Autoimmunity Reviews 18 (2019) 814–824

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

The gut microbiota perspective for interventions in MS

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ARTICLE INFO

Keywords:
Gut microbiota
Experimental autoimmune encephalomyelitis
Probiotics
Multiple sclerosis
Akkermansia muciniphila
Mucosal immunity

ABSTRACT

The heritable genetic variation that explains phenotypic differences in a population fluctuates for different autoimmune disorders. Particularly in multiple sclerosis (MS) etiology, modest genetic and major environmental effects emerge.

Increasingly recognized as a major environmentally shaped contributor to disease and treatment outcomes are gut microbiota.

As discussed here, the observed impact of gut microbiome on MS pathophysiology, involves both quantitative and functional changes in composition, metabolism, gut permeability, homeostasis and modulation of the immune system. Although the first supplementary therapeutic interventions have been approached in general autoimmune disorders they are relatively cruder and a translation of knowledge from other pathologies is valuable but still required.

Consequently initial therapeutic interventions with microbiota for autoimmune disorders could be correspondingly improved.

1. Introduction

MS as other autoimmune diseases is triggered by largely unknown environmental factors in genetically susceptible individuals. The genetic contribution to MS has been inferred from heightened disease risk among related individuals [1–5]. A contribution of human leukocyte antigen system (HLA) variability and over 200 independent associations in genome wide association studies (GWAS) have confirmed that observation [6–19,17]. However, each GWAS associated locus has only small effect size, and disease concordance among monozygotic twins is only 20-30%, so that environmental effects are paramount in susceptibility [20–22].

Thus a contemporary view of the disease consist of organ specific, central nervous system directed, autoimmune process that is cell mediated and likely initiates in periphery, triggered by environment. Indeed the genetic findings shared between the autoimmune disorders confirm conceivably the erroneous interaction of host with environment contributing to the disease etiopathology [23,24]. That interaction guides immune cell levels, differentiation and activation showing characteristics for autoimmunity time-space specific manner through the precise genomic enhancers regulation pathomechanism that is a molecular feature of corresponding host-environment interactions [25,26].

Both adaptive and innate immune cells participate to the pathogenesis; abnormal vascular permeability of the blood barrier has been observed before inflammatory demyelination and subsequent activation of microglia and adaptive immunity [27–29].

Notably, disease prevalence differs in geographically distinct populations and children who migrate from high-risk to low-risk environments acquire the risk level at the new location [30,31]. But triggered immune system in an adult maintains the risk of the country of origin [32,33]. Correspondingly, the disease is rare in Africans but affects African Americans, with a more severe course in second generation migrants [34,35]. The dynamics of higher prevalence of autoimmune disease in specific populations have been seen for other autoimmune disorders as Systemic Lupus Erythematous (SLE) [36].

Abbreviations: MS, multiple sclerosis; HLA, human leukocyte antigen system; GWAS, genome wide association studies; SLE, systemic lupus erythematous; T1D, type 1 diabetes; EAE, experimental autoimmune encephalomyelitis; IL-17A, interleukin 17 alpha; Th17, IL17 – producing CD4 + T cells; SBF, segmented filamentous bacteria; CNS, central nervous system; IgA, immunoglobulin A; iNKT, invariant natural killer T; LPSA, lipopolysaccharide A; IgA + PC, plasma cells of secretory immunoglobulin A; IgG, immunoglobulin G; 16S rRNA, 16S ribosomal RNA bacterial gene; OTUs, operational taxonomic units; SCFAs, short-chain fatty acids; HLA-DR, Human Leukocyte antigen – DR isotype; Th1, Type 1 T helper; Th2, Type 2 T helper; TLR7, toll-like receptor 7; TRPV1, Capsaicin Receptor; PAR, pregnane X receptor; IBD, inflammatory bowel disease; CTLA-4, cytotoxic T lymphocyte – associated antigen; PD-1, programmed death 1

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https://doi.org/10.1016/j.autrev.2019.03.016
Received 14 March 2019; Accepted 19 March 2019
Available online 07 June 2019

Therefore the autoimmune diseases risk factors as viral infections, vitamin D deficiency, and smoking have all been proposed as environmental contributors also to MS pathogenesis [37–42]. The other variables, both causing prolonged inflammation or metabolic deficits, included high salt intake, melatonin deficiency, diet, obesity, and stress [43–48].

The technological progress on whole genome sequencing allows dissection of a contribution of the gut microbiome to the autoimmune diseases etiopathology including MS [49–51]. Those studies showed that gut microbiome modulate human host innate and adaptive immune responses by quantitative or qualitative changes of microbial taxa composition with different role in host metabolism, but also by the control of immune system development and function, and at the end through an antigenic interaction [52,53].

A recent study in mice microbiome supports MS microbiota findings and underline the critical role of environment and microbial variations on host health. Indeed, germ-free laboratory mice - C57BL/6 reconstituted with natural microbiota from wild *Mus musculus domesticus* exhibited reduced inflammation, increased survival after influenza virus infection, and resistance to mutagens [54].

Factors initiating anomalous immune response or interfering into restraining self-reacting mechanisms as viral infections, could cause prolonged inflammation with exhaustion of components of immune response and when intersected with permissive genetic background contributes to autoimmune responses [29].

But also absence of specific infective immune stimulation correlates with improved socioeconomic conditions and a parallel rise in autoimmunity [55,56]. Immune system education can be related to gut colonization differences temporally and geographically, with Gram-negative bacteria that carry immunomodulating lipopolysaccharide, coffering protection from MS, appearing later in children gut in developed countries [55,57,58]. Moreover recent large-scale multiethnic metagenomic studies confirm spatial phylogenetic and functional diversity of population-specific characteristics of microbiota composition [59–61].

Although a strong adaptive feature of the postnatal immune system development in a presence of variable microbiome has been described in a recent study [52]. Could it illustrate how changed should be an ecosystem to conduct to an autoimmune state or that extreme adaption to new environmental settings to trigger the pathology?

2. Microbial interactions in EAE mouse models

A quantitative deficiency of microbiota biodiversity – dysbiosis, has been associated with metabolic (obesity), autoimmune (type 1 diabetes (T1D), inflammatory bowel disorder, Crohn's disease, celiac disease, SLE, psoriasis) disorders, malnutrition and cancer therapy response [49,62–65].

But in MS gut microbiota well powered studies a dysbiosis state has not been observed, instead the association has been attributed to the quantitative shifts in abundance of specific species [66–68].

The main evidence of imbalance in gut ecosystems in MS came from a gnotobiotic (mice with defined microbiota), transgenic, germ-free mice models of relapsing remitting MS, expressing a rearranged T cell receptor, specific for myelin basic protein, that developed to different extend and depending on the mouse genome and transgene used, genetically controlled, spontaneous experimental autoimmune encephalomyelitis (EAE) when housed in non-sterile conditions [69–73]. Consistently, in such conditions another germ-free mice model developed attenuated EAE, along with lower levels of the proinflammatory cytokines - interferon gamma, interleukin 17A (IL-17A) and an increase in CD4 + CD25 + FOXP3 + regulatory T cells. Instead intestinal colonization with segmented filamentous bacteria (SFB) in those animals promoted IL17 production in the gut comparably to enteric infections and induced IL17A- producing CD4 + T cells (Th17) in the central nervous system (CNS) [74]. SBF comprise predominantly Firmicutes – Bacilli and Clostridia prompting immunomodulatory responses including B cell activation and immunoglobulin A (IgA) secretion. In particular the spore-forming *Clostridia* with more abundant *Clostridium perfringens*, overrepresented in MS patients impaired anti-inflammatory responses *in vitro* causing dampened CD4 + CD25 + FOXP3 + regulatory T cells differentiation and IL-10 secretion and brain blood breakdown [75,76].

Those studies demonstrated that autoimmune adaptive mechanisms triggered by microorganisms present in ecosystem or shifts in specific gut microbiota are accompanied by, altered immune state in gut, peripheral immune cells activation and EAE exacerbation.

Additionally innate mechanisms responded after gut flora antibiotic manipulation by altering function of natural killer T (iNKT) and suppressing EAE. Specifically - V alfa 14 invariant cells induced the production of the mesenteric lymph nodes proinflammatory Th17 cells [77].

While those first experiments indicate the immunogenic and inflammatory role of gut microbiota in triggering of autoimmune attack on myelin, presentation of specific antigen could be beneficial for protection from MS. It has been shown that expression of sphingolipids, particularly lipopolysaccharide A (LPSA), by *Bacteroides fragilis* had immunomodulatory effects, inducing the adaptive immune system by increasing intestinal, protective regulatory T cells differentiation. In parallel, NK T cells were reduced in mice carrying that bacterium [78]. When neonatal iNKT cell proliferation was limited and restricted in adulthood, defended the host against experimental iNKT induced colitis suggesting existence of early in life autoimmune mechanisms [79].

Successively, human microbiota studies confirmed that observations, specifically the transfer of gut microbiota from a twin pair discordant for MS to transgenic mice expressing a myelin autoantigen specific T cell receptor induced EAE more frequently than microbiota from the unaffected sibling along with decreased IL10 production in MS cases [66]. Correspondingly, microbiota transplants including *A. muciniphila* and *Acinetobacter calcoaceticus* monocolonization from MS patients into germ-free mice caused more severe EAE exacerbation and reduced proportions of anti-inflammatory IL10 producing, regulatory T cells compared to fecal transfers from healthy controls [67]. Concomitantly a reduction of *Parabacteroides distasonis* that induce of regulatory T cells was reduced in MS patients demonstrating that specific stain overrepresentation and deficiency between gut microbiota contribute to MS etiopathology.

3. Mucosal immunity in MS

The gastrointestinal tract carries the largest mucosal barrier in a human organism with important role in protecting from environmental factors, including immune defensive responses. Specifically by mucosa associated lymphoid tissue producing plasma cells of secretory immunoglobulin A (IgA + PC) [80]. When impaired as in IgA knock out mice model alter serum IgG levels initiating systemic immunity responses [50]. Similarly in human selective secretory IgA deficiency is characterized by recurrent intestinal infections [81]. Moreover changes in intestinal permeability have been widely described in gastrointestinal autoimmune disorders and T1D [82–85]. Moreover the same mechanism has been inferred from genetic associations for psoriatic arthritis [23].

IgA protects the mucosal epithelium from invading pathogens, toxins and food-derived antigens but also regulate gut microbial composition. The importance of B cells in mediating neuroinflammation in MS has been underlined by the effective anti-B therapies and recent genetic studies [72,86,87]. In the mucosal tissue B cells guided by the cytokines undergo class switch recombination of their immunoglobulin receptor to IgA and differentiate to PC. B lineage –specific expression of the innate effector molecules is required for IgA + PC homeostasis in the basal state and during pathogen infection [88]. The number and distribution of IgA + PC cells and IgA secretion in gut is regulated by

commensal microbial ecosystem, and in germ-free animals is diminished [88].

Interestingly, it has been demonstrated in EAE mice model that IgA+ PC originated from gut, suppressed neuroinflammation in an IL-10 dependent manner, rising importance of mucosal immunity impairment in MS [89]. The IgA+PC gut derived intraepithelial lymphocytes migrated from gut to periphery and then to inflamed CNS suppressing neuroinflammation, preventing EAE and reducing granulocyte-macrophage colony-stimulating factor producing CD4+T cells. A parallel reduction of IgA -bound fecal bacteria has been observed in MS patients during relapse [89]. Concomitantly decrease levels of secreting IgA altered diversification of gut microbiota and reduced elimination of gut – tropic pathogens [80]. Thus, corroborated by compromised intestinal barrier function in EAE and MS, it has been postulated that imbalances in mucosal PC regulation allow their egress from gut. Moreover IL10 production by B cells is required to prevent various autoimmune disorders including EAE recovery.

Mobilization of immunosuppressive IgA+ PB or PC is a valuable therapeutic target; those cells are not depleted during Rituximab anti-CD20 therapy against memory B cells while Atacicept therapy depleting both B and PC cells was damaging in MS [88–90].

The second large mucus host –environment interactive barrier - human lung has been studied for lesser extend in MS. It has been acknowledged that relapses in MS are initiated by local inflammation of the respiratory tract [91]. The migration study of T cells from bronchus associated lymphoid tissues and lung-draining lymph nodes that reentered to peripheral blood and then CNS, demonstrated that colonization by microbiota can cause local inflammation and formation of autoreactive T cells [92]. Those cells up regulated the locomotion molecules, membrane receptors that guided cells out from lymph nodes concomitantly with chemokine receptors and adhesion molecules toward inflamed tissues and transgressed endothelial barriers including CNS initiating EAE.

That observations open novel perspectives on the development of novel therapeutics as recombinant chimeric secreting IgA/IgG antibodies designed against recolonization of the pathogenic species or mucosal vaccination [93]

4. 16S rRNA and metagenome MS gut microbiome studies

Recent multidisciplinary studies using whole genome sequencing technology characterized of broad taxonomic variability, including species that are difficult to grow in culture or rare. Also the composition of the MS gut microbiome has been approached along with putative metabolic and immunogenic functions.

Although contemporary view of MS gut microbiota is based mainly on mouse studies, and primarily analyses of bacteria, the mammalian gut microbiota is a complex and diverse ecosystem including the bacterial and archaeal microbiome, but also the virome (bacteriophages, eukaryotic viruses), the mycobiome, and the immune-stimulating meiofauna of which involvement can not be excluded [94].

Most human microbiome studies are modestly powered and largely based on operational definition of the variants in 16S ribosomal RNA bacterial gene by sequencing, but provide an overview that has highlighted higher abundances in MS patients of *Methanobrevibacter* (Archaea), *Akkermansia muciniphila* (Verrucomicrobia) and a reduction in butyrate-producing and regulatory T cell-inducing Butyricimonas confirming gut microbiota involvement [67,68,70]. However larger multiethnic studies are needed to define precise impact of specific strains of MS gut microbiome.

5. Importance of technical issues in proper assessing MS gut microbial composition

Although fecal samples do not represent microbial mucosal composition of the gut, still their components are adequate for gastrointestinal system microbiome reconstitution, providing potential marker or state of the gut colonization and offering an intervention target [95–97].

Between 10^{23}-10^{24} microbial cells present in human gut, *Bacteroidetes* are most represented phylum and it is conceivably that this gives the higher power to detect possible shifts in abundance even in small studies. Indeed lower abundances of that phylum members, *Bacteroides* and *Prevotella*, have been consistently shown even in lesser powered MS gut microbiota studies [76,98,99]. Interestingly, *Bacteroidetes* and *Prevotella spp* have been shown enriched in non-Westernized populations [59,61].

Importantly, gut microbiota studies require: i) sufficient coverage of sequenced microbial reads; ii) an adequate study design, including environmentally matched control group, adjusted for gender, age and therapy; iii) elimination or correcting of confounding factors and batch effects; iv) implementation of improved reference annotation with use of well established catalogues; iv) and adequate association analysis, with correction for multiple testing in association analyses.

Because of the tens of trillions of microbial cells and the variety of species with different combinations of frequencies, it is important to establish the power of the study, especially in a context of the small size effect. Correction for multiple testing in case-control studies is challenging but critical. It has been established that species with genomes that reached 20x coverage in metagenomic studies are significantly abundant, but that is difficult to obtain for all microbiome members present in a sample [100]. Besides it has been argued that the number of sequence reads from unobserved Operational Taxonomic Units (OTUs) should be subtracted from the total number of reads when estimating the coverage of a species based on the composition and size of their genome. Although the unobserved OTUs significantly affect the alpha-diversity distribution of a given metagenome and the genomic coverage of rare species, they do not affect the genomic coverage of common species. While representative current experiments target 1-10Gb of data, no published rules prescribe correct coverage and these depths may be either unnecessary or insufficient depending on the sensitivity required to detect rare members targeted in a gut ecosystem. Overall, at least 7Gb of sequence are required to enumerate the gene content of prokaryotes with a relative abundance of more than 1% in a sample of human faecal microbiota genome [100–103]. Although, a mappability of human gut metagenomes increases over 87%, annotation has been limited to the reference genome catalogues, leaving cryptic unread part of the data specially of rare species [59,104]. Similarly the effect size for each strain is not well defined and it is unknown whether rarer OTUs large effects exist and if the common MS associated strains tag more associated subtypes.

Moreover extreme inter-individual and between population variability of gut microbiome could limit computational analysis. But also substantial strain level heterogenetity has to be characterized. The microbial phenotypes are specific and for example the species *Escherichia coli* includes strains that are physiologically commensal, pathogenic or carcinogenic [105]. Similarly, subtypes of *Prevotella copri* are associated with low-fat diet and others with arthritis and only specific subtypes of *Staphylococcus epidermitis* contribute to psoriasis or *Bifidobacterium breve* strains used as anti-inflammatory probiotics could cause meningitis [105–107].

Because of the major effect of obesity, high caloric diet, diet itself on microbiota composition and concomitant impact of those factors on autoimmunity it is important though challenging to consider it as covariates in association analyses. That would be possible implementing anthropometric measures, dietary questionnaire and food diaries with use of daily used devices [94,108].

6. Host genetic determination of gut microbial composition

Remarkably, gut microbiota composition can be shaped predominantly by environmental factors - not significantly associated with

genetic variability; a major portion of inter-person microbiome variability has been connected to diet, parallel treatment, and anthropometric measurements [94]. Correspondingly, gut microbiota composition had an average heritability of only 1.9% in the UK twins cohort [109]. Environment also explained larger portion of the observed variability of specific immune system components that inevitably interact with gut microbiota [110–113]. Impacts of exposure to infections, vaccination, nutrition and microbiome composition have an additive effect during human life [110,114]. Thus, significant microbiome similarity exists among relatives who share a household [115]. Indeed, it has been recently estimated that the average genetic heritability of the gut microbiota is only about 2-8 % [94]. Consistently, the gut microbiomes of healthy twins become dissimilar over time when they live in different households [116,117].

Interestingly, changed abundances of some strains of microbiota genetically determined in humans including *Blautia*, *Methanobacteriaceae*, *Bifidobacterium*, were also associated with MS. Notably, the concordance rate for carriage of *Methanobrevibacter smithii* methanogen is higher for monozygotic twins.

Previous studies have identified several heritable bacterial taxa, though the combined bacterial abundance accounted for has not been quantified. Other studies have even found associations between host genetic variability and individual bacterial taxa or pathways, but most reported associations are not statistically significant after multiple testing correction [94,118].

7. Probiotic candidates and supplementary therapy in MS

While the human microbiome provides a well studied diagnostic and therapeutic target, interventions on the microbiome to alleviate MS are still largely conjectural.

The dynamic character of probiotic action could explain that problematic. A probiotic has been defined as live miroorganisms that, when administrated in adequate amounts, confer a health benefit on the host [119,120]. Possibly because of a living supplementary therapeutics - probiotic dynamics are more complex than previously thought, with enormous variability of gut taxa functions and opposite effects of specific strains on different pathologies. Competitive, metabolic gut ecosystem interactions among the probiotic-gut microbiota and -gut epithelium involve synergistic or antagonistic mechanisms that affect clinical trials [32,73]. Minimally invasive methods such as dietary changes to reestablish a growth of favorable strains or oral administration of probiotics to retrieve gut immune balance are conceived.

General mechanisms of probiotic favorable effects on host include: i) inhibition of bacterial toxins as for example *Saccharomyces boulardii*; ii) dampening intestinal pH by production of acetic, lactic and propionic acids by *Lactobacillus spp*. that inhibit growth of pathogenic strains as *Escherichia coli* and *Clostridium spp*.; iii) presence of probiotics in intestinal tract that physically or chemically concurrent for an ecologic niche impeding adhesion and colonization of pathogenic bacteria; iv) inducing, educating during development or enhancing host immune responses directly in gut or indirectly in periphery [121].

Specifically, the observed protective effect of the immunomodulatory polysaccharide A from *Bacteroides fragilis* on EAE suggests the potential in MS [78,79]. Also administration of specific strains *Lactobacillus spp.* and *Bifidobacterium,* which were underrepresented in MS patient's gut, ameliorated EAE in mice and increased regulatory T cell levels [122,123].

The common studies use mix of live microbial therapeutics belonging to the Bifidobacterium, Lactobacillus, Enterococcus, Streptococcus, Propionibacterium and Bacillus strains because they are depleted in many conditions but also are present in dietary yogurt or kefir supplements [124]. Supplementation of those species have been associated with reduced risk of islet autoimmunity in highest genetic risk children with T1D [125]. Human origin probiotic cocktails successfully increased short-chain fatty acids (SCFAs) production and ameliorated gut microbiome dysbiosis. In a recent trial the most commonly tested probiotic VSL3 was associated with increased abundance of several taxa including *Lactobacillus*, *Streptococcus* and *Bifidobacterium* in MS patients. That supplement, induced an anti-inflammatory peripheral immune response characterized by decreased frequency of CD14++CD16+ intermediate monocytes, maturation of classical CD14++CD16- monocytes and HLA-DR antigen expression on dendritic cells [122,126]. However, the microbial community returned to baseline levels after supplementation, suggesting homeostasis and resilience of personal microbiota and possible utility of combined intervention - for example, with diet.

Interestingly, *Clostridium immunis* administration to colitis susceptible mice protected against disease associated death in a chemically induced mice model of inflammatory bowel disease [127]. Similarly a combination of 17 Clostridial species reduced severity of experimental colitis by increasing the regulatory T cells levels [128]. This approach could be extended to supplement deficient abundances of particular organisms in MS patients – or to provide critical metabolites to substitute for microbial defective function and improve bowel dysfunction [51].

Butyrate producers *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Roseburia* spp. have been recognized valuable probiotic target. *F. prausnitzii*, a major member of the Firmicutes, affecting butyrate production and gut mucosa, has been considered as a potential probiotic in ulcerative colitis patients [129], Underrepresentation of immunomodulating *F. prausnitzii* has been associated with autoimmune and metabolic impairment including Crohn's disease, ulcerative colitis, inflammatory bowel disease, diabetes, obesity, asthma, eczema and atopic dermatitis and negatively associated with inflammatory markers [130].

Conversely, just as some gut microbiota are resilient to pathogens, they can also be resistant to probiotics [131]. The specific microbial communities along gastrointestinal system could compete for a niche and could interfere into reconstitution of microbiota with exogenous species. A review of randomized clinical trials found no effects of probiotics on microbiota composition and no indication for probiotic engraftment [132]. Also, information from clinical trials of probiotics often does not reveal confounding factors [133,134]. Thus the probiotics manufacturing norms have to preclude supplements from contaminants and incorrect species of bacteria, considering the risks associated with introducing new genes in host microbiome conferring antibiotic resistance. For example in rodent model probiotic *Lactobacillus plantarum* M345 can transfer erythromycin resistance genes to *Listeria monocytogenes* [134].

Inferring from irritable bowel syndrome disease studies, where agents other than probiotics are proposed – like SYN-010 decreasing methane production by blocking cell membrane synthesis and considering concomitant association of *Methonbrevibacter smithi* with MS the strategy could be reused [135].

An alternative to wide spectrum antibiotic therapy could be bacteria-derived, biological activity: antibiotics, bacteriocins and thiopeptides. For example lantibiotics produced by *Lactococcus* species, small molecules are directed against specific bacterial species without destroying commensals [136–138].

The complexity of optimal oral probiotic supplementation effects in an established ecosystem can include delayed microbiota recovery back to baseline after antibiotic therapy - while autologous fecal transplantation is effective [95]. Thus it has been demonstrated that the post-antibiotic microbiome reconstitution of gut in mice have been delayed by probiotic use. Moreover healthy humans recover faster their gut microbiome after autologous fecal transfer or spontaneously. Additionally, characterization of the human gut-mucosa – associated microbiome show preferential engraftment by an existing host ecosystem than probiotics colonization [96].

Likewise immune and metabolic conditions in the gut regulated colonization during probiotic engraftment; the nutrients coming from

diet, metabolic cross-feeding, host-microbiota and inter-microbiota signaling had an important role in niche competition and settlement [97].

Importantly, beneficial manipulation of human microbiome could include prebiotics –non-viable selectively fermented substrates that serve as nutrients for beneficial microorganisms carried by host including resident or probiotics microorganisms conferring benefits to host health [119]. The definition includes non-digestible by host fructans-fructooligosaccharides, inulin and galactans-galactooligosaccharides that are fermented by gut microbiota known to promote enrichment of *Lactobacillus* and *Bifidobacterium* spp [139].

Similarly, the targeted strain specific microbiome manipulation recall broader knowledge on strain-specific functions and the first biologic specifically targeting *Clostridium difficile* toxin B has been found to limits epithelial damage and augmenting microbiome recovery [140].

8. Potential impact of a diet on MS microbiota composition and function

The relevant effects of diet on MS are partially understood. Diet contains compounds used by the body directly or modified by microbes that at the same time shape microbiome composition. Plant carbohydrates indigestible for humans are, fermented by colon microbiota to produce SCFAs like acetate, propionate and butyrate, which provide energy, modulate inflammation and vasodilatation, participate in motility and tissue injure healing [141]. High fiber diet modulate mucosal immunity through the microbial fermentation to anti-inflammatory SCFAs that maintain mucosal barrier, production of secretory IgA stratifying microbiota on luminal part of epithelium and regulatory T cell differentiation [52].

The modern diet in most industrialized countries i.e. “Western diet” – low –fiber, high-fat diet, accompanied with use of antibiotics in healthcare and agriculture, contains massively produced, highly refined food selects for microbiota that lack resilience and diversity required for balanced immune responses. Moreover, the micobiome from different lifestyle societies differs quantitatively with specific species for non-Western style, in composition but also functionally; sulfur energy metabolism, vitamin B12 recover, homeostasis of sodium dependent energy production, antibiotic biosynthesis functions, are significantly enriched in Western microbial ecosystems [59].

Dietary intervention improve microbial richness [142]. A fiber diet promotes colonization of phylogenetically diverse bacterial species in a gut and subsequent butyrate production [143]. Indeed a fiber – rich diet combined with probiotics shifted gut microbiome functional composition and contributed to weight loss in obese children [144]. While long-term diet influences structure and activity of gut microbiota communities, temporary changes to specific diet can reverse microbial composition, hinting at intervention in MS [145]. Indeed, radical switches to all-plant or animal based diets could have greater impact [145]. Certainly, individuals on a higher fiber diet present more phylogenetically diverse bacterial species producing more butyrate. Indeed, dietary long-chain fatty acids promote differentiation and proliferation of Th1 and Th2 cells, whereas short-chain fatty acids expand gut regulatory T cells ameliorating EAE, with potential MS therapeutic implications [47].

Shifts in abundance of taxa in MS patients that belong to the *Firmicutes* phylum, proficient in metabolism of dietary fiber, could indicate a dietary insufficiency. Also in the absence of dietary fiber the mucin-degrading species expand [146]. Butyrate producing families were also underrepresented in MS patients [68]. Indeed, *Prevotella,* a marker of long-term fiber intake, and *Christensenellaceae,* which alters metabolism and reduces obesity, were less abundant in MS patients and EAE model [147].

Dissection of dietary effects on gut microbiota in SLE mice model pointed to the possible dietary interventions in human. A *Lactobacillus reuteri* previously known to activate epithelial barrier and studied as a probiotic, trigger autoimmune disease in Toll-like receptor 7 (TLR7) –dependent mouse model of SLE when feed with starch free diet increasing plasmocytoid dendritic cells and interferon signaling [148]. Moreover starch present in diet prevented migration of *L. reuteri* from gut to the periphery. Likewise subset of SLE patients carried similar pattern of gut microbiota composition as observed in mice models with starch deficient diet.

Importantly, recent studies showed that in geographically different societies harbor distinct, culturally shaped microbiome and populations experiencing shifting to other dietary habits for example specific spice as cholekinetic spice-turmeric in Asian populations undergo the changes in gut microbiome [60]. Indeed various components of the diet have an impact on the immune system and autoimmunity thus exploring diet of different world population could bring the possible examples of therapeutic supplements. Specifically, anti-inflammatory dietary components as capsaicin derived from chili peppers acting on capsaicin receptor (TRPV1) regulated T cell activation with effector cytokine production in autoimmunity [149].

Further studies on effects of diet in MS patients could help to devise favorable probiotics and interventions on gut microbiota.

9. Gut microbial metabolites impact on MS

Microbial communities produce metabolites that are released to the blood regulating host metabolic and immune-inflammatory pathways [150]. Microbiome status significantly affects prediction accuracy for human traits, such as metabolic - glycemic and obesity parameters compared to models that use only host genetic and environmental data [94].

Although most of the contemporary MS microbiome studies based on microbial DNA genomes sequencing demonstrate that gut ecosystem biodiversity impact host immunity, the rare metabolomic studies brought in light the metabolic deficiencies importance. Interestingly, in concurrent with observed obesity impact on MS, depletion of members of *Bacteroidetes* has been shown to cause aberrant host-microbiota interactions in obese individuals [62,68,151].

Moreover the predominant anaerobic archaeon in human gut - *Methanobrevibacter* enriched among MS gut microbiota - combine hydrogen oxidation with CO_2 reduction to produce methane [68]. It cannot be replaced by alternative orders providing microbial biomarker. Interestingly, methane producing *Methanobrevibacter* are associated with constipation and irritable bowel syndrome and may be relevant to abnormal gut function in MS patients [152].

Similarly, microbial metabolites limit pathogenic activities of microglia astrocytes suppressing central nervous system inflammation. Tryptophan-derived metabolites levels are controlled by gut microbiota; genetic manipulation of a gut commensal in experimental model altered levels of indolepropionic acid altering host immunity pronounced by higher levels of myeloid and activated T cells in periphery and increasing of secreted IgA in gut [153]. Indolepropionic acid produced from tryptophan by microbiota could enforce intestinal barrier acting on pregnane X receptor PAR and has neuroprotective properties [153,154]. A diet depleted in tryptophan produced by commensal flora controlled microglial activation, tumor necrosis factor alpha and vascular endothelial growth factor production, modulating astrocyte transcriptional activity and CNS inflammation through a mechanism mediated by the aryl hydrocarbon receptor. That process exacerbated EAE in mice, whereas diet enriched in tryptophan ameliorated disease [155,156]. A recent study comparing Western with non –Westernized lifestyles human microbial ecosystems found the differences in presence of genes of tryptophan metabolism pathway differentially present in two groups underlying an evolutionary importance of adaptation for availability of important molecules [59].

10. *Akkermansia muciniphila*: an established pro-inflammatory component of MS gut microbiota?

Three recent studies have shown induction of pro-inflammatory responses by the *Verrucomicrobia* member *Akkermansia muciniphila* in "not under therapy" MS patients [66–68,75].

The frequency of different mucus degrading, sulfate releasing genus of *A. muciniphila* reaches 1-5% in gut of European descent individuals providing power to detect associations during pathologic shifts [157,158].

Complex interactions of microbiota and host are illustrated by opposite trends in the relative abundance of *A. muciniphila* in autoimmunity: increased in MS but reduced in T1D and inflammatory bowel disorder [159,160]. Whereas several studies have shown positive associations between *A. muciniphila* on host metabolism, direct administration - has shown protection for obesity, type 2 diabetes, and atherosclerosis conversely, obesity has been shown as a risk factor in MS. Moreover purified membrane protein from *A. muciniphila* or pasteurized bacteria improves metabolism in obese, diabetic mice model preventing systemic inflammation [161]. That founding is in line with recent mutiethinc microbiome study that showed *A. muciniphila* significantly associated in Westernized populations [59]. In all studies reporting *A. muciniphila* positive association with MS mixed cohorts consisted of untreated and under therapy patients in a period of disease remission, potentially illustrating benefits of the immune modifying therapy. However the *A. muciniphila* association with MS remained significant after correction for therapy [66]. Still, the *Akkermansia* genus contains various strains that could be differently classified in various studies therefore subtyping is required to reveal strain/effect differences among pathologies.

Mucosal layers minimize exposure of resident bacteria to the systemic immune system but some bacteria penetrate to cause specific immune responses. Coherently to increasing abundance of mucus degrading species of *Akkermansia* that is observed in the absence of diet fiber indicate possible dietary deficiencies in MS [146]. Accordingly, hypothetically in gut microbial disequilibrium, pathobionts could contribute to the mucosal barrier destabilization and interacting with immunogenic spore forming bacteria, SBF exacerabrate MS with function of *A. muciniphila* in decompartmetizing immune barrier and molecular-mimicry, downregulating regulatory T responses in gut - Clostridium [75]. Moreover, intestinal barrier malfunctioning and distorted permeability has been shown in MS patients and EAE [162,163]. Importantly, the gaps in epithelial cells of the gut villi have been detected in EAE mice model corroborating that observation [89].

Massive antigenic load in gut has to be compartmentalized to prevent immune system anomalous activation, destabilization of mucosal barrier could thus contribute to beneficial or pathogenic phenotypes.

11. Gut microbiome predators (bacteriophages) could alter the gut ecosystem equilibrium

Challenging to measure even with metagenomic approaches, viruses are potent immunomodulators that can contribute to immune-mediated diseases [114,164]. It has been known that in imbalanced ecological conditions destabilizing bacteria-phage gut dynamics could lead to the consequent disappearance [165]. The blood virome including Epstein Barr virus has been extensively studied for MS, but little almost nothing is known about predator-prey dynamics among gut communities of bacteria and their phages in MS.

The bacteriophages are most abundant among gut microbiota providing also higher power to detect possible effects. They significantly control eubiosis of the human microbiome. But in general phage infection/expansion decreases bacterial prey, leaving open a niche that competitive bacterial taxa populate. Although some phage-interventions were implemented to modulate the diversity of gut bacteria, more information on the MS gut microbiome is needed before supplementation could be considered.

As an example expansion of *Caudovirales* bacteriophages with decreased diversity in individuals with Crohn's disease or IBD contributes to intestinal inflammation and bacterial shifts, with potential as a biomarker [166]. Intriguingly, that phage order was enriched in mouse models fed a Western diet, lowered by low-fat diet [167]. Moreover quantitative and qualitative bacteriophage changes were associated with T1D development [168].

Considering beneficial or opportunistic influences, such viruses represent an alternative to broad-spectrum antibiotic therapy.

12. Gut microbiota and melanoma therapy outcome – lessons for MS

Inter-individual microbiome variation has been linked to differential response to cancer therapy. Similarly to autoimmune disorders melanoma pathogenesis includes the presentation of novel autoantigens however fighting cancer immune system requires augmentation rather than depression of immune response that is desirable in MS. Treatment strategies included immune checkpoint inhibitors against the cytotoxic T lymphocyte – associated antigen (CTLA-4) and programmed death 1 (PD-1) protein both with function in self-tolerance and prevention of autoimmunity that downregulation potentiates of immune response.

Moreover some of MS therapies as natalizumab, a humanized monoclonal antibody against the cell adhesion molecule alpha 4 integrin, could trigger melanoma while cancer treatment could activate autoimmune disorder [169]. Therefore all factors modulating immunity including gut microbiome can differently influence cancer therapeutic responses. For example a recent study found that only mice carrying *Bifidobacterium* responded to immune checkpoint therapy of melanoma [170]. Indeed in melanoma patients treated with anti-PD1 immunotherapy the significant differences in gut mirobiota diversity and composition was observed. Interestingly, enhanced gut microbiome diversity was associated with improved response to the therapy. Specifically, *Faecalibacterium* genus and Akkermansia were significantly enriched in melanoma immunomodulating therapy responders while *Bacteroidales* including *Bacteroides thetaiomicron*, *Escherichia coli* and *Anaerotruncus colihominis* augmented in non - responders [171,172]. Moreover patients with high abundance of Clostridiales, Ruminococcaceae or *Faecalibacterium* in gut presented higher effector CD4+ and CD8+ T cell levels and markers of antigen presentation in periphery concomitantly responding to the therapy. A higher abundance of Bacteroidales had higher levels of regulatory T cells and myeloid derived suppressor cells with diminished cytokine response thus showing possible microbiome use in modulation of immune response. Congruently, in a small study, *Faecalibacterium prausnitzii* together with Akkermansia have been enriched in MS patients responders to vitamin D treatment [98]. It will be interesting to see whether different probiotic regimens have comparable or antagonistic actions for autoimmune disease and particular cancers.

Moreover study on melanoma therapeutic strategies underlined unpredictable effects of antibiotics on microbial function; anti PD-1 blocking combined with antibiotic therapy was associated with less effective outcome [171].

From the other side blocking the activation of T cells by CTLA-4 extracellular domain fused with immunoglobulin IgG1, Abatacept has been effective in autoimmune disorders including rheumatoid arthritis and T1D [173–175].

That studies underline that the immune response could be regulated by gut microbiota composition and at the same time physiological microbiota has impact on human disease.

13. Parallel treatment impact on gut microbiota and microbiome studies

Intuitively, MS modifying therapies like glatiramer acetate and

dimethyl fumarate but also immune-modulating interventions change gut microbial composition constituting confounding factor in gut microbiota studies [176,177]. Moreover dimethyl fumarate supplementation used previously in psoriasis significantly reduced relapses in MS through the immunomodulatory proprieties however with unknown impact on gut microbiota studied in present clinical trials [178–180].

Moreover the EAE reduced severity has been observed after antibiotics administration [181]. Therefore, published MS gut microbiota study design usually excluded possible impact of antibiotics. However non-antibiotic treatments like anti-diabetics (metformin), proton pump inhibitors, antipsychotics, non-steroidal, and anti-inflammatory drugs are not usually considered when enrolling study participants thought they also have an impact on gut microbiota composition. In general pharmaceuticals used regularly may contribute to decrease microbiome diversity in modern Western societies [182].

Conversely, oral antibiotics like vancomycin and ampicillin extended the lifespan of mice prone to the development of SLE. Gnotobiotic (NZWxBXSB)F1 lupus models lived longer and had lower titers of autoantibodies to auto-antigens than untreated mice. Interestingly, after the passage of bacterial products from the bowel into the circulation, bacterial endotoxins and peptidoglycans were recognized by host receptors inducing inflammation. In particular, *Enterococcus gallinarum* escaped the mouse intestine, spread to veins, gut-draining lymph nodes, and the liver, inducing plasmocytoid dendritic cells, that produce interferon gamma, implicated in lupus erythematous pathogenesis [183,184]. Moreover a presence of bacterial DNA in peripheral blood that was associated with systemic inflammation has been observed in psoriasis [185].

Although the specific antibiotic/bacteria pair have not been tested for MS, treatment with kanamycin, colistyn and vancomycin - antibiotics that act specifically in the human gut - protected mouse from EAE, modulating their innate immune system [77]. Furthermore, the treatment of mice model with a mixture of commensal bacteria can protect against EAE [123]. Likely those findings reflect the relative specificity of different microbes in autoimmune pathology or protection.

14. Conclusions

The study of human microbiota has extensive prospects for improvements of the human health and disease. Considering the biogeographic patterns in microbiota taxonomic composition, that could also reflect an overlapping function, likely next step is to confirm and extend the findings in well powered, carefully designed studies in various ethnic groups to refine the identification of functional changes in MS patients.

Microbiota impact on the host could be multifocal, modulating immune response or gut barrier defense however it has been difficult to distinguish which observed gut microbiota – MS patient interactions are markers of the disease or causal.

Although gut microbiome is becoming a biomarker of intestine associated disorders, metabolic, cardiovascular traits and therapy response in cancer, it is difficult to apply it as a diagnostic tool because of demographic factors that cause enormous variability - social position, age, diet, inflammation and obesity [186].

An open vital question is, can a change of dietary habits or modification of the physiological gut flora by probiotics or potential fecal microbiota transplantation contribute to lower risk or severity of MS?

Competing interests

The author declares she has no competing interests.

Acknowledgements

The author would thank to Dr. David Schlessinger, Retired Distinguished Scientist at Laboratory of Genetics and Genomics at NIA, NIH, Baltimore, USA, and Prof. Sergio Baranzini, University of California, San Francisco, USA for the useful comments and discussion on the manuscript; to the Sardinian voluntiers of Sardinian Autoimmunity Case - Control Study and SardiNIA cohort who generously participate to the study; to National Multiple Sclerosis Society, USA for awarding Pilot Project 6003279; to AISM society and PI and CNR-IRGB Director Prof. Francesco Cucca for continuous support.

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