THE POWER TO REVEAL

JOIN US IN SHAPING THE FUTURE OF CELL ANALYSIS

With Cytek's innovative Aurora and Northern Lights cell analysis technology, scientists are able to see more highly resolved and multiplexed data than ever before, all from a single sample tube. Accelerate your research in immunotherapy, cancer, and cell biology with 40 colors per sample.

Cytek Bio's cell analysis solutions are accelerating the pace of scientific discovery by revealing more answers per sample. Together with our unique flow cytometers, we are proud to offer top tier service, a highly knowledgeable applications support, and a variety of support tools – all to help scientists achieve the best results the first time around.

Reveal more from your cells with Cytek.

See for yourself at go.cytekbio.com/immunology

CYTEK
TRANSCEND THE CONVENTIONAL

www.cytekbio.com

 DOI: 10.1002/eji.201948322 Eur. J. Immunol. 2020. 50: 326–3

European Journal of Immunology

HIGHLIGHTS

REVIEW

The microbiota and immune-mediated diseases: Opportunities for therapeutic intervention

Gillian Fitzgibbon and Kingston H. G. Mills

School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland

A multitude of diverse microorganisms, termed the microbiota, reside in the gut, respiratory tract, skin, and genital tract of humans and other animals. Recent advances in metagenomic sequencing and bioinformatics have enabled detailed characterization of these vital microbial communities. Studies in animal models have uncovered vital previously unrecognized roles for the microbiota in normal function of the immune responses, and when perturbed, in the pathogenesis of diseases of the gastrointestinal tract and lungs, but also at distant sites in the body including the brain. The composition of gut and respiratory microbiota can influence systemic inflammatory responses that mediate asthma, allergy, inflammatory bowel disease, obesity-related diseases, and neurodevelopmental or neurodegenerative conditions. Experiments in mouse models as well as emerging clinical studies have revealed that therapeutic manipulation of the microbiota, using fecal microbiota transplantation, probiotics, or engineered probiotics represent effective nontoxic approaches for the treatment or prevention of *Clostridium difficile* infection, allergy, and autoimmune diseases and may enhance the efficacy of certain cancer immunotherapeutics. This review discusses how commensal bacteria can influence immune responses that mediate a range of human diseases and how the microbiota are being targeted to treat these diseases, especially those resistant to pharmacological therapies.

Keywords: Allergy · autoimmune disease · cancer · microbiota · neurological disease

Introduction

The human microbiota comprise the microorganisms that live in or are associated with a variety of human tissues including the gut, lungs, skin, nasal cavity, and genital tract [1, 2]. It has been estimated that there are 3.8×10^{13} bacteria in an average 70 kg man, roughly equivalent to the total number of human cells in the body [3]. Although bacteria of the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria are the main constituents of the gut microbiota, it also includes archaea, viruses, and eukaryotic microbes [4, 5]. The microbial community includes both commensal and symbiotic microbes, reflecting the coevolution of the host and its microbiome.

Correspondence: Prof. Kingston H. G. Mills
e-mail: kingston.mills@tcd.ie

The gut microbiome resembles an endocrine organ, exerting its effects on sites remote from the origin such as the lung and the brain. These microbes harbored along the length of the intestinal tract interact with a myriad of host systems and cells resulting in a symbiosis between microbiota and the host [6–10]. This relationship is mutually beneficial, with the microbiota contributing to important host processes, such as vitamin synthesis, food processing, and maintenance of immunity, while the host in turn provides an environment that allows for microbial survival and proliferation [11, 12]. Advances in high-throughput DNA/RNA sequencing technologies have enabled significant advances in microbiome analysis and have helped in elucidating a role of these microbes in disease processes.

The National Institutes of Health Human Microbiome Project was established in 2008 with the aim of generating a data base that would provide a comprehensive characterization of the human

microbiome to help investigate its role in human health and disease [13]. The metagenomic approach analyzed the genomes from microbial communities that were sampled in its natural environment rather than from isolated strains cultivated in vitro [14, 15]. The main body of data initially obtained came from 242 screened and phenotyped "healthy" male and female adults who were sampled at 15 and 18 body habitats three times to gain a total of 4788 specimens. This project focused on the upper respiratory tract (oral cavity and nasal passage), the skin, the gastrointestinal (GI) tract, and the urogenital tract, sites in the body that are each known to harbor many distinct microbial communities [15]. Emerging data have demonstrated that bacterial colonization of these sites is vital to human health but can also contribute to disease processes.

The microbiota of the respiratory tract, especially the lower respiratory tract, because it is more difficult to sample, have received much less attention than the gut and the lower respiratory microbiome was thus excluded from the human microbiome project [16]. Humans take in a multitude of nonnative microbes with each inhalation, and these pass through the nasal cavity and oropharynx and colonize the nasal tissue and lungs [17]. Bronchoalveolar lavage techniques have allowed characterization of the lung microbiota [18] and showed that it contains approximately 2000 bacterial genomes per cm^2 surface [19]. *Prevotella,* anaerobic Gram-negative bacterial species of the phylum Bacteroidetes, are common commensal microbes of the healthy respiratory microbiome, found in oral cavity and upper and lower respiratory tract [20, 21]. The composition of the lung microbiota is determined by microbial immigration, acquired through microaspiration and inhalation, their elimination and transient growth, which is dependent on local nutrients [22]. The nutrient sources in the lungs include mucins, high molecular weight glycoproteins that provide bioavailable carbohydrates. It has been suggested that humans have coevolved with *Prevotella* [20], or may evade protective immunity by inhibiting the function of neutrophils [23]. However, an abundance of *Prevotella* has also been linked with inflammatory disorders; it appears that some strains may exhibit pathobiontic properties [20].

The influence of the microbiota on immune responses

There is growing evidence that the gut microbiota can influence many of the vital homeostatic processes that maintain a healthy human immune system. Evidence from zebrafish and germ-free mouse models has demonstrated that the gut microbiota have a major influence on the development of immune responses. Studies with the larvae of zebrafish have demonstrated that the microbiota can enhance protective innate immune responses before adaptive immunity has developed. Colonization of newborn zebrafish by commensal bacteria was found to prime neutrophils and induce expression of antiviral and proinflammatory molecules including IL-1β [6]. It has also been reported that the gut microbes of zebrafish can induce expression of intestinal alkaline phosphatase at the gut lumen brush border, which dephosphorylates the toxic Lipid A component of lipopolysaccharide (LPS). Detoxification of this proinflammatory mediator promotes immune tolerance to gut microbes and regulates the numbers of neutrophils present in the gut in the steady state. Young zebrafish that lack intestinal alkaline phosphatase, and cannot therefore detoxify LPS, have an excessively large influx of neutrophils into the intestine [9, 10]. Further evidence of the crucial role of gut microbiota in the development of the immune system has come from studies in germ-free mice. These mice have abnormal intestinal epithelial cells, reduced numbers of Peyer's patches, and lack mesenteric lymph nodes [7, 8].

Studies on germ-free mice and antibiotic-treated mice have generated complementary information on the role of the microbiota in shaping the immune response and when deregulated in the development of inflammatory diseases [24]. Antibiotics, even broad-spectrum cocktails, are unlikely to completely eliminate bacteria from all surfaces in the body, whereas germ-free mice should in theory be free of all living microorganisms. Therefore, germ-free mice are more useful for reconstitution experiments to examine the effect of mono-colonization with specific bacteria on modulation of immune responses or disease progression. On the other hand, antibiotic treatment will allow transient reduction in microbiota levels at specific time points and may be more translatable to studies in humans.

Dysbiosis or disruption of the microbial balance in the gut can increase susceptibility to a range of immune-mediated diseases including inflammatory bowel diseases (IBDs), asthma/allergy, and obesity-related diseases. Specific gut microbiota can have pro- or anti-inflammatory properties (Fig. 1). Littman and colleagues found that the balance between Th17 cells and Treg cells in the lamina propria in mice was influenced by the intestinal microbiota [25]. Furthermore, they demonstrated that a single commensal microbe, segmented filamentous bacteria (SFB), could drive the development of Th17 cells [26]. These SFB-induced Th17 cells can promote development of autoimmune diseases including arthritis [27]. Although SFB colonization of the ileum is associated with activation of Th17 cells in mice, the levels of SFB are low in human gut microbiota [28]. However, bacteria that bind to epithelial cells in the gut, including SFB, but also *Citrobacter rodentium* and *Escherichia coli* O157, promote Th17-inducing gene-expression program [29]. Furthermore, a diversity of bacterial strains in fecal samples of a patient with ulcerative colitis (UC) that had epithelial cell adhesive characteristics were capable of inducing Th17 cells in mouse colon. [29]. Therefore, SFB may not be the major bacterial strain that influences the development of Th17 cells in gut-associated lymphoid tissue in humans.

Manipulating the intestinal microbiota, with for example broad-spectrum antibiotics, may be an effective approach for inhibiting innate immune responses that drive pathogenic T cells in autoimmune diseases. However, microbiota-driven Th17 cells, which produced IL-17 and IL-22, protected against the intestinal pathogen *C. rodentium* [26]. Furthermore, specific gut commensal can also mediate immunosuppressive effects, dampening inflammation [30], (Fig. 1). Resident intestinal microbes can mediate anti-inflammatory effects through production of metabolites, including short chain fatty acids (SCFAs), from indigestible fibers, which can suppress immune responses [31]. The

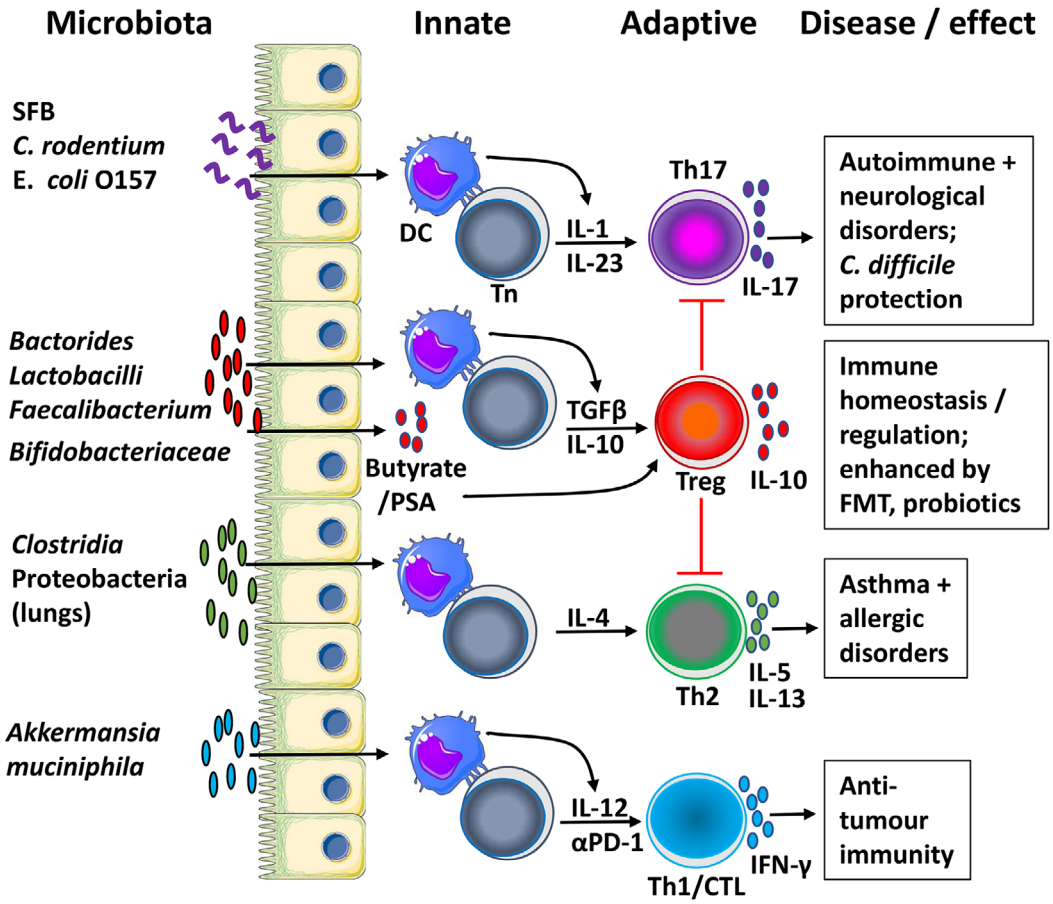

Figure 1. The microbiota influence a range of human disease by modulating innate and adaptive immune responses. Certain commensal bacteria of the gut and airways (bacterial species named are examples only) influence innate immune responses, including dendritic cells (DC), which promote differentiation of naïve T (Tn) cells into Th17 cells that mediate pathology in certain autoimmune disease (e.g. psoriasis, multiple sclerosis and rheumatoid arthritis) and neurological disorder (e.g. autism spectrum disorder) or Th2 cells that mediate pathology in asthma and allergy. For example, segmented filamentous bacteria (SFB), *Citrobacter rodentium*, and *E. coli* O157 promote Th17 responses by binding to epithelial cells and by enhancing innate IL-1 and IL-23 production. Th17 responses are protective against *C. difficile* infection. Other commensal bacteria activate innate IL-12 production, which drives Th1 cells and cytotoxic T lymphocytes and in the context of anti-PD1 treatment, promote anti-tumor immunity. Finally, distinct commensal bacteria that produce polysaccharide A or short chain fatty acids, such as butyrate, promote regulatory T (Treg) cells that help to maintain immune homeostasis and regulate Th2 and Th17 cells that mediate allergy/asthma and autoimmune diseases, respectively. Fetal microbiota transplantation (FMT) or probiotic treatment with anti-inflammatory bacteria can enhance Treg responses and are therefore protective against Th2- and Th17-mediated diseases.

receptors for SCFA, GPR43, or GPR41 are expressed on epithelial cells, macrophages, and dendritic cells (DCs). Mice deficient in GPR43 were unable to suppress commensal bacterial invasion into colonic tissue and develop chronic Th17-driven inflammation and intestinal carcinogenesis [32]. Butyrate a SCFA produced by commensal bacteria has anti-inflammatory properties, stimulating DCs to induce tolerogenic responses in naïve T cells. Human monocyte derived DCs stimulated with butyrate promoted induction of IL-10-producing type 1 regulatory T (Tr1) cells from naïve T cells [33, 34]. Another metabolite, polysaccharide A, produced by the ubiquitous gut microbe *Bacteroides fragilis* can trigger TLR2 signaling and expansion of Treg cells that produce the anti-inflammatory cytokine IL-10 [35]. Furthermore, in an inflammatory state, Ly6C^{hi} inflammatory monocytes respond to gut microbiota derived metabolites, such as SCFAs and polysaccharide A, to produce prostaglandin E_2, which inhibits neutrophil activation thus controlling inflammation and neutrophil-induced tissue damage [36].

The majority of bacteria in the GI tract are in the large intestine, but studies in mice [37] and pigs [38] have shown that the diversity of the microbiota are greater in the stomach and duodenum compared with the jejunum and ileum. Furthermore, treatment with antibiotic early in life induced dramatic changes in bacterial diversity, especially in the foregut, with an increase in abundances of potential pathogens and a reduction in bacterial strains known to play a beneficial regulatory role in control of Crohn's disease (CD) in humans. [38]. Therefore, frequent antibiotics treatment, especially early in life when the immune system is developing, may have a negative impact on the immune homeostasis in the gut and may increase susceptibility to immune-mediated diseases of the GI tract and at other sites in the body. A better understanding of the mechanisms involved in this dysbiosis is allowing the emergence

of new treatment approaches for inflammatory and other diseases based on approaches for manipulating the microbiome including fecal microbiota transplantation (FMT).

Clostridium difficile infection and fecal microbiota transplantation

Clostridium difficile is an anaerobic Gram-positive bacterium that produces two harmful toxins that cause colitis and severe diarrhea [39]. *C. difficile* infection is usually treated using a standard course of antibiotics, such as vancomycin or metronidazole, which is successful in controlling the initial infection, but fails to prevent its recurrence. Indeed antibiotic use can deplete the healthy gut microbial community, leading to dysbiosis and enable further outgrowth of *C. difficile* [40]. In contrast, FMT was reported to be more effective in the treatment of recurrent *C. difficile* infections than antibiotic treatment [41]. FMT is a process by which microbes are retrieved from the stool of a healthy individual and then transplanted into the gut of the recipient in an attempt to restore symbiosis in the intestine of that individual [42]. Patients who receive FMT were found to have an increased fecal bacterial diversity, with an increase in the abundance of Bacteroidetes species and a decrease in Proteobacteria [41]. Recent meta-analysis of clinical studies has demonstrated that FMT is a highly effective treatment for *C. difficile* [43]. However, a recent safety alert issued by the US Food and Drug Administration reported that two immunocompromised *C. difficile* infected patients contracted multidrug-resistant *E. coli* infections and one died after receiving an FMT from the same donor. (https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse). Therefore, while FMT has considerable promise in the treatment of *C. difficile* infection, donor microbiota need to be stringently screened for potentially pathogenic bacteria.

Inflammatory bowel diseases

IBDs, an umbrella term for UC and CD, are chronic inflammatory disease of the gut, where symptoms include recurrent diarrhea, vomiting, and abdominal pain [44]. The development of IBD is thought to involve complex interactions between genetics, the host immune system, and environmental factors, including the gut microbiome [45]. Defects in immune regulation, including reduced Treg cells, and enhanced Th1/Th17 cells and innate immune cells that produce IFN-γ, IL-17, or IL-22 have been implicated in the pathogenesis of IBD [46]. Studies in the naïve effector T-cell transfer to $RAG^{-/-}$ mouse model of colitis have demonstrated that microbiota-induced T-cell activation appears to be a key pathway in the development of intestinal inflammation [47]. Th1 and Th17 and IL-23-driven IL-17^{+}IFN-γ^{+} T cells play a pathogenic role in this model [48] and the role of T-bet and Th1 cells is influenced by the composition of the intestinal microbiota [49].

There is growing evidence of an imbalance in the gut microbiota in IBD patients [50, 51]. A comprehensive longitudinal study of microbial species using 16S RNA gene sequencing involving 49 patients with CD, 60 with UC, and 9 healthy controls showed significant differences in the diversity of the gut microbes of IBD patients versus controls [52]. *Enterobacteriaceae* were consistently associated with IBD patients compared with the abundant presence of *Ruminococcaceae* in control subjects [52]. Consistent with other studies, *Prevotella copri* and *Faecalibacterium prauznnitzii* were much less ubiquitous in IBD patients. Interestingly, *F. prauznnitzii* produces the anti-inflammatory metabolite butyrate [51]. A study on multiple molecular features of the gut microbiome in IBD patients performed as part of Human Microbiome Project 2 project revealed that patients with IBD have an increase in facultative anaerobes and a reduction of obligate anaerobes [53]. Interestingly, the severity of disease reflected a variability in the gut microbiota, with a high disease activity associated with a reduction in species that typically produce butyrate [53, 54].

On the basis that dysbiosis is a key characteristic of IBD, FMT has been explored as an alternative to conventional treatment approaches [55–57]. A rigorous meta-analysis of 18 studies in total amounting to 122 IBD patients showed a 45% remission rate [30]. A separate analysis of cohort studies revealed a success rate of 36% [58]. In a study by Moayyedi et al. where 25% of 75 UC patients treated with FMT went into remission, fecal microbiota from a particular donor induced remission in 39% of patients, while fecal microbiota from another five donors only induced remission in 10% of the subjects [59]. This effective donor was found to be colonized with high levels of *Ruminococcus* [59], highlighting the need for stringent screening of the microbiota from FMT donors.

The emerging data from analysis of microbiome in IBD patients together with the positive results from treatment with FMT provide convincing evidence that the gut microbiota may be key environmental factors in the development of chronic inflammatory pathology in the gut and/or triggering of disease flares in IBD patients. While the mechanism has not been established in humans, it is likely to involve microbial activation of innate immune responses through pattern recognition receptors on macrophages and DCs, leading to activation of pathogenic Th1/Th17 cells. Studies in the DSS-induced murine colitis model have shown that therapeutic FMT promotes IL-10-dependent suppression of APC and CD4 T cells that mediate colon inflammation [60]. Therefore, FMT in humans may also function through introduction of bacteria to the gut that promote regulatory immune responses, which suppress colon inflammation mediated by innate immune cells and T cells.

Obesity and associated diseases

Obesity is a major contributing factor in cardiovascular disease and type 2 diabetes (T2D). Long-term nutrient excess in obese individuals promotes chronic low-grade inflammation mediated by M1-type adipose tissue macrophages, Th1 cells, and CD8 T cells, leading to decreased insulin sensitivity and development of T2D [61]. Obesity has also been impacted in a range of other diseases,

including cancer, through modulation of immune cell function. For example, the anti-tumor function of NK cells is suppressed in obese individuals by modulation of cellular metabolism following lipid accumulation [62]. Evidence is emerging that the increased disease susceptibility, at least in part, reflects dysbiosis of the gut microbiota [63, 64] and as a consequence, modulated immune responses in obese individuals.

There is an imbalance in the composition of the microbiota in the gut of obese individuals, with a higher ratio of *Firmicutes* than *Bacteroidetes* compared with nonobese subjects [65]. There is also significantly fewer *Methanobrevibacter smithii* in an obese gut compared with the lean gut [66]. However, sampling of the upper GI tract in numerous studies failed to show an association among the microbial community with obesity, suggesting that the lower GI tract could play a role in obesity-related diseases [67, 68].

The microbiota can affect adaptive thermogenesis, which is regulated by brown adipose tissue, but is reduced in obesity [69]. Depletion of the microbiota with a cocktail of antibiotics suppressed the thermogenic capacity of brown adipose tissue by inhibiting expression of uncoupling protein 1 and reducing the browning of white adipose tissue [69]. This defect was reversed by oral administration of butyrate, suggesting that this anti-inflammatory metabolite of certain gut bacteria can help to maintain thermogenesis. Another microbiota product, indole, a metabolite of tryptophan metabolism, controls expression of white adipocytes mir-181, which protects against high fat diet (HFD) induced insulin resistance [70]. It has also been demonstrated that gut microbes can protect against HFD-induced inflammation and insulin resistance by metabolizing omega-6 polyunsaturated PUFAs that are abundant in Western diets and have been linked with obesity-related metabolic diseases [71]. Furthermore, administration of the gut-microbial metabolite, 10-hydroxy-*cis*-12-octadecenoic acid (HYA), reversed HFD-induced obesity and insulin resistance in mice. This was also achieved by colonization with HYA-expressing *Lactobacillus*.

The observation of altered gut microbiota in obesity suggested that FMT could have therapeutic effect in individuals suffering from associated metabolic disorders such as insulin resistance. Studies in mice have shown that antibiotic treatment alters genes involved with SCFA production, resulting in increased levels of tissue fat [72]. Furthermore, colonization of germ-free mice with gut microbes from obese, but not lean, mice resulted in an increase in body fat [73]. A human-mouse FMT study showed that transfer of fecal microbes from an obese twin led to a high level of body fat in the germ-free mouse, whereas the FMT from the lean twin did not [74]. Furthermore, when the obese FMT recipient mice were housed with the lean FMT recipient mice, they were found to be resistant to further weight gain. A study in T2D patients found improved insulin sensitivity, increased bacterial diversity in the intestine, and increased levels of SCFA-producing bacteria in obese subjects who received an FMT from lean donors [75]. Currently, there are a number of clinical trials evaluating the efficacy of FMT in obesity and metabolic disorders (https://clinicaltrials.gov). FMT is unlikely to be the treatment of choice for obesity-related metabolic disorders including T2D. However, long-term caloric restriction and exercise coupled with dietary interventions that promote diversity of the microbiota, especially an increase in "anti-inflammatory" species, may help to prevent or reverse the development of obesity-induced inflammation and associated metabolic disorders.

Influence of microbiome on neurodevelopmental and neurodegenerative disorders

Studies in mice have shown that viral infection during pregnancy can result in offspring displaying abnormal behavioral phenotypes that are associated with defects arising during neural development [76–78] and characteristic of those displayed in humans with autism spectrum disorder [79]. Furthermore, offspring from pregnant mice injected with poly(I:C), a TLR3 agonist and a mimic of viral infection, exhibit behavioral symptoms that were mediated by IL-17 cells and reversed by treatment of the mothers with anti-IL-17 [80]. Treatment of mice with broad-spectrum antibiotics before injection of poly(I:C) suppressed Th17 responses and IL-17A production and prevented development of behavioral abnormalities in the offspring [81]. SFB, which are highly susceptible to the broad-spectrum antibiotic vancomycin and stimulate Th17 cell production in the small intestine, were found to have a significant role in producing offspring with autism spectrum disorder like disorder [81]. Mice from Taconic Biosciences that had abundant SFB in their gut and thus a high level of Th17 cells displayed maternal immune activation associated behavior, whereas mice from Jackson Laboratories that lacked SFB and had fewer intestinal Th17 cells did not. This highlights the proinflammatory effect of the maternal intestinal SFB on the behavioral abnormalities of the offspring. Furthermore, it was shown that poly(I:C) stimulates intestinal $CD11c^+$ DCs in the pregnant mice to secrete IL-1β, IL-6, and IL-23, which promoted IL-17A production from intestinal Th17 cells, which mediated the maternal immune activation associated behavioral phenotypes [81].

Evidence is also emerging that neurological disorders in adult humans, including Parkinson's disease (PD) [82] and Alzheimer's disease (AD) [83], may be associated with changes in the gut microbiota. PD patients have altered colonic microbiota and microbiota metabolism [84] and this can influence the efficacy of L-Dopa, a drug commonly used to treat PD [85]. Recent evidence suggests that L-Dopa can be metabolized by gut bacteria and this can be inhibited by a drug that prevented L-Dopa decarboxylation [85]. Mouse studies with transgenic models of AD showed that perturbation of the gut microbiota with antibiotic led to a reduction in Aβ deposition in the brains of $APP_{SWE}/PS1_{\Delta E9}$ mice and this was associated with enhanced brain-resident Treg cells. [86]. Gut microbiota dysbiosis has been reported in patients with AD or mild cognitive impairment [87] and this may reflect reduced microbial diversity leading to enhanced inflammation in the elderly. One study found less diverse microbiota in older people in long-stay care and that this was associated with increased markers of inflammation and increased frailty and poorer health [88]. It is tempting to speculate that the proinflammatory effects of certain bacteria

in the gut or other mucosal surfaces may be an important environmental influence on the development of a range of neurological diseases. Therefore, dietary interventions that enhance gut microbial diversity, especially species that induce regulatory immune response, may help to delay the onset of certain age-related neurogenerative conditions in humans.

Asthma and allergic diseases

Asthma and a number of allergic diseases, including food allergies, anaphylaxis, rhinoconjunctivitis and eczema, are becoming increasingly prevalent, especially in developed countries where the incidence of allergy has more than doubled in the last 20 years [89]. Atopic individuals with a predisposition to becoming IgE sensitized now represent a quarter of the population in the developed world [90]. Dysbiosis of the gut microbiota [91, 92] as well as an absence of helminth infections [93] has been implicated in these diseases. A study of gut microbiome on infants' stool samples showed that low intestinal microbial diversity during the first month of life was associated with the development of atopic eczema at 2 years of age. [94]. A high level of *Clostridium* species and a lack of *Bifidobacterium* colonization have been found in the intestine of infants that would go on to develop allergies including food allergy [95–97]. Furthermore, the development of asthma, food allergy, or allergic rhinitis in 3-6-year-old children was associated with production of high levels of butyrate in the gut early in life [98]. Corresponding studies in mice showed that oral treatment with SCFAs attenuated allergic airway inflammation [98].

Recent findings have suggested that a mother's exposure to microbes during her pregnancy can prove to be more important in training the child's immune system to prevent the development of allergy than the child's own exposure later in life. In accordance with the ever-changing "hygiene hypothesis," investigations have focused on discrepancies between pregnancies that occur in agricultural settings versus nonfarmland environments. A study by Ege et al. showed that the development of allergies, including asthma, eczema, and rhinoconjunctivitis, was strongly influenced by prenatal exposure to farmland, whereas exposure to the agricultural environment postnatally had minimal effects [99]. An animal study where mice were exposed to dust from households with or without dogs found that mice exposed to dust from household with pets had reduced airway Th2 responses and inflammation in response to airway challenge with a cockroach allergen [100]. Analysis of the gut microbiomes revealed that the pet-exposed mice had high levels of *Lachnospiraceae, Bacillaceae, Peptococcaceae,* and *Lactobacillaceae* bacterial taxa, whereas *Lactobacillus johnsonii* were dominant in the control mice. Administration of *L. johnsonii* conferred protection against airway allergens in the susceptible mice [100]. The link between the microbiome and allergy is clear especially with reference to prenatal microbial exposure and suggests that prenatal manipulation of the microbiome could hold significant promise in the prevention of allergic disease.

Attempts to treat or prevent allergic diseases by manipulating the microbiota have largely though not exclusively focused on probiotics, live organisms that when administered in adequate amounts confer a health benefit to the host [101]. A study in a murine model of asthma showed that oral administration of the probiotic *Lactobacillus rhamnosus* GG prevented inflammation of the airways induced with a model allergen, ovalbumin (OVA); probiotic treatment reduced OVA-specific IgE in the serum [102]. The results of a double-blind, randomized placebo-controlled trial showed that administration of *Lactobacillus* GG prenatally to mothers who had a close relative or partner with atopic eczema, allergic rhinitis, or asthma, and postnatally for 6 months to their infants significantly reduced recurring atopic eczema in the first year of life [103]. However, a meta-analysis of literature in this area showed that prenatal or postnatal administration of probiotics alone had no effect on atopic diseases such as eczema. [104]. Indeed, there are conflicting data and concerns around the efficacy, mode of action, and methods for testing the effectiveness of probiotic in preventing or treating allergic and other diseases. One study showed that self-administration of probiotics during breastfeeding and directly to the infants enhanced the abundance of *Bifidobacterium* in the infant gut microbiota for 1 week only, but did not alter SCFA production or immune markers in breast milk, and surprisingly was associated with a higher incidence of mucosal infections in toddlers [105]. Further rigorous, controlled follow-up studies are required to determine the safety and efficacy of probiotic supplementation, in particular more stringent protocols and documentation are necessary to generate valid and conclusive data on their efficacy.

An alternative approach to probiotics for treating allergic disease has involved interventions with high-fiber diets that alter the microbiota of the gut and the lungs leading to reduced lung inflammation [106]. Ingested dietary fermentable fibers that are metabolized by bacteria like *Bifidobacteriaceae* in the gut of mice produced a higher concentration of SCFAs in circulation, which in turn inhibit Th2-mediated allergic airway inflammation [106]. Studies in mice have shown that probiotics can adapt in vivo, with more bacterial evolution occurring in mice fed a Western-type high-fat human diet, and less adaption in mice with a diverse microbiome following feeding a diet supplemented with the prebiotics inulin [107]. Therefore, the efficacy and safety of probiotics or engineered probiotics in treating asthma/allergy and other human diseases is likely to be influenced by the diversity of the gut microbiota in the individual patient, which in turn can be influenced by diet and antibiotic treatment.

The respiratory microbiome—asthma and respiratory diseases

The symbiosis of the lung microbial community is disturbed in certain respiratory diseases, such as asthma, where the abundance of *Prevotella* spp. is lower while the prevalence of Proteobacteria is higher [19]. When compared with the *Prevotella* bacteria, the Proteobacteria, *Hemophilus influenzae* B and *Moraxella catarrhalis* produce higher IL-23 and IL-12 from human DCs. Co-culture experiments revealed that the *Prevotella* spp. had

anti-inflammatory effects, down regulating IL-12 production in response to *H. influenzae* [108]. Complementary studies in a murine model revealed that *H*. influenzae induced severe chronic obstructive pulmonary disease like inflammation, with airway neutrophilia and inflammatory cytokine production in the lungs, whereas colonization with *Prevotella,* suppressed IL-8 and TNF-α production in the lung [109]. The difference in stimulatory capacity is may be due to differences in expression of microbe-associated molecular patterns, with *Prevotella* expressing penta-acylated LPS, whereas proteobacteria producing both hexa-acylated and hepta-acylated LPS; the stimulatory capacity of the hexa-acylated LPS via TLR4 was 100 times that of the penta-acylated LPS [110]. The outgrowth of pathogenic bacteria is a common marker for dysbiosis in respiratory disease. However, these studies illustrate that respiratory diseases may be influenced by a local gain of pathogenic species as well as a loss of commensal bacteria.

The emerging evidence of a relationship between lung microbiome dysbiosis and respiratory diseases suggests that manipulating the respiratory microbiome may have therapeutic promise in these diseases. However, it has been questioned whether a reduced abundance of *Prevotella* is a risk factor for disease onset or a product of an inhospitable environment caused by disease [20]. This ambiguity as well as more limited research on the respiratory microbiome in general has constrained attempts to treat asthma and related diseases such as chronic obstructive pulmonary disease by manipulation of the local microbiota. There is also growing evidence that gut-lung axis and gut microbiota induced inflammation may lead to the development of chronic inflammatory disorders of the respiratory as well as GI tract [111]. It has been demonstrated that autoimmunity of the lungs can be driven by Th17 cells that are induced by SFB in the gut by promoting inducible bronchus associated lymphoid tissues, a type of ectopic lymphoid tissues in the lung of rheumatoid arthritis patients [112]. Therefore, manipulating the gut microbiota may also be useful in the treatment of respiratory diseases. Furthermore, in acute respiratory distress syndrome, as well as in sepsis, the lung microbiome is enriched with gut-associated bacteria [113]. Not only does this provide evidence of shared mechanism of pathogenesis of these diseases, it suggests that their treatment could be enhanced by manipulating the microbiota at all mucosal surfaces.

Gut microbiome influences efficacy of cancer immunotherapy

Immune checkpoint inhibitors that target programmed cell death protein 1 (PD-1) and CTLA-4 are successful immunotherapeutics against melanoma and other cancers [114]. It has been reported that the composition of the gut microbiota may influence patient responses to these immune checkpoint inhibitors [115]. Administration of broad-spectrum antibiotics to patients with either advanced nonsmall cell lung cancer, renal cell carcinoma, or urothelial carcinoma, before or 1 month after receiving PD-1 blockade treatment, reduced progression-free survival and overall survival [116]. Quantitative shotgun metagenomic sequencing of intestinal microbiota from patients undergoing PD-1 treatment showed the presence of the commensal bacteria *Akkermansia muciniphila* was consistently found to be indicative of a more positive clinical outcome. Studies in mice involving FMT from the stools of responsive patient to antibiotic-treated mice with MCA-205 tumors conferred responsiveness treatment with anti-PD-1 [116]. Mice that received FMT from nonresponding patients did not respond to anti-PD1, suggesting that the intestinal microbiota of a patient can affect their responsiveness to immune checkpoint inhibitor immunotherapies. Moreover, administration of *A. muciniphila* into germ-free or antibiotic-treated mice with established melanoma that received FMT from nonresponding patients restored the responsiveness PD-1 blockade (Fig. 1). The reduction in tumor growth in mice that received FMT from response patients was associated with increases in tumor-infiltrating $CCR9^+CXCR3^+CD4^+$ T cells and was mediated by induction of IL-12 [116].

However, other studies in mice and humans have demonstrated that a range of microbial species in the gut, including Bacteroidetes and Bifidobacteria, can enhance the effectiveness of immune checkpoint inhibitors [117]. Furthermore, there does not appear to be as a single mechanism involved, although stimulation of DCs through pathogen recognition receptors, including the cGAS-STING [118] or TLR pathways, may explain the immune-enhancing function of certain gut microbiota.

As well as the influence of gut microbiota on the response to immune checkpoint inhibitors, the local microbiota in the tumor can influence progression of cancer as well as the patient responses to therapies. Bacterial colonization of the pancreas is enhanced in patients with pancreatic cancer [119]. A high proportion of patients with pancreatic ductal adenocarcinoma had intratumor bacteria, predominantly Gammaproteobacteria [120]. These intratumor bacteria can metabolize the chemotherapeutic drug gemcitabine, thereby contributing to drug resistance. Studies in mouse model of colon cancer showed that gemcitabine resistance mediated by intratumor Gammaproteobacteria could be reversed by antibiotic treatment [120]. It has also been demonstrated that the composition of intratumor microbiota may determine the immune response to the tumor and thereby influence patient survival. Long-term survivors of resected pancreatic adenocarcinoma had high alpha diversity within the intratumoral microbiome signature, including *Pseudoxanthomonas, Streptomyces, Saccharopolyspora* and *Bacillus clausii*, and these bacteria may enhance the recruitment and activation of anti-tumor $CD8^+$ T cells. [121]. Human to mouse FMT from long-term, but not short-term, human survivors of pancreatic cancer reduced tumor growth in mice. These findings suggest that antibiotic treatment combined with FMT may be a viable approach for enhancing the efficacy of chemotherapy or immunotherapies against pancreatic and other cancers.

Future Perspectives

Probiotic therapy has had rather limited success in treating immune-mediated diseases. As consequence researchers have

turned to other approaches for altering the gut microbiota, including FMT, but also commensal bacteria that have been genetically engineered to express anti-inflammatory molecules including IL-10. Treatment of mice with a genetically engineered strain of *Lactococcus lactis* capable of secreting IL-10 resulted in a 50% reduction in intestinal inflammation. [122]. The results of a phase 1 clinical trial with IL-10-secreting *L. lactis* in human CD patients revealed a clinical benefit in 8 of 10 patients, with 5 patients going into complete remission without inducing serious side effects [123]. In contrast, the results of a larger phase 2 trials failed to show any clinical benefit in UC patients (https://clinicaltrials.gov/ct2/show/NCT00729872)

IL-2 also has a protective role in murine colitis [124], and expression of IL-2 in the commensal bacteria *Bacteroides ovatus* has been assessed as a potential treatment for IBD. *B. ovatus* has a putative xylan promoter and insertion of the IL-2 gene into the xylanase operon allowed control of IL-2 production by dietary xylan [125]. The same bacterium was engineered to secrete human keratinocyte growth factor-2, which helps to maintain epithelial cell homeostasis in the gut and aids its repair [126]. Therapeutic studies in mice with colitis showed the recombinant bacteria reduced inflammation, accelerated repair of the gut epithelium, and improved stool consistency [126]. However, the translation of these to the treatment of human IBD will not be straightforward. There are safety concerns around the possible spread of the transgenic bacteria in vivo and horizontal transfer of the transgenes to WT bacteria in the GI tract [127].

Bacteriophages have also been explored as a therapeutic approach for manipulating the gut microbiome in order to remove pathogenic bacteria that promote inflammation in IBD. A cocktail of three virulent bacteriophages administered to mice expressing human carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), a host receptor for *E. coli*, significantly reduced the adherent invasive *E. coli,* which are prevalent in the mucosal lining of the ileum of CD patients [128]. Bacteriophages have also been assessed for their effect on multidrug-resistant strains of the opportunistic pathogen *Enterococcus faecalis;* treatment of mice with the lytic phage EF-P29 prevented infection with a vancomycin-resistant *E. faecalis* strain [129]. These studies suggest that phage therapy is a feasible approach for manipulating microbiota to restore symbiosis. For example, phages could be engineered to selectively eliminate the pathogenic proteobacteria implicated in asthma, enabling the outgrowth of the anti-inflammatory commensal *Prevotella* species. Although bacteria lysis may stimulate innate immune responses in the GI tract, phages have potential as a nontoxic therapeutic approach for diseases caused by dysbiosis of the intestinal microbiota [130].

Concluding remarks

There is a clear association between the composition of gut and respiratory microbiota and asthma, allergy, IBD, and obesity-related diseases and evidence is emerging of a link with neurodegenerative diseases. Although the mechanisms are not fully understood, evidence from mouse models has suggested that commensal and pathogenic bacteria in the gut and other mucosal surfaces can promote or regulate systemic inflammatory responses that mediate these diseases. FMT, engineered probiotics, and phage therapy have emerged as viable approaches for successful manipulation of the microbiome in the prevention as well as treatment of immune-mediated diseases.

Acknowledgements: This work was supported by grants from Science Foundation Ireland (16/IA/4468 and 12/RI/2340).

Conflict of interest: The authors declare no commercial or financial conflict of interest.

References

1 **Ursell, L. K., Metcalf, J. L., Parfrey, L. W. and Knight, R.**, Defining the human microbiome. *Nutr. Rev.* 2012. **70**(Suppl 1): S38–S44.

2 **Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R. and Gordon, J. I.**, The human microbiome project. *Nature* 2007. **449**: 804–810.

3 **Sender, R., Fuchs, S. and Milo, R.**, Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* 2016. **14**: e1002533.

4 **Shreiner, A. B., Kao, J. Y. and Young, V. B.**, The gut microbiome in health and in disease. *Curr. Opin. Gastroenterol.* 2015. **31**: 69–75.

5 Johnson, L. R., Ghishan, F. K., Kaunitz, J. D., Merchant, J. L., Said, H. M. **and** Wood, J. D. (Eds.) *Physiology of the gastrointestinal tract* (Fifth Edition). Academic Press, Boston 2012, pp I1-I31.

6 **Galindo-Villegas, J., García-Moreno, D., de Oliveira, S., Meseguer, J. and Mulero, V.**, Regulation of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development. *Proc. Natl. Acad. Sci. U S A* 2012. **109**: E2605-E2614.

7 **Round, J. L. and Mazmanian, S. K.**, The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 2009. **9**: 313–323.

8 **Macpherson, A. J. and Harris, N. L.**, Interactions between commensal intestinal bacteria and the immune system. *Nat. Rev. Immunol.* 2004. **4**: 478.

9 **Bates, J. M., Mittge, E., Kuhlman, J., Baden, K. N., Cheesman, S. E. and Guillemin, K.**, Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Develop. Biol.* 2006. **297**: 374–386.

10 **Bates, J. M., Akerlund, J., Mittge, E. and Guillemin, K.**, Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell Host Microbe* 2007. **2**: 371–382.

11 **Littman, D. R. and Pamer, E. G.**, Role of the commensal microbiota in normal and pathogenic host immune responses. *Cell Host Microbe* 2011. **10**: 311–323.

12 **Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. F. and Dinan, T. G.**, Minireview: gut microbiota: the neglected endocrine organ. *Mol. Endocrinol.* 2014. **28**: 1221–1238.

13 Human Microbiome Project, C. Structure, function and diversity of the healthy human microbiome. *Nature* 2012. **486**: 207–214.

14 **Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., Gonzalez, A. et al.**, Best practices for analysing microbiomes. *Nat. Rev. Microbiol.* 2018. **16**: 410–422.

15 Human Microbiome Project, C. A framework for human microbiome research. *Nature* 2012. **486**: 215–221.

16 **Dickson, R. P., Erb-Downward, J. R. and Huffnagle, G. B.**, The role of the bacterial microbiome in lung disease. *Expert Rev. Respir. Med.* 2013. **7**: 245–257.

17 **Singh, N., Vats, A., Sharma, A., Arora, A. and Kumar, A.**, The development of lower respiratory tract microbiome in mice. *Microbiome* 2017. **5**: 61.

18 **Radha, S., Afroz, T., Prasad, S. and Ravindra, N.**, Diagnostic utility of bronchoalveolar lavage. *J. Cytol.* 2014. **31**: 136–138.

19 **Hilty, M., Burke, C., Pedro, H., Cardenas, P., Bush, A., Bossley, C., Davies, J. et al.**, Disordered microbial communities in asthmatic airways. *PloS One* 2010. **5**: e8578.

20 **Larsen, J. M.**, The immune response to Prevotella bacteria in chronic inflammatory disease. *Immunology* 2017. **151**: 363–374.

21 **Huffnagle, G. B., Dickson, R. P. and Lukacs, N. W.**, The respiratory tract microbiome and lung inflammation: a two-way street. *Mucosal Immunol.* 2016. **10**: 299.

22 **Dickson, R. P. and Huffnagle, G. B.**, The lung microbiome: new principles for respiratory bacteriology in health and disease. *PLoS Pathog.* 2015. **11**: e1004923.

23 **Uriarte, S. M., Edmisson, J. S. and Jimenez-Flores, E.**, Human neutrophils and oral microbiota: a constant tug-of-war between a harmonious and a discordant coexistence. *Immunol. Rev.* 2016. **273**: 282–298.

24 **Kennedy, E. A., King, K. Y. and Baldridge, M. T.**, Mouse microbiota models: comparing germ-free mice and antibiotics treatment as tools for modifying gut bacteria. *Front. Physiol.* 2018. **9**: 1534.

25 **Ivanov, 2nd, Frutos Rde, L., Manel, N., Yoshinaga, K., Rifkin, D. B., Sartor, R. B., Finlay, B. B. et al.**, Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 2008. **4**: 337–349.

26 **Ivanov, 2nd, Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., Wei, D. et al.**, Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009. **139**: 485–498.

27 **Wu, H. J., Ivanov, 2nd, Darce, J., Hattori, K., Shima, T., Umesaki, Y., Littman, D. R. et al.**, Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 2010. **32**: 815–827.

28 **Chen, B., Chen, H., Shu, X., Yin, Y., Li, J., Qin, J., Chen, L. et al.**, Presence of segmented filamentous bacteria in human children and its potential role in the modulation of human gut immunity. *Front. Microbiol.* 2018. **9**: 1403.

29 **Atarashi, K., Tanoue, T., Ando, M., Kamada, N., Nagano, Y., Narushima, S., Suda, W. et al.**, Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell* 2015. **163**: 367–380.

30 **Belkaid, Y. and Hand, T. W.**, Role of the microbiota in immunity and inflammation. *Cell* 2014. **157**: 121–141.

31 **Canani, R. B., Costanzo, M. D., Leone, L., Pedata, M., Meli, R. and Calignano, A.**, Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World J. Gastroenterol.* 2011. **17**: 1519–1528.

32 **Kim, M., Friesen, L., Park, J., Kim, H. M. and Kim, C. H.**, Microbial metabolites, short-chain fatty acids, restrain tissue bacterial load, chronic inflammation, and associated cancer in the colon of mice. *Eur. J. Immunol.* 2018. **48**: 1235–1247.

33 **Davie, J. R.**, Inhibition of histone deacetylase activity by butyrate. *J.Nutr.*2003. **133**: 2485S–2493S.

34 **Kaisar, M. M. M., Pelgrom, L. R., van der Ham, A. J., Yazdanbakhsh, M. and Everts, B.**, Butyrate conditions human dendritic cells to prime type 1 regulatory T cells via both histone deacetylase inhibition and G protein-coupled receptor 109A signaling. *Front. Immunol.* 2017. **8**: 1429-1429.

35 **Round, J. L., Lee, S. M., Li, J., Tran, G., Jabri, B., Chatila, T. A. and Mazmanian, S. K.**, The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 2011. **332**: 974–977.

36 **Grainger, J. R., Wohlfert, E. A., Fuss, I. J., Bouladoux, N., Askenase, M. H., Legrand, F., Koo, L. Y. et al.**, Inflammatory monocytes regulate pathologic responses to commensals during acute gastrointestinal infection. *Nat. Med.* 2013. **19**: 713–721.

37 **Gu, S., Chen, D., Zhang, J. N., Lv, X., Wang, K., Duan, L. P., Nie, Y. et al.**, Bacterial community mapping of the mouse gastrointestinal tract. *PLoS One* 2013. **8**: e74957.

38 **Mu, C., Yang, Y., Su, Y., Zoetendal, E. G. and Zhu, W.**, Differences in microbiota membership along the gastrointestinal tract of piglets and their differential alterations following an early-life antibiotic intervention. *Front. Microbiol.* 2017. **8**: 797.

39 **Ofosu, A.**, Clostridium difficile infection: a review of current and emerging therapies. *Ann. Gastroenterol.* 2016. **29**: 147–154.

40 **Dethlefsen, L., Huse, S., Sogin, M. L. and Relman, D. A.**, The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol.* 2008. **6**: e280.

41 **van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E. G., de Vos, W. M., Visser, C. E. et al.**, Duodenal infusion of donor feces for recurrent Clostridium difficile. *New Engl. J. Med.* 2013. **368**: 407–415.

42 **Chanyi, R. M., Craven, L., Harvey, B., Reid, G., Silverman, M. J. and Burton, J. P.**, Faecal microbiota transplantation: where did it start? what have studies taught us? where is it going? *SAGE Open Med.* 2017. **5**: 2050312117708712.

43 **Quraishi, M. N., Widlak, M., Bhala, N., Moore, D., Price, M., Sharma, N. and Iqbal, T. H.**, Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. *Alimentary Pharmacol. Ther.* 2017. **46**: 479–493.

44 **Fakhoury, M., Negrulj, R., Mooranian, A. and Al-Salami, H.**, Inflammatory bowel disease: clinical aspects and treatments. *J. Inflamm. Res.* 2014. **7**: 113–120.

45 **Khor, B., Gardet, A. and Xavier, R. J.**, Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011. **474**: 307–317.

46 **Friedrich, M., Pohin, M. and Powrie, F.**, Cytokine networks in the pathophysiology of inflammatory bowel disease. *Immunity* 2019. **50**: 992–1006.

47 **Feng, T., Wang, L., Schoeb, T. R., Elson, C. O. and Cong, Y.**, Microbiota innate stimulation is a prerequisite for T cell spontaneous proliferation and induction of experimental colitis. *J. Exp. Med.* 2010. **207**: 1321–1332.

48 **Ahern, P. P., Schiering, C., Buonocore, S., McGeachy, M. J., Cua, D. J., Maloy, K. J. and Powrie, F.**, Interleukin-23 drives intestinal inflammation through direct activity on T cells. *Immunity* 2010. **33**: 279–288.

49 **Zimmermann, J., Durek, P., Kuhl, A. A., Schattenberg, F., Maschmeyer, P., Siracusa, F., Lehmann, K. et al.**, The intestinal microbiota determines the colitis-inducing potential of T-bet-deficient Th cells in mice. *Eur. J. Immunol.* 2018. **48**: 161–167.

50 **Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L. G., Gratadoux, J.-J., Blugeon, S. et al.**, Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut

microbiota analysis of Crohn's disease patients. *Proc. Natl. Acad. Sci. U S A* 2008. **105**: 16731–16736.

51 **Rajca, S., Grondin, V., Louis, E., Vernier-Massouille, G., Grimaud, J.-C., Bouhnik, Y., Laharie, D. et al.**, Alterations in the intestinal microbiome (dysbiosis) as a predictor of relapse after infliximab withdrawal in Crohn's disease. *Inflamm. Bowel Dis.* 2014. **20**: 978–986.

52 **Halfvarson, J., Brislawn, C. J., Lamendella, R., Vázquez-Baeza, Y., Walters, W. A., Bramer, L. M., D'Amato, M. et al.**, Dynamics of the human gut microbiome in inflammatory bowel disease. *Nat. Microbiol.* 2017. **2**: 17004-17004.

53 **Lloyd-Price, J., Arze, C., Ananthakrishnan, A. N., Schirmer, M., Avila-Pacheco, J., Poon, T. W., Andrews, E. et al.**, Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019. **569**: 655–662.

54 **Morgan, X. C., Tickle, T. L., Sokol, H., Gevers, D., Devaney, K. L., Ward, D. V., Reyes, J. A. et al.**, Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol.* 2012. **13**: R79.

55 **Kunde, S., Pham, A., Bonczyk, S., Crumb, T., Duba, M., Conrad, H., Jr., Cloney, D. et al.**, Safety, tolerability, and clinical response after fecal transplantation in children and young adults with ulcerative colitis. *J. Pediatr. Gastroenterol. Nutr.* 2013. **56**: 597–601.

56 **Suskind, D. L., Singh, N., Nielson, H. and Wahbeh, G.**, Fecal microbial transplant via nasogastric tube for active pediatric ulcerative colitis. *J. Pediatr. Gastroenterol. Nutr.* 2015. **60**: 27–29.

57 **Cui, B., Feng, Q., Wang, H., Wang, M., Peng, Z., Li, P., Huang, G. et al.**, Fecal microbiota transplantation through mid-gut for refractory Crohn's disease: safety, feasibility, and efficacy trial results. *J. Gastroenterol. Hepatol.* 2015. **30**: 51–58.

58 **Colman, R. J. and Rubin, D. T.**, Fecal microbiota transplantation as therapy for inflammatory bowel disease: a systematic review and meta-analysis. *J. Crohn's Colitis* 2014. **8**: 1569–1581.

59 **Moayyedi, P., Surette, M. G., Kim, P. T., Libertucci, J., Wolfe, M., Onischi, C., Armstrong, D. et al.**, Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* 2015. **149**: 102–109.e106.

60 **Burrello, C., Garavaglia, F., Cribiu, F. M., Ercoli, G., Lopez, G., Troisi, J., Colucci, A. et al.**, Therapeutic faecal microbiota transplantation controls intestinal inflammation through IL10 secretion by immune cells. *Nat. Commun.* 2018. **9**: 5184.

61 **Shu, C. J., Benoist, C. and Mathis, D.**, The immune system's involvement in obesity-driven type 2 diabetes. *Semin. Immunol.* 2012. **24**: 436–442.

62 **Michelet, X., Dyck, L., Hogan, A., Loftus, R. M., Duquette, D., Wei, K., Beyaz, S. et al.**, Metabolic reprogramming of natural killer cells in obesity limits antitumor responses. *Nat. Immunol.* 2018. **19**: 1330–1340.

63 **Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T. et al.**, A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010. **464**: 59–65.

64 **Ley, R. E., Turnbaugh, P. J., Klein, S. and Gordon, J. I.**, Human gut microbes associated with obesity. *Nature* 2006. **444**: 1022.

65 **Kasai, C., Sugimoto, K., Moritani, I., Tanaka, J., Oya, Y., Inoue, H., Tameda, M. et al.**, Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol.* 2015. **15**: 100.

66 **Million, M., Maraninchi, M., Henry, M., Armougom, F., Richet, H., Carrieri, P., Valero, R. et al.**, Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. *Int. J. Obesity* 2012. **36**: 817–825.

67 **Lin, S.-W., Freedman, N. D., Shi, J., Gail, M. H., Vogtmann, E., Yu, G., Klepac-Ceraj, V. et al.**, Beta-diversity metrics of the upper digestive tract microbiome are associated with body mass index. *Obesity* 2015. **23**: 862–869.

68 **Angelakis, E., Armougom, F., Carrière, F., Bachar, D., Laugier, R., Lagier, J.-C., Robert, C. et al.**, A metagenomic investigation of the duodenal microbiota reveals links with obesity. *PloS One* 2015. **10**: e0137784.

69 **Li, B., Li, L., Li, M., Lam, S. M., Wang, G., Wu, Y., Zhang, H. et al.**, Microbiota depletion impairs thermogenesis of brown adipose tissue and browning of white adipose tissue. *Cell Rep.* 2019. **26**: 2720–2737.e2725.

70 **Virtue, A. T., McCright, S. J., Wright, J. M., Jimenez, M. T., Mowel, W. K., Kotzin, J. J., Joannas, L. et al.**, The gut microbiota regulates white adipose tissue inflammation and obesity via a family of microRNAs. *Sci. Transl. Med.* 2019. **11**: https://doi.org/10.1126/scitranslmed.aav1892.

71 **Miyamoto, J., Igarashi, M., Watanabe, K., Karaki, S. I., Mukouyama, H., Kishino, S., Li, X. et al.**, Gut microbiota confers host resistance to obesity by metabolizing dietary polyunsaturated fatty acids. *Nat. Commun.* 2019. **10**: 4007.

72 **Cho, I., Yamanishi, S., Cox, L., Methé, B. A., Zavadil, J., Li, K., Gao, Z. et al.**, Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 2012. **488**: 621–626.

73 **Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R. and Gordon, J. I.**, An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006. **444**: 1027.

74 **Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., Griffin, N. W. et al.**, Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013. **341**: 1241214.

75 **Vrieze, A., Van Nood, E., Holleman, F., Salojärvi, J., Kootte, R. S., Bartelsman, J. F. W. M., Dallinga–Thie, G. M. et al.**, Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012. **143**: 913–916.e917.

76 **Minakova, E., Lang, J., Medel-Matus, J.-S., Gould, G. G., Reynolds, A., Shin, D., Mazarati, A. et al.**, Melanotan-II reverses autistic features in a maternal immune activation mouse model of autism. *PloS One* 2019. **14**: e0210389.

77 **Bergdolt, L. and Dunaevsky, A.**, Brain changes in a maternal immune activation model of neurodevelopmental brain disorders. *Prog. Neurobiol.* 2019. **175**: 1–19.

78 **Wang, X., Yang, J., Zhang, H., Yu, J. and Yao, Z.**, Oral probiotic administration during pregnancy prevents autism-related behaviors in offspring induced by maternal immune activation via anti-inflammation in mice. *Autism Res.* 2019. **12**: 576–588.

79 **Barak, B. and Feng, G.**, Neurobiology of social behavior abnormalities in autism and Williams syndrome. *Nat. Neurosci.* 2016. **19**: 647–655.

80 **Choi, G. B., Yim, Y. S., Wong, H., Kim, S., Kim, H., Kim, S. V., Hoeffer, C. A. et al.**, The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science* 2016. **351**: 933–939.

81 **Kim, S., Kim, H., Yim, Y. S., Ha, S., Atarashi, K., Tan, T. G., Longman, R. S. et al.**, Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* 2017. **549**: 528–532.

82 **Li, C., Cui, L., Yang, Y., Miao, J., Zhao, X., Zhang, J., Cui, G. et al.**, Gut microbiota differs between Parkinson's disease patients and healthy controls in northeast China. *Front. Mol. Neurosci.* 2019. **12**: 171.

83 **Cattaneo, A., Cattane, N., Galluzzi, S., Provasi, S., Lopizzo, N., Festari, C., Ferrari, C. et al.**, Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol. Aging* 2017. **49**: 60–68.

84 **Bedarf, J. R., Hildebrand, F., Coelho, L. P., Sunagawa, S., Bahram, M., Goeser, F., Bork, P. et al.**, Functional implications of microbial and viral

gut metagenome changes in early stage L-DOPA-naive Parkinson's disease patients. *Genome Med*. 2017. **9**: 39.

85 **Maini Rekdal, V., Bess, E. N., Bisanz, J. E., Turnbaugh, P. J. and Balskus, E. P.**, Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. *Science* 2019. **364**: https://doi.org/10.1126/science.aau6323

86 **Minter, M. R., Hinterleitner, R., Meisel, M., Zhang, C., Leone, V., Zhang, X., Oyler-Castrillo, P. et al.**, Antibiotic-induced perturbations in microbial diversity during post-natal development alters amyloid pathology in an aged APPSWE/PS1DeltaE9 murine model of Alzheimer's disease. *Sci Rep*. 2017. **7**: 10411.

87 **Li, B., He, Y., Ma, J., Huang, P., Du, J., Cao, L., Wang, Y. et al.**, Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota. *Alzheimers Dement*. 2019. **15**: 1357–1366.

88 **Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., Harris, H. M. et al.**, Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012. **488**: 178–184.

89 **Galli, S. J., Tsai, M. and Piliponsky, A. M.**, The development of allergic inflammation. *Nature* 2008. **454**: 445–454.

90 **Giangrieco, I., Rafaiani, C., Liso, M., Palazzo, P., Pomponi, D., Tuppo, L., Crescenzo, R. et al.**, Allergens in allergy diagnosis: a glimpse at emerging new concepts and methodologies. *Transl. Med. UniSa*. 2012. **4**: 27–33.

91 **Bisgaard, H., Li, N., Bonnelykke, K., Chawes, B. L. K., Skov, T., Paludan-Müller, G., Stokholm, J. et al.**, Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J. Allergy Clin. Immunol*. 2011. **128**: 646–652.e645.

92 **McAleer, J. P. and Kolls, J. K.**, Contributions of the intestinal microbiome in lung immunity. *Eur. J. Immunol*. 2018. **48**: 39–49.

93 **Finlay, C. M., Walsh, K. P. and Mills, K. H.**, Induction of regulatory cells by helminth parasites: exploitation for the treatment of inflammatory diseases. *Immunol. Rev*. 2014. **259**: 206–230.

94 **Abrahamsson, T. R., Jakobsson, H. E., Andersson, A. F., Björkstén, B., Engstrand, L. and Jenmalm, M. C.**, Low diversity of the gut microbiota in infants with atopic eczema. *J. Allergy Clin. Immunol*. 2012. **129**: 434–440.e432.

95 **Björkstén, B., Sepp, E., Julge, K., Voor, T. and Mikelsaar, M.**, Allergy development and the intestinal microflora during the first year of life. *J. Allergy Clin*. 2001. **108**: 516–520.

96 **Woodcock, A., Moradi, M., Smillie, F. I., Murray, C. S., Burnie, J. P. and Custovic, A.**, Clostridium difficile, atopy and wheeze during the first year of life. *Pediatr. Allergy Immunol*. 2002. **13**: 357–360.

97 **Tanaka, M., Korenori, Y., Washio, M., Kobayashi, T., Momoda, R., Kiyohara, C., Kuroda, A. et al.**, Signatures in the gut microbiota of Japanese infants who developed food allergies in early childhood. *FEMS Microbiol. Ecol*. 2017. **93**: https://doi.org/10.1093/femsec/fix099.

98 **Roduit, C., Frei, R., Ferstl, R., Loeliger, S., Westermann, P., Rhyner, C., Schiavi, E. et al.**, High levels of butyrate and propionate in early life are associated with protection against atopy. *Allergy* 2019. **74**: 799–809.

99 **Ege, M. J., Bieli, C., Frei, R., van Strien, R. T., Riedler, J., Üblagger, E., Schram-Bijkerk, D. et al.**, Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J. Allergy Clinic. Immunol*. 2006. **117**: 817–823.

100 **Fujimura, K. E., Demoor, T., Rauch, M., Faruqi, A. A., Jang, S., Johnson, C. C., Boushey, H. A. et al.**, House dust exposure mediates gut microbiome Lactobacillus enrichment and airway immune defense against allergens and virus infection. *Proc. Natl. Acad. Sci. U S A* 2014. **111**: 805–810.

101 **Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N. and Fakiri, E. M.**, Health benefits of probiotics: a review. *ISRN Nutr*. 2013. **2013**: 481651.

102 **Wu, C.-T., Chen, P.-J., Lee, Y.-T., Ko, J.-L. and Lue, K.-H.**, Effects of immunomodulatory supplementation with Lactobacillus rhamnosus on airway inflammation in a mouse asthma model. *J. Microbiol. Immunol. Infect*. 2016. **49**: 625–635.

103 **Kalliomäki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P. and Isolauri, E.**, Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001. **357**: 1076–1079.

104 **Zhang, G.-Q., Hu, H.-J., Liu, C.-Y., Zhang, Q., Shakya, S. and Li, Z.-Y.**, Probiotics for prevention of atopy and food hypersensitivity in early childhood: a PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine* 2016. **95**: e2562-e2562.

105 **Quin, C., Estaki, M., Vollman, D. M., Barnett, J. A., Gill, S. K. and Gibson, D. L.**, Probiotic supplementation and associated infant gut microbiome and health: a cautionary retrospective clinical comparison. *Sci. Rep*. 2018. **8**: 8283.

106 **Trompette, A., Gollwitzer, E. S., Yadava, K., Sichelstiel, A. K., Sprenger, N., Ngom-Bru, C., Blanchard, C. et al.**, Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med*. 2014. **20**: 159.

107 **Crook, N., Ferreiro, A., Gasparrini, A. J., Pesesky, M. W., Gibson, M. K., Wang, B., Sun, X. et al.**, Adaptive strategies of the candidate probiotic E. coli nissle in the mammalian gut. *Cell Host Microbe* 2019. **25**: 499–512.e498.

108 **Larsen, J. M., Steen-Jensen, D. B., Laursen, J. M., Søndergaard, J. N., Musavian, H. S., Butt, T. M. and Brix, S.**, Divergent pro-inflammatory profile of human dendritic cells in response to commensal and pathogenic bacteria associated with the airway microbiota. *PloS One* 2012. **7**: e31976.

109 **Larsen, J. M., Musavian, H. S., Butt, T. M., Ingvorsen, C., Thysen, A. H. and Brix, S.**, Chronic obstructive pulmonary disease and asthma-associated Proteobacteria, but not commensal Prevotella spp., promote toll-like receptor 2-independent lung inflammation and pathology. *Immunology* 2015. **144**: 333–342.

110 **Brix, S., Eriksen, C., Larsen, J. M. and Bisgaard, H.**, Metagenomic heterogeneity explains dual immune effects of endotoxins. *J.Allergy Clin. Immunol*. 2015. **135**: 277–280.

111 **Budden, K. F., Gellatly, S. L., Wood, D. L., Cooper, M. A., Morrison, M., Hugenholtz, P. and Hansbro, P. M.**, Emerging pathogenic links between microbiota and the gut-lung axis. *Nat. Rev. Microbiol*. 2017. **15**: 55–63.

112 **Bradley, C. P., Teng, F., Felix, K. M., Sano, T., Naskar, D., Block, K. E., Huang, H. et al.**, Segmented filamentous bacteria provoke lung autoimmunity by inducing gut-lung axis Th17 cells expressing dual TCRs. *Cell Host Microbe* 2017. **22**: 697–704.e694.

113 **Dickson, R. P., Singer, B. H., Newstead, M. W., Falkowski, N. R., Erb-Downward, J. R., Standiford, T. J. and Huffnagle, G. B.**, Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. *Nat. Microbiol*. 2016. **1**: 16113.

114 **Dyck, L. and Mills, K. H. G.**, Immune checkpoints and their inhibition in cancer and infectious diseases. *Eur. J. Immunol*. 2017. **47**: 765–779.

115 **Gong, J., Chehrazi-Raffle, A., Placencio-Hickok, V., Guan, M., Hendifar, A. and Salgia, R.**, The gut microbiome and response to immune checkpoint inhibitors: preclinical and clinical strategies. *Clin. Transl. Med*. 2019. **8**: 9.

116 **Routy, B., Le Chatelier, E., Derosa, L., Duong, C. and Dailere, R.**, Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science* 2017. **359**: 91–97.

117 **Frankel, A. E., Deshmukh, S., Reddy, A., Lightcap, J., Hayes, M., McClellan, S., Singh, S. et al.**, Cancer immune checkpoint inhibitor therapy and the gut microbiota. *Integr. Cancer Ther*. 2019. **18**: 1534735419846379.

118 **Wang, H., Hu, S., Chen, X., Shi, H., Chen, C., Sun, L. and Chen, Z. J.**, cGAS is essential for the antitumor effect of immune checkpoint blockade. *Proc. Natl. Acad. Sci. U S A* 2017. **114**: 1637–1642.

119 **Pushalkar, S., Hundeyin, M., Daley, D., Zambirinis, C. P., Kurz, E., Mishra, A., Mohan, N. et al.**, The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. *Cancer Discov.* 2018. **8**: 403–416.

120 **Geller, L. T., Barzily-Rokni, M., Danino, T., Jonas, O. H., Shental, N., Nejman, D., Gavert, N. et al.**, Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* 2017. **357**: 1156–1160.

121 **Riquelme, E., Zhang, Y., Zhang, L., Montiel, M., Zoltan, M., Dong, W., Quesada, P. et al.**, Tumor microbiome diversity and composition influence pancreatic cancer outcomes. *Cell* 2019. **178**: 795–806.e712.

122 **Steidler, L., Hans, W., Schotte, L., Neirynck, S., Obermeier, F., Falk, W., Fiers, W. et al.**, Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* 2000. **289**: 1352.

123 **Braat, H., Rottiers, P., Hommes, D. W., Huyghebaert, N., Remaut, E., Remon, J. P., van Deventer, S. J. et al.**, A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin. Gastroenterol. Hepatol.* 2006. **4**: 754–759.

124 **Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A. C. and Horak, I.**, Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 1993. **75**: 253–261.

125 **Farrar, M. D., Whitehead, T. R., Lan, J., Dilger, P., Thorpe, R., Holland, K. T. and Carding, S. R.**, Engineering of the gut commensal bacterium Bacteroides ovatus to produce and secrete biologically active murine interleukin-2 in response to xylan. *J. Appl. Microbiol.* 2005. **98**: 1191–1197.

126 **Hamady, Z. Z. R., Scott, N., Farrar, M. D., Lodge, J. P. A., Holland, K. T., Whitehead, T. and Carding, S. R.**, Xylan-regulated delivery of human keratinocyte growth factor-2 to the inflamed colon by the human anaerobic commensal bacterium *Bacteroides ovatus*. *Gut* 2010. **59**: 461.

127 **Wegmann, U., Carvalho, A. L., Stocks, M. and Carding, S. R.**, Use of genetically modified bacteria for drug delivery in humans: revisiting the safety aspect. *Sci. Rep.* 2017. **7**: 2294-2294.

128 **Galtier, M., Sordi, L. D., Sivignon, A., de Vallée, A., Maura, D., Neut, C., Rahmouni, O. et al.**, Bacteriophages targeting adherent invasive Escherichia coli strains as a promising new treatment for Crohn's disease. *J. Crohn's Colitis* 2017. **11**: 840–847.

129 **Cheng, M., Liang, J., Zhang, Y., Hu, L., Gong, P., Cai, R., Zhang, L. et al.**, The bacteriophage EF-P29 efficiently protects against lethal vancomycin-resistant Enterococcus faecalis and alleviates gut microbiota imbalance in a murine bacteremia model. *Front. Microbiol.* 2017. **8**: 837-837.

130 **Loc-Carrillo, C. and Abedon, S. T.**, Pros and cons of phage therapy. *Bacteriophage* 2011. **1**: 111–114.

Abbreviations: **AD**: Alzheimer's disease · **CD**: Crohn's disease · **FMT**: fecal microbiota transplantation · **GI**: gastrointestinal · **HFD**: high fat diet · **IBD**: inflammatory bowel disease · **PD**: Parkinson's disease · **PD-1**: programmed cell death protein 1 · **SCFA**: short chain fatty acid · **SFB**: segmented filamentous bacteria · **T2D**: type 2 diabetes · **UC**: ulcerative colitis

Full correspondence: Prof. Kingston Mills, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, D02 R590, Ireland.
e-mail: kingston.mills@tcd.ie

Received: 1/10/2019
Revised: 3/12/2019
Accepted: 27/1/2020
Accepted article online: 28/1/2020

