Emerging Concepts on the Gut Microbiome and Multiple Sclerosis

Justin D. Glenn and Ellen M. Mowry

Microbiota of the human body perform fundamental tasks that contribute to normal development, health, and homeostasis and are intimately associated with numerous organ systems, including the gut. Microbes begin gut inhabitation immediately following birth and promote proper gut epithelial construction and function, metabolism and nutrition, and immune system development. Inappropriate immune recognition of self-tissue can lead to autoimmune disease, including conditions such as multiple sclerosis (MS), in which the immune system recognizes and attacks central nervous system tissue. Preclinical studies have demonstrated a requirement of gut microbiota for neuroinflammatory autoimmune disease in animal models, and a growing number of clinical investigations are finding associations between MS status and the composition of the gut microbiota. In this review, we examine current undertakings into better understanding the role of gut bacteria and their phages in MS development, review associations of the gut microbiota makeup and MS, and discuss potential mechanisms by which the gut microbiota may be manipulated for therapeutic benefit.

Introduction

THE HUMAN BODY is an exquisite composition of cells derived from every taxonomic domain. Over time, human eukaryotic cells, and those of most other higher-level mammals, have evolved to acquire diverse structural and functional phenotypes that are grouped into higher-level organs and systems that work in concert to promote a stable well-being (homeostasis) and survival of the organism. These cell-based systems vary in anatomical space, and as such may be relatively sessile (ie, central nervous system) or dispersed throughout the organism (ie, the immune system). Interspersed on and within various human cell-based systems are also collections of diverse nonhuman microbial organisms and entities.

The total microbial collection within a specific anatomical niche is known as microbiota, while the entirety of genes derived from the microbiota comprises its microbiome. It is estimated that microbes of the collective microbiota outnumber human somatic and germline cells by a factor of 10 and inhabit vast stretches of human skin and mucosa, especially of the lung and genital microenvironments, though the largest collection (~70%) resides in the gut (Savage 1977; Backhed and others 2005). Of the different microbial domains composing the microbiome, we will focus on bacteria and viruses, as these are the most numerous and highly investigated members. Current methods to study bacteria in the gut consist of collecting DNA from stool and using high-

throughput gene sequencing of bacterial 16S rRNA and bioinformatics analyses (Backhed and others 2005; Jandhyala and others 2015). Bacteriophages, which are the most abundant viral entities of the gut, are studied by viral particle concentration from stool, elimination of contaminating cells and nucleic acids, amplifying viral sequences and metagenomics analyses (Dalmasso and others 2014).

The gut microbiota lies in close association with the host immune system and both play crucial roles in regulating gut development and immunity. Altered host immunogenetics and environmental factors can result in disease pathogenesis as in the case of autoimmune disease. A growing number of studies are exploring the contributions of the gut microbiota to the development of multiple sclerosis (MS) and suggest this field as a potential novel target for MS therapeutics and prevention.

The Gut Microbiome

Bacteria

The adult human intestine is populated by an astonishing 100 trillion microbes, and its microbiome contains at least 100 times the number of genes of the human genome (Savage 1977; Backhed and others 2005). Indeed, bacteria within this environment reach the highest cellular densities of any ecosystem studied (Whitman and others 1998). Given the enormity of this population, it is surprising that at the division level, bacterial diversity diminishes. Only 8 of 55

Division of Neuroimmunology and Neurological Infections, Department of Neurology, Johns Hopkins University, School of Medicine, Baltimore, Maryland.

known bacterial divisions have been observed in the gut (Hugenholz and others 1998; Eckburg and others 2005). Those of the divisions cytophaga-flavobacterium-bacteroides and Firmicutes reach considerably large percentages at ~30% of bacteria each, while Proteobacteria and Actinobacteria are observed but at low abundance; it is this bacteria divisional makeup that suggests a healthy human gut (Backhed and others 2005; Eckburg and others 2005; Jandhyala and others 2015).

Infants are rapidly colonized by bacteria shortly after birth from the mother and environment, and infant mode of delivery is thought to impact bacterial colonization. Babies born by vaginal delivery (Andersch-Bjorkman and others 2007) are exposed to maternal vaginal and intestinal microbiota, while those born via caesarian section delivery (CS) lack these contacts (Neu and Rushing 2011). Studies have shown that infants born by CS have delayed bacterial colonization, which may even last for 6 months (Gronlund and others 1999). Furthermore, delivery-based differences in the intestinal microbial makeup has been observed in 7-year old children, with those by vaginal delivery having significantly higher numbers of Clostridia (Salminen and others 2004). Long term, those delivered via CS may exhibit an abnormal development of the immune system due to abnormal infant GI tract colonization (Neu and Rushing 2011).

Bacteria of the gut microbiota contribute tremendously to maintaining healthy human physiology, with many functions that can only be achieved by bacterial intervention. These functions largely consist of (a) complex carbohydrate metabolism and nutrient extraction; (b) xenobiotic metabolism; (c) antimicrobial protection; (d) promoting gut barrier function; and (e) contributing to shape gut immunity (Fig. 1).

Viruses

In addition to bacteria, viruses are even more bountiful in the human gut and comprise both eukaryotic and prokaryotic types. We will highlight prokaryotic viruses, particularly the bacteriophages, as these are the most numerous biological entities of the human gut and engage in complex

interactions with gut bacteria of the microbiome. Bacteriophages (phages) can be extracted from human feces at 10^9 virus-like particles (VLP) per gram and may inhabit up to 10^{14} bacterial cells in the gut (Rohwer 2003; Breitbart and others 2008). Phages vary in the types of bacterial surface molecules that they target for binding, and thus viral tropisms exist for certain strains of bacteria for a given bacterial species. Generally, phages exhibit 2 lifecycles: the lytic phase/lifecycle, in which a phage infects the bacterial cell and kills it upon replication and viral release, and the lysogenic phase/lifecycle, in which the phage infects the bacterial cell and integrates its viral genome into the host bacterial genome without inducing lysis and simultaneously maintains its genome in a latent form (prophage) (Dalmasso and others 2014). Under a range of stimuli, the prophage may exit latency and enter the lytic phase.

The principle intestinal phages consist of (1) the tailed, double-stranded DNA viruses of the order Caudovirales (Podoviridae, Siphoviridae, and Myoviridae) and (2) the tailless, cubic or filamentous, single-stranded DNA viruses of Microviridae (Ackermann 2009; Minot and others 2011; Foca and others 2015). Phages appear in the newborn human gut as early as 1 week after birth at 10^8 VLP per gram of feces and exhibit low diversity, as in the case of bacteria (Breitbart and others 2008). However, it is possible that more viral genotypes remain to be discovered, as a large number of assembled contigs from a recent metagenomics investigation matched no known public sequences (Reyes and others 2010; Minot and others 2011; Wagner and others 2013).

As these are viruses that infect bacteria, most of their effects are manifested in changes to bacterial phenotype, and they thus represent an extra dimension of regulation on bacterial dynamism within the gut microenvironment. Bacteria may utilize a form of immunity known as CRISPR (clustered regularly interspaced short palindromic repeats) to evade phage infection (Gasiunas and others 2014). Here, the bacterial genome contains a region of short DNA repeats that are regularly spaced and contain variable, foreign phage DNA between them. Upon recognition of foreign nucleic acid, the CRISPR system matches the foreign nucleic acid to

FIG. 1. Functions of bacteria in the human intestinal tract. Gut bacteria perform a range of functions in the human gut, including participating in metabolism of complex biopolymers, metabolism of drugs and foreign agents, promoting the production of AMPs, and stimulating proper development of intestinal structures and parenchymal functions. AMPs, antimicrobial molecules.

its complementary sequence saved between 2 spacers and proceeds to cleave the intruding sequence, thus thwarting infection. However, phages have also been shown to counter bacterial immunity (Seed and others 2013; Villion and Moineau 2013).

Regulation of the Gut Microbiome

Bacteria–bacteriophage interaction

Most human gut phages exhibit a temperate lifecycle in which the phage is able to undergo lytic or lysogenic lifecycles (Dalmasso and others 2014). When phages incorporate viral DNA into bacterial chromosomes, they may transfer exogenous segments or whole gene clusters into the host bacterial chromosome by gene transfer (horizontal gene transfer if exogenous bacterial DNA is introduced), which may occur via transduction or lysogenic conversion (Brussow and others 2004).

In transduction, a phage introduces DNA from previous bacteria into a new bacterial chromosome, as exhibited in some *Streptomyces* spp. (Burke and others 2001). Importantly, lysogenic conversion can wield crucial power in determining bacterial pathogenicity through the introduction of genes that increase bacterial fitness, such as preventing lytic infection and endowing bacteria with toxins. In some cases, phage DNA integration may even disrupt host bacterial genes. The outcome of phage-mediated gene transfer in bacteria is dynamic and heavily dependent on environmental stress. In a recent example, antibiotic treatment led to an enrichment of phage-encoded resistance genes in gut bacteria, which promoted host colonization and growth adaptation, ultimately strengthening connections between the phageome and gut bacteria (Reyes and others 2013); such an observation may represent 1 mechanism of bacterial adaptation and potential drug resistance.

Bacteriophages are also thought to exert beneficial influences on the human host outside of the bacterium, as they are populous in the gut mucus layer and bind to mucin glycoproteins with immunoglobulin-like domains on their capsids (Barr and others 2013). This interaction contributes to gut epithelial protection from bacteria.

The bacteria and their phages are in constant interaction within the gut, and each strives to regulate the other. However, it is possible that phages may negatively affect bacterial communities in the host by promoting dysbiosis, in which the balance of bacterial symbionts and pathogens has been skewed, which may ultimately translate into disease (Foca and others 2015). Though there is scant investigation into the contribution of gut phages to bacterial dysbiosis, several rational scenarios have been proposed (De Paepe and others 2014).

In the “*Kill the winner*” mechanism, phages target and kill only the dominant bacterial species, thus indicating viral predation based on bacterial density. This has been explored in a study of GF mice colonized with 15 known human symbiotic bacteria and subsequently infected with a combination of phages (Reyes and others 2013). Changes in the community structure were observed though transient. The “*biological weapon*” model has also been suggested in which commensal bacteria would use its own phage to kill competitive bacteria by inducing lysis (Bossi and others 2003; Brown and others 2006). “*Community shuffling*” holds that stressful scenarios such as antibiotic treatment, inflammation, and oxidative stress may lead to the induction of prophages

and eventually alter the symbiont/pathobiont relationship (De Paepe and others 2014). Evidence for this model comes from studies in *Escherichia coli* (Zhang and others 2000) and *Clostridium difficile* (Meessen-Pinard and others 2012). As discussed earlier, lateral gene transfer is potentially a critical mechanism of dysbiosis, in which viral virulent genes are endowed to bacteria, as in the case of *E. coli* strain O104:H4, in which an *E. coli* strain acquired Shiga-toxin via lysogenic conversion (Muniesa and others 2012).

Host gut immunity and microbiome

Commensal bacteria and their phages perform indispensable tasks for the human organism, yet their very foreign identity, along with their potential virulence and the existence of pathogenic bacteria and viruses, necessitates a fully functional immune system that continuously interacts with this microbiome (Fig. 2). This interaction is bidirectional; the gut microbiota elicits a specific immune micro-environment in the gut for the sake of gut homeostasis and optimal parenchymal function, while the immune system controls the species composition of the gut microbiota for optimal growth and nutritional benefit. Dysfunction on either end of this transaction can lead to disease.

First, bacteria (and presumably their phages) are absolutely required for the development of the intestinal immune system, as documented by studies in axenic (GF) mice (Smith and others 2007). Axenic mice exhibit a range of intestinal immune structural and cellular defects, including general reduction in lymphoid tissue size, smaller mesenteric lymph nodes, which contain fewer cells and no germinal centers; reduction in the number and size of Peyer’s patches; and a reduction in the number of intraepithelial lymphocytes, all in combination with defects to aforementioned epithelial cellular defects. Interestingly, these defects can be reversed with bacterial colonization and touts the potential therapeutic use of commensal administration for corrective defects in intestinal immune defects (Smith and others 2007; Palm and others 2015).

Furthermore, bacteria can be classified into 2 distinct categories, each of which is dependent on the response they elicit from the immune system: immunosuppression or proinflammation.

Extensive investigation into the proinflammatory effects of the immune system in the gut have been done with a limited group of bacteria. Studies using segmented filamentous bacteria (SFB) have by far revealed much about induction of inflammatory responses (Schnupf and others 2013). SFB (*Candidatus Arthromatis*) represent a unique clade of commensals in mice, and perhaps in humans, that are most related to the Type I Clostridia (Kuwahara and others 2011). They are intimately associated with the gut epithelium and are primarily recognized as strong drivers of immune responses. Studies in mice mono-colonized with SFB demonstrated that they dramatically induce the number of intestinal IgA⁺ B cells and increase the concentration of secretory IgA in the intestinal lumen (Klaasen and others 1993). Underlying this observation was a short-lived but strong activation of germinal centers in Peyer’s patches by SFB (Talham and others 1999). SFB also strongly expand the number of intraepithelial lymphocytes, including $\gamma\delta$ T cells and CD8⁺ T cells (Schnupf and others 2013). Of all proinflammatory immune cells stimulated by SFB, T_H17

FIG. 2. Cross-regulation among bacteriophages, bacteria, and immune system in the gut. Bacteria, their phages, and the gut epithelia and immune system engage in complex cross-regulations to maintain gut homeostasis. Bacteriophages infect bacteria and through a largely temperate lysogeny can horizontally transfer genes or disrupt genes to affect bacterial colonization and growth. Bacteria also limit phage infection through CRISPR immunity. Bacteria promote proper gut lymphoid tissue development, AMP production, and induce the development of immunosuppressive and proinflammatory immune cell types, dependent on the bacterial species. These very cell types then work together with AMPs to limit bacterial contact to gut epithelia. Bacteriophages are known to bind mucin and also aid in limiting bacteria-epithelial adhesion but how gut immune cells and epithelia directly affect bacteriophages remains largely unknown. As each component is situated as an intermediary among the other 2, and all are connected, indirect regulation among the 3 components also occurs. ↑, promote; ↓, suppress.

cells have garnered the most attention. Intestinal dendritic cell presentation of SFB antigen is required for T_H17 development in the gut mucosa (Gaboriau-Routhiau and others 2009; Ivanov and others 2009). SFB colonization also protects against pathogenic infection by *Citrobacter rodentium* (Ivanov and others 2009); this is a prime example of the importance of indigenous bacterial colonization as a means of combating pathogenic bacterial infection.

T_H17 cells mainly produce the cytokines IL-17A, IL-17F, IL-21, and IL-22, which are critical to controlling fungal and bacterial infections and maintaining antimicrobial gut function. Both IL-17 cytokines recruit neutrophils to the infection site for bacterial phagocytosis and induce epithelial production of the chemokine CCL20, which recruits even more T_H17 cells to the site via binding to the chemokine receptor CCR6 on the T_H17 cell surface (Huber and others 2012). These cytokines also increase the production of β -defensin by epithelial cells (Liang and others 2006; Ishigame and others 2009). In addition to the IL-17 cytokines, T_H17 -produced IL-22 promotes epithelial layer integrity and antimicrobial homeostasis by inducing the expression of antimicrobial molecules (AMPs) from the epithelium, promoting epithelial cell proliferation, survival, and tissue repair (Huber and others 2012).

In addition to SFB, the Gram-negative bacterium *Helicobacter hepaticus* has been shown to promote T_H17 responses and those of T_H1 cells, which are marked by

production of the proinflammatory cytokine interferon- γ (IFN γ) and are important for defense against intracellular pathogens such as intracellular bacteria and viruses (Palm and others 2015).

As bacteria of the microbiome do represent a foreign yet beneficial entity to the host, their presence also elicits immunosuppressive immune responses to tolerate commensal gut microbes. Mechanisms of immunosuppression include induction of immune cell subsets that dampen immune responses and the suppression of proinflammatory immune cells. One of the most prominent immunosuppressive cells of the intestine is the regulatory $CD4^+$ T cell (Treg), which is induced by the activation of $CD4^+$ T cells in the presence of the immunosuppressive cytokine transforming growth factor- β (TGF- β). These cells produce the anti-inflammatory cytokines IL-10 and TGF- β , which potently decrease proinflammatory cytokine production and cell proliferation. GF mice colonized with a microbiota known as Altered Schaedler's Flora induce Treg to the intestine and are required for homeostasis and prevention of colitis (Geuking and others 2011). Furthermore, a consortium of bacteria from the genus *Clostridium* from conventionally reared mice was shown to induce mobilization of Treg to the intestine (Atarashi and others 2011). A rational selection of 17 *Clostridia* from human fecal samples that enhance Treg abundance in the gut was shown to attenuate mouse models of colitis and allergic diarrhea after oral administration

(Atarashi and others 2013). Furthermore, Clostridia are major producers of short chain fatty acids (SCFAs), which have been demonstrated to induce Tregs (Palm and others 2015). SCFAs, especially butyrate, induce Treg formation via epigenetic regulation in the form of increased acetylation of the locus of *Foxp3*, which is the master transcription factor of TGF- β production (Arpaia and others 2013; Furusawa and others 2013; Smith and others 2013). In addition to bacteria of the genus *Clostridia*, *Bacteroides fragilis* induces Treg development by its production of capsular polysaccharide A (PSA), which is recognized by TLR2 on Tregs (Round and Mazmanian 2009; Round and others 2011).

Other immune cell types that are affected by intestinal microbiota include invariant natural killer T cells (iNKT cells) and innate lymphoid cells (ILCs). iNKT cells are a T-cell subset that expresses the invariant T-cell receptor α -chain (Sommer and Backhed 2013). These cells are proinflammatory, as following activation they produce the cytokines IFN γ , IL-2, IL-17A, and tumor necrosis factor (TNF) among others, which promote T_H1 and T_H17 immune responses. Importantly, GF mice have increased numbers of these cells in the intestine, which suggests that the gut microbiota serve to limit their potentially exacerbated proinflammatory presence (Olszak and others 2012). ILCs rapidly respond to inflammatory cues and develop along effector pathways parallel to those of CD4⁺ T cells; for instance, Type 3 ILCs express the transcription factor retinoid-related orphan receptor γ t (ROR γ t, which promotes T_H17 development) and produce IL-17A and IL-22 (Jandhyala and others 2015). Studies have shown that the bacterial metabolite indole-3-aldehyde stimulates ILC production of IL-22 via the aryl hydrocarbon receptor, which is expressed in type 3 ILCs (Zelante and others 2013). Interestingly, loss of MHC-II on ILC3 induces T_H17 development independently of SFB, which may indicate a regulatory role of ILC3 on gut T_H17 differentiation (Goto and others 2014).

The interplay of the gut bacterial microenvironment with the gut immune system is further compounded by the crosstalk of bacteria-bacteriophage and direct interactions of bacteriophages and the immune system. It is conceivable that bacteriophages could interact indirectly with the gut immune system by manipulating gut bacteria via the aforementioned mechanisms discussed, and bacteriophages are presumed to be strong drivers of intestinal bacterial biodiversity. Although the phages predominate over other microbes of the gut, how they directly interact with the intestinal immune system is very poorly understood and understudied.

In nonimmunized humans and animal sera, a low level of phage antibodies are detectable, and most are neutralizing against the phage tail (Jerne 1956; Kamme 1973; Kucharewicz-Krukowska and Slopek 1987). Few studies have addressed the effects of phages on T cells; Srivastava and others (2004) reported no significant difference in phage clearance between WT and T cell-deficient mice, thus suggesting an insignificant T cell-mediated role in antiphage immune responses. However, phages can exert an immunosuppressive effect on T-cell function, as purified T4 phage has been shown to suppress human T-cell proliferation (Gorski and others 2006). Studies investigating bacteriophage treatment in tuberculous guinea pigs showed that mycobacteriophages suppressed phytohemagglutinin-induced activation of T lymphocytes in a dose-dependent fashion (Mankiewicz and others 1974). Unpublished data

from Gorski and others suggest that phages inhibit some dendritic cell functions and NF κ B activation. Phagocytosis and generation of reactive oxygen species (ROS) are critical functions of innate immune cells that maintain appropriate bacterial densities in the gut, though excessive activity can also drive pathology. Phagocytes, including neutrophils and monocytes, preincubated with T4 and F8 (a *Pseudomonas aeruginosa* phage) exhibited a reduction in phagocytosis of *E. coli* (Przerwa and others 2006). However, *in vivo* phage-affected neutrophil phagocytosis varied depending on pretreatment with bacteria. ROS can be quickly generated by respiratory bursts in phagocytes. T4 phages can suppress *E. coli*-induced respiratory burst with preincubation with the bacteria (Przerwa and others 2006). Other reports suggest that staphylococcal A3/R purified phage or lysates may have no effect on phagocytic respiratory burst, thus suggesting that this effect may be bacteriophage dependent (Borysowski and others 2010).

In the context of cytokine production, phages display largely mixed results, which may reflect the differential effects of bacteriophage cell tropism and/or mode of phage preparation. While purified *Staphylococcus aureus* phage can increase IL-6 production in activated splenocytes, it may also decrease it, along with decreasing TNF α production (Zimecki and others 2003). The preparation staphage lysate induced the cytokines TNF α , IL-1 β , IFN γ , and IL-10 in human mononuclear cultures (Gorski and others 2012). Moreover, purified preparations of the T4 phage decrease IL-2 and IL-6 production in these cells, but its *lysate* increases that of IL-6 (Gorski and others 2012). These observations show the complexity of phage effects on immune cells and the potential confounding effects phage use may have in treatment depending on the therapeutic outcome desired. Purified phage preparations appear to elicit more immunosuppressive outcomes, which could depend on direct phage immunomodulation or reducing the burden of bacteria and thus driving down bacterial antigen load. On the contrary, lytic phage infection could cause an increase in bacterial antigen and exposure to the immune system.

The Gut Microbiome, Autoimmune Disease, and MS

The gut microbiota is a direct product of the environment and is situated topologically outside of the host; as such, it is in constant contact with external environmental factors against which it must maintain equilibrium. However, it must also maintain normal, physiologic associations with the host gut stroma, parenchyma, and immune system. Numerous metagenomic investigations show that there exists limited physiologically acceptable temporo-spatial changes in the dynamics of the gut microbiota (including microbial composition, specific bacterial densities and ratios, and phenotypes) that are compatible with overall healthy intestinal function. On the other side, the immune system, in concert with gut epithelia, must keep commensal bacteria and other microbiota at acceptable distance from the epithelium, contribute to bacterial homeostasis, and preserve the epithelial border from insult while allowing proper nutritional absorption. Alterations to various parameters on either side of the gut host-microbiome microcosm can result in serious complications, from improper nutrition to autoimmune disease.

Multiple sclerosis

Broadly, autoimmune pathogenesis defines conditions in which the host immune system is activated to mount inappropriate inflammatory immune responses against host cells, and includes conditions such as systemic lupus erythematosus, type-1 diabetes, rheumatoid arthritis, and MS. MS is a debilitating, inflammatory autoimmune disease of the central nervous system characterized by axonal demyelination and neurodegeneration (Wekerle and Kuchars 2006). It is thought to be mainly a disease of the Western world and, like most autoimmune diseases, has an increased female: male disease ratio (Trojano and others 2012). Although its etiology remains unknown, MS development is multifactorial, involving both heritable, genetic components and environmental components.

Epidemiological and genome-wide association studies have identified common MS risk-associated loci, mostly immune related, including 2 human leukocyte antigen (HLA) alleles DRB1*1501 and DQ6, *IL2RA*, *IL7R*, *IRF8*, *TNFRSF1A*, and *CD40* (Bush and others 2010). In addition, defects in tolerance mechanisms have been identified in MS (Gonsette 2012). It is possible that the associated HLA molecules may be more apt at presenting antigen to T cells in the process of MS development, there is change in the activation threshold of certain components of the overall immune response associated with certain immune susceptibility loci, and there is an abnormal escape of self-antigen-specific T cells from the thymus in MS. These factors in some combination could greatly contribute to MS development.

Environmental associations with the risk of MS development are greatly varied; those confirmed include lower vitamin D levels, cigarette smoking, viral infection (Epstein-Barr virus), and obesity, although this list is suspected to be incomplete (Hedstrom and others 2012; Summerday and others 2012; Wingerchuk 2012; Pender and Burrows 2014). Since the incidence of MS has increased in many locations throughout the world, there is increased attention focused on identifying other environmental risk factors for MS. Given its association with the risk of other autoimmune diseases, studies of the gut microbiota are critical (Ho and others 2015).

The gut microbiome and MS

The gut microbiome and models of MS. The impact of the gut microbiome on MS development has its roots in several seminal preclinical investigations on the role of bacteria in experimental autoimmune encephalomyelitis (EAE). EAE is a murine model of MS induced by immunization with a myelin protein/peptide in combination with complete Freund's adjuvant with/out pertussis toxin (Stromnes and Goverman 2006). This immunization activates myelin-reactive CD4⁺ T cells and leads to their differentiation into pathogenic, encephalitogenic T_H1 and T_H17 cells that mediate neuropathology and ascending paralysis.

It was first observed that GF mice are resistant to spontaneous and inducible EAE and fail to generate pathogenic T cells, especially T_H17 cells (Berer and others 2011; Lee and others 2011). This was mechanistically attributed to a failure of gut DC activation and production of cytokines crucial for T-cell polarization. The failure to generate pathogenic T cells was concomitant with an increase in Treg development. Furthermore, EAE induction could be restored

with colonization with SFB, which drove T_H17 differentiation in the gut and T-cell encephalitogenicity. Gut bacteria also produce extracellular adenosine 5'-triphosphate, which induces CD11c⁺ DC in the gut lamina propria to produce the cytokines IL-6 and IL-23, both important for T_H17 differentiation (Atarashi and others 2008); this mechanism of T_H17 induction is thought to also contribute to T-cell encephalitogenicity in EAE. Gut microbiota can also affect blood-brain barrier permeability, as GF mice exhibit disrupted tight junctions (Braniste and others 2014).

In the context of antibiotic treatment, mice also exhibit suppression of EAE induction, which is marked by an increase in Treg formation and IL-10-producing B cells (Ochoa-Reparaz and others 2010b; Mielcarz and Kasper 2015). Specifically, it has been shown that the product PSA by *B. fragilis* exerts a protective effect against EAE and involves a decrease in IL-17 production and increase in IL-10-producing CD4⁺ T cells, which was dependent on TLR2 signaling (Wang and others 2014). Treatment of EAE with bacterial formations has also been done. *Bifidobacterium bifidum*, *Lactobacillus*, and *Streptococcus thermophilus* treatment reduces EAE severity with decreases in T_H1 and T_H17 cells and Treg induction (Kwon and others 2013).

Importantly, these studies portray the immunomodulatory effects of bacteria and speak to the necessity of gut bacterial presence in EAE. All of these bacteria are represented in the phyla that are most commonly observed in the healthy mammalian gut, and these investigations hearken to the potential outcomes for EAE induction and possibly contribution to MS development when there is imbalance of bacterial species in the gut. It would be interesting to evaluate the outcome of EAE induction with mice harboring similar bacterial phyla and species compositions as the human gut with controlled alterations to specific bacterial populations within the microflora to more reflectively query the role of bacterial dysbiosis on EAE development.

Bacteriophages have not been evaluated for association with MS/EAE, though there has been postulation about the association of bacteriophages and autoimmunity (Riley 2004; Dalmaso and others 2014; Ogilvie and Jones 2015). As dysbiosis has been hypothesized as a risk factor for many autoimmune diseases, one method of causing underlying dysbiosis could be bacteriophage infection in which destabilization of bacterial populations occurs.

To our knowledge, there has been one investigation thus far to demonstrate a difference in bacteriophage abundance and autoimmune disease. Crohn's disease is an autoimmune disease of the digestive tract. Lepage and others (2008) showed that CD patients harbored significantly more bacteriophage VLP in colonic mucosa than non-CD counterparts, and these phages were of morphotypes consistent with the order *Caudovirales*. Whether this is due to increased bacterial burden or persistence of these phages in CD versus non-CD persons is unknown but warrant further investigation into the role of phages on bacterial dysbiosis and autoimmune disease.

The gut microbiome and MS. To date few, but highly informative, studies have examined the possible link between gut microbiota and MS. First, CS as a mode of infant delivery perturbs normal gut bacterial colonization dynamics and could possibly result in abnormal immune system development (Neu and Rushing 2011). Case-controlled studies reveal that CS delivery may be associated with increased

risk of MS development. In the context of infant feeding, human milk is a rich source of bacteria, including staphylococci, streptococci, lactic acid bacteria, propionic bacteria, and bifidobacteria, and mother-to-infant transfer of strains belonging to *Lactobacillus*, *Staphylococcus*, *Enterococcus*, and *Bifidobacterium* through breastfeeding has been shown (Fernandez and others 2013). Breastfed bacteria are thus an important early source of infant gut bacteria, and these bacteria may contribute to normal immune system development by bacterial competitive exclusion, enhancing AMP production, and improving intestinal barrier function, among other mechanisms. Interestingly, Pisacane and others (1994) reported that persons with MS on average were breastfed for significantly shorter duration than their unaffected counterparts, and suggests a possible link between breastfed bacteria and risk of MS development that merits further inquiry. An exploratory investigation by Cantarel and others (2015) revealed overall decreased levels of the bacterial family *Bacteroidaceae* (phylum Bacteroidetes) and those of the genera *Faecalibacterium* and *Ruminococcus* (both of Firmicutes phylum) in persons with MS compared to Non-MS Counterparts (Table 1). In this same study, some MS patients were placed on vitamin D treatment, which led to increases in Firmicutes phylum members *Faecalibacterium* and *Coproccoccus*. Importantly, *Faecalibacterium* is thought to be immunosuppressive via production of the SCFA butyrate and may be protective in MS. As also mentioned previously, *Bacteroides*, such as *B. fragilis*, are also known to be immunosuppressive, though via PSA production.

Archaea, which form a kingdom separate from prokaryotes and eukaryotes and compose 10% on colonic anaerobes, were found at higher levels in the gut of MS versus control persons, with specificity to the species *Methanobacter smithii* (Jhangi and others 2014). It is thought that the structural components of this *Archaea* species make it especially immunogenic. In a very recent report, Miyake and others (2015) investigated the gut microbiota of Japanese MS and healthy control cohorts and found an overall difference in the gut microbiota structure and greater inter-individual gut microbiota variability with MS versus healthy control comparison. They noted more prevalent Actinobacteria and less prevalent Bacteroidetes and Firmicutes phyla. At the genera echelon, MS subjects harbored lower levels of *Bacteroides*, *Faecalibacterium*, *Prevotella*, and *Anaerostipes*, though increased *Bifidobacterium* and *Streptococcus*. There were great differences in the levels of 21 species, 19 of which depletion was exhibited and 14 from the Clostridia clusters XIVa and IV, which are distinguished as heavy producers of SCFAs. *Streptococcus thermophilus* and *Eggerthella lenta* were more prevalent in MS versus healthy control subjects. Thus far, it appears from the cur-

rent studies that there is gut microbial dysbiosis associated with MS development, with common findings including decreases in Bacteroidetes and Firmicutes phyla. These bacterial agents are crucial for immunoregulation and SCFA production. However, these studies have all been small and typically involve prevalent cases, making it difficult to know if the results are reproducible across various populations or to know if the differences are a cause or a result of MS.

Bacterial toxins may also be associated with MS development, according to a recent finding from Rumah and others (2013). Here, they report identification of *Clostridium perfringens* type B from an MS patient, which was the first reported identification of this strain from humans. This strain produces its unique epsilon toxin, which has known ability to cross the blood-brain barrier and bind myelin and oligodendrocytes. Sera and cerebrospinal fluid analyses from a well-studied MS cohort showed higher immunoreactivity to epsilon toxin than healthy controls, suggesting prior exposure to epsilon toxin in these MS patients and the potential of a bacterial toxin to contribute to MS pathogenesis, especially in the absence of inflammation.

MS Gut Microbiome Manipulation for Therapeutic Benefit

There are currently no approved treatments for MS through manipulating the gut microbiome, though preclinical studies and ongoing clinical trials show great promise for therapeutic modulation of the gut microflora in MS. Vitamin D supplementation has extensively been shown to ameliorate EAE, presumably via downregulating DC and B-cell activation and Treg induction (Correale and Gaitan 2015). Investigative clinical studies show association of vitamin D treatment with MS patients and changes in the gut microbiota, specifically those of the phylum Firmicutes which exhibited increased abundance (Cantarel and others 2015). There are now several ongoing randomized controlled trials of vitamin D supplementation for MS treatment, though many small studies have been done. Positive outcomes include reports of fewer relapse events, decreases in the number of Gadolinium-enhancing regions per patient, suppression of T-cell proliferation, and increases in the percentage of peripheral IL-10⁺CD4⁺ T cells and decreases in the ratio of the cytokines IFN γ : IL-4 in T cells (Kimball and others 2007; Burton and others 2010; Smolders and others 2010). Overall, it is conceivable that vitamin D may affect the gut microbiome by rectifying dysbiosis and enforcing the immunosuppressive properties exerted by the microflora.

Probiotics are classified as live microorganisms that exert beneficial health on the host, and are heavily used in the management of intestinal illness (Jandhyala and others

TABLE 1. COMPLETED STUDIES INVESTIGATING GUT MICROBIOTA IN MULTIPLE SCLEROSIS SUBJECTS

Study design	Findings	Reference
Case-controlled	Overall ↓ <i>Bacteroidaceae</i> levels; ↓ <i>Faecalibacterium</i> , <i>Ruminococcus</i> ; Vitamin D treatment ↑ <i>Faecalibacterium</i> and <i>Coproccoccus</i>	Cantarel and others, 2015
Case-controlled	↑ in <i>Archaea</i> (<i>Methanobacter smithii</i>)	Jhangi and others, 2014
Case-controlled	↑ in Actinobacteria; ↓ in Bacteroidetes, Firmicutes; ↓ in <i>Bacteroides</i> , ↓ <i>Faecalibacterium</i> , ↓ <i>Prevotella</i> , ↓ <i>Anaerostipes</i> ; ↑ in <i>Bifidobacterium</i> , ↑ <i>Streptococcus</i>	Miyake and other, 2015

↑, increased; ↓, decreased.

2015). Their use in EAE has yielded mixed results, with some groups showing increased T_H1 responses associated with exacerbated EAE, some showing lack of effects on disease symptomology, and 1 showing a protective effect (von Geldern and Mowry 2012). Specific species of bacteria have resulted in beneficial outcomes in EAE, including oral *B. fragilis* administration, which associated with IL-17 reduction and IL-10 induction, and *Lactobacillus casei* in which the mechanism of action is thought to be strain specific and enhance IL-10 production (Maassen and others 2000; Ochoa-Reparaz and others 2010a).

To date, there are no published clinical studies of bacterial probiotic administration for MS treatment, but several factors must be kept in mind for future studies. It is possible that the particular bacterial combination and amount must be patient-tailored since inter-individual variability in the gut microbiota may exist. Preexisting gut pathobionts may also prevent proper colonization of administered probiotics and present a null effect (Ventura and others 2011). Generally, any strain that increases its numbers relative to that of other bacteria will be eliminated by phage predation, which thus highlights the potential importance of phages in bacterial therapeutic intervention. As patients most likely already exhibit gut dysbiosis, a potential testable intervention may include the introduction of a combination of the potentially beneficial species of *Bacteroidetes* and *Firmicutes*, along with a tailored concoction of lysogenic phages that have specific tropism to these bacterial species and confer a survival advantage to these bacterial hosts. Moreover, administration of phages with tropisms specific for certain bacterial species already in the gut may also be considered, for instance, in conferring survival and proliferation of potentially beneficial bacteria and lysis of pathogenic bacteria, and may especially be important for antibiotic-resistant bacterial strains. So far there has been one phase II clinical trial to show safety and efficacy of phage administration for disease-Biophage-PA bacteriophage preparation for antibiotic-resistant *P. aeruginosa* in chronic otitis patients (Wright and others 2009).

Moreover, diet is known to play a major role in gut microbiota makeup (Mielcarz and Kasper 2015). Many studies show differences in the gut microbial composition of lean versus obese individuals, and this is highly associated with the adoption of the “Western diet,” which is based on proteins, animal fat, large sugar intake, and refined carbohydrates (Le Chatelier and others 2013; Manzel and others 2014; Cresci and Bawden 2015). This diet is associated with an increase in proinflammatory fatty acids and increased EAE severity (Mielcarz and Kasper 2015). Contrarily, calorie restriction is associated with decreased EAE severity; lowered inflammation, demyelination, and axon injury; increased corticosterone and adiponectin; and lowered IL-6 and leptin (Piccio and others 2008). A high ratio of eubiotic *Bacteroidetes*: *Firmicutes* is thought to be eubiotic, as *Bacteroidetes* including *Prevotella* heavily consume complex glycans and produce butyrate, which again is linked to increased Treg formation and proinflammatory NF κ B down-regulation (Jandhyala and others 2015). A fascinating recent study by Haghikia and others (2015) found that dietary fatty acids exert profound effects of T cells in the gut. Middle- and long-chain fatty acids (LCFAs) promoted T_H1 and T_H17 development, while SCFAs promoted Treg differentiation. Furthermore, LCFAs worsened EAE while SCFAs ameliorated this disease. This study in particular demonstrates the

fundamental importance of gut bacterial composition and influence on EAE and possibly MS development, and suggests that dietary changes that favor gut SCFA production may have beneficial outcomes for MS therapy.

In sum, early small studies have already shown associations of gut bacterial makeup and MS, but larger, confirmatory studies are needed. Further, more research will need to be undertaken to also determine the gut virome makeup in these persons and how the overall microbiota changes with current therapeutics. Probiotics may give only partial coverage as an add-on treatment to some individuals, and phage therapy may also need to be explored. Thus, more investigation should also be invested in the bacteria–bacteriophage interaction in the gut and how that may factor into MS development and its importance in treatment. Additionally, changes in diet may also have beneficial effects on ameliorating the MS course, which may involve increasing intake of consumables that promote SCFA production (especially butyrate) by gut bacteria and immunosuppression. Therefore, the avenues of gut bacterial modulation and phage therapy are open for further exploration in MS therapy.

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References

- Ackermann HW. 2009. Phage classification and characterization. *Methods Mol Biol* 501:127–140.
- Andersch-Bjorkman Y, Thomsson KA, Holmen Larsson JM, Ekerhovd E, Hansson GC. 2007. Large scale identification of proteins, mucins, and their O-glycosylation in the endocervical mucus during the menstrual cycle. *Mol Cell Proteomics* 6(4):708–716.
- Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffey PJ, Rudensky AY. 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504(7480):451–455.
- Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, Yagita H, Ishii N, Evans R, Honda K, Takeda K. 2008. ATP drives lamina propria T(H)17 cell differentiation. *Nature* 455(7214):808–812.
- Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. 2013. Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* 500(7461):232–236.
- Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K. 2011. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* 331(6015):337–341.
- Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. 2005. Host-bacterial mutualism in the human intestine. *Science* 307(5717):1915–1920.
- Barr JJ, Auro R, Furlan M, Whiteson KL, Erb ML, Pogliano J, Stotland A, Wolkowicz R, Cutting AS, Doran KS, Salamon P, Youle M, Rohwer F. 2013. Bacteriophage adhering to

- mucus provide a non-host-derived immunity. *Proc Natl Acad Sci U S A* 110(26):10771–10776.
- Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johnner C, Wekerle H, Krishnamoorthy G. 2011. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* 479(7374):538–541.
- Borysowski J, Wierzbicki P, Klosowska D, Korczak-Kowalska G, Weber-Dabrowska B, Gorski A. 2010. The effects of T4 and A3/R phage preparations on whole-blood monocyte and neutrophil respiratory burst. *Viral Immunol* 23(5):541–544.
- Bossi L, Fuentes JA, Mora G, Figueroa-Bossi N. 2003. Prophage contribution to bacterial population dynamics. *J Bacteriol* 185(21):6467–6471.
- Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, Korecka A, Bakocevic N, Ng LG, Kundu P, Gulyas B, Halldin C, Hultenby K, Nilsson H, Hebert H, Volpe BT, Diamond B, Pettersson S. 2014. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* 6(263):263ra158.
- Breitbart M, Haynes M, Kelley S, Angly F, Edwards RA, Felts B, Mahaffy JM, Mueller J, Nulton J, Rayhawk S, Rodriguez-Brito B, Salamon P, Rohwer F. 2008. Viral diversity and dynamics in an infant gut. *Res Microbiol* 159(5):367–373.
- Brown SP, Le Chat L, De Paepe M, Taddei F. 2006. Ecology of microbial invasions: amplification allows virus carriers to invade more rapidly when rare. *Curr Biol* 16(20):2048–2052.
- Brussow H, Canchaya C, Hardt WD. 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiol Mol Biol Rev* 68(3):560–602, table of contents.
- Burke J, Schneider D, Westpheling J. 2001. Generalized transduction in *Streptomyces coelicolor*. *Proc Natl Acad Sci U S A* 98(11):6289–6294.
- Burton JM, Kimball S, Vieth R, Bar-Or A, Dosch HM, Cheung R, Gagne D, D'Souza C, Ursell M, O'Connor P. 2010. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. *Neurology* 74(23):1852–1859.
- Bush WS, Sawcer SJ, de Jager PL, Oksenberg JR, McCauley JL, Pericak-Vance MA, Haines JL. 2010. Evidence for polygenic susceptibility to multiple sclerosis—the shape of things to come. *Am J Hum Genet* 86(4):621–625.
- Cantarel BL, Waubant E, Chehoud C, Kuczynski J, DeSantis TZ, Warrington J, Venkatesan A, Fraser CM, Mowry EM. 2015. Gut microbiota in multiple sclerosis: possible influence of immunomodulators. *J Investig Med* 63(5):729–734.
- Correale J, Gaitan MI. 2015. Multiple sclerosis and environmental factors: the role of vitamin D, parasites, and Epstein-Barr virus infection. *Acta Neurol Scand Suppl* 132(199):46–55.
- Cresci GA, Bawden E. 2015. Gut microbiome: what we do and don't know. *Nutr Clin Pract* 30(6):734–746.
- Dalmasso M, Hill C, Ross RP. 2014. Exploiting gut bacteriophages for human health. *Trends Microbiol* 22(7):399–405.
- De Paepe M, Leclerc M, Tinsley CR, Petit MA. 2014. Bacteriophages: an underestimated role in human and animal health? *Front Cell Infect Microbiol* 4:39.
- Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. 2005. Diversity of the human intestinal microbial flora. *Science* 308(5728):1635–1638.
- Fernandez L, Langa S, Martin V, Maldonado A, Jimenez E, Martin R, Rodriguez JM. 2013. The human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res* 69(1):1–10.
- Foca A, Liberto MC, Quirino A, Marascio N, Zicca E, Pavia G. 2015. Gut inflammation and immunity: what is the role of the human gut virome? *Mediators Inflamm* 2015:326032.
- Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504(7480):446–450.
- Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N. 2009. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 31(4):677–689.
- Gasiunas G, Sinkunas T, Siksnys V. 2014. Molecular mechanisms of CRISPR-mediated microbial immunity. *Cell Mol Life Sci* 71(3):449–465.
- Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, McCoy KD, Macpherson AJ. 2011. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* 34(5):794–806.
- Gonsette RE. 2012. Self-tolerance in multiple sclerosis. *Acta Neurol Belg* 112(2):133–140.
- Gorski A, Kniotek M, Perkowska-Ptasinska A, Mroz A, Przerwa A, Gorczyca W, Dabrowska K, Weber-Dabrowska B, Nowaczyk M. 2006. Bacteriophages and transplantation tolerance. *Transplant Proc* 38(1):331–333.
- Gorski A, Miedzybrodzki R, Borysowski J, Dabrowska K, Wierzbicki P, Ohams M, Korczak-Kowalska G, Olszowska-Zaremba N, Lusiak-Szelachowska M, Klak M, Jonczyk E, Kaniuga E, Golas A, Purchla S, Weber-Dabrowska B, Letkiewicz S, Fortuna W, Szufnarowski K, Pawelczyk Z, Rogoz P, Klosowska D. 2012. Phage as a modulator of immune responses: practical implications for phage therapy. *Adv Virus Res* 83:41–71.
- Goto Y, Panea C, Nakato G, Cebula A, Lee C, Diez MG, Laufer TM, Ignatowicz L, Ivanov, II. 2014. Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. *Immunity* 40(4):594–607.
- Gronlund MM, Lehtonen OP, Eerola E, Kero P. 1999. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr* 28(1):19–25.
- Haghikia A, Jorg S, Duscha A, Berg J, Manzel A, Waschbisch A, Hammer A, Lee DH, May C, Wilck N, Balogh A, Ostermann AI, Schebb NH, Akkad DA, Grohme DA, Kleinewietfeld M, Kempa S, Thone J, Demir S, Muller DN, Gold R, Linker RA. 2015. Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. *Immunity* 43(4):817–829.
- Hedstrom AK, Olsson T, Alfredsson L. 2012. High body mass index before age 20 is associated with increased risk for multiple sclerosis in both men and women. *Mult Scler* 18(9):1334–1336.
- Ho JT, Chan GC, Li JC. 2015. Systemic effects of gut microbiota and its relationship with disease and modulation. *BMC Immunol* 16:21.
- Huber S, Gagliani N, Flavell RA. 2012. Life, death, and miracles: Th17 cells in the intestine. *Eur J Immunol* 42(9):2238–2245.
- Hugenholtz P, Goebel BM, Pace NR. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* 180(18):4765–4774.
- Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, Fujikado N, Tanahashi Y, Akitsu A, Kotaki H, Sudo K, Nakae S, Sasakawa C, Iwakura Y. 2009. Differential

- roles of interleukin-17A and -17F in host defense against mucocutaneous bacterial infection and allergic responses. *Immunity* 30(1):108–119.
- Ivanov, II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139(3):485–498.
- Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. 2015. Role of the normal gut microbiota. *World J Gastroenterol* 21(29):8787–8803.
- Jerne NK. 1956. The presence in normal serum of specific antibody against bacteriophage T4 and its increase during the earliest stages of immunization. *J Immunol* 76(3):209–216.
- Jhingi S, Ganghi R, Glanz B, Cook S, Nejad P, Ward D, Li N, Gerber G, Bry L, Weiner H. 2014. Increased archaea species and changes with therapy in gut microbiome of multiple sclerosis subjects (S24.001). *Neurology* 2014: 82.
- Kamme C. 1973. Antibodies against staphylococcal bacteriophages in human sera. I. Assay of antibodies in healthy individuals and in patients with staphylococcal infections. *Acta Pathol Microbiol Scand B Microbiol Immunol* 81(6): 741–748.
- Kimball SM, Ursell MR, O'Connor P, Vieth R. 2007. Safety of vitamin D3 in adults with multiple sclerosis. *Am J Clin Nutr* 86(3):645–651.
- Klaasen HL, Van der Heijden PJ, Stok W, Poelma FG, Koopman JP, Van den Brink ME, Bakker MH, Eling WM, Beynen AC. 1993. Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. *Infect Immun* 61(1):303–306.
- Kucharewicz-Krukowska A, Slopek S. 1987. Immunogenic effect of bacteriophage in patients subjected to phage therapy. *Arch Immunol Ther Exp (Warsz)* 35(5):553–561.
- Kuwahara T, Ogura Y, Oshima K, Kurokawa K, Ooka T, Hirakawa H, Itoh T, Nakayama-Imaohji H, Ichimura M, Itoh K, Ishifune C, Maekawa Y, Yasutomo K, Hattori M, Hayashi T. 2011. The lifestyle of the segmented filamentous bacterium: a non-culturable gut-associated immunostimulating microbe inferred by whole-genome sequencing. *DNA Res* 18(4):291–303.
- Kwon HK, Kim GC, Kim Y, Hwang W, Jash A, Sahoo A, Kim JE, Nam JH, Im SH. 2013. Amelioration of experimental autoimmune encephalomyelitis by probiotic mixture is mediated by a shift in T helper cell immune response. *Clin Immunol* 146(3):217–227.
- Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jorgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clement K, Dore J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T, Bork P, Wang J, Ehrlich SD, Pedersen O. 2013. Richness of human gut microbiome correlates with metabolic markers. *Nature* 500(7464):541–546.
- Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. 2011. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 108 Suppl 1:4615–4622.
- Lepage P, Colombet J, Marteau P, Sime-Ngando T, Dore J, Leclerc M. 2008. Dysbiosis in inflammatory bowel disease: a role for bacteriophages? *Gut* 57(3):424–425.
- Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA. 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 203(10):2271–2279.
- Maassen CB, van Holten-Neelen C, Balk F, den Bak-Glashouwer MJ, Leer RJ, Laman JD, Boersma WJ, Claassen E. 2000. Strain-dependent induction of cytokine profiles in the gut by orally administered *Lactobacillus* strains. *Vaccine* 18(23):2613–2623.
- Mankiewicz E, Kurti V, Adomonis H. 1974. The effect of mycobacteriophage particles on cell-mediated immune reactions. *Can J Microbiol* 20(9):1209–1218.
- Manzel A, Muller DN, Hafler DA, Erdman SE, Linker RA, Klei-newietfeld M. 2014. Role of “Western diet” in inflammatory autoimmune diseases. *Curr Allergy Asthma Rep* 14(1):404.
- Meessen-Pinard M, Sekulovic O, Fortier LC. 2012. Evidence of in vivo prophage induction during *Clostridium difficile* infection. *Appl Environ Microbiol* 78(21):7662–7670.
- Mielcarz DW, Kasper LH. 2015. The gut microbiome in multiple sclerosis. *Curr Treat Options Neurol* 17(4):344.
- Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, Lewis JD, Bushman FD. 2011. The human gut virome: inter-individual variation and dynamic response to diet. *Genome Res* 21(10):1616–1625.
- Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, Chihara N, Tomita A, Sato W, Kim SW, Morita H, Hattori M, Yamamura T. 2015. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVa and IV clusters. *PLoS One* 10(9):e0137429.
- Muniesa M, Hammerl JA, Hertwig S, Appel B, Brussow H. 2012. Shiga toxin-producing *Escherichia coli* O104:H4: a new challenge for microbiology. *Appl Environ Microbiol* 78(12): 4065–4073.
- Neu J, Rushing J. 2011. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. *Clin Perinatol* 38(2):321–331.
- Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, Burroughs AR, Begum-Haque S, Dasgupta S, Kasper DL, Kasper LH. 2010a. Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression. *J Immunol* 185(7):4101–4108.
- Ochoa-Reparaz J, Mielcarz DW, Haque-Begum S, Kasper LH. 2010b. Induction of a regulatory B cell population in experimental allergic encephalomyelitis by alteration of the gut commensal microflora. *Gut Microbes* 1(2):103–108.
- Ogilvie LA, Jones BV. 2015. The human gut virome: a multifaceted majority. *Front Microbiol* 6:918.
- Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, Glickman JN, Siebert R, Baron RM, Kasper DL, Blumberg RS. 2012. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 336(6080): 489–493.
- Palm NW, de Zoete MR, Flavell RA. 2015. Immune-microbiota interactions in health and disease. *Clin Immunol* 159(2):122–127.
- Pender MP, Burrows SR. 2014. Epstein-Barr virus and multiple sclerosis: potential opportunities for immunotherapy. *Clin Transl Immunology* 3(10):e27.
- Piccio L, Stark JL, Cross AH. 2008. Chronic calorie restriction attenuates experimental autoimmune encephalomyelitis. *J Leukoc Biol* 84(4):940–948.
- Pisacane A, Impagliazzo N, Russo M, Valiani R, Mandarini A, Florio C, Vivo P. 1994. Breast feeding and multiple sclerosis. *BMJ* 308(6941):1411–1412.
- Przerwa A, Zimecki M, Switala-Jelen K, Dabrowska K, Krawczyk E, Luczak M, Weber-Dabrowska B, Syper D, Miedzybrodzki R, Gorski A. 2006. Effects of bacteriophages

- on free radical production and phagocytic functions. *Med Microbiol Immunol* 195(3):143–150.
- Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F, Gordon JI. 2010. Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature* 466(7304):334–338.
- Reyes A, Wu M, McNulty NP, Rohwer FL, Gordon JI. 2013. Gnotobiotic mouse model of phage-bacterial host dynamics in the human gut. *Proc Natl Acad Sci U S A* 110(50):20236–20241.
- Riley PA. 2004. Bacteriophages in autoimmune disease and other inflammatory conditions. *Med Hypotheses* 62(4):493–498.
- Rohwer F. 2003. Global phage diversity. *Cell* 113(2):141.
- Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. 2011. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 332(6032):974–977.
- Round JL, Mazmanian SK. 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 9(5):313–323.
- Rumah KR, Linden J, Fischetti VA, Vartanian T. 2013. Isolation of *Clostridium perfringens* type B in an individual at first clinical presentation of multiple sclerosis provides clues for environmental triggers of the disease. *PLoS One* 8(10):e76359.
- Salminen S, Gibson GR, McCartney AL, Isolauri E. 2004. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut* 53(9):1388–1389.
- Savage DC. 1977. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 31:107–133.
- Schnupf P, Gaboriau-Routhiau V, Cerf-Bensussan N. 2013. Host interactions with segmented filamentous bacteria: an unusual trade-off that drives the post-natal maturation of the gut immune system. *Semin Immunol* 25(5):342–351.
- Seed KD, Lazinski DW, Calderwood SB, Camilli A. 2013. A bacteriophage encodes its own CRISPR/Cas adaptive response to evade host innate immunity. *Nature* 494(7438):489–491.
- Smith K, McCoy KD, Macpherson AJ. 2007. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin Immunol* 19(2):59–69.
- Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS. 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341(6145):569–573.
- Smolders J, Peelen E, Thewissen M, Cohen Tervaert JW, Menheere P, Hupperts R, Damoiseaux J. 2010. Safety and T cell modulating effects of high dose vitamin D3 supplementation in multiple sclerosis. *PLoS One* 5(12):e15235.
- Sommer F, Backhed F. 2013. The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol* 11(4):227–238.
- Srivastava AS, Kaido T, Carrier E. 2004. Immunological factors that affect the in vivo fate of T7 phage in the mouse. *J Virol Methods* 115(1):99–104.
- Stromnes IM, Goverman JM. 2006. Active induction of experimental allergic encephalomyelitis. *Nat Protoc* 1(4):1810–1819.
- Summerday NM, Brown SJ, Allington DR, Rivey MP. 2012. Vitamin D and multiple sclerosis: review of a possible association. *J Pharm Pract* 25(1):75–84.
- Talham GL, Jiang HQ, Bos NA, Cebra JJ. 1999. Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect Immun* 67(4):1992–2000.
- Troiano M, Lucchese G, Graziano G, Taylor BV, Simpson S, Jr., Lepore V, Grand'maison F, Duquette P, Izquierdo G, Grammond P, Amato MP, Bergamaschi R, Giuliani G, Boz C, Hupperts R, Van Pesch V, Lechner-Scott J, Cristiano E, Fiol M, Oreja-Guevara C, Saladino ML, Verheul F, Sleen M, Paolicelli D, Tortorella C, D'Onghia M, Iaffaldano P, Drenzo V, Butzkueven H. 2012. Geographical variations in sex ratio trends over time in multiple sclerosis. *PLoS One* 7(10):e48078.
- Ventura M, Sozzi T, Turrone F, Matteuzzi D, van Sinderen D. 2011. The impact of bacteriophages on probiotic bacteria and gut microbiota diversity. *Genes Nutr* 6(3):205–207.
- Villion M, Moineau S. 2013. Virology: phages hijack a host's defence. *Nature* 494(7438):433–434.
- von Geldern G, Mowry EM. 2012. The influence of nutritional factors on the prognosis of multiple sclerosis. *Nat Rev Neurol* 8(12):678–689.
- Wagner J, Maksimovic J, Farries G, Sim WH, Bishop RF, Cameron DJ, Catto-Smith AG, Kirkwood CD. 2013. Bacteriophages in gut samples from pediatric Crohn's disease patients: metagenomic analysis using 454 pyrosequencing. *Inflamm Bowel Dis* 19(8):1598–1608.
- Wang Y, Telesford KM, Ochoa-Reparaz J, Haque-Begum S, Christy M, Kasper EJ, Wang L, Wu Y, Robson SC, Kasper DL, Kasper LH. 2014. An intestinal commensal symbiosis factor controls neuroinflammation via TLR2-mediated CD39 signalling. *Nat Commun* 5:4432.
- Wekerle H, Kuchus FC. 2006. Animal models of multiple sclerosis. *Drug Discov Target Dis Models* 3(4):359–367.
- Whitman WB, Coleman DC, Wiebe WJ. 1998. Prokaryotes: the unseen majority. *Proc Natl Acad Sci U S A* 95(12):6578–6583.
- Wingerchuk DM. 2012. Smoking: effects on multiple sclerosis susceptibility and disease progression. *Ther Adv Neurol Disord* 5(1):13–22.
- Wright A, Hawkins CH, Anggard EE, Harper DR. 2009. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clin Otolaryngol* 34(4):349–357.
- Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, Zecchi R, D'Angelo C, Massi-Benedetti C, Fallarino F, Carvalho A, Puccetti P, Romani L. 2013. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* 39(2):372–385.
- Zhang X, McDaniel AD, Wolf LE, Keusch GT, Waldor MK, Acheson DW. 2000. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J Infect Dis* 181(2):664–670.
- Zimecki M, Weber-Dabrowska B, Lusiak-Szelachowska M, Mulczyk M, Boratynski J, Pozniak G, Syper D, Gorski A. 2003. Bacteriophages provide regulatory signals in mitogen-induced murine splenocyte proliferation. *Cell Mol Biol Lett* 8(3):699–711.

Address correspondence to:

Dr. Justin D. Glenn

Division of Neuroimmunology and Neurological Infections

Department of Neurology

Johns Hopkins University

School of Medicine

Meyer Building, Room 6-138

600 North Wolfe Street

Baltimore, MD 21287

E-mail: jglenn7@jhmi.edu

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