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REVIEW

Bugging inflammation: role of the gut microbiota

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The advent of vaccination and improved hygiene have eliminated many of the deadly infectious pathogens in developed nations. However, the incidences of inflammatory diseases, such as inflammatory bowel disease, asthma, obesity and diabetes are increasing dramatically. Research in the recent decades revealed that it is indeed the lack of early childhood microbial exposure, increase use of antibiotics, as well as increase consumption of processed foods high in carbohydrates and fats, and lacking fibre, which wreak havoc on the proper development of immunity and predispose the host to elevated inflammatory conditions. Although largely unexplored and under-appreciated until recent years, these factors impact significantly on the composition of the gut microbiota (a collection of microorganisms that live within the host mucosal tissue) and inadvertently play intricate and pivotal roles in modulating an appropriate host immune response. The suggestion that shifts in the composition of host microbiota is a risk factor for inflammatory disease raises an exciting opportunity whereby the microbiota may also present as a potential modifiable component or therapeutic target for inflammatory diseases. This review provides insights into the interactions between the microbiota and the immune system, how these affect disease phenotypes, and explore current and emerging therapies that target the gut microbiota as potential treatment for inflammatory diseases.

The increasing incidence of inflammatory conditions, such as inflammatory bowel disease (IBD, including ulcerative colitis (UC) and Crohn's disease (CD)),[1] diabetes[2] and asthma[3], is creating a new wave of diseases. For many of these diseases, such as IBD, the cause is unknown, and the complex biological interactions that result in disease pathology are only starting to be unravelled. Current disease management strategies for some of these diseases, such as insulin injections for diabetes patients and Salbutamol (Ventolin) for asthma sufferers, are long-term and poses a significant financial burden on individuals and on the health system. Other first-line treatment strategies generally involve immunosuppressant drugs (that is, corticosteroids) that may predispose patients to increased risk of acquiring infections. Therefore, there is an urgent need for a better understanding into disease progression, with the aim to develop effective and targeted treatment for inflammatory diseases.

For the past half a century, the advent of vaccinations, antibiotics such as penicillin and the increasingly improved hygiene have dramatically decreased, even eliminated, the incidence of some infectious diseases (Figure 1).[4] Within this period, there has also been drastic changes to diet, with increases in consumption of carbohydrates and fats in highly processed foods, and decreases in the intake of dietary fibre.[5] This forms the signature 'Western diet',[5] and translates to the average person living in a developed country ingesting about half of the 30 g of daily recommended intake of fibre.[6] Both decreases in early-life microbe exposure owing to increased hygiene, and decreases in dietary fibre parallel major increases in the incidence of inflammatory diseases (Figure 1).[4,7] Owing to this

association, researchers have proposed two hypotheses to describe the recent drastic increase in the incidence of inflammatory diseases.

The hygiene hypothesis was proposed as an explanation for the increasing prevalence of inflammatory diseases in the Western world. A study in 1989 found that individual's hygiene and the number of older siblings were factors associated with hay fever development.[8] This notion was controversial at the time, but gained support in later studies that differentiated between type 1 and type 2 helper T (T_H1 and T_H2, respectively) cells.[9] Importantly, T_H1 cytokine interferon (IFN)-γ inhibits a T_H2 response, and T_H2 cytokine interleukin (IL)-4 inhibits a T_H1 response,[10] supporting the concept that the lack of early-life infections (T_H1 response) polarised the host immunity towards a T_H2 response and elevated the risk of developing diseases such as asthma. This forms the basis of the hygiene hypothesis.

The hygiene hypothesis is hinged on the proposition that early-life exposure to diverse microbes help the immune system develop and differentiate infectious from harmless agents. Previous studies have shown that children raised in rural areas have more frequent microbial exposures and lower incidences of asthma,[11] leading to the belief that a cleaner environment, such as with improved hygiene, results in a dysregulated immune response and consequent development of inflammatory diseases.[12] As Parker pointed out, the definition of 'hygiene' has changed from being increases in better sanitisation infrastructures (such as sewer systems) in the past to now being associated with handwashing and cleaning.[12] This suggests that although the term 'hygiene hypothesis' has been retained, it encompasses an older perspective where increased sanitisation may indeed be

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microbiota-depleting. This opposes the current perspectives where being clean may only be microbiota-skewing. Indeed, research on general and personal hygiene may not be sufficient to link environmental cleanliness to the development of inflammatory diseases.[13] Therefore, research may need to target factors that have more prominent effects on the skewing or depletion of microbiota. One such factor is the use of antibiotics. Antibiotics are not effective against all bacteria, and will therefore skew the composition of the host microbiota towards one that is antibiotic-resistant.[14] In addition, different antibiotics target different bacterial components, and may be effective only on restricted groups of bacteria, such as anaerobes or Gram-positive strains.[14,15] This creates a lack of diversity in the microbiota, and is thought to cause an underdeveloped immune system, predisposing the host to a range of diseases. Therefore, the contribution of both urban/rural setting and antibiotic use have been shown to influence microbiota composition and diversity, induce a dysregulated immune response and leads to the development of inflammatory diseases.[11,16]

Another proposed explanation for the increasing prevalence of inflammatory diseases is the diet hypothesis. The inverse correlation between the amount of fibre intake and the development of
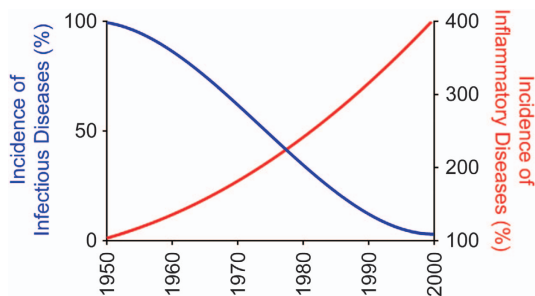

Figure 1 The inverse relationship between the incidences of infectious diseases (blue) and inflammatory diseases (red) over 1950–2000. Modified from publication.[4]
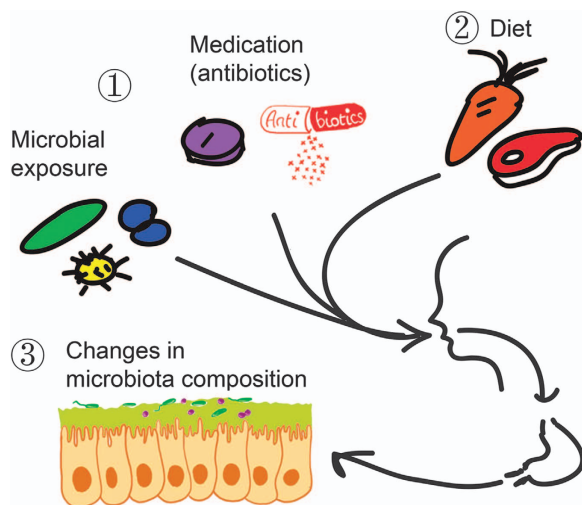

Figure 2 Factors in both the 'hygiene hypothesis' and the 'diet hypothesis' converge on microbiota modulation. (1) The factors proposed by the hygiene hypothesis (such as early childhood exposure to microorganisms and the use of antibiotics) and (2) the diet hypothesis (components and bacterial fermented products of food) both culminate in interactions with the microbiota (3). The changes in microbiota composition due to these and other factors may underlie their associations with inflammatory diseases.

inflammatory diseases has sparked extensive research. Fibre intake is protective for many inflammatory diseases,[17–20] and is also associated with an increase in longevity.[19] As fibre cannot be digested by the human body, we rely on fermentation by the gut microbiota to result in the generation of short-chain fatty acids (SCFAs) as metabolites, which includes predominantly acetate, butyrate and propionate.[21] Not only can SCFAs be absorbed into the circulation and have systemic anti-inflammatory effects, butyrate is also a local energy source for colonic epithelial cells.[21,22] Some of the anti-inflammatory actions of SCFAs and their associated receptors include promoting the production of immunoglobulin (Ig)A and immunosuppressive cytokines such as IL-10, the full extent of which has been reviewed elsewhere.[21] Indeed, there is accumulating evidence to strongly suggest that dietary fibre positively shape the composition of the gut microbiota and immunity, whereby its metabolites and associated receptors are potential links between diet, gut microflora and the host's inflammatory response.

It is becoming increasingly clear that both of the aforementioned hypotheses inadvertently influence the composition of the host gut microbiota/microbiome (Figure 2). Direct sequencing of genetic material of the human gut microbiota revealed that the gut consists of a complex community of commensal archaeal and bacterial cells from >1000 species.[23] The microbiota also harbour essential genes required for the metabolism of food intake,[23] indicating an additional role of the microbiota in energy harvest and homeostasis. Many factors in the two hypotheses, such as antibiotics use[24] and dietary components,[25] influence significantly on the composition of the host gut microbiota. The resultant dysbiotic microbiota could prove to merge both the hygiene hypothesis and the diet hypothesis into one, and contribute to the risk of inflammatory disease development.[26–29] However, this also raises an exciting opportunity whereby altering the microbiota may also present as a potential modifiable component or therapeutic target for inflammatory diseases.

This review will discuss the interactions between the microbiota and the immune system and how this affects subsequent immune responses and the development of inflammatory diseases. The latter parts of the review will examine in depth both the local and the systemic effect of the gut microbiota, and discuss the current and potential microbiota-modulating therapies for IBD, and its emerging uses in treating other inflammatory diseases.

INTERACTIONS BETWEEN MICROBIOTA AND THE IMMUNE SYSTEM

Recent research suggests that there is a symbiotic relationship between the 'non-self' microbiota and 'self' immune system, and both should be considered as one in the superorganism theory.[30] The microbiota has been shown to have profound effects in the development of gut-associated lymphoid tissue, the differentiation of gut immune cells, and production of immune mediators such as Igs (IgA) and antimicrobial peptides (defensins), as reviewed by Sommers and Bäckhed.[31] Importantly, the microbiota has modulatory effects on important regulatory immune cells, including invariant natural killer T (iNKT) cells and regulatory T cells (Tregs).[32–34] The findings from these studies highlighted the tight and proper control of the interaction between the host immune system and microbiota provide mutual benefit and regulation. Indeed, cells of the gut and the immune system have continual interactions to sample and distinguish between non-pathogenic commensal microflora, harmless foodstuff and pathogenic microorganisms. An appropriate balance between inflammatory and anti-inflammatory state is needed to achieve intestinal homeostasis.

A key tool in gaining insights into the importance of the microbiota in regulating the immune response is through studies of germ-free (GF) mice.[35] Unlike specific pathogen-free (SPF) counterparts, GF mice are born and reared without exposure to any live microbes, thus they are devoid of microbiota. Although different to the skewing of microbiota seen with a change in hygiene or diet, the use of GF mice in an experimental research setting has many benefits. It can be used to examine the extent to which a lack of microbiota influences the immune system, among other systems of the organism. Inoculating GF mice with single strains of bacteria also provides a valuable tool in studying specific microbial species and its effect on the immune response.

GF mice have an underdeveloped immune system, especially in the mucosal compartments.[36] There are inherent deficiencies of T cells in the lamina propria of the intestine, with GF mice having fewer numbers of CD3+ T cells.[37] Such deficiency in T cells has been shown to result in increased bacterial translocation, such as *Escherichia coli*.[38] In addition, other studies have demonstrated that the T cell deficiencies and a T_H1/T_H2 imbalance is rescued by the addition of commensal bacteria *Bacteroides fragilis*, bacterial molecule polysaccharide A, or lipopolysaccharide produced by Gram-negative bacteria.[34,39] These studies clearly indicate an important reciprocal regulation between microbiota and T cell development. The interactions between host microbiota and immune response extend beyond T cells. The expression of angiogenin-4, a selective yet potent antimicrobial peptide, is diminished in GF mice, and that inoculation of a single strain of *Bacteroides thetaiotaomicron* can induce its expression.[40] Furthermore, bacterial colonisation of the commensal *Morganella morganii* in the gut of GF mice also stimulates IgA production that in turn prevents bacterial translocation.[41] This may represent further mechanisms whereby the microbiota promotes the development of host immune responses, and also the capacity of the host immune system to prevent excessive bacterial translocation and inflammation. Together, these point towards an important role of the microbiota in shaping different facets of the immune system.[42]

In mice lacking microbiota, studies have shown a defective Treg population with lowered numbers and reduced capacity to function. Not only were there less CD4+CD25+ T cells, GF mice also had reduced expression of Treg transcription factor forkhead box P3 (FoxP3), IL-10 and IL-13.[43] Interestingly, Tregs with increased suppressive capacity can be generated *in vitro* by co-incubation with bacterial antigens and antigen presenting cells (APC),[44] and *in vivo* by administration of *Clostridium*-related segmented filamentous bacterium.[45] A separate study suggests that microbiota-induced generation of Treg in the periphery have a unique set of T-cell receptor repertoire, different to those of thymic origin.[46] These results demonstrate an important role of the microbiota in the control of regulatory immune cells. In GF non-obese diabetic mice, the expression of FoxP3 was only reduced in the gut, suggesting a restricted effect of the microbiota on Treg biology.[47] Interestingly, the addition of SCFAs acetate, butyrate or propionate reversed the deficiencies of colonic Treg numbers and function in GF mice.[48] This supports a pathway whereby the generation of SCFAs through fermentation of fibre by the gut microbiota have direct effects on Tregs, and highlights the anti-inflammatory functions of fibre and its metabolites. Despite this, there is also evidence suggesting that DNA of commensal microbiota limits Treg and promotes T cell differentiation through TLR9.[49] These studies clearly further indicate the interaction between microbiota and the host immune system can result in a multitude of consequences. In addition, it advances the idea that the interplay and balance between gut microbiota and the immune system is crucial for

intestinal homeostasis, and that dysbiosis may cause excessive inflammation that contributes to disease pathology.

The second part of the review will highlight recent findings on the influence of microbiota changes in the development of inflammatory diseases, and the potential of harnessing the gut microbiota to provide opportunities for translation from research to clinical management. Intriguingly, recent studies into the GF mice demonstrated an upregulation of genes involved in cell growth and signalling, signal transduction and metabolism with oncogenic implications, whereas a downregulation of immune-related genes, reflecting an underdeveloped immune system.[50] This finding indicates that the impact of the microbiota is not limited to the local immune function and inflammation, but also influence the host metabolic homeostatic state. Therefore, in addition to our primary focus on the localised effects of dysbiotic microbiota on the development of IBD, we will explore recent findings from clinical and animal studies that demonstrate the systemic effects of the dysbiotic microbiota on asthma, obesity and diabetes, and some of the emerging uses of microbiota modification as a target for therapeutic intervention.

THE LOCAL EFFECTS OF GUT MICROBIOTA ON IBD

Expectedly, changes in the gut microbiota has associations with inflammation and inflammatory diseases in the gut. IBD patients have increased Enterobacteriaceae (such as *E. coli*) and Bacteroidetes (such as *B. fragilis*) and reduced Firmicutes (Clostridia-related clusters), and also consistently elevated bacterial load in the colon.[51,52] This suggests that the increase in bacteria directly promotes gut inflammation. Patients with UC also have reduced *Faecalibacterium prausnitzii*, a bacterium that has anti-inflammatory properties.[53] Similar trends can be observed in patients with CD.[54] In an experimental setting, *F. prausnitzii* has been shown to protect against murine model of colitis, through induction of regulatory cytokine IL-10, and not T_H1 cytokines IL-12 or IFN-γ.[55] In addition, purified polysaccharide A, a product from *B. fragilis*, can prevent gut pathology through the modulation of IL-10-producing T cells in mice.[56] Despite the fact that a change in microbiota composition is associated with IBD development, changes in specific bacterial species have not been identified across patients.[51] It is likely that a combination of elevated bacterial load and a shift away from bacteria with anti-inflammatory properties that have an accumulative effect in initiating or aggravating the pathology of disease. However, the current research does not elucidate a causal effect. In a mouse model, despite commensal Enterobacteriaceae (primarily *E. coli*) are enriched in mice with colitis, transfer of these microorganisms did not induce disease in antibiotic-pre-treated mice, whereas transfer of commensal Bacteroides did.[57] This suggests a clear distinction between microorganisms that induce disease and those that change as a result of disease. It will be a difficult feat to identify any specific microorganisms that promotes the pathogenesis of IBD given the retrospective nature of human studies, and the fact that not all animal studies successfully translate to humans. Nevertheless, associations in human studies, and temporal studies in animals will allow greater insight into the immunopathogenesis of IBD.

GF mice have been an invaluable tool used to study the function of microbiota in animal models of inflammatory diseases. GF mice have inherently elevated levels of colonic inflammation. There are increased relative and absolute numbers of iNKT cells that forms a stable population in the lamina propria, although their activation markers remain unaltered.[58] GF mice are more susceptible to iNKT-dependent oxazolone-induced colitis, and have increased expression of IL-13 and IL-1β, whereby this disease phenotype can be reversed with CD1d

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blockade.[58] This clearly indicates that antigen presentation and subsequent activation of iNKT cells is critical to maintain gut homeostasis. In a 2% dextran sulphate sodium (DSS)-induced colitis model, GF mice showed increase mortality compared with SPF mice.[59] However, disease severity was lessened in GF mice with the addition of B. fragilis, with improved survival rates, disease activity scoring, histopathological scoring, reduced TNF-α and increased IL-10 production.[59] The results of this study revealed the immunomodulatory role of B. fragilis and the effects this bacterium could have on disease progression. Therefore, the appropriate modulation of gut microbiota may be a therapeutic target for localised inflammatory conditions.

Microbiota as immunotherapy for IBD

One method of altering the gut microbiota is to restrict bacterial growth in the patients' gut microbiome by the use of antibiotics. Studies have shown that in certain subgroups, and indeed during certain stages of disease, treatment with antibiotics was successful in inducing remission and preventing relapse.[60] In a multicentre trial, oral administration of amoxicillin, tetracycline and metronidazole reduced clinical score, increased clinical remission and also promoted corticosteroid discontinuation in patients with UC who were dependent on it.[61] Interestingly, it seems that as the more time progresses, the less significance there was between treatment and control groups. This could be, at least partially, explained by the resilient nature of the gut microbiota in a study with ciprofloxacin for 5 days.[62] The use of antibiotics was shown to result in an altered, yet stable, microbiota and the persistence of antibiotic-resistant genes.[14] The propagation of antibiotic resistance and the distribution of bacteria beneficial or detrimental for IBD are potentially important determinants of the level of response to antibiotic therapies. Since IBD has microbial complications, if not infectious origins, the use of antibiotics seem more than appropriate.[54] The effects of antibiotics are not restricted to killing bacteria, with Rifaximin shown to have effects on epithelial cells and immunological transcription factors such as nuclear factor-κB (NF-κB).[63] The findings of this study demonstrated the potential actions of certain antibiotics to act beyond an antibacterial agent, which could have synergistic roles in dampening inflammation, though the mechanisms behind the beneficial effects of antibiotics in IBD is still unclear. On the other hand, the tipping of microbiota composition may not always favour commensals. The reduction in gut microbiota diversity may promote the growth of bacteria that may not benefit, if not worsen, the IBD phenotype. Therefore, there is great potential for antibiotic therapy in IBD, with possible benefits beyond that of modulating the microbiota,[64] but details of the mechanisms of action need to be elucidated.

Animal models provides us with a controlled environment for the study of specific aspects of disease. Studies suggest that antibiotics therapy with the combined dose of ampicillin, metronidazole, neomycin and vancomycin, or bacitracin and neomycin, increases bacterial translocation, induces mild inflammation and the risk of developing DSS-induced colitis.[65,66] However, different studies using CIN-102, a natural cinnamon oil composition-like mixture with broad-spectrum antibacterial activity, and cathelicidin from *Bungarus fasciatus* decreased intestinal bacterial load, and improved symptoms of DSS-induced colitis.[67,68] Ciprofloxacin and metronidazole have also been shown to reduce ileitis in genetically modified mice through the downregulation of pro-inflammatory cytokines IFN-γ and TNF, and T-cell activation.[69] Although some antibiotics such as ampicillin and ciprofloxacin have broad-spectrum activities, others have specific activities, with vancomycin targeting Gram-positive bacteria, and

metronidazole targeting anaerobes.[15] These highlight the importance in choosing the right antibacterial compounds and combinations of antibiotics are crucial to the control of disease. Furthermore, with the natural antibacterial therapy having shown no resistance in the study, it may have advantages over conventional antibiotics in its spectrum of control of the gut microbiota.[67] Antibiotics can change the outcome of colitis even when given antepartum. The use of antibiotics during pregnancy also decreases bacterial richness and increases the risk of colitis for the offspring.[70] Therefore, much considerations are needed in choosing a specific antibacterial compound, and its timing of use, especially with the increases in antibiotic-resistant strains in humans. Nonetheless, these animal studies have outlined important microbiota-modulating roles of antibiotics in controlled and less genetically diverse animal models, and successful therapies could lead to future human research and development into clinical trials.

To investigate further into the association of specific bacteria and the progression of IBD, addition of bacteria to the patients' gut microbiome can be achieved in the form of probiotics. Probiotics are live microorganisms that are beneficial to gastrointestinal and general health. In most cases, patients with IBD have increased Bacteroides, Enterobacteriaceae (mainly E. coli) and decreased Lactobacillus and Bifidobacterium.[71] In a systematic review of clinical trials, lactic acid bacteria and Bifidobacteria, and even Bifidobacteria-fermented milk, had the potential to treat patients with IBD, with greater efficacy in patients with UC than CD.[72] A mixture named VSL#3 containing Lactobacillus, Bifidobacteria and Streptococcus was also successful in inducing and maintaining remission of paediatric UC.[73] In addition, a non-pathogenic strain of E. coli was effective in maintaining remission in patients with UC.[74] The greater benefit observed in patients with UC could be the restricted nature of UC pathology to the mucosal layer of the colon, whereas CD can affect any layer of any part of the gastrointestinal tract, and thus have more variance in its pathology.[75] Nevertheless, these evidence suggest a therapeutic role of probiotic Lactobacillus and Bifidobacteria in patients with IBD that should be further pursued.

Similar results were achieved using mouse models. A study found that a probiotic mixture can improve disease scoring of DSS-induced colitis.[76] *Bifidobacterium longum* can also reduce colitis severity by preserving tight junction proteins and improving intestinal epithelial integrity. However, this protective effect was observed in a specific strain (CCM7952) and not in others, suggesting the different genetic make up and specific bacteria-host interactions even between different strains are important to confer protection.[77] Another animal study showed that the combined effects of *Clostridium butyricum* and *Bifidobacterium infantis* protected against colitis, by restoring the gut microbiota.[78] *Lactobacillus casei* can also modulate the microbiota and protect against colitis, but it requires the dairy delivery matrix of milk for this effect.[79] F. prausnitzii and the extracellular polymeric matrix isolated from its biofilm both had anti-inflammatory effects through TLR-2 signalling and modulation of IL-10 and IL-12, ameliorating DSS-induced colitis.[80] A recent study suggests that live Bifidobacteria can also protect against DSS-induced colitis, although it only stably colonises the gut in GF but not SPF mice.[81] This is likely due to the fact that there is no competing colonising strains in GF mice, and raises an interesting question of how well bacteria given as a probiotic can colonise the gut. Human studies have shown that although the probiotics have minimal effects on the composition of the gut microbiota, and is usually undetectable after 2 weeks post-ingestion, they do promote bacterial gene expressions related to plant-polysaccharide and other carbohydrate metabolism pathways.[82,83] There could also be immunomodulatory effects during the period of

probiotic consumption that polarise DCs. *In vitro* studies suggest probiotics promote a regulatory DC phenotype, either by direct interactions or through epithelial expression of TGF-β and thymic stromal lymphopoietin.[84] Therefore, it is possible that given sufficient dosage and length of probiotic therapy, there can be bias towards a less inflammatory gut environment that benefit patients with IBD.

Another concept is to replace an 'unhealthy' gut microbiota with a 'healthy' one, in what is termed faecal microbiota transplant (FMT). FMT has been successful in treatment of *Clostridium difficile* infections,[85] and given the dysbiotic nature of IBD, FMT seems suitable as a treatment option. Indeed, in a recent systematic review of FMT therapy in adults and children with IBD (along with other gastrointestinal infection and inflammatory diseases),[85] active UC[86] or refractory CD,[87] the results were positive without adverse effects. Notably, FMT was more successful for treating patients with UC rather than CD,[85] again likely due to the heterogeneity of CD. However, FMT for IBD is much less effective than for *C. difficile* infections,[88] which may be because of a collection of different risk factors in disease development, and in the differences of donor microbiota.[89,90] The primary reason might be the difference between one causative bacteria (*C. difficile*) compared with an unknown cause and contributing microorganisms for IBD. Even the differences in study design and methodology may affect the study outcomes.[91] For example, the amount of processing time may affect the populations of anaerobic and aerobic bacteria, changing the composition and affecting the outcome of transplantation. Moreover, the donor–recipient match is very important for successful FMTs. The microbiota following FMT varies significantly, and patients whose microbiota shifted towards that of the donors' had better outcomes.[92,93] The richness of donor microbiota, and the enrichment of butyrate-producing bacteria also correlated with successes in FMT.[94,95] Interestingly, the latter enrichment suggests that an increased utilisation of butyrate may be a pathway benefiting patients with IBD, possibly through restoration of epithelial cell function and colonic integrity.[22] Although FMT are generally well-tolerated by patients, both children[96] and the elderly,[97] there are limited studies that involve long-term follow-up. Given the increasing research outlining roles of the microbiota in many aspects of host physiology, long-term safety of FMT is an important aspect that should be included in future studies.

One method of modelling FMT and studying the effects of microbiota transfers experimentally is the use of co-housed animals. A study found that a genetic knockout strain harboured altered microbiota containing higher populations of Firmicutes and probiotic bacteria.[98] Interestingly, this altered microbiota was transferred to wild-type mice when co-housed, and subsequently transferred protection to DSS-induced colitis. This indicated that a transferred microbiota, if well tolerated, can transfer protection, giving support to the use of FMT. Similarly, when the genetic knockout mice were inherently protected from DSS-induced colitis, co-housing with wild-type mice increased their sensitivity to disease,[99] while the vice versa was also true; when the genetic knockout mice were susceptible to colitis, co-housing conferred protection.[100] In addition, when using the same Helicobacter-induced model of colitis on mice in two different facilities, researchers showed that differing compositions of gut microbiota had significant and opposing effects on the outcomes of disease.[101] This hints at the possibility that modulating the microbiota may override genetic susceptibilities, adding further support to the capacity of microbiota transfers as a therapeutic strategy. However, the same environmental variations presents obstacles in the translation of microbiota research to human studies, where

there are vastly more variations to be considered. Taken together, it is evident that microbiota transfers have the potential to modulate disease outcomes, and supports the notion that FMT is beneficial to patients with IBD. The important difference between animal models and clinical IBD is the known cause of disease in animal models. Indeed, the unknown cause of IBD in humans and how to increase the compatibility of donor–recipient gut microbiota are immediate hurdles for research into the clinical effectiveness of FMT in IBD.

Last but not least, prebiotics are types of fibre that promote the growth of beneficial microorganisms which contribute to the health of the host, and is therefore an option for therapeutic intervention. Different prebiotics have different effects, with inulin and fructo-oligosaccharides promoting the growths of Bifidobacteria and Lactobacilli, respectively.[102] A few studies on the effects of prebiotics in patients with IBD showed it lowered inflammation and clinical activity score, and maintained remission in patients with UC.[103–105] Interestingly, it was found that patients with active CD had a lower intake of inulin-type fructans and oligofructose than patients with inactive CD and healthy controls.[106] Therefore, the findings from these clinical studies suggest that improvements in disease activity may be due to the anti-inflammatory properties of fermented products of prebiotics, such as SCFAs, they are produced by bacteria such as *F. prausnitzii*, Bifidobacteria and Lactobacilli.[102] When assessed for the capacity of the colonic mucosa to utilise butyrate, glucose and glutamate, it was found that patients with UC were less effective at metabolising butyrate, while there were increased utilisation of glucose and glutamate, possibly as a compensatory mechanism.[107]

In animal studies, prebiotics alter the gut microbiota and have anti-inflammatory effects. Soluble dextrin from different sources (corn or tapioca) differentially alters the gut microbiota, thus it is safe to assume the complexity of prebiotic fermentation and the effects of prebiotics on the gut microbiota colonies.[108] When fed a high-fibre diet or SCFA acetate, mice had a reduced risk of developing colitis.[109,110] This could be due to the actions of butyrate on the colonic epithelium, but also the capacity of SCFAs in promoting the numbers and functions of colonic Tregs, thus enabling a state that is more resistant to inflammation.[48] However, when given during DSS-induced colitis as a model of treatment, both fibre (with or without the addition of *B. longum*) and acetate failed to alter disease progression, indicating a role of prebiotics in prevention rather than treatment of disease.[109] This further supports the association between consumption of a Western diet and increase in risk for the later development of inflammatory diseases. It also suggests the importance in timing of prebiotic treatments, and that such therapies are more effective during remission and inactive inflammatory responses.

THE SYSTEMIC EFFECTS OF ALTERING GUT MICROBIOTA

Dysbiotic microbiota also exerts systemic effects, and is implicated in extra-gastrointestinal diseases such as asthma and diabetes. Microbiota research in asthma has centred more on the hygiene hypothesis, whereby early-life exposure to microorganisms has been a large part of the focus. Studies suggest that babies born through vaginal delivery when compared with those born via caesarean section, and also infants fed with breast milk when compared with those fed formula, have increased Bifidobacteria and decreased Bacteroides, among other differences in gut microbiota.[111] In addition, having older siblings also increased bacterial diversity and bacterial richness in 18-month-old individuals.[112] These findings indicate that interactions between the offspring and parents or siblings are associated with beneficial outcomes, possibly through having similar composition of the gut microbiota. Children living in rural areas were exposed to an increased

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variety of microorganisms (in bacteria and fungi) and this microbial diversity rendered them less likely to develop asthma, though the study was unable to identify the specific species involved.[11] These findings support the hypothesis that there is a window of opportunity for microbial modulation during early childhood, and when there are reductions in gut microbial diversity and altered airway microbiota during this time, it may present as a risk factor for chronic wheeze and asthma,[113] though there have also been studies that failed to find any associations.[112] Unlike the gut, increased bacterial load and diversity in the lung were correlated with bronchial hyperresponsiveness and disease activity in patients with asthma. This may be due to inappropriate colonisations of potentially pathogenic bacteria, such as by Sphingomonadaceae, which elicit an iNKT cell-driven production of IL-4 and IL-13, and induction of airway hyperresponsiveness.[114] These results from the animal study showed that even though an increase in bacterial load increases recruitment of immune cells, there needs to be in depth clarification of bacterial strains to understand how bacterial diversity affects the host immune response and subsequent development of inflammation.

Another aspect of microbiota research in asthma has been the use of antibiotics. In a study using ovalbumin (OVA)-induced allergic airways disease (AAD), a murine model of asthma, vancomycin treatment in early-life stages exacerbated disease.[24] Here, the researchers showed that vancomycin during early-life stages, but not streptomycin, reduced microbial diversity and percentages of Tregs in the colon, but not in the lung. In addition, Actinobacteria and Bacteroidetes were almost completely depleted, while there is an overgrowth of Lactobacillus.[24] Another study found that components of *Streptococcus pneumoniae* increased the number of Tregs in the lung, which were required for the suppression of iNKT cells and AAD.[115] Taken together, these results advocate for the hygiene hypothesis in the development of asthma. More specifically, reduced antibiotic use and increased gut microbial diversity and richness (especially during early life) can induce the number and function of regulatory immune cells to protect against asthma.

The gut microbiota also has crucial functions in harvesting energy, and its alterations have profound effects on host systemic physiology and metabolic homeostasis.[116] Previous studies suggest that Firmicutes have a greater capacity for energy harvest when compared with Bacteroidetes.[117] In fact, the positive association between increased ratio of Firmicutes:Bacteroidetes and obesity was found in *ob/ob* mice.[117] Similar result was observed in humans, and as such, this Firmicutes-predominant microbiota composition has been colloquially termed an 'obese microbiota'.[118] However, this is not always true,[119] and even the inverse relationship has been found in other studies.[120] The confounding factor could be diet, with differential use of fat-restricted or carbohydrate-restricted diet, or no dietary restrictions at all in the different studies.[118–120] Another possibility may be the lack of dependency of obesity on the ratio of bacterial phyla, but rather specific species and their capacity to produce SCFAs. Acetate, the most abundant SCFAs in the gut, was found to be increased in patients on the carbohydrate-restricted diet and in lean individuals.[119,120] Furthermore, there are studies that suggest an obese individual can be distinguished from a lean individual by analysing the difference in the number of microbiota genes.[121] A reduction of faecal Bifidobacteria in children may predict overweight, with Bifidobacteria being associated with breast feeding and reduced risk of obesity.[122] A potential pathway for the beneficial effects of Bifidobacteria is the fermentation of breast milk and prebiotic fibre to produce SCFAs.[123] In studies using rats fed on a high-fat diet, those prone to obesity had increased TLR4 expression, decreased epithelial integrity and

increased serum lipopolysaccharide levels.[124] Furthermore, when compared with rats fed a low-fat diet, consumption of a high-fat diet decreased total bacterial load, but increased the relative abundance of Bacteroides, and, to a greater extent, Clostridia and Enterobacteriaceae in the composition of the gut microbiota.[124] These animal studies provided evidence that different phyla of bacteria have differential effects on energy harvest and fibre fermentation, though there are still discrepancies between studies. More importantly, it highlights varying effects of different species of bacteria on host physiology. Indeed, it may be these differences that contribute to the contrasting results from phyla analysis. Together, the findings from these studies reveal a change in microbiota composition and encoded genes that promotes energy harvest and inflammation play a more prominent role in obesity, and shed light on the potential of SCFAs and SCFA-producing bacteria as a therapeutic.

The microbiota of diabetic patients and mouse models also hints at how changes in microbiota affects metabolic diseases. In patients with pre-type II diabetes (T2D), there were reduced butyrate-producing bacteria (such as *F. prausnitzii*) and decreased Bacteroides.[125] However, in a different study, an increase in Bacteroidetes and decrease in Firmicutes were observed in patients with T2D.[126] As with obesity, a confounding factor could be diet, as the studies were conducted in China[125] and Denmark,[126] with inherently different diets and lifestyle factors. Sample handling may also contribute to alterations of microbial compositions, with the Chinese study freezing samples at − 80 °C within 2 h of collection, a shorter time than the 24 h in the Danish study.

Animal studies have provided insights into the positive association between lipopolysaccharide, a product of Gram-negative bacteria such as Bacteroidetes, and the induction of T2D.[127] The mechanism for this is likely to be mediated through toll-like receptor (TLR) sensing, as TLR9-deficient mice in a type I diabetes (T1D) model had increased Actinobacteria (Bifidobacteria) and Firmicutes, and decreased Bacteroidetes.[128] Although changes in the Firmicutes:Bacteroidetes population in animal studies contrast that of the aforementioned human studies, it may be due to differences between mice and humans, or the different nature of disease. Mice deficient in TLR3 or myeloid differentiation primary response gene 88 (MyD88) (dependent signalling molecule of other TLRs) were protected from T1D,[128] suggesting differential but important roles of TLRs, and consequently, the microbiota, in the initiation and development of diabetes. Interestingly, studies in GF and SPF non-obese diabetic mice found no difference in the incidence of T1D,[47,129] though the addition of *Bacillus cereus* resulted in a delayed and lowered incidence.[129] Although a very good model of human T1D, non-obese diabetic mouse models are still not perfect in translating murine studies to humans.[130]

Furthermore, a study into GF mice that was fed a 'Western-like' diet was shown to be protected from developing obesity, and this was found to be mediated through increased expression of phosphorylated AMP-activated protein kinase in muscle and liver, which promotes fatty acid oxidation.[131] However, in GF mice deficient in fasting-induced adipocyte factor, there were reductions in enzymes involved in fatty acid oxidation and increased weight gain, even though muscle and liver phosphorylated AMP-activated protein kinase remained similar.[131] This study showed that the microbiota may directly impact on fatty acid metabolism, or indirectly, through the regulation of genes that modulate metabolism. Taken together, the use of various strains of mice and types of disease models under normal SPF or GF conditions reveal important roles of the microbiota in modulating inflammatory conditions and metabolic diseases.

Emerging uses of microbiota as a therapy

There is a growing body of research on the use of probiotics for the treatment of respiratory diseases, including asthma and other airway hypersensitivity conditions. A randomised, double-blinded and placebo-controlled trial of oral *Lactobacillus gasseri* capsule in asthmatic children found the treatment group had lower bronchial hyper-reactivity, better pulmonary function, and greater improvements in day time asthmatic symptoms.[132] This treatment also reduced *in vitro* production of TNF, IL-12, IL-13 and IFN-γ in peripheral blood mononuclear cells. This study provided strong evidence for the systemic regulatory effects of orally administered probiotics. Similar results were observed with oral inoculation of *Lactobacillus rhamnosus* in mice being protective of the OVA model of AAD.[133] The group of mice treated with probiotics had decreased clinical score, airway hyperresponsiveness and OVA-specific T cells in the spleen, and increased proportions of Tregs in the spleen.[133] This could be in part mediated through the modulation of macrophages, with *L. rhamnosus* strains previously shown to have an effect outside of the gut in mice.[134] Similarly, administration of a probiotics mixture was found to exert effects on regulatory DCs and Tregs in the respiratory tract and protect against asthma.[135] Interestingly, although intragastric administration of *Lactobacillus paracasei* reduced symptoms of OVA-induced AAD in mice, it was significantly more effective when introduced intranasally (direct to the lungs).[136] This finding indicates that different routes of entry for probiotics can elicit different extent of responses. However, it is noteworthy that with current technology, it is implausible to perform routine intranasal administrations on human patients. In addition, a randomised, double-blinded and placebo-controlled trial concluded that *Lactobacillus GG* is protective of upper respiratory tract infections in children, with infections lasting a significantly shorter amount of time.[137] These studies showed that orally taken probiotics have the capacity to induce both local and systemic effects on regulatory cells and cytokines, and consequently modulate respiratory tract conditions.

The modulation of microbiota also has the capacity to affect diseases such as diabetes that involve tissues other than the mucosa. The effects of prebiotics, probiotics, symbiotics (the combination of pre- and probiotics), antibiotics and FMT on obesity and T2D have been comprehensively reviewed by Kootte and Vrieze.[138] This group also showed the effectiveness of transferring lean donor microbiota to obese subjects with metabolic syndrome in a double-blinded randomised controlled study.[139] It is important to note that in research on obesity, diabetes and other extra-gastrointestinal diseases, there are currently limited human studies, and trials have been small-scale.

Moreover, dysbiosis may not be restricted to the gut. Recent research has shown that places previously thought to be sterile, such as the urinary tract[140] and the placental environment,[141] are in fact colonised by microbiota but not necessarily infectious.[140] In addition,
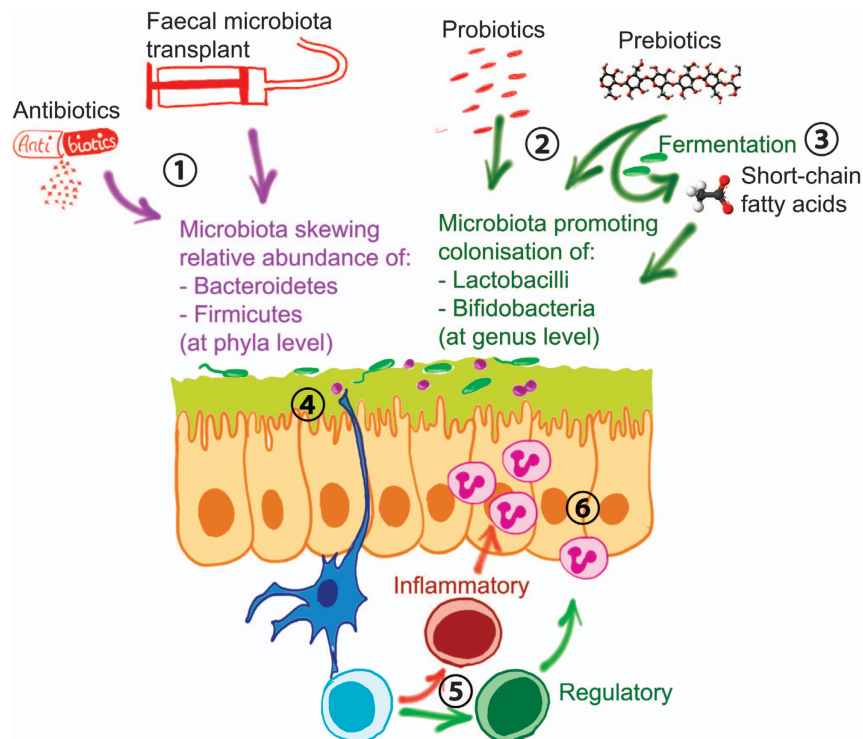

Figure 3 Therapeutic modulation of the microbiota influences immune responses and inflammatory diseases, a perspective of the gut environment. (1) Therapies such as antibiotics and FMTs shift the composition of the whole microbiota, altering the relative abundance of the main phyla Bacteroidetes and Firmicutes. (2) Other therapies such as probiotics and prebiotics promote the growth and colonisation of selective genus of bacteria, such as Lactobacilli and Bifidobacteria. (3) Prebiotic fibre can also be fermented to SCFAs by certain bacteria. SCFAs such as butyrate is a preferred energy source for colonic epithelial cells, and SCFAs can also modulate immune cell functions. (4) It is now known that the microbiota is not only essential for the development of the immune system, but may also modulate inflammatory responses. (5) Dysbiosis may lead to polarised induction of immune cells. (6) Increased pro-inflammatory T cells may increase inflammatory effector cells, leading to an increased inflammatory state, and may pose as a risk factor for inflammatory diseases, or fuel disease development and severity. On the other hand, induction of regulatory T cells dampens the inflammatory response, and alleviate inflammatory disease phenotype. Finally, excessive inflammation decreases the gut epithelial integrity, which leads to increased bacterial translocation and further induction of inflammation (not shown in figure).

it is intriguing to examine whether or not the placenta microbiota may mediate 'inheritance' of the maternal gut microbiota and risks of diseases, or impact on the microflora of other sites, such as the offspring's skin and in the mouth, ultimately influencing their immune responses. This may then be able to explain the benefits of closer parent-offspring or sibling interactions. Although current research are lacking in this area, recent evidence suggests an epigenetic effects of the maternal gut microbiota in regulating the development of asthma in the offspring.[20] Further research in this area will provide significant insight on the important role of the gut microbiota in the regulation of immune responses and the development of inflammatory diseases in not only the host, but perhaps also their offspring.

CONCLUDING REMARKS

The increasing incidences of inflammatory disease have had scientists striving for an explanation. Proposals of how early childhood hygiene, widespread antibiotic use and the increase in processed food and lack of fibre consumption culminates in what was an under-appreciated part of us: our microbiota. Extensive studies in GF mice have elucidated important interactions between bacteria and immune cells that enables proper development and function of our immune system. This has profound impact as the relative abundance of Bacteroidetes and Firmicutes, and the proportions of Bifidobacteria and Lactobacilli are all important determinants in gut homeostasis and whole-body health. A summary figure of the complex interplay of how therapeutics affect the gut microbiota, immune response and inflammatory conditions is shown in Figure 3. Although we still do not know the exact cause of a number of inflammatory diseases such as IBD, and whether or not the gut microbiota changes or dysbiosis are the cause or effect, we are starting to understand the protective and pathological effects of some of these changes. Furthermore, by modulating the total population of the gut microbiota using antibiotics or FMT, or by specifically targeting one or more bacterial species using prebiotics and/or probiotics, we are gaining significant insights into the effects of specific bacteria on disease pathology. The combined use of antibiotics and FMT, of prebiotics and probiotics, or even all four of these therapies may 1 day provide effective treatment for many inflammatory diseases. There is emerging data to suggest that the gut microbiota affects not only the inflammatory state of mucosal surfaces, but also metabolic disorders such as diabetes. Therefore, harnessing and manipulating the gut microbiota will provide widespread therapeutic opportunities not restricted to simply a single-organ system.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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