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Review Article

Immunometabolism of obesity and diabetes: microbiota link compartmentalized immunity in the gut to metabolic tissue inflammation

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Abstract

The bacteria that inhabit us have emerged as factors linking immunity and metabolism. Changes in our microbiota can modify obesity and the immune underpinnings of metabolic diseases such as Type 2 diabetes. Obesity coincides with a low-level systemic inflammation, which also manifests within metabolic tissues such as adipose tissue and liver. This metabolic inflammation can promote insulin resistance and dysglycaemia. However, the obesity and metabolic disease-related immune responses that are compartmentalized in the intestinal environment do not necessarily parallel the inflammatory status of metabolic tissues that control blood glucose. In fact, a permissive immune environment in the gut can exacerbate metabolic tissue inflammation. Unravelling these discordant immune responses in different parts of the body and establishing a connection between nutrients, immunity and the microbiota in the gut is a complex challenge. Recent evidence positions the relationship between host gut barrier function, intestinal T cell responses and specific microbes at the crossroads of obesity and inflammation in metabolic disease. A key problem to be addressed is understanding how metabolite, immune or bacterial signals from the gut are relayed and transferred into systemic or metabolic tissue inflammation that can impair insulin action preceding Type 2 diabetes.

Key words: antibiotics, insulin resistance, glucose, metabolic inflammation, microbiome.

INTRODUCTION

Bacteria inhabit many niches, including multicellular animals. The assemblage of a bacterial community is termed the microbiota, and the collection of bacterial genes associated with this community is often called the microbiome. Symbiotic, commensal and parasitic relationships all contribute to aspects of health and disease. Dysbiosis, an imbalance in the bacterial community, disrupts these relationships. Dysbiosis in oral, skin or vaginal niches has been associated with periodontitis, psoriasis and vaginosis respectively [1–3]. The gastrointestinal tract is also a key site of bacterial colonization, and intestinal dysbiosis has been linked to gut-derived diseases. Antibiotic-mediated intestinal dysbiosis is a key factor in susceptibility to *Clostridium difficile*-dependent colitis and may be a factor in susceptibility to many other forms of enteropathogenesis, including susceptibility to chronic disease such as inflammatory bowel disease [4,5]. Although it is still difficult to separate the cause and consequence of intestinal inflammation and dysbiosis, alterations in the intestinal microbiome are a factor in other chronic diseases that extend beyond the gut, including metabolic diseases. Increasingly, dysbiosis has been implicated in the regulation of the metabolic state of the host and a role for intermediate signals through the immune system are also emerging as important mediators contributing to metabolic disease. The present review highlights how the connection between specific microbes and gut immune responses relate to hormonal and metabolic responses in tissues that help to control blood glucose. As such, we compare gut and metabolic tissue inflammatory environments during obesity-related insulin resistance.

Obesity is a predictor for many metabolic diseases, which represent a significant global disease burden. Obesity coincides

Abbreviations: DIO, diet-induced obesity; HFD, high-fat diet; IFNγ, interferon γ; IL, interleukin; ILC, innate lymphoid cell; LDP, low doses of penicillin; LPS, lipopolysaccharide; LTβR, lymphotoxin-β receptor; MAM, microbial anti-inflammatory molecule; PPAR, peroxisome-proliferator-activated receptor; RORγ, retinoic acid receptor-related orphan receptor γ; RYGB, Roux-en-Y gastric bypass; SFB, segmented filamentous bacteria; T2D, Type 2 diabetes; TLR, Toll-like receptor; T_{reg}, regulatory T cell.

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with a low-level chronic inflammation in metabolic tissues such as the adipose tissue, liver and muscle, which are key regulators of whole-body glucose homoeostasis. This obesity-related 'metabolic inflammation' drives aspects of diseases, including ineffective insulin-mediated lowering of blood glucose (i.e. insulin resistance) that can contribute to T2D (Type 2 diabetes). The links between metabolic inflammation and impaired insulin action are well established. For example, obesity-induced inflammation within metabolic tissues (such as adipose tissue and liver) can manifest as pro-inflammatory cytokine and stress kinase-mediated insulin resistance [6]. The connections between post-insulin receptor signalling defects and various inflammatory signals continue to grow. However, the sources or key instigators of metabolic inflammation associated with obesity are still ill-defined. Emerging evidence links specific communities of the intestinal microbiota, diet and the compartmentalized regulation of immune responses within the gut to discordant inflammatory responses in metabolic tissues and metabolic dysfunction. The paradigm that microbiota influence immunity appears to be more developed than models that link microbiota to altered endocrine or metabolic responses. The present review outlines the emerging concept that communication between the microbiota and immunity alter metabolic responses relevant to obesity-induced disease. In particular, the present review highlights how dysbiosis associated with obesity, insulin resistance and T2D is linked to specific immune responses in the gut, which do not necessarily 'carry-over' or parallel circulating immune responses or those in the tissues that help to control blood glucose.

Inflammation in vascular and metabolic tissues occurs in many forms during obesity, and the immunological underpinnings of insulin resistance and cardiovascular disease have been reviewed in [7,8]. In general, metabolic inflammation involves accumulation and pro-inflammatory skewing/polarization of professional immune cells in metabolic tissues. The resultant pro-inflammatory environment impairs insulin action in tissues that help to control blood glucose. The participation of innate and adaptive immune responses from various cell populations have been shown during obesity-induced insulin resistance, including (but not limited to) neutrophils, mast cells, macrophages, dendritic cells, B-cells and T cells. Deleting specific immune responses or the chemoattraction of cells that mediated these responses can attenuate obesity-induced inflammation and insulin resistance. As an example, blocking IL (interleukin)-1 receptor signalling attenuates metabolic inflammation and promotes insulin sensitivity in a rodent model of T2D [9]. Furthermore, deletion of a major recruitment factor for monocytes, MCP-1 (monocyte chemoattractant protein 1)/CCL2 (CC chemokine ligand 2) or the receptor, CCR2 (CC chemokine receptor 2), in mice equates to partial protection from HFD (high-fat diet)- and obesity-mediated inflammation [10].

Obesity is associated with skewing toward T_H1/T_H17 immune profiles in the liver, adipose and muscle tissue at the relative expense of T_H2 and anti-inflammatory T_{reg} (regulatory T cell) responses. DIO (diet-induced obesity) promotes an expansion of the T_H17 lineage in the spleen and this proinflammatory bias can be communicated to other organs to alter disease responses involved in autoimmunity [11]. Insulin resistance in humans has been associated with increased levels of T_H1 cells and decreased T_H2 cells in adipose tissue [12]. In humans, obesity is also characterized by increased T_H17 and T_H22 cells in adipose tissue and T cell effectors (IL-17 and IL-22) promote insulin resistance in muscle and liver cells [13]. In addition, during DIO, T_H1 cells accumulate more rapidly in the adipose tissue compared with T_H2 and T_{reg} populations, which normally counterbalance metabolic inflammation during obesity, contribute to insulin sensitivity and confer responsiveness to anti-diabetic drugs. These T_{reg} cells harbour tissue-distinct characteristics and have been reviewed recently [14]. Another fundamental aspect of metabolic inflammation during obesity is the skewing of tissue-resident macrophages towards pro-inflammatory M1/classically activated characteristics away from M2/alternatively activated macrophages that have higher anti-inflammatory potential. It has become clear that the pro-inflammatory environment in metabolic tissues involves the integration of innate and adaptive immunity, which has been expertly reviewed in [15]. There is strong evidence for a pro-inflammatory environment in metabolic tissues contributing to insulin resistance. However, in adipose tissue, the induction of specific inflammatory responses with adipocytes during dietary stress, such as an HFD, is imperative for tissue remodelling, and certain immune responses can be protective in terms of systemic metabolism, including glucose tolerance [16]. Hence there is still much to be learned about the immunometabolism within insulin responsive metabolic tissues during obesity.

Much less is known about the triggers for obesity-related immune responses in different parts of the host. The microbiota are positioned as instigators that can drive changes in immunity. Responses to microbes or microbial components may be very different in the well-tolerized gut environment compared with naïve metabolic tissues. It has been shown that the composition of the intestinal microbiota can influence both the population of immune cells present in metabolic tissues as well as their activation state. A number of individual dietary and microbiota-derived molecules can shape the specific T cell polarization and alter intestinal barrier function, which ultimately affects glucose metabolism during obesity. Compared with inflammation in metabolic tissues, the connection between intestinal inflammation and metabolic disease is ill-defined. Various models of obesity and T2D diabetes coincide with a pro-inflammatory state in various metabolic tissues, but there are some discrepancies in reports describing how the inflammatory state in the intestinal tract relates to obesity and metabolic disease. We propose that DIO is associated with a permissive gut inflammatory environment and mediates a barrier/inflammatory deficit in the intestinal epithelium, which permits dysbiosis and microbiota-derived molecules to enter the bloodstream at an increased rate, thereby altering host metabolic status and control of blood glucose.

THE MICROBIOTA AND IMMUNITY

The role of the microbiota in modifying immunity and infectious disease is well-established. Germ-free mice have deficiencies in development of secondary and tertiary lymphoid tissue, reduced

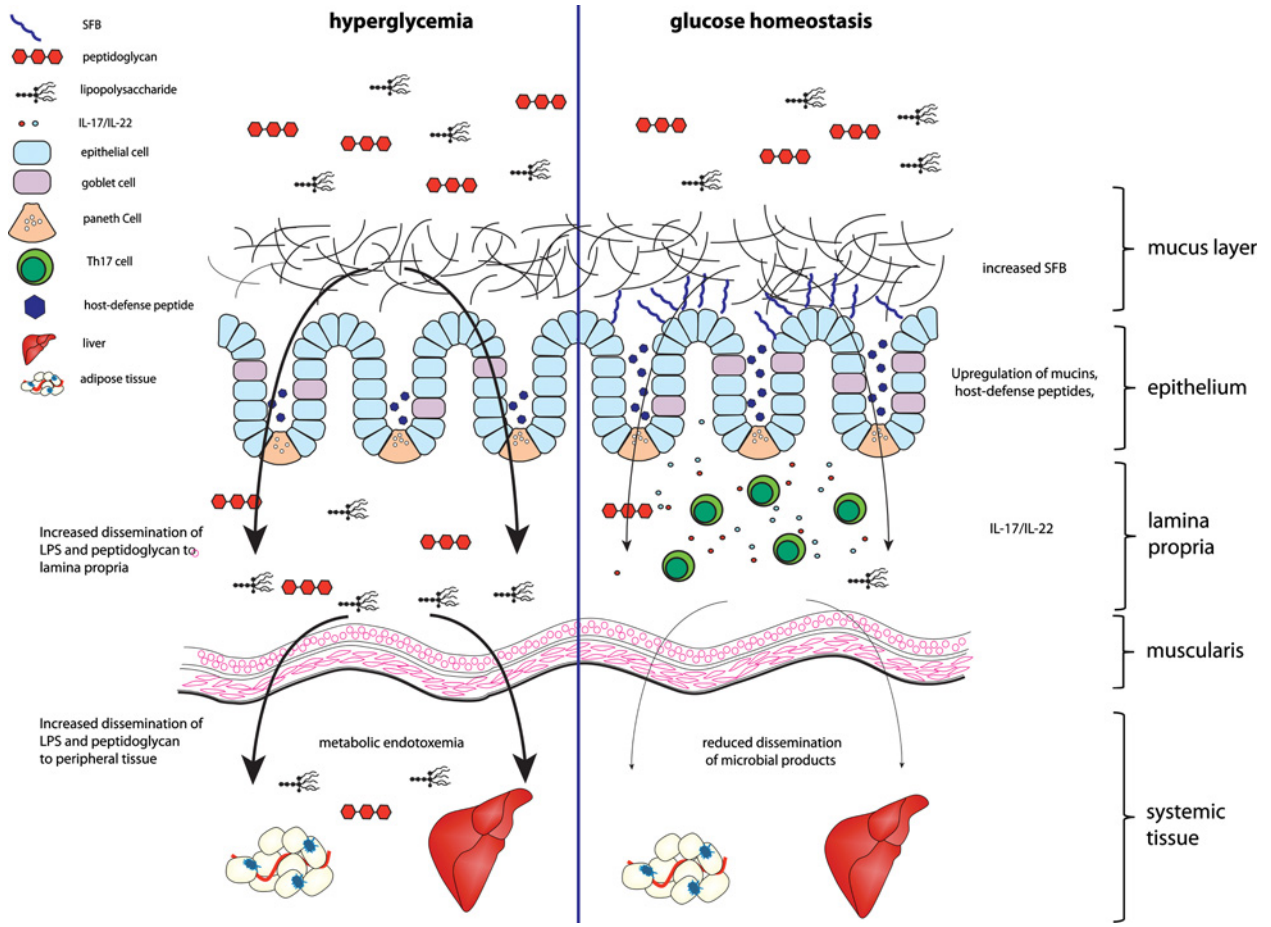

Figure 1 **Segmented filamentous bacteria (SFB) improve intestinal barrier function**

In mice with SFB, the bacteria form a tight attachment with ileal epithelial cells. The presence of SFB leads to up-regulation of IL-17 and IL-22 from lamina propria T_H17 cells. IL-17 and IL-22, in turn can drive the up-regulation of host-defence peptides from Paneth cells, which are critical for controlling bacterial numbers at the epithelial cell surface. This reduction in surface bacteria, in turn, results in reduced dissemination of luminal bacterial products to the lamina propria and distal metabolic tissues. This reduced dissemination to metabolic tissue results in lower metabolic endotoxaemia, lower metabolic tissue inflammation, improved insulin action and better glucose control.

adaptive immune responses to antigen challenge, and reduced expression of antimicrobial lectins and defensins that are involved in innate immunity [17]. Germ-free mice are more susceptible to infection by enteric pathogens such as *Salmonella*, *Shigella* or *Listeria* compared with conventionally raised animals [18–20]. This susceptibility could be decreased by colonization with some members of the commensal microbiota, but not others [21], demonstrating that the modulation of host immunity occurs in response to specific components of the microbiota. Signals from the intestinal microbiota are transmitted to the immune system via intestinal epithelial cells, dedicated M-cells, interdigitating dendritic cells and goblet cells that constitute the epithelial barrier [17]. Immune cell development is influenced by components of the microbiota, since germ-free animals have reduced numbers and sizes of intestinal Peyer's patches, less differentiated gastrointestinal-associated lymphoid tissue and mesenteric lymph nodes, as well as reduced levels of intestinal IgA [17].

The barrier function of the intestinal epithelium is critical for mediating resistance to infectious disease. This function is mediated by secreted factors from Paneth cells, columnar epithelial cells and mucin-secreting goblet cells. Paneth cells are a specialized secretory cell that lie at the base of ileal crypts and secrete a number of antimicrobial molecules, including α-defensins, lysozyme, antimicrobial lectins (RegIIIβ and RegIIIγ) and other host defence molecules [22]. Although the production of the α-defensins is thought to be constitutive [23], germ-free animals express little to no RegIIIγ, and expression can be restored by exposure to specific components of the microbiota, particularly those that are able to intimately associate with the intestinal epithelium [24]. RegIIIγ binds peptidoglycan and is directly antimicrobial with specificity for Gram-positive organisms, whereas RegIIIβ recognizes lipid A and has activity towards Gram-negative bacteria [25]. Similarly, production of β-defensins and mucus by the colonic epithelium is important for the barrier function of this site [26]. This inducible antimicrobial response is regulated by IL-22 and it modulates both susceptibility to infection and metabolic disorders [27]. In addition to the barrier function, signals from epithelial cells also regulate the

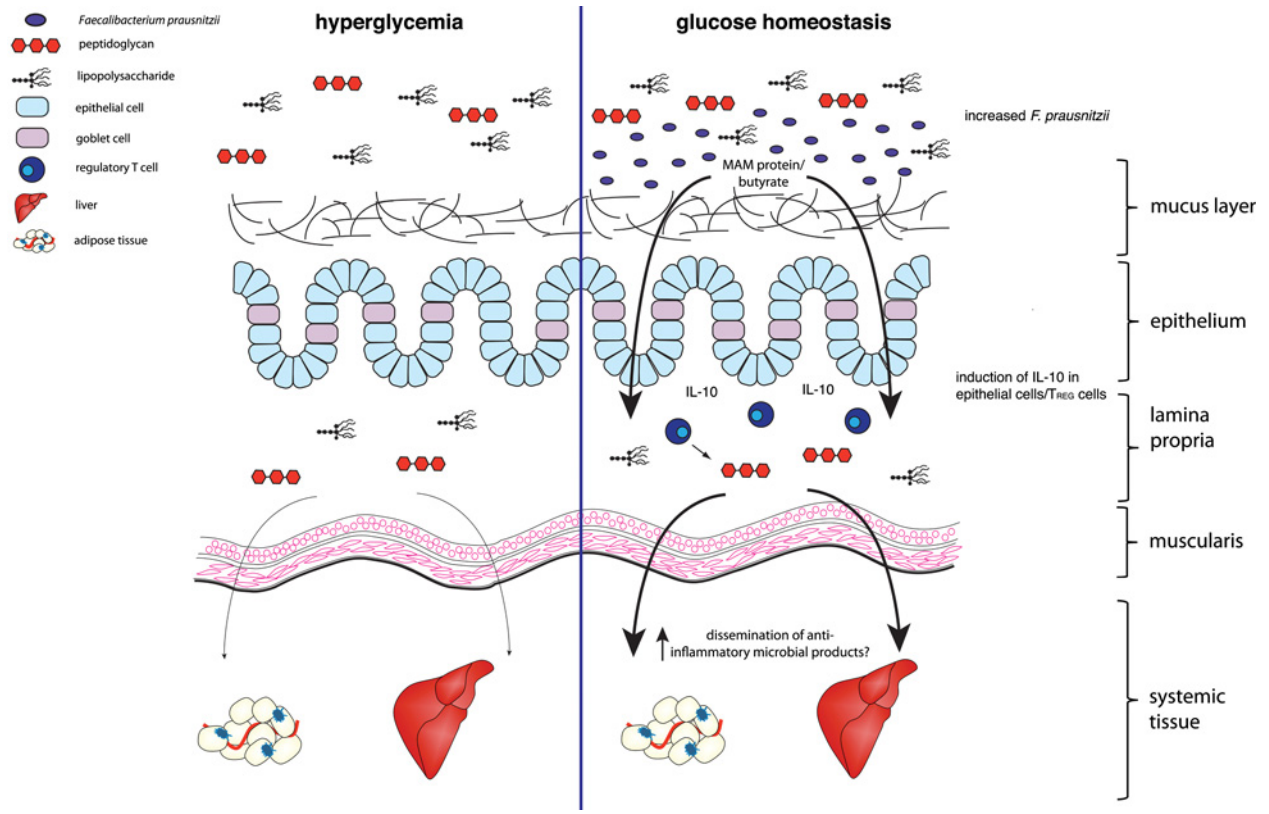

Figure 2 ***Faecalibacterium prausnitzii*** **in the intestinal tract dampens inflammation in the gut**

The presence of *F. prausnitzii* (and other bacteria) leads to the increased production of butyrate, a short-chain fatty acid that contributes to dampening of inflammation in the gut. In addition, this bacterium produces a molecule, MAM, which exhibits direct anti-inflammatory effects in intestinal epithelial cells. This increased abundance of a demonstrated anti-inflammatory molecule in the gut may also disseminate to metabolic tissues. This has the potential to lower metabolic tissue inflammation, improve insulin action and promote better glucose control.

early innate immune responses via ILCs (innate lymphoid cells), a population of lymphoid cells that do not display antigen-specific responses. ILCs can be grouped based on their similarity to T cell population cytokine profiles. Group 3 ILCs resemble T_H17 and T_H22 cells. Within this group, LTi (lymphoid tissue inducer) cells promote the development of lymphoid tissues, whereas ILC3 cells are mainly found in the mucosal sites of the gut. ILC3s respond to stimulation from epithelial factors, which helps to craft both pro-inflammatory and regulatory signals from different components of the microbiota. In conventional animals, ILC3s are responsible for high-level production of IL-22-stimulated host defence [28]. Thus there is a strong interrelationship between the composition of the intestinal microbiota and the function of the mucosal immune system. We highlight the emerging link between microbiota and metabolic diseases, including those shaped by gut barrier function and T cell-mediated responses. These gut immune responses may also represent participation of ILCs.

THE MICROBIOTA AND METABOLISM

Although it is clear that microbes play a critical role in the development of host immunity, bacteria are not required for host survival, and a germ-free state can promote a longer lifespan, reduced inflammatory responses in metabolic tissue and resistance to DIO [29,30]. This was an early indication that the bacterial composition of the host could alter host metabolism. Following colonization with microbiota from a conventionally raised mouse, germ-free animals increase their adiposity by ~40–60 % despite decreased food intake and an increase in energy expenditure [30]. Hence microbiota alter energy harvest capacity. This goes beyond conventionalization of germ-free mice, since, compared with lean counterparts, the microbiota from obese humans can be transferred to mice and promotes increased energy extraction, nutrient absorption and increased adiposity, independently of donor or recipient host genetics [31].

Metabolic endotoxaemia involves a modest increase in lipopolysaccharide (endotoxin) in the circulation and metabolic tissues that coincides with the low-grade inflammation during obesity. This is generally associated with increased intestinal permeability following acute or chronic HFD feeding or genetic obesity models, which generate an intestinal environment that permits increased translocation of microbiota-derived endotoxin from the intestinal lumen into the host [32]. Although originally described solely with respect to circulating LPS (lipopolysaccharide), a number of studies have demonstrated that animals with defective detection of other microbial innate immune system

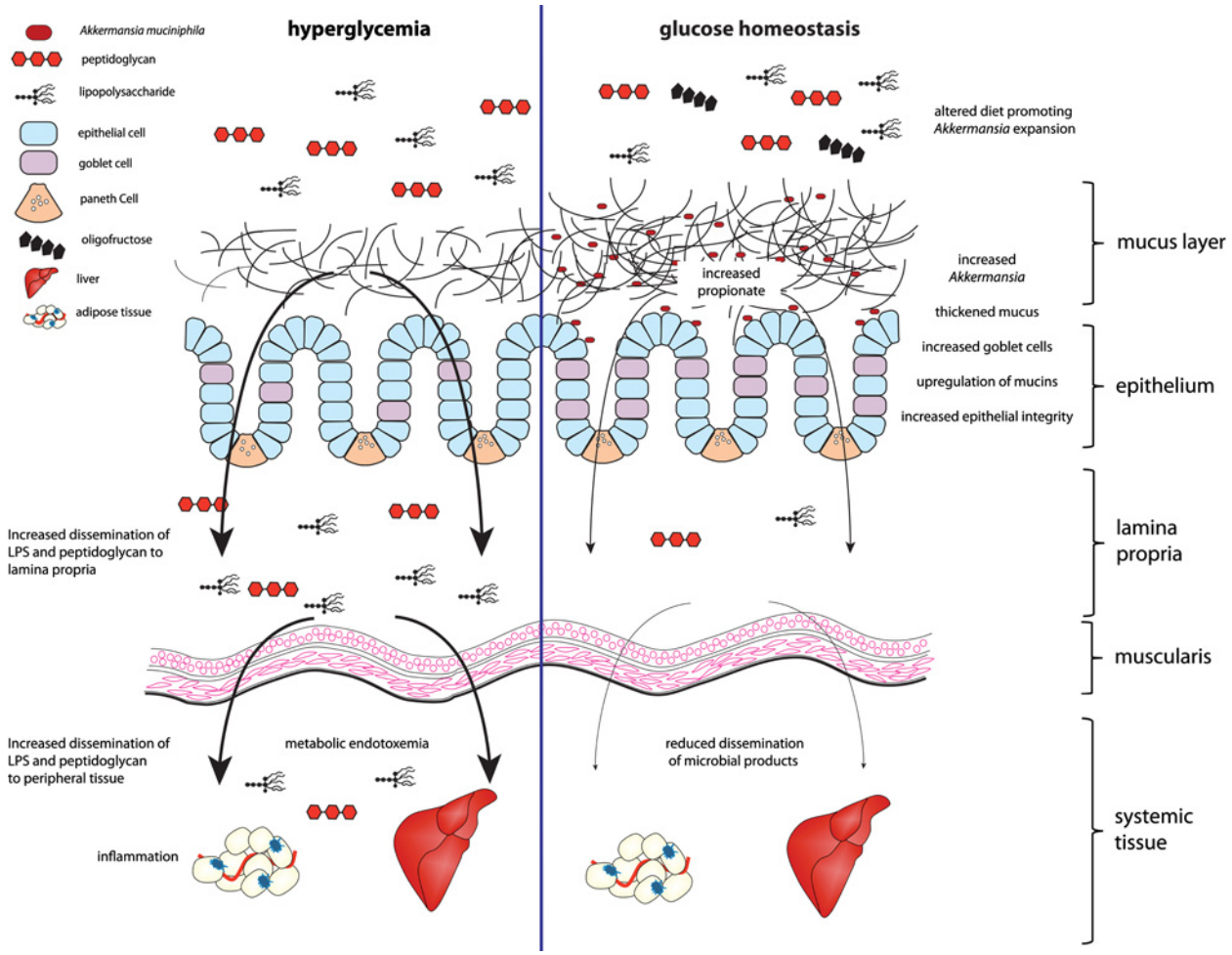

Figure 3 **A number of dietary interventions such as oligofructose can promote the growth of *Akkermansia muciniphila***

A. muciniphila signals to intestinal stem cells, leading to increased production of mucin-secreting goblet cells and the up-regulation of host mucin. These mucins, as well as increasing epithelial cell integrity, increase the barrier function of the intestinal epithelium, leading to reduced dissemination of microbial products to the lamina propria and to distal metabolic tissues. This reduction in metabolic endotoxaemia leads to lower metabolic tissue inflammation, improved insulin action and better glucose control.

agonists have altered host metabolic status [33–36], suggesting that other microbe-derived factors can alter host status in metabolic tissue.

An important avenue of study involves characterizing the constituents of the intestinal microbiome, which are affected by host genotype, diet and other environmental factors, and understanding how they play a role in host immunometabolism. Various models of obesity have shown an increased proportion of Firmicutes and a reduction in Bacteroidetes [37,38]. This phylum-level change is not always observed, but reduced microbial diversity as well as a reduction in predicted metabolic (metagenomic) capacity based on a lower number of unique genes during obesity appears to be important [39,40]. From this type of study, a number of bacterial genera have been associated with a leaner phenotype and reduced susceptibility of insulin resistance including *Faecalibacterium*, *Bifidobacterium*, *Lactobacillus* and *Akkermansia*. This work has been extended to the species level. Some of the most interesting association of microbiota changes during obesity and weight loss have also been associated with the remarkable weight loss and glucose-lowering effects of surgical gastric bypass [41,42]. Microbiota changes associated with RYGB (Roux-en-Y gastric bypass) can be observed as soon as 3 months after surgery, and, interestingly, increases in *Faecalibacterium prausnitzii* were negatively associated with markers of systemic inflammation such as CRP (C-reactive protein) and IL-6 [43]. *Escherichia* spp. and *Akkermansia* were enriched 1 week after a mouse model of RYGB and accompanied reductions in adiposity, liver triacylglycerols and blood insulin levels [44]. Furthermore, transfer of the intestinal microbiota from RYGB animals to germ-free animals was sufficient to transfer the resistance to DIO to the recipient animals.

Diet is a major contributor to obesity, which is the largest risk factor for T2D. The microbiota can be rapidly altered due to changes in diet with associated effects on host metabolism. In response to a 5-day diet swap, rapid changes in the composition of the faecal microbiome occurred in humans who were fed on

diets consisting of entirely vegetarian or entirely animal-derived nutrients [45]. The vegetarian diet that contains higher fibre and lower protein correlated with a rapid increase in saccharolytic bacteria and sugar-derived short-chain fatty acids (acetate and butyrate). The animal-based diet (higher in fat and protein) was associated with increases in bile salts, increases in amino-acid-derived metabolites, as well as up-regulation of bacterial genes involved in protein metabolism and aromatic detoxification. Although rapid changes in the relative abundance of specific bacterial operational taxonomic units were observed, no significant changes in α-diversity were seen during short-term dietary shifts. It remains unclear whether this maintenance of α-diversity would occur with longer-term dietary changes, as has been suggested on the basis of dietary questionnaires [46].

In general, this work demonstrates that diet is a key driver of changes in the microbiome and that the 'obese microbiome' promotes obesity, but it is also now appreciated that changes in the microbiome due to external stimuli (in addition to food, such as antibiotics) can alter metabolism. Recently, seminal papers demonstrated that early life treatment of conventionally raised animals with low doses of antibiotics has profound and lifelong consequences for host metabolic function [47,48]. Mice that were exposed to LDP (low doses of penicillin) during an early-life developmental window (at birth or weaning in mice), followed by exposure to an HFD were characterized by having reduced levels of ileal T_H1 and T_H17 cells with concomitant reduction of IFNγ (interferon γ), IL-17 and the genes for the antimicrobial molecules β-defensin 1 and RegIIIγ. This antibiotic/microbiota-mediated change in intestinal barrier function and host immunometabolism resulted in increased adiposity and hepatic lipogenesis. LDP treatment had lasting effects including reductions of SFB (segmented filamentous bacteria) and *Lactobacillus* species. Altered development of the gut immune system is one way that early-life LDP could have lasting effects on the microbiota and metabolism. Strikingly, this effect is transmissible, and the microbiota of LDP-treated mice is sufficient to supress intestinal T_H17 responses and promote increased adiposity. Similarly, Hong et al. [49] reported that during an HFD, the number of T_H17 cells found in the small intestine were decreased relative to controls. This is consistent with results showing that an HFD suppressed IL-17 in ileal tissue and IL-22 in colonic tissue compared with chow-fed controls (W. Khan and J. Schertzer, unpublished work and [50]). Furthermore, an HFD decreases antigen-presenting cell T_H17 differentiation. Thus an HFD and even microbiota during obesity supresses T_H17 effectors in the lamina propria of the small intestine. This is important because the absence of IL-17/RORγ (retinoic acid receptor-related orphan receptor γ)-expressing $CD4^+$ T cells is sufficient to promote obesity, infiltration of microbial components in metabolic tissues and metabolic dysfunction [50]. Although these studies provide some evidence of selective intestinal immunosuppression due to HFD/antibiotic treatment in mice, other excellent studies have documented that obesity is associated with an increased intestinal inflammation and elevated levels of intestinal T_H1 and T_H17 cells and reduced T_{reg} levels following 12–16 weeks of HFD in mice [51–53]. In fact, oral delivery of anti-inflammatory drugs such as 5-ASA (5-aminosalicylic acid) was sufficient to reduce intestinal inflammation, reduce metabolic endotoxaemia and improve metabolic parameters such as glucose tolerance without any appreciable change in adiposity [51]. Consistent with these results, the numbers of T_H1 cells correlated positively and T_{reg} cells correlated negatively with BMI (body mass index) in surgical explants of human patients [51]. There appears to be some conflicting evidence regarding the association of obesity and intestinal inflammation. Different environmental microbiota is a possible explanation for these discrepancies. Also, some experiments have focused on specific types of cells within the gut, but the contribution to overall gut tissue segment cytokine levels from various cell populations such as ILCs and T cells should be considered. Furthermore, a recent study has documented phenotypic switching of T_H17 cells to form T_{reg} cells in a number of models of T_H17-mediated pathogenesis [54]. It is tempting to speculate that the discrepancies in published reports of intestinal immune status during HFD feeding are due to diet and microbiota (or other unknown factors) interacting to generate divergent T_H17 outcomes. This warrants further investigation. Although the specific status of various immune cell populations in the intestine of HFD-fed animals remains unclear, immune receptors for flagellin [TLR5 (Toll-like receptor 5)], lipoproteins (TLR2) and peptidoglycan [NOD1/2 (nucleotide-binding and oligomerization domain 1/2)] have been shown to link obesity-related dysbiosis and metabolic inflammation [35,55–57]. DIO promotes a reduction in gut barrier function, with concomitant dissemination of bacterial products such as LPS [32]. More work needs to be done to determine which bacterial components penetrate into the circulation and metabolic tissues during obesity. This is important because these bacterial factors can have direct effects on metabolic cells that are a source of metabolic inflammation in tissues that control blood glucose [58,59]. These studies provide clear and compelling evidence that alterations to the microbiome can have lasting metabolic effects on the host and have revealed some of the potential immune responses involved.

BACTERIAL SPECIES MODIFY HOST IMMUNOMETABOLISM RELEVANT TO DIABETES

Understanding the host–microbe relationship in the gut and how it alters chronic disease risk are key challenges. This is a rapidly changing field, and a number of studies have demonstrated that specific taxonomic changes in the microbiota are associated with host risk for atherosclerosis, colorectal cancer and the regulation of neurotransmission between the gut and the brain [60–62]. Similarly, it is enticing to hypothesize that specific communities or species of the microbiota can modify hormonal (i.e. insulin) action and thereby modify the risk of host diabetic risk. There is some evidence that the intestinal microbial composition is a factor in the immunometabolic underpinnings of diseases such as T2D, but several key problems remain unresolved. How are changes in the gut communicated to the key metabolic tissues controlling blood glucose? Are there obesity/adiposity-independent factors that can alter glucose metabolism? What are the physiological and immunological underpinnings that regulate communication

between the intestinal environment and systemic sites? Although this field is still in its infancy, we highlight some of the best-characterized pathways in which specific microbes shape immune responses that link to metabolism since these provide interesting opportunities for prebiotic/probiotic strategies to improve glucose control and possibly the use of narrow-spectrum antibiotics that could improve, or at least not promote, metabolic disease.

Segmented filamentous bacteria ('*Candidatus* Savagella'): T_H17/T_H22 responses

SFB were first described as a constituent of the mammalian gut over 100 years ago. Light and electron microscopy have demonstrated that these bacteria have long segmented filaments that form an intimate attachment with cells of the ileal epithelium [63,64]. The presence of this type of bacteria was associated with altered resistance to infectious *Salmonella* [65], as well as with specific stimulation of the intestinal intraepithelial lymphocyte mucosal immune response [66]. Mice that are monocolonized with SFB have permitted genomic analysis of several SFB strains [67,68]. These bacteria have been termed '*Candidatus* Arthromitus' in some publications, but the name was later changed to '*Candidatus* Savagella' [69]. Recently, the co-culture of SFB with immortalized epithelial cells has been described thereby permitting *in vitro* analysis of SFB physiology for the first time [70].

SFB have frequently been associated with the hypothesis that altered microbiota can affect immunity. This is based on the observation that genetically similar (sometimes identical) mice treated with antibiotics or sourced from different suppliers or even with different penetrance of SFB within animal facilities can have different susceptibility to infectious disease and (Type 1) diabetes [4,71–74]. This altered susceptibility was associated with the presence or absence of SFB influencing intestinal T_H17 responses [71]. Ivanov et al. [75] demonstrated that SFB were required to develop IL-17- and IL-22-producing small intestinal lamina propria T_H17 cells, and that, in their absence, there was an enrichment of T_{reg} cells. This development was dependent on intact TGFβ (transforming growth factor β) signalling, and germ-free animals or those that were treated with vancomycin, ampicillin or neomycin/metronidazole had a significant reduction in the development of lamina propria T_H17 cells. The ability to mount a strong innate immune response to the murine enteropathogen *Citrobacter rodentium* requires a robust IL-22-mediated response [76], and SFB were both necessary and sufficient for T_H17 lamina propria cells development and increased resistance to *C. rodentium* [71]. The link between SFB and gut T_H17 responses in humans is not as clear, which may reflect the amount, prevalence or importance of SFB colonization in different mammals at different times. Molecular analysis has shown that mice, chickens and humans contain genomic markers of SFB [77]. In humans, SFB markers generally disappeared below detection levels after age 3. As outlined by others [77,78], we should consider how the human diet, use of antibiotics and environmental factors shape SFB and gut immunity.

Although the data describing how SFB affect innate immune function are increasingly well-established, the role of SFB in obesity remains less well-understood. Some have found no association between SFB colonization and obesity [79]. In contrast, mice that lacked LTβR (lymphotoxin-β receptor), LTα (lymphotoxin α), IL-23a or RORγ were protected from DIO [80]. In both LTβR- and RORγ-deficient animals, this protection was associated with overgrowth of SFB in mice fed on an HFD. Exogenous delivery of IL-22 or of IL-23 was sufficient to reduce this SFB-mediated overgrowth, and delivery of IL-22 was sufficient to increase body mass gained when mice were fed on an HFD. Interestingly, when LT$\beta R^{+/-}$ heterozygous and LT$\beta R^{-/-}$ homozygous littermates were co-housed, the heterozygous animals were protected from DIO due to coprophagous continued exposure to the SFB from the homozygous animals.

SFB regulation of a pro-inflammatory T_H17 response in the intestine is positioned as a factor at the crossroads of susceptibility to obesity, but there is still much to solve. Dysbiosis and immune insults during developmental windows should be carefully considered, given the association of increased obesity with decreased intestinal T_H17 due to early-life antibiotics [47,48]. This is interesting because markers of SFB are higher in the first 2 years of life in humans [75]. Furthermore, it was shown recently that injecting mice with the T_H17/T_H22 effector IL-22 was sufficient to reduce both diet- and hyperphagy-induced mouse obesity and the related dysglycaemia, an effect that was not transmissible in co-housing experiments [27]. Importantly, intraperitoneal injection of IL-22 is likely to be very different to gut luminal SFB regulation of intestinal T_H17 responses. Nevertheless, injection of IL-22 lowered metabolic endotoxaemia during obesity, and the insulin-sensitizing effects of this cytokine may still involve improved gut barrier function in addition to direct IL-22R1 (IL-22 receptor 1)-mediated signalling that could increase lipid oxidation in metabolic cells. These results using injection of IL-22 do not negate the potential role of SFB in obesity, but highlight the importance of site-specific (compartmentalized) inflammation in the gut compared with metabolic tissues. For example, decreased T_H17 responses in the gut, like that observed during low-dose antibiotic treatment [47,48], may modify obesity-modifying microbiota, but this may be in stark contrast with increased T_H17 within metabolic tissues or the spleen, which can promote insulin resistance during obesity. Segregating the gut immune responses from those in metabolic tissues appears to be important. Indeed, a common theme that is emerging is that the immunological underpinnings that promote enhanced intestinal barrier function can reduce metabolic inflammation and improve glucose homoeostasis by limiting dissemination of microbiota-derived factors (such as those contributing to metabolic endotoxaemia). Gut immunity is likely to be more tolerized to a given bacterial trigger compared with metabolic tissues. An illustration of the role of SFB in intestinal and systemic inflammation is shown in Figure 1.

Faecalibacterium prausnitzii: T_H2 cell and T_{reg} responses

At sites with high levels of commensal colonization, the specific T cell repertoire contributes to the balance between pro- and anti-inflammatory environments and prevents an inappropriate response to microbes that colonize a niche without posing any particular danger. Thus a healthy gut is characterized by an adequately tolerogenic environment, characterized by the presence of T_H2 cells and/or T_{reg} cells producing factors such as IL-4 and

IL-10. Germ-free mice have reduced levels of T_{reg} cells and even those T_{reg} cells that are present exhibit defective immunosuppressive capacity [81]. A number of bacterial species have been associated with intestinal T_{reg} levels [82,83], indicating that there is functional redundancy in the pathways leading to T_{reg} development, perhaps underlining the importance of this immune axis. Nevertheless, much less is known about how anti-inflammatory or immune-resolving factors in the gut are related to obesity and diabetes. This is contrasted by solid evidence for T_{reg} and T_H2 cell responses co-operating with macrophage polarization in liver and adipose tissue, thereby mitigating risk of obesity-induced insulin resistance and diabetes [84,85]. Again, a key problem to solve in this paradigm of microbes that can alter metabolic outcomes is understanding compartmentalization and the route of immune responses driven by changes in the gut.

F. prausnitzii was identified as a species that was specifically reduced in inflamed sections of Crohn's disease patients undergoing ileal resection [86]. A lower proportion of *F. prausnitzii* in the gut was associated with increased risk of relapse. Other groups have confirmed this association between decreased *F. prausnitzii* and increased gut inflammation [87]. *In vitro* studies demonstrated that a secreted factor of *F. prausnitzii* was able to reduce the pro-inflammatory cytokine IL-8 from monocytes as well as inhibiting pro-inflammatory signalling via NF-κB (nuclear factor κB) activation in a reporter cell line. This inhibition was associated with stimulation of IL-10 production, and the bacterium was shown to reduce inflammation in a chemically induced model of colitis [88]. This may be relevant to metabolism since SNPs (single nucleotide polymorphisms) in the *IL10* gene are known risk factors for T2D [89–91]. It is not clear how IL-10 levels in the intestine would relate to those in metabolic tissues, but rescue of intestinal inflammation and colitis pathology could also be accomplished via intraperitoneal delivery of the *F. prausnitzii* supernatant to the animals. This suggests that factors from *F. prausnitzii* could be anti-inflammatory independently of actions on gut-resident immune cells. Recent work has also identified an *F. prausnitzii* protein, MAM (microbial anti-inflammatory molecule), that is capable of suppressing inflammation in intestinal epithelial cells [92]. These observations are intriguing in the light of the relationship between metabolic inflammation, T2D and *F. prausnitzii*, and studies identifying potential mediators such as short-chain fatty acids and cytokines/chemokines are warranted.

A metagenomic analysis of T2D patients showed that two species, *F. prausnitzii* and *Roseburia intestinalis*, were significantly associated with protection from T2D in a European cohort, and a Chinese study found that the same genera, as well as other butyrate-producing Clostridial species, were depleted in the T2D cohort [93,94]. Intriguingly, in faecal microbiota-transfer studies from healthy to insulin-resistant individuals, the authors observed increased levels of the butyrate producer *R. intestinalis* and that this was associated with improved peripheral insulin sensitivity [95]. *F. prausnitzii* was also identified as one of the discriminating bacterial genera that differentiate between first- and third-trimester microbiomes in women with gestational insulin resistance [96]. This association of *F. prausnitzii*-mediated butyrate production and protection from metabolic disease provides interesting insights and potential therapeutic interventions for future development [97]. Although it remains unclear what the mechanism is by which *F. prausnitzii* and other butyrate-producing bacteria elicit a potential diabetes protective effect, it is enticing to speculate that certain microbes such as *F. prausnitzii* generate factors that can penetrate the gut barrier to counterbalance metabolic endotoxaemia. Furthermore, it is possible that certain microbe-derived anti-inflammatory responses represent a marker of a robust (less permissive) intestinal inflammatory environment and are more relevant to promoting intestinal barrier function or influence host metabolism through second messengers such as short-chain fatty acids. A model for the role of *F. prausnitzii* in host susceptibility to T2D is shown in Figure 2.

Akkermansia muciniphila: intestinal barrier function

Goblet cells are largely responsible for the production and maintenance of a protective mucin layer between the epithelial surface and the lumen of the gastrointestinal tract. Mucin is composed of a complex series of hydrated highly glycosylated proteins, and there is a reciprocal relationship between mucin production/composition and the microbiota [98]. An anaerobic Gram-negative bacterial species named *Akkermansia muciniphila* was isolated from human faeces and it can utilize intestinal mucins as a sole carbon and nitrogen source [99]. This species can adhere to intestinal epithelial cells as well as host matrix proteins [100]. This organism constitutes ~1–5% of the total intestinal human microbiome, and genome analysis suggests that a large proportion of secreted proteins are proteins predicted to be important for mucin degradation [101].

Several studies have linked genus level changes in *Akkermansia* to metabolism through relative changes based on 16S sequencing. In animal studies, DIO resulted in a significant reduction in the proportion of *Akkermansia* found in the faecal microbiota, demonstrating an association between host metabolic status and the microbiome [102,103]. Similarly, *Akkermansia* prevalence inversely correlated with obesity in a small study of 4–5-year-old children [104]. Changes in *Akkermansia* do not necessarily depend on the fat content of the diet or in the intestine since hyperphagic genetically obese leptin-deficient mice also have a reduction in the amount of *Akkermansia* found in faeces [103,105]. Further, *Akkermansia* has also been linked to ingestion of metformin, one of the most widely used anti-diabetic drugs. Treatment of DIO mice with metformin altered the type and number of ileal Goblet cells and, strangely, did not result in the expected improvement in insulin sensitivity when the mice were also treated with broad-spectrum antibiotics [102]. It was then found that metformin was associated with significant increases in the levels of *Akkermansia* found in the faeces of these animals compared with DIO animals. Delivery of live *Akkermansia* to DIO mice improved glucose tolerance and reduced adipose tissue inflammation evinced by decreased IL-6 and IL-1β and increased T_{reg} cells in the stromal vascular fraction of adipose tissue. These effects required live *Akkermansia*, and understanding how immune responses in the gut epithelium are communicated to T_{reg} responses in the adipose tissue resulting in improved glucose control are important future directions.

Feeding mice with a sugar-alternative sweetener and recognized prebiotic supplement, oligofructose, also promotes

expansion of *Akkermansia* and improves metabolic parameters associated with DIO or genetically obese mice [103,106]. This seminal work showed a relationship between *Akkermansia* and improved gut barrier function, suggesting limiting the entry of an abundant constituent of the microbiota (i.e. LPS) that can promote insulin resistance. Importantly, it has been shown that delivery of live *A. muciniphila* reduced the (chronic low level) rise in serum endotoxin levels associated with obesity (i.e. metabolic endotoxaemia) and was also associated with reduced adiposity and macrophage infiltration of adipose tissue and in DIO mice [103]. Similarly, feeding DIO animals with a polyphenol-rich cranberry juice extract significantly increased the proportion of *Akkermansia* in their guts and resulted in reduced adiposity, better glucose homoeostasis and reduced insulin resistance [107]. Importantly, delivery of live (but not heat-killed) *Akkermansia* to DIO animals was sufficient to decrease adiposity, serum endotoxin levels and insulin resistance [103]. *Akkermansia* also reduced adiposity in hyperphagic genetically obese mice and it will be important to understand energy balance during obesity, particularly since *Akkermansia* was associated with higher expression of PPARα (peroxisome-proliferator-activated receptor α) and other markers of lipid oxidation in adipose tissue.

Transcriptional analysis of *Akkermansia* monocolonized animals demonstrated that the presence of this organism altered transcription regulation of host T- and B-cell maturation and the activation of PPARα [108]. The latter gene is particularly interesting, because PPAR$\alpha^{-/-}$ animals exhibit increased adiposity, increased markers of inflammation and increased hepatic triacylglycerols [109]. Conversely, treatment of mice with agonists of PPARα has been demonstrated to attenuate DIO-mediated hyperinsulinaemia, hyperglycaemia and increased adiposity [110]. *In vitro* grown ileal organoids have been used to probe *Akkermansia* metabolite–epithelial cell interactions and demonstrated that a number of host genes were highly modulated by this exposure [111]. These included down-regulation of GPR43 (G-protein-coupled receptor 43) and PPARγ, two proteins with a role in regulation of host adiposity in response to the microbiota [112,113]. Similar regulatory effects could also be observed by exposing ileal organoids to short-chain fatty acids that match those produced by *Akkermansia*, suggesting that the production of specific types and amounts of short-chain fatty acids combined with spatial proximity to the host epithelium may be a crucial factor in the ability of a particular bacterium to modulate host metabolism. It is noteworthy that dysbiosis is also involved in diabetes development in non-obese diabetic mice, a model of Type 1 diabetes [73,114,115]. The Gram-positive-targeting antibiotic vancomycin significantly expanded the levels of *Akkermansia* and increased intestinal CD4$^+$ T cells secreting IFNγ and TNFα (tumour necrosis factor α). Intriguingly, vancomycin-treated neonatal mice developed diabetes at a lower rate, whereas vancomycin treatment of adult mice had a less profound effect, only lowering blood glucose [74]. These results suggest that expansion of *Akkermansia* was protective in this autoimmunity model. Comparable studies in obesity and T2D are warranted, particularly those that compare the type of antibiotics and resultant changes in gut immunity relevant to obesity-promoting dysbiosis observed within the early-life period [48].

These are important results, but a poorly understood concept is how *Akkermansia* regulates intestinal barrier function, and whether this alteration reduces energy extraction through absorption or digestibility of specific nutrients, since increased amounts of this species and these prebiotic strategies reduced adiposity and body mass. Recent results suggest that the effect is mediated through direct interaction with intestinal epithelial cells rather than the mucous layer, but the precise mechanism remains poorly understood [100]. There is some anecdotal evidence that *Akkermansia* LPS may be less pro-inflammatory than that of *Escherichia coli* [100]. This leads to the hypothesis that alterations in systemic inflammation may be due to changes in the type, rather than the amount of LPS found systemically. Since the original (and all subsequent) discovery of metabolic endotoxaemia was based on biological measurements (as opposed to biochemical ones) of LPS activity, it is possible that modifying the type of LPS present at the epithelial surface, may lead to reductions in systemic inflammatory responses. Understanding these host–bacterial interactions are critical if we are to develop other strategies to modulate the host intestinal barrier or metabolic endotoxaemia for the treatment of metabolic diseases. A model for the role of *A. muciniphila* in host metabolism is shown in Figure 3.

CONCLUSIONS

A paradigm is emerging whereby the connection between microbes and immunity influences host metabolism. This may provide opportunities to alter host metabolism by active management of the intestinal microbiome. Transfer of faecal microbiota has been done as treatment strategy for recalcitrant *C. difficile* infections with profound effects on host disease [116]. Although it is clear that obesity or HFD are associated with a pro-inflammatory state in the metabolic tissues, there is emerging evidence that these treatments have divergent effects in the gut. This suggests that there may be pre- or pro-biotic strategies to modify and ameliorate the divergent intestinal immune response to HFD/obesity, including engineering bacteria to modify or secrete a specific (immune) factor, consumption of supplements that promote or inhibit specific bacterial taxa, or utilization of very-narrow-spectrum antimicrobial molecules that can alter the microbial community in a desired manner. Indeed, a small study has already demonstrated that faecal microbiota transfer (by infusion into the intestine) from lean donors can also result in short-term improvements in peripheral insulin sensitivity in obese patients [95].

Although it is clear that specific bacteria can induce either pro-inflammatory or regulatory immune responses, it is important to note that, with respect to intestinal function, either response can increase or decrease intestinal barrier function, depending on the specific composition of the intestinal microbiome or the diet of the individual. Generally, during homoeostasis (that is, in the absence of acute inflammation) an increased T_H17/T_H22 response leads to increased barrier function, increased production of host-defence peptides and mucin, and generally better

metabolic parameters due to reduced dissemination of microbial/diet-derived inflammatory molecules. Conversely, having a highly tolerogenic intestinal tract, which has a tendency to reduce intestinal barrier function, could lead to higher levels of metabolic inflammation, due to the increased dissemination of microbial molecules from the gut to metabolically active tissues, such as adipose tissue, muscle and liver. Therefore the immune response characteristics in the gut compared with those in the metabolic tissues are not necessarily the same in response to dietary- or obesity-related stimuli (Figure 1). This relationship is complicated by the recent description of transdifferentiation of inflammatory T_H17 cells to T_{reg}s during both steady-state and acute infection [54]. Despite these complications, we believe that by understanding the relationship among the intestinal microbiota, intestinal immune responses and systemic immune responses will reveal new avenues towards the treatment of metabolic disorders.

It appears likely that many bacteria are capable of affecting host function in a similar manner and that, although there may be genetic heterogeneity in the microbes that induce a specific response, there is also great functional redundancy. A number of classical and forward genetic approaches could be applied to this problem, and we see great potential in using these approaches for determining how the microbiome affects host metabolism. Recent papers have described an elegant solution to the problem of identifying bacterial strains that produce a given effect in germ-free mice [82,117]. Systematic refinement of colonization and phenotyping using subsets of the microbiota may prove very useful in cataloguing many effector strains from a given population or to identify rarer species that give rise to more specific responses.

Microbes could also be used as vehicles to alter metabolism. For example, a *Lactobacillus* strain engineered to secrete leptin reduced food intake and obesity in mice that lacked functional leptin [118]. Similarly, using leptin-secreting *Lactococcus lactis* as a probiotic improved glucose tolerance in diet-induced obese mice [119]. This shows that engineered bacteria in the gut can deliver a therapeutic factor that is normally produced in a metabolic tissue. It is highly likely that as our understanding of the microbial species (and ultimately microbial products) increases, we may uncover more specific molecules with potential for treatment of metabolic diseases. If we are to harness the potential of modulating the microbiota to improve metabolic diseases, the immune implications should be considered. This will be important to ascertain any unwanted immunological side effects, but also many of the beneficial metabolic outcomes may be derived from immune responses that relay microbe to host metabolic effects.

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REFERENCES

1 Abusleme, L., Dupuy, A.K., Dutzan, N., Silva, N., Burleson, J.A., Strausbaugh, L.D., Gamonal, J. and Diaz, P.I. (2013) The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J. **7**, 1016–1025 CrossRef PubMed
2 Alekseyenko, A.V., Perez-Perez, G.I., De Souza, A., Strober, B., Gao, Z., Bihan, M., Li, K., Methé, B.A. and Blaser, M.J. (2013) Community differentiation of the cutaneous microbiota in psoriasis. Microbiome **1**, 31 CrossRef PubMed
3 Ravel, J., Brotman, R.M., Gajer, P., Ma, B., Nandy, M., Fadrosh, D.W., Sakamoto, J., Koenig, S.S., Fu, L., Zhou, X. et al. (2013) Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. Microbiome **1**, 29 CrossRef PubMed
4 Sekirov, I., Tam, N.M., Jogova, M., Robertson, M.L., Li, Y., Lupp, C. and Finlay, B.B. (2008) Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. Infect. Immun. **76**, 4726–4736 CrossRef PubMed
5 Sartor, R.B. (2008) Microbial influences in inflammatory bowel diseases. Gastroenterology **134**, 577–594 CrossRef PubMed
6 Hotamisligil, G.S. (2005) Role of endoplasmic reticulum stress and c-Jun NH_2-terminal kinase pathways in inflammation and origin of obesity and diabetes. Diabetes **54**, S73–S78 CrossRef PubMed
7 Fullerton, M.D., Steinberg, G.R. and Schertzer, J.D. (2013) Immunometabolism of AMPK in insulin resistance and atherosclerosis. Mol. Cell. Endocrinol. **366**, 224–234 CrossRef PubMed
8 Steinberg, G.R. and Schertzer, J.D. (2014) AMPK promotes macrophage fatty acid oxidative metabolism to mitigate inflammation: implications for diabetes and cardiovascular disease. Immunol. Cell Biol. **92**, 340–345 CrossRef PubMed
9 Ehses, J.A., Lacraz, G., Giroix, M.-H., Schmidlin, F., Coulaud, J., Kassis, N., Irminger, J.-C., Kergoat, M., Portha, B., Homo-Delarche, F. et al. (2009) IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat. Proc. Natl. Acad. Sci. U.S.A. **106**, 13998–14003 CrossRef PubMed
10 Weisberg, S.P., Hunter, D., Huber, R., Lemieux, J., Slaymaker, S., Vaddi, K., Charo, I., Leibel, R.L. and Ferrante, A.W. (2006) CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J. Clin. Invest. **116**, 115–124 CrossRef PubMed
11 Winer, S., Paltser, S., Chan, G., Tsui, Y., Engleman, H., Winer, E., Dosch, D. and -M., H. (2009) Obesity predisposes to Th17 bias. Eur. J. Immunol. **39**, 2629–2635 CrossRef PubMed
12 McLaughlin, T., Liu, L.-F., Lamendola, C., Shen, L., Morton, J., Rivas, H., Winer, D., Tolentino, L., Choi, O., Zhang, H. et al. (2014) T cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. Arterioscler. Thromb. Vasc. Biol. **34**, 2637–2643 CrossRef PubMed
13 Fabbrini, E., Cella, M., McCartney, S.A., Fuchs, A., Abumrad, N.A., Pietka, T.A., Chen, Z., Finck, B.N., Han, D.H., Magkos, F. et al. (2013) Association between specific adipose tissue $CD4^+$ T cell populations and insulin resistance in obese individuals. Gastroenterology **145**, 366–374 CrossRef PubMed
14 Cipolletta, D. (2014) Adipose tissue-resident regulatory T cells: phenotypic specialization, functions and therapeutic potential. Immunology **142**, 517–525 CrossRef PubMed
15 Chawla, A., Nguyen, K.D. and Goh, Y.P.S. (2011) Macrophage-mediated inflammation in metabolic disease. Nat. Rev. Immunol. **11**, 738–749 CrossRef PubMed
16 Wernstedt Asterholm, I., Tao, C., Morley, T.S., Wang, Q.A., Delgado-Lopez, F., Wang, Z.V. and Scherer, P.E. (2014) Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. Cell Metab. **20**, 103–118 CrossRef PubMed

17 Round, J.L. and Mazmanian, S.K. (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. **9**, 313–323 CrossRef PubMed
18 Carter, P.B. and Collins, F.M. (1974) The route of enteric infection in normal mice. J. Exp. Med. **139**, 1189–1203 CrossRef PubMed
19 Collins, F.M. and Carter, P.B. (1978) Growth of salmonellae in orally infected germfree mice. Infect. Immun. **21**, 41–47 PubMed
20 Zachar, Z. and Savage, D.C. (1979) Microbial interference and colonization of the murine gastrointestinal tract by *Listeria monocytogenes*. Infect. Immun. **23**, 168–174 PubMed
21 Sonnenburg, J.L., Chen, C.T.L. and Gordon, J.I. (2006) Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol. **4**, 2213–2226 CrossRef
22 Clevers, H.C. and Bevins, C.L. (2013) Paneth cells: maestros of the small intestinal crypts. Annu. Rev. Physiol. **75**, 289–311 CrossRef PubMed
23 Pütsep, K., Axelsson, L.G., Boman, A., Midtvedt, T., Normark, S., Boman, H.G. and Andersson, M. (2000) Germ-free and colonized mice generate the same products from enteric prodefensins. J. Biol. Chem. **275**, 40478–40482 CrossRef PubMed
24 Cash, H.L., Whitham, C.V., Behrendt, C.L. and Hooper, L.V. (2006) Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science **313**, 1126–1130 CrossRef PubMed
25 Miki, T., Holsts, O. and Hardt, W.D. (2012) The bactericidal activity of the C-type lectin regIIIβ against gram-negative bacteria involves binding to lipid A. J. Biol. Chem. **287**, 34844–34855 CrossRef PubMed
26 Peterson, L.W. and Artis, D. (2014) Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat. Rev. Immunol. **14**, 141–153 CrossRef PubMed
27 Wang, X., Ota, N., Manzanillo, P., Kates, L., Zavala-Solorio, J., Eidenschenk, C., Zhang, J., Lesch, J., Lee, W.P., Ross, J. et al. (2014) Interleukin-22 alleviates metabolic disorders and restores mucosal immunity in diabetes. Nature **514**, 237–241 PubMed
28 Korn, L.L., Thomas, H.L., Hubbeling, H.G., Spencer, S.P., Sinha, R., Simkins, H.M.A., Salzman, N.H., Bushman, F.D. and Laufer, T.M. (2014) Conventional CD4$^+$ T cells regulate IL-22-producing intestinal innate lymphoid cells. Mucosal Immunol. **7**, 1045–1057 CrossRef PubMed
29 Gordon, H.A., Bruckner-Kardoss, E. and Wostmann, B.S. (1966) Aging in germ-free mice: life tables and lesions observed at natural death. J. Gerontol. **21**, 380–387 CrossRef PubMed
30 Bäckhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Semenkovich, C.F. and Gordon, J.I. (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. U.S.A. **101**, 15718–15723 CrossRef PubMed
31 Ridaura, V.K., Faith, J.J., Rey, F.E., Cheng, J., Duncan, A.E., Kau, A.L., Griffin, N.W., Lombard, V., Henrissat, B., Bain, J.R. et al. (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science **341**, 1241214 CrossRef PubMed
32 Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A.M., Fava, F., Tuohy, K.M., Chabo, C. et al. (2007) Metabolic endotoxemia initiated obesity and insulin resistance. Diabetes **56**, 1761–1772 CrossRef PubMed
33 Henao-Mejia, J., Elinav, E., Jin, C., Hao, L., Mehal, W.Z., Strowig, T., Thaiss, C.A., Kau, A.L., Eisenbarth, S.C., Jurczak, M.J. et al. (2012) Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature **482**, 179–185 PubMed
34 Vijay-Kumar, M., Aitken, J.D., Carvalho, F.A., Cullender, T.C., Mwangi, S., Srinivasan, S., Sitaraman, S.V., Knight, R., Ley, R.E. and Gewirtz, A.T. (2010) Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science **328**, 228–231 CrossRef PubMed
35 Denou, E., Lolmède, K., Garidou, L., Pomie, C., Chabo, C., Lau, T.C., Fullerton, M.D., Nigro, G., Zakaroff-Girard, A., Luche, E. et al. (2015) Defective NOD 2 peptidoglycan sensing promotes diet-induced inflammation, dysbiosis, and insulin resistance. EMBO Mol. Med. **7**, 259–274 CrossRef PubMed
36 Shi, H., Kokoeva, M.V., Inouye, K., Tzameli, I., Yin, H. and Flier, J.S. (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J. Clin. Invest. **116**, 3015–3025 CrossRef PubMed
37 Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R. and Gordon, J.I. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature **444**, 1027–1031 CrossRef PubMed
38 Ley, R.E., Turnbaugh, P.J., Klein, S. and Gordon, J.I. (2006) Microbial ecology: human gut microbes associated with obesity. Nature **444**, 1022–1023 CrossRef PubMed
39 Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.-M., Kennedy, S. et al. (2013) Richness of human gut microbiome correlates with metabolic markers. Nature **500**, 541–546 CrossRef PubMed
40 Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P. et al. (2009) A core gut microbiome in obese and lean twins. Nature **457**, 480–484 CrossRef PubMed
41 Corrodi, P., Wideman, P.A., Sutter, V.L., Drenick, E.J., Passaro, E. and Finegold, S.M. (1978) Bacterial flora of the small bowel before and after bypass procedure for morbid obesity. J. Infect. Dis. **137**, 1–6 CrossRef PubMed
42 Bjørneklett, A., Viddal, K.O., Midtvedt, T. and Nygaard, K. (1981) Intestinal and gastric bypass: changes in intestinal microecology after surgical treatment of morbid obesity in man. Scand. J. Gastroenterol. **16**, 681–687 CrossRef PubMed
43 Furet, J.P., Kong, L.C., Tap, J., Poitou, C., Basdevant, A., Bouillot, J.L., Mariat, D., Corthier, G., Doré, J., Henegar, C. et al. (2010) Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. Diabetes **59**, 3049–3057 CrossRef PubMed
44 Liou, A.P., Paziuk, M., Luevano, J.-M., Machineni, S., Turnbaugh, P.J. and Kaplan, L.M. (2013) Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci. Transl. Med. **5**, 178ra41 CrossRef PubMed
45 David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A. V, Devlin, A.S., Varma, Y., Fischbach, M.A. et al. (2014) Diet rapidly and reproducibly alters the human gut microbiome. Nature **505**, 559–563 CrossRef PubMed
46 Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R. et al. (2011) Linking long-term dietary patterns with gut microbial enterotypes. Science **334**, 105–108 CrossRef PubMed
47 Cho, I., Yamanishi, S., Cox, L., Methé, B.A., Zavadil, J., Li, K., Gao, Z., Mahana, D., Raju, K., Teitler, I. et al. (2012) Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature **488**, 621–626 CrossRef PubMed
48 Cox, L.M.M., Yamanishi, S., Sohn, J., Alekseyenko, A.V.V., Leung, J.M.M., Cho, I., Rogers, A.B.B., Kim, S.G.G., Li, H., Gao, Z. et al. (2014) Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell **158**, 705–721 CrossRef PubMed
49 Hong, C.P., Yang, B.G., Kim, J.-H., Jang, M.S., Lee, E.-J., Jeun, E.J., Kim, C., Seoh, J.-Y. and Jang, M.H. (2013) High fat diet-induced obesity affects CD4$^+$ T cell differentiation in the small intestine (P3176). J. Immunol. **190**, 61.13

50 Garidou, L., Pomié, C., Klopp, P., Waget, A., Charpentier, J., Aloulou, M., Giry, A., Serino, M., Stenman, L., Lahtinen, S. et al. (2015) The gut microbiota regulates intestinal CD4 T cells expressing RORγt and controls metabolic disease. Cell Metab **22**, 100–112 CrossRef PubMed

51 Luck, H., Tsai, S., Chung, J., Clemente-Casares, X., Ghazarian, M., Revelo, X.S., Lei, H., Luk, C.T., Shi, S.Y., Surendra, A. et al. (2015) Regulation of obesity-related insulin resistance with gut anti-inflammatory agents. Cell Metab. **21**, 527–542 CrossRef PubMed

52 Monk, J.M., Hou, T.Y., Turk, H.F., Weeks, B., Wu, C., McMurray, D.N. and Chapkin, F.S. (2012) Dietary n-3 polyunsaturated fatty acids (PUFA) decrease obesity-associated Th17 cell-mediated inflammation during colitis. PLoS One. **7**, e49739 CrossRef PubMed

53 Kim, K.A., Gu, W., Lee, I.A., Joh, E.H. and Kim, D.H. (2012) High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. PLoS One. **7**, e47713 CrossRef PubMed

54 Gagliani, N., Vesely, M.C.A., Iseppon, A., Brockmann, L., Xu, H., Palm, N.W., de Zoete, M.R., Licona-Limón, P., Paiva, R.S., Ching, T. et al. (2015) Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. Nature **523**, 221–225 CrossRef PubMed

55 Vijay-Kumar, M., Aitken, J.J.D., Carvalho, F.A.F.F.A., Cullender, T.C., Mwangi, S., Srinivasan, S., Sitaraman, S.V., Knight, R., Ley, R.E. and Gewirtz, A.T. (2010) Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science **328**, 228–231 CrossRef PubMed

56 Caricilli, A.M., Picardi, P.K., de Abreu, L.L., Ueno, M., Prada, P.O., Ropelle, E.R., Hirabara, S.M., Castoldi, Â., Vieira, P., Camara, N.O.S. et al. (2011) Gut microbiota is a key modulator of insulin resistance in TLR 2 knockout mice. PLoS Biol **9**, e1001212 CrossRef PubMed

57 Chassaing, B., Ley, R.E. and Gewirtz, A.T. (2014) Intestinal epithelial cell Toll-like receptor 5 regulates the intestinal microbiota to prevent low-grade inflammation and metabolic syndrome in mice. Gastroenterology **147**, 1363–1377 CrossRef PubMed

58 Pekkala, S., Munukka, E., Kong, L., Pöllänen, E., Autio, R., Roos, C., Wiklund, P., Fischer-Posovszky, P., Wabitsch, M., Alen, M. et al. (2015) Toll-like receptor 5 in obesity: the role of gut microbiota and adipose tissue inflammation. Obesity **23**, 581–590 CrossRef PubMed

59 Chi, W., Dao, D., Lau, T.C., Henriksbo, B.D., Cavallari, J.F., Foley, K.P. and Schertzer, J.D. (2014) Bacterial peptidoglycan stimulates adipocyte lipolysis via NOD1. PLoS One **9**, e97675 CrossRef PubMed

60 Koeth, R.A., Wang, Z., Levison, B.S., Buffa, J.A., Org, E., Sheehy, B.T., Britt, E.B., Fu, X., Wu, Y., Li, L. et al. (2013) Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat. Med. **19**, 576–585 CrossRef PubMed

61 Cuevas-Ramos, G., Petit, G., Marcq, C.R., Boury, I., Oswald, M., Nougayrède, E. and -P., J. (2010) *Escherichia coli* induces DNA damage *in vivo* and triggers genomic instability in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. **107**, 11537–11542 CrossRef PubMed

62 Bercik, P., Verdu, E.F., Foster, J.A., MacRi, J., Potter, M., Huang, X., Malinowski, P., Jackson, W., Blennerhassett, P., Neufeld, K.A. et al. (2010) Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology **139**, 2102–2112 CrossRef PubMed

63 Reimann, H.A. (1965) Microbic phagocytosis by enteric epithelial cells. JAMA **192**, 1130–1132 CrossRef PubMed

64 Davis, C.P. and Savage, D.C. (1974) Habitat, succession, attachment, and morphology of segmented, filamentous microbes indigenous to the murine gastrointestinal tract. Infect. Immun. **10**, 948–956 PubMed

65 Garland, C.D., Lee, A. and Dickson, M.R. (1982) Segmented filamentous bacteria in the rodent small intestine: their colonization of growing animals and possible role in host resistance to *Salmonella*. Microb. Ecol. **8**, 181–190 CrossRef PubMed

66 Umesaki, Y., Setoyama, H., Matsumoto, S., Imaoka, A. and Itoh, K. (1999) Differential roles of segmented filamentous bacteria and clostridia in development of the intestinal immune system. Infect. Immun. **67**, 3504–3511 PubMed

67 Sczesnak, A., Segata, N., Qin, X., Gevers, D., Petrosino, J.F.F., Huttenhower, C., Littman, D.R.R. and Ivanov, I.I.I. (2011) The genome of Th17 cell-inducing segmented filamentous bacteria reveals extensive auxotrophy and adaptations to the intestinal environment. Cell Host Microbe **10**, 260–272 CrossRef PubMed

68 Prakash, T., Oshima, K., Morita, H., Fukuda, S., Imaoka, A., Kumar, N., Sharma, V.K., Kim, S.-W., Takahashi, M., Saitou, N. et al. (2011) Complete genome sequences of rat and mouse segmented filamentous bacteria, a potent inducer of Th17 cell differentiation. Cell Host Microbe **10**, 273–284 CrossRef PubMed

69 Thompson, C.L., Vier, R., Mikaelyan, A., Wienemann, T. and Brune, A. (2012) "*Candidatus* Arthromitus" revised: segmented filamentous bacteria in arthropod guts are members of Lachnospiraceae. Environ. Microbiol. **14**, 1454–1465 CrossRef PubMed

70 Schnupf, P., Gaboriau-Routhiau, V., Gros, M., Friedman, R., Moya-Nilges, M., Nigro, G., Schnupf, P., Cerf-bensussan, N. and Sansonetti, P.J. (2015) Growth and host interaction of mouse segmented filamentous bacteria *in vitro*. Nature **520**, 99–103 CrossRef PubMed

71 Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V. et al. (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell **139**, 485–498 CrossRef PubMed

72 Brugman, S., Klatter, F.A., Visser, J.T.J., Wildeboer-Veloo, A.C.M., Harmsen, H.J.M., Rozing, J. and Bos, N.A. (2006) Antibiotic treatment partially protects against type 1 diabetes in the Bio-Breeding diabetes-prone rat: is the gut flora involved in the development of type 1 diabetes? Diabetologia **49**, 2105–2108 CrossRef PubMed

73 Kriegel, M.A., Sefik, E., Hill, J.A., Wu, H.-J., Benoist, C. and Mathis, D. (2011) Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proc. Natl. Acad. Sci. U.S.A. **108**, 11548–11553 CrossRef PubMed

74 Hansen, C.H.F., Krych, L., Nielsen, D.S., Vogensen, F.K., Hansen, L.H., Sørensen, S.J., Buschard, K. and Hansen, a K. (2012) Early life treatment with vancomycin propagates *Akkermansia muciniphila* and reduces diabetes incidence in the NOD mouse. Diabetologia **55**, 2285–2294 CrossRef PubMed

75 Ivanov, I.I., Frutos, R.D.L., Manel, N., Yoshinaga, K., Rifkin, D.B., Sartor, R.B., Finlay, B.B. and Littman, D.R. (2008) Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe **4**, 337–349 CrossRef PubMed

76 Ota, N., Wong, K., Valdez, P.A., Zheng, Y., Crellin, N.K., Diehl, L. and Ouyang, W. (2011) IL-22 bridges the lymphotoxin pathway with the maintenance of colonic lymphoid structures during infection with *Citrobacter rodentium*. Nat. Immunol. **12**, 941–948 CrossRef PubMed

77 Yin, Y., Wang, Y., Zhu, L., Liu, W., Liao, N., Jiang, M., Zhu, B., Yu, H.D., Xiang, C. and Wang, X. (2013) Comparative analysis of the distribution of segmented filamentous bacteria in humans, mice and chickens. ISME J **7**, 615–621 CrossRef PubMed

78 Ivanov, I.I. and Littman, D.R. (2010) Segmented filamentous bacteria take the stage. Mucosal Immunol. **3**, 209–212 CrossRef PubMed

79 Harley, I.T.W., Giles, D.A., Pfluger, P.T., Burgess, S.L., Walters, S., Hembree, J., Raver, C., Rewerts, C.L., Downey, J., Flick, L.M. et al. (2013) Differential colonization with segmented filamentous bacteria and *Lactobacillus murinus* do not drive divergent development of diet-induced obesity in C57BL/6 mice. Mol. Metab. **2**, 171–183 CrossRef PubMed

80 Upadhyay, V., Poroyko, V., Kim, T., Devkota, S., Fu, S., Liu, D., Tumanov, A.V., Koroleva, E.P., Deng, L., Nagler, C. et al. (2012) Lymphotoxin regulates commensal responses to enable diet-induced obesity. Nat. Immunol. **13**, 947–953 CrossRef PubMed

81 Ostman, S., Rask, C., Wold, A.E., Hultkrantz, S. and Telemo, E. (2006) Impaired regulatory T cell function in germ-free mice. Eur. J. Immunol. **36**, 2336–2346 CrossRef PubMed

82 Faith, J.J., Ahern, P.P., Ridaura, V.K., Cheng, J. and Gordon, J.I. (2014) Identifying gut microbe–host phenotype relationships using combinatorial communities in gnotobiotic mice. Sci. Transl. Med. **6**, 220ra11 CrossRef PubMed

83 Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K. et al. (2013) T_{reg} induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature **500**, 232–236 CrossRef PubMed

84 Odegaard, J.I., Ricardo-Gonzalez, R.R., Eagle, A.R., Vats, D., Morel, C.R., Goforth, M.H., Subramanian, V., Mukundan, L., Ferrante, A.W. and Chawla, A. (2008) Alternative (M2) activation of Kupffer cells by PPARδ ameliorates obesity-induced insulin resistance. Cell Metab **7**, 496–507 CrossRef PubMed

85 Kang, K., Reilly, K., Karabacak, S.M., Gangl, V., Fitzgerald, M.R., Hatano, K., Lee, B. and -H., C. (2008) Adipocyte-derived Th2 cytokines and myeloid PPARδ regulate macrophage polarization and insulin sensitivity. Cell Metab **7**, 485–495 CrossRef PubMed

86 Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L.G., Gratadoux, J.-J., Blugeon, S., Bridonneau, C., Furet, J.-P., Corthier, G. et al. (2008) *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc. Natl. Acad. Sci. U.S.A. **105**, 16731–16736 CrossRef PubMed

87 Zhang, M., Qiu, X., Zhang, H., Yang, X., Hong, N., Yang, Y., Chen, H. and Yu, C. (2014) *Faecalibacterium prausnitzii* inhibits interleukin-17 to ameliorate colorectal colitis in rats. PLoS One **9**, e109146 CrossRef PubMed

88 Qiu, X., Zhang, M., Yang, X., Hong, N. and Yu, C. (2013) *Faecalibacterium prausnitzii* upregulates regulatory T cells and anti-inflammatory cytokines in treating TNBS-induced colitis. J. Crohns Colitis **7**, e558–e568 CrossRef PubMed

89 Hua, Y., Shen, J., Song, Y., Xing, Y. and Ye, X. (2013) Interleukin-10 -592C/A, -819C/T and -1082A/G polymorphisms with risk of Type 2 diabetes mellitus: a HuGE review and meta-analysis. PLoS One **8**, e66568 CrossRef PubMed

90 Saxena, M., Srivastava, N. and Banerjee, M. (2013) Association of IL-6, TNF-α and IL-10 gene polymorphisms with type 2 diabetes mellitus. Mol. Biol. Rep. **40**, 6271–6279 CrossRef PubMed

91 Bai, H., Jing, D., Guo, A. and Yin, S. (2014) Association between interleukin 10 gene polymorphisms and risk of type 2 diabetes mellitus in a Chinese population. J. Int. Med. Res. **42**, 702–710 CrossRef PubMed

92 Quevrain, E., Maubert, M.A., Michon, C., Chain, F., Marquant, R., Tailhades, J., Miquel, S., Carlier, L., Bermudez-Humaran, L.G., Pigneur, B. et al. (2015) Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. Gut CrossRef

93 Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D. et al. (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature **490**, 55–60 CrossRef PubMed

94 Karlsson, F.H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C.J., Fagerberg, B., Nielsen, J. and Bäckhed, F. (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature **498**, 99–103 CrossRef PubMed

95 Vrieze, A., Van Nood, E., Holleman, F., Salojärvi, J., Kootte, R.S., Bartelsman, J.F.W.M., Dallinga-Thie, G.M., Ackermans, M.T., Serlie, M.J., Oozeer, R. et al. (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology **143**, 913–916 CrossRef PubMed

96 Koren, O., Goodrich, J.K., Cullender, T.C., Spor, A., Laitinen, K., Bäckhed, H.K., Gonzalez, A., Werner, J.J., Angenent, L.T., Knight, R. et al. (2012) Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell **150**, 470–480 CrossRef PubMed

97 Lin, H.V., Frassetto, A., Kowalik, E.J., Nawrocki, A.R., Lu, M.M., Kosinski, J.R., Hubert, J.A., Szeto, D., Yao, X., Forrest, G. et al. (2012) Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PLoS One. **7**, 1–9 CrossRef PubMed

98 McGuckin, M.A., Lindén, S.K., Sutton, P. and Florin, T.H. (2011) Mucin dynamics and enteric pathogens. Nat. Rev. Microbiol. **9**, 265–278 CrossRef PubMed

99 Derrien, M., Vaughan, E.E., Plugge, C.M. and de Vos, W.M. (2004) *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int. J. Syst. Evol. Microbiol. **54**, 1469–1476 CrossRef PubMed

100 Reunanen, J., Kainulainen, V., Huuskonen, L., Ottman, N., Belzer, C., Huhtinen, H., de Vos, W.M. and Satokari, R. (2015) *Akkermansia muciniphila* adheres to enterocytes and strengthens the integrity of epithelial cell layer. Appl. Environ. Microbiol. **81**, 3655–3662 CrossRef PubMed

101 Van Passel, M.W.J., Kant, R., Zoetendal, E.G., Plugge, C.M., Derrien, M., Malfatti, S.A., Chain, P.S.G., Woyke, T., Palva, A., de Vos, W.M. et al. (2011) The genome of *Akkermansia muciniphila*, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. PLoS One **6**, e16876 CrossRef PubMed

102 Shin, N.-R., Lee, J.-C., Lee, H.-Y., Kim, M.-S., Whon, T.W., Lee, M.-S. and Bae, J.-W. (2014) An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut **63**, 727–735 CrossRef PubMed

103 Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J.P., Druart, C., Bindels, L.B., Guiot, Y., Derrien, M., Muccioli, G.G., Delzenne, N.M. et al. (2013) Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. Proc. Natl. Acad. Sci. U.S.A. **110**, 9066–9071 CrossRef PubMed

104 Karlsson, C.L.J., Önnerfält, J., Xu, J., Molin, G., Ahrné, S. and Thorngren-Jerneck, K. (2012) The microbiota of the gut in preschool children with normal and excessive body weight. Obesity **20**, 2257–2261 CrossRef PubMed

105 Ellekilde, M., Krych, L., Hansen, C.H.F., Hufeldt, M.R., Dahl, K., Hansen, L.H., Sørensen, S.J., Vogensen, F.K., Nielsen, D.S. and Hansen, A.K. (2014) Characterization of the gut microbiota in leptin deficient obese mice: correlation to inflammatory and diabetic parameters. Res. Vet. Sci. **96**, 241–250 CrossRef PubMed

106 Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G.M., Neyrinck, A.M., Possemiers, S., Van Holle, A., François, P., De Vos, W.M. et al. (2011) Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes **60**, 2775–2786 CrossRef PubMed

107 Anhê, F.F., Roy, D., Pilon, G., Dudonné, S., Matamoros, S., Varin, T.V., Garofalo, C., Moine, Q., Desjardins, Y., Levy, E. et al. (2014) A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased *Akkermansia* spp. population in the gut microbiota of mice. Gut **64**, 872–883 PubMed

108 Derrien, M., Van Baarlen, P., Hooiveld, G., Norin, E., Müller, M. and de Vos, W.M. (2011) Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader *Akkermansia muciniphila*. Front. Microbiol. **2**, 1–14 PubMed

109 Stienstra, R., Mandard, S., Patsouris, D., Maass, C., Kersten, S. and Müller, M. (2007) Peroxisome proliferator-activated receptor α protects against obesity-induced hepatic inflammation. Endocrinology **148**, 2753–2763 CrossRef PubMed

110 Guerre-Millo, M., Gervois, M., Raspé, P., Madsen, E., Poulain, L., Derudas, P., Herbert, B., -M., J., Winegar, J., Willson, D.A., Fruchart, T.M., -C., J. et al. (2000) Peroxisome proliferator-activated receptor α activators improve insulin sensitivity and reduce adiposity. J. Biol. Chem. **275**, 16638–16642 CrossRef PubMed

111 Lukovac, S., Belzer, C., Pellis, L., Keijser, B.J., de Vos, W.M., Montijn, R.C. and Roeselers, G. (2014) Differential modulation by *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. MBio **5**, e01438–14 CrossRef PubMed

112 Maslowski, K.M., Vieira, A.T., Ng, A., Kranich, J., Sierro, F., Yu, D., Schilter, H.C., Rolph, M.S., Mackay, F., Artis, D. et al. (2009) Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature **461**, 1282–1286 CrossRef PubMed

113 Alex, S., Lange, K., Amolo, T., Grinstead, J.S., Haakonsson, A.K., Szalowska, E., Koppen, A., Mudde, K., Haenen, D., Al-Lahham, S. et al. (2013) Short-chain fatty acids stimulate angiopoietin-like 4 synthesis in human colon adenocarcinoma cells by activating peroxisome proliferator-activated receptor γ. Mol. Cell. Biol. **33**, 1303–1316 CrossRef PubMed

114 Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, P.B., Avanesyan, L., Stonebraker, A.C., Hu, C., Wong, F.S., Szot, G.L., Bluestone, J.A. et al. (2008) Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature **455**, 1109–1113 CrossRef PubMed

115 King, C. and Sarvetnick, N. (2011) The incidence of type-1 diabetes in NOD mice is modulated by restricted flora not germ-free conditions. PLoS One **6**, e17049 CrossRef PubMed

116 Mattila, E., Uusitalo-Seppälä, R., Wuorela, M., Lehtola, L., Nurmi, H., Ristikankare, M., Moilanen, V., Salminen, K., Seppälä, M., Mattila, P.S. et al. (2012) Fecal transplantation, through colonoscopy, is effective therapy for recurrent *Clostridium difficile* infection. Gastroenterology **142**, 490–496 CrossRef PubMed

117 Ahern, P.P., Faith, J.J. and Gordon, J.I. (2014) Mining the human gut microbiota for effector strains that shape the immune system. Immunity **40**, 815–823 CrossRef PubMed

118 Bermúdez-Humarán, L.G., Nouaille, S., Zilberfarb, V., Corthier, G., Gruss, A., Langella, P. and Issad, T. (2007) Effects of intranasal administration of a leptin-secreting *Lactococcus lactis* recombinant on food intake, body weight, and immune response of mice. Appl. Environ. Microbiol. **73**, 5300–5307 CrossRef PubMed

119 Amar, J., Chabo, C., Waget, A., Klopp, P., Vachoux, C., Bermúdez-Humarán, L.G., Smirnova, N., Bergé, M., Sulpice, T., Lahtinen, S. et al. (2011) Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO Mol. Med. **3**, 559–572 CrossRef PubMed

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