetate-induced increase in levels of peroxisomal acyl-coenzyme A oxidase 1 [79]. In addition, butyrate was shown to improve insulin sensitivity and to increase energy expenditure by the induction of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 alpha) expression in brown adipose tissue. In studies where investigators introduced butyrate dietary supplements to obese mice, a significant lowering of adiposity and enhanced insulin sensitivity was observed [80]. In addition, feeding of SCFAs was found to directly regulate GPR41-mediated sympathetic nervous system activity and thereby also control body energy expenditure by mechanisms involving Gβγ-PLCβ-MAPK signaling [81]. Butyrate was reported to induce cAMP-dependent intestinal gluconeogenesis, whereas propionate was shown to activate intestinal gluconeogenesis by a mechanism involving free fatty acid receptor 3 (FFAR3). These data thus infer another mechanism of host-microbial cross-talk where SCFAs generated by bacteria from soluble fiber are involved in the generation and regulation of glucose in gut epithelial cells [82]. At the organismal level, SCFAs can influence the levels of food intake by inducing the release of satiety hormones in the gut which enter the circulation and act on receptors in the brain. These include peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) which are produced by enteroendocine

cells, and subdue hunger by dampening neuropeptide Y (NPY) activity and by activating proopiomelanocortin (POMC) neurons in the hypothalamus [83-85]. GLP-1 has also been reported to slow solid gastric secretion and emptying in humans [86,87].

Acetate has been extensively studied for its influences on lipolysis, which mechanistically involves the hydrolysis of triglycerides into glycerol and fatty acids. Indeed, the administration of acetate was shown to significantly reduce serum free fatty acids (FFA), showing that colonic SCFAs can have a major influence on host metabolism and lipid synthesis [88]. Mechanisms of SCFA-induced lipolysis have been shown to require GPRs, where it was shown that GPR43 was necessary to mediate a reduction in host lipolytic activity following acetate and propionate administration [89]. Other evidence include the observation that SCFAs can modulate the expression of the fasting-induced adipose factor (FIAF), which is a regulator fat metabolism [90], as well as the report of a novel mechanism of gene regulation in the colon which showed that SCFAs activate peroxisome proliferator-activated receptor γ (PPARγ) expression and FIAF synthesis [91]. Lactic acid bacteria that produce SCFAs were shown to be inhibitory toward the accumulation of large adipocytes [92], and in a recent study, GPR41 was identified as a regulator of host energy balance through a gut microbiota-dependent mechanism [93]. Together, these studies point towards emerging evidence that SCFAs can mechanistically induce molecules that function in host energy expenditure and in lipolysis (Figure 3).

In the liver, hepatic fat accumulation and chronic inflammation are strongly linked with insulin resistance and obesity. Thus, the potential for microbiome derived factors, such as SCFAs to be used as positive modulators of liver metabolism is of great interest in treating these conditions. In rat hepatocytes, it was found that acetate is a lipogenic substrate, acting by a mechanism that involved a reduction in fatty acid synthase activity [94]. SCFAs can also influence hepatic lipid metabolism by a mechanism involving the enzyme 5' AMP-activated protein kinase (AMPK), which functions in cellular energy homeostasis [95]. SCFAs were also shown to activate AMPK signaling and stimulate an increase in lipid oxidation, and a decrease in lipid synthesis in bovine hepatocytes [96]. Furthermore, administration of SCFAs in models of obesity decreased the accumulation of fats in the liver and improved insulin resistance by mechanisms involving gluconeogenesis, lipogenesis, the expression of PPARα target genes, and AMPK phosphorylation [97]. In addition, treatment of rats with SCFA producing bacteria prevented nonalcoholic fatty liver disease and lowered triglyceride concentrations [98]. Finally, as well as in enterocytes, the influences of SCFAs in the liver also occurs by a mechanism that involves GPR41 and GPR43 in hepatic cells [99]. In summary, SCFAs are direct substrates of gluconeogenesis and lipogenesis in the liver, with the cell signaling pathways that mediate these influences only beginning to be discovered. Together, these are promising initial observations that may act as basis for a mechanistic explanation for the positive effects of direct SCFA supplementation, or as a rational for the supplementation of SCFA producing lactic acid bacteria as treatment for the control of body weight and obesity.

THE REGULATION OF IMMUNOLOGICAL RESPONSES BY SHORT CHAIN FATTY ACIDS

SCFAs have been extensively reported as regulators of immune responses. One well-studied effect of SCFAs on the immune system is via the regulation of T cell activity which plays a central role in controlling immune tolerance and adaptive immunity. T cell maturation is regulated by a number of cytokines that control T cell differentiation into specialized effector and regulatory types. An increasing body of literature include publications that report that SCFAs stimulate T cell differentiation [100-103]. This is significant because effector Th1 (T helper type 1) and Th17 cells function in the response to pathogens and can cause tissue inflammation, whereas regulatory T-cells (Tregs) such as IL-10$^+$ T cells and FoxP3$^+$ T cells balance the activities of effector immune cells [104]. Relevant to this review is the established notion that effector and regulatory T cell differentiation is strongly influenced by the gut microbiota and the SCFAs they generate [105,106]. SCFAs have been shown to selectively support the development of Th1 and Th17 effector cells and IL-10$^+$ regulatory T cells by the suppression of histone deacetylases and the modulation of mTOR–S6K pathway signaling [107]. In addition, SCFAs can induced the expansion of colonic Tregs that function in immune tolerance. For example, butyrate has an inhibitory effect on cytokine production by lymphocytes [108,109] and has inhibitory effects on the production of interleukins [110]. Furthermore, SCFAs produced by commensal bacteria were shown to promote peripheral regulatory T-cell generation [111,112], and germ-free mice inoculated with SCFA-producing Clostridia induced IL-10 production in FoxP3$^+$ T cells [113], altogether showing that SCFAs generated as a result of metabolism of complex carbohydrates by lactic acid bacteria in the gut have potent effects on T cell activity and immunity.

As mentioned, the most well-studied mechanism of SCFA activity is through binding to the GPCRs such as GPR41, GPR43, and GPR109A [102]. However, negligible expression of these receptors occur in T cells, thus pointing to the likelihood that other pathways are responsible for mediating SCFA-induced modulation of T cell responses. Indeed, pathways proposed to function in T-cell responses to SCFAs include those involved in metabolism. For example, SCFAs are known to be metabolized to acetyl-CoA, which is a central energy storing molecule.

Further metabolism of Acetyl-CoA during energy production results in the activation of mTOR pathway [114], which was shown to be involved in SCFA-regulation of T cell lineage commitment [115]. In addition to metabolism, SCFAs can indirectly influence T cells through their effects on dendritic cells. For example, SCFAs suppress functional maturation of dendritic cells *in vitro* [116,117], and increase IL-23 production from stimulated dendritic cells [118]. Furthermore, the SCFA valproate, which is a strong inhibitor of histone deacetylases, was shown to block maturation of dendritic cells and inhibit the production of T-cell activating molecules [119].

Interestingly, transcriptional analysis revealed specific effects of each SCFA species on gene activity in human dendritic cells. This study showed that acetate exerted negligible effects on dendritic cells, whereas both butyrate and propionate potently activated gene expression. Pathway analysis suggested that propionate and butyrate also modulated leukocyte trafficking genes, as both strongly reduced the release of several pro-inflammatory chemokines including CCL3, CCL4, CCL5, CXCL9, CXCL10, and CXCL11. Additionally, butyrate and propionate were shown to inhibit the production of inflammatory markers that are induced by lipopolysaccharide (LPS) binding to TLR4, such as IL-6 and IL-12p40 [120]. Together, these influences of SCFAs have the general effect of dampening inflammation. Thus, the accumulation of this impressive body of data on the effects if SCFAs on immunity clearly warrants further study, as it may offer inexpensive alternatives to, or at least augmentation of expensive immunotherapy approaches. Additional studies are necessary to establish the full extent of SCFA-induced activation of immunity, in particular the identification of the cell types and cell signaling pathways within immune cells that respond to SCFAs.

THE GENERATION OF ANTIMICROBIAL PROTEINS BY PROBIOTIC STRAINS

Another mechanism by which the gut luminal microbiome, and the supplementation of the live microorganisms can influence health is through the production of antimicrobial factors that modulate the viability of other bacteria within the microbiome. Examples of antimicrobial compounds generated by bacteria are hydrogen peroxide [121], short-chain fatty acids [122], and bacteriocins, which are the focus of the remainder of this review on the mechanisms of host-commensal bacterial interactions [123-125]. The production of bacteriocins by bacteria has been shown to improve the bacteria's capacity to contest with other microbes in the gastrointestinal tract for an ecological niche. Thus, the capacity to generate bacteriocins has been an important consideration in assessing the probiotic potential of a bacterial strains. Indeed, bacteriocin production means that that microbe develops a specific immunity against bacteria that are the target of the bacteriocin [126,127]. However, only in a few investigations has it been conclusively established that bacteriocin generation can positively influence a strain's ability to compete with other microbes in the gastrointestinal lumen, and highlights from this field of research will now be discussed.

All bacteria and archaea can produce bacteriocins which suggests that they are fundamentally necessary for bacteria to establish within their given niche. How bacteriocins exactly alter population diversity is still an open question. There are three leading hypotheses, each not mutually exclusive, that propose how bacteriocins function. Firstly, they may function as inter-bacterial signaling intermediates through quorum sensing. Secondly, they may function as colonizing factors establishing the dominance of a given bacteria within a niche. Thirdly, they function as antimicrobial peptides exclusively against pathogens, thereby protecting the host from infection [128-130]. Here, examples and the importance of bacteriocins within microbial communities of the gastrointestinal tract will be discussed in relation to their influence on bacterial pervasiveness, as well as on the survival of pathogens and modulation of microbial diversity.

Microbes within the gut luminal contents must co-operatively exist while also ensuring that they are not outcompeted from the niche. One mechanism bacteria employ to contest for a niche is through the production of bacteriocins. Bacteriocins generated within bacteria may either by transmitted by contact-dependent mechanisms, or may be secreted and function as diffusible molecules that have cytotoxic activity that is independent of direct cell to cell contact. For example, early investigations in this field included studies showing that *Escherichia coli* generating the bacteriocin Colicin could persist in the colon of streptomycin-treated mice for longer than isogenic *E. coli* that could not produce Colicin [131]. In addition, the production of mutacin, a bacteriocin produced by streptococci facilitated the persistence of this bacteria in oral cavities [132], and a study of the bacteriocin BlpMN generated by *S. pneumoniae*, showed that this bacteriocin facilitated colonization of *Streptococcus* in the murine nasopharynxthe [133].

As stated above, bacteriocin production is emerging as an important element in the assessment of the probiotic potential of bacterial strains. As probiotics, it is envisaged that bacteriocins would function to preserve the ratio of advantageous to potentially adverse components of the human gastrointestinal microbiota [134]. However, relatively few investigations have described the impact of bacteriocins on health a disease, and their apparent potential as probiotic agents. Bacteria that secrete bacteriocins are attractive candidates as stabilizers of microbial diversity within niches due to their potential bactericidal activity against competing or invading bacteria that may enter their environment [135,136]. Particularly well-studied are lactic acid bacteria which are known to produce an exten-

sive repertoire of bacteriocins [137]. For example, some *Lactobacillus salivarius* strains were reported to produce a bacteriocin essential for its protective influence on mice infected with the foodborne pathogen *Listeria monocytogenes* [138,139]. Furthermore, examination of bacteriocin production and total DNA genome comparison of several other *L. salivarius* isolates of intestinal origin revealed a conserved gene cluster of plasmid origin that was postulated to function in the secretion of bacteriocins in this bacterium [140]. In studies where pigs were given a probiotic mixture of five LABs, the only bacteriocin producer, *L. salivarius* DPC6005, outcompeted the other administered strains within the intestine [141]. In addition, bacteriocin production was shown to facilitate *Bifidobacterium longum* subsp. longum DJO10A survival and competition against strains of *Clostridium difficile* and *E. coli* in the gastrointestinal tract [142]. Mice receiving *Enterococcus faecium* KH24, a bacteriocin-producing strain for 12 days, were found to have significantly increased numbers of lactobacilli in the intestine [143]. Other studies into bacteriocins involved assessing the activity of peptides secreted by the well-studied probiotic *Lactobacillus rhamnosus* GG. Several anti-microbial peptides have already been isolated from *L. rhamnosus* GG culture media which were reported to exhibit bactericidal activity against both Gram-negative and Gram-positive microbes [144]. Furthermore, exopolysaccharides produced by *L. rhamnosus* GG inhibited the cytotoxic effect of Bacillus cereus extracellular factors on colonic epithelial cells [145], and *L. rhamnosus* GG was also reported to have anti-microbial influences against *S. typhiumrium* 1344 via the production of lactic acid and other molecules [146].

Outside of lactic acid bacteria and classic probiotics, a well-studied microbe in relation to bacteriocin production is *E. coli* H22. *E. coli* H22 produces several bacteriocins that impede the prevalence of some pathogenic enterobacteria *in vitro* [147]. In addition, *E. coli* H22 was shown to impede *Shigella flexneri* pathogenesis within 6 days of administration, while having no influence on the growth of the resident gut commensal microbiota [147]. Furthermore, *E. coli* strains that produce Colicin E7 were found to have anti-*E. coli* O157:H7 activity in cattle [148], thus establishing a compelling body of data emphasizing the importance of bacteriocins in the elimination of pathogenic bacteria from the intestinal microbial population.

A recent study showed that bacteriocin production augments niche competition by *Enterococcus faecalis* in the mammalian gastrointestinal tract. This study investigated *E. faecalis* pathogenesis in the context of the molecular mechanisms that it employs to contest with other bacteria and establish itself within the gut. Previously, plasmids harboring genes that encode bacteriocins were found to be common among enterococcal strains which modulate niche competition between enterococci and the intestinal microbiota. The study showed how *E. faecalis* harboring the pPD1 plasmid that expresses bacteriocin 21 [149], outcompetes *E. faecalis* lacking the same plasmid when both are introduced into the same murine gut. The study also showed that within the intestine, pPD1 is transferred to other *E. faecalis* strains by conjugation, and that colonization with an *E. faecalis* strain carrying a conjugation-defective pPD1 mutant cleared vancomycin-resistant enterococci in the gut [150]. This study is an example of how bacteriocin expression by resident intestinal bacteria can influence niche competition in the gastrointestinal tract and substantiates the notion that bacteriocins secreted by probiotic bacteria may be an effective therapeutic approach to selectively eliminate intestinal colonization by pathogens.

As stated above, some bacteriocin molecules are transmitted by direct cell-cell contact, and recent evidence has shown that this occurs by the action of a type VI secretion system (T6SS), which is a structure that can transfer DNA or proteins to either eukaryotic or bacterial cells [151,152]. Indeed, transposon insertion site sequencing (Tn-seq) analysis in *Vibrio cholerae* identified a mutant strain with a colonization defect that had an insertional inactivation in tsiV3 gene, which encodes immunity in *V. cholerae* against the bacteriocidal effects of the T6SS effector protein VgrG3. It was shown that *tsiV3* mutants exhibited reduced survival *in vivo* only when cocolonized with bacteria expressing *vgrG3* and T6SS structural genes, thus showing evidence that T6SS mediates antagonistic inter-bacterial communications [153]. In addition, bioinformatic and functional analysis in Bacteroidetes, which along with Firmicutes are the two most highly abundant phyla in the human intestines [154,155], revealed that T6SS-dependent mechanisms function in inter-bacterial antagonism in this taxa. This study suggested putative mechanisms that may explain the high prevalence of Bacteroidetes in polymicrobial population within the intestine, where they demonstrated that specific T6SS-like mechanisms in Bacteroidetes function in exporting antibacterial proteins that target rival bacteria [156]. Further information about the ecological role of T6SS in Bacteroidetes was gleaned where the incidence of T6SS-contact events was calculated using an approach that combined gnotobiotic animals, microbial genetics, and mathematical modeling. In this study, it was estimated that Bacteroidetes effector proteins transmission rates exceed 1 billion events per minute in each gram of luminal colonic contents [157]. These investigations underscore the significance of T6SS in human gut Bacteroidetes as a crucial mechanism by which they are able to successfully out-compete many rival commensals and pathogens for the nutrient rich environment of the mammalian intestine. Furthermore, metagenomic analysis of human luminal contents revealed that more than 50 percent of gut Bacteroidales encode T6SSs, which could be classified into three subgroups based on distinct genetic architectures [158]. This finding was corroborated in a contemporary study that similarly identified three subgroups of T6SSs

in Bacteroidales. Moreover, this study also showed that one of these subgroups, which they named genetic architecture 3 (GA3) harbored novel effector and immunity proteins which they demonstrated to function in conferring a competitive advantage to *B. fragilis* in the mammalian gut [159].

Altogether, these studies on bacteriocin function are exiting initial findings in the quest to identify mechanisms that mediate the influence microbiome diversity on host health. Nevertheless, this field remains largely untapped and is still in its infancy. Yet undoubtedly, the initial investigations establish a proof of principle that bacteriocins may be exploited to have positive influences on health and disease in the gastrointestinal tract. For example, bacteriocins have the potential to facilitate and establish colonization, or prolong the duration by which a probiotic bacteria is a guest resident in the gut. In addition, the microbe that produces the bacteriocin may well obstruct the incursion of pathogens or promote the establishment of a bacterial community that enhances and educates host immune system responses. Furthermore, beyond the scope of this review, but certainly noteworthy, is the fact that bacteriocins are now considered the next wave of conventional antibiotics [160,161], as antiviral molecules [162] and even as potential anticancer agents [163].

CONCLUSIONS AND OUTLOOK

Probiotic bacterial-induced gut epithelial generation of ROS is a conserved process with many known well characterized downstream responses. This is a mechanism by which a gut microbiome mechanistically activates a wide range of host signaling and homeostatic processes. A complete characterization of signaling pathways that mediate these responses will advance our knowledge of mechanisms by which probiotics promote health. ROS-induced oxidation of sensor proteins has increasingly been appreciated as a fundamental element of signal transduction. Advanced Mass Spectrometry techniques can identify reactive cysteines within the proteome, including those oxidized in response to microbial contact with the cell. Corroborating the functions of these proteins *in vivo* will be challenging future work.

Pertaining to studies involving SCFAs, these illustrate that bacterial metabolites remote from the site of their production can modulate physiological responses, providing mechanistic insights into host-microbiome interactions. Many lactobacilli and bifidobacteria are ingested as supplements due to their well-established beneficial effects on the host. Both genus harbor genes that encode for metabolic pathways that ferment carbohydrates into SCFAs. Future studies must focus on discovering the physiological activities of SCFAs in animal models or within clinical settings. These approaches are expected to yield information that will contribute towards an understanding of chemical cross-talk between the microbiota and metazoan tissues, and by extension, contribute to the understanding of human diseases associated with metabolites generated by the gut microbiota.

Despite the extensive progress made in our understanding of bacteriocin functions within the gastrointestinal tract, future directions must see the development of consistent protocols of measuring bacteriocin activity in order to resolve the experimental variability and discrepancies, especially within mammalian hosts. Establishing these consistencies in protocol will be the springboard to the ultimate goal of testing bacteriocin producing probiotics in human clinical trials for their beneficial influents on health and disease. Analysis of the factors influencing bacteriocin production, activity and survival is essential to establish connections between *in vitro* and *in vivo* results. Further investigations will reveal the role of to date uncharacterized bacteriocin-producing strains in the gastrointestinal tract, with the promise of creating enhanced probiotics that control and modulate microbiome diversity that favors optimal health.

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