A clinical update on the significance of the gut microbiota in systemic autoimmunity

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Abstract

Systemic lupus erythematosus (SLE) is a complex autoimmune disease where a loss of tolerance to nuclear antigens leads to inflammation in multiple organ systems. The cause of SLE remains ill defined, although it is known that a complex interplay between genes and environment is necessary for disease development. In recent years, case studies have reported that the incidence of SLE in the USA, for example, has increased by approximately 3 fold. Although the reason for this is likely to be multifactorial, it has been hypothesized that the increasing incidence of autoimmune disease is due to considerable shifts in the bacterial communities resident the gut, collectively known as the gut microbiota, following a change in diet and the widespread introduction of antibiotics. Furthermore, a growing body of evidence suggests that the gut microbiota plays a role in the development of a range of autoimmune diseases including inflammatory bowel disease, multiple sclerosis, type one diabetes and rheumatoid arthritis. In this review, we summarize how advances in DNA-based sequencing technologies have been critical in providing baseline information concerning the gut microbiota in health and how variation amongst individuals in controlled by multiples factors including age, genetics, environment and the diet. We also discuss the importance of the gut microbiota in the development of a healthy immune system and how changes in particular bacterial phyla have been associated with immune abnormalities in animal models of autoimmune disease. Finally, in order to place the data in a clinical context, we highlight recent findings showing that abnormalities in the gut microbiota can be detected in patients with SLE, which provides the rationale for greater investigation into whether

microbiota-targeted therapies could be used for the treatment/prevention of disease.

Key words: gut microbiota, dysbiosis, inflammation, autoimmunity

Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease, where a loss tolerance to nuclear antigens leads to pathology that can affect multiple organ systems. The diverse clinical presentations include rashes, arthritis, nephritis, seizures, serotosis, thrombocytopenia and psychosis [1]. Furthermore, progressive disability and systemic complication lead to high socioeconomic costs, with an unmet need for drugs that re-establish immunological tolerance [1]. While the precise aetiology of SLE remains unknown, it is hypothesized that disease development is dependent upon a complex interplay between genetic predisposition and environmental factors (Figure 1). The identification of environmental factors that play a role in the development SLE may shed light on new therapeutic avenues for disease prevention/treatment, with growing evidence suggesting that one such factor may be the commensal bacteria that colonize the gastrointestinal tract [2,3].

In mammals trillions of commensal microbes, including bacteria, archaea, viruses and unicellular eukaryotes, collectively known as the microbiota, colonize the skin and mucosal surfaces. Whilst the role of viruses, archaea, and unicellular eukaryotes is relatively under studied, in recent years the bacterial components of the microbiota and its role in modulating immune responses has attracted intense investigation. The largest community of commensal bacteria is located in the gastro-intestinal tract, thought to total as many as 10^{14} individual bacterium [4]. An intimate relationship between the host and the gut microbiota has developed following millions of years of co-evolution, leading to a mutualistic relationship allowing for microbial survival, whilst preventing the colonization of pathogens [5]. Other contributions of the gut microbiota to the host include help with metabolism of indigestible dietary components, protection against the colonisation of pathogenic bacteria, the production of certain vitamins, as well as the development of mature and diverse immune responses.

The combined genomes of the gut microbiota, known collectively as the gut microbiome, are thought to contain 100-fold more genes than the human genome [6]. However, whilst the human genome is rarely modified by environmental factors, the gut microbiome is easily altered by infectious pathogens, antibiotic-treatment, diet, or other non-specific changes in environment, making it an attractive target for potential therapeutic intervention. Changes in the composition of the gut microbiota or changes to the abundance of certain phyla over others, generally defined as dysbiosis, have been implicated as a potential trigger for numerous disorders including systemic autoimmunity [4]. In order to understand how the gut microbiota can be targeted for therapy, first we must investigate the role of the commensal microflora during health and how this is altered by disease. In this review, we will discuss current knowledge concerning how changes in the composition of the gut microbiota may contribute to the onset of systemic autoimmunity in animal models and in humans. In addition, we will highlight novel findings describing the effect that environmental factors have on the stability of the gut microbiota and consequently to the immune system. A glossary of common terms used in the study of the gut microbiota can be found in Table 1.

Study of the gut microbiota and gut microbiome

The development of DNA-based culture-independent methods has been fundamental for deepening our understanding of the bacterial species that constitute the gut microbiota. To date, the majority of studies investigating the taxonomic identity and function of the gut microbiota have used two DNA-based sequencing methods. The first focuses on sequencing the 16S ribosomal RNA (rRNA) gene. The 16S rRNA gene contains sequences that are highly conserved amongst all bacteria that can be targeted by universal PCR primers [7]. It also contains hypervariable regions (V1 to V9) that display considerable sequence diversity and which can therefore be used to identify particular bacterial phylotypes [7]. 16S rRNA sequencing has been extremely useful for phylogeneic classification of particular species within the gut microbiota. However, analysis of the 16S rRNA gene alone does not provide any information about the functional capacity of the gut microbiome. The second widely-used technique, whole-genome shotgun next-generation sequencing (NGS), has been used to overcome this problem. Whole-genome shotgun sequencing analyzes every gene within a given sample, allowing functional profiles to be assigned to the gut microbiota based on the genes that are present, in a process also known as metagenomics.

Considering there is large inter-individual diversity in the gut microbiota, large-scale collaborative studies using NGS such as the European Metagenomics of the Human Intestinal Tract (MetaHIT) project [6] and the US Human gut microbiome Project (HMP) [8] have been instrumental in providing base-line information about microbial identify and functionality in the gut. We now know that although the gut microbiota is densely populated, there is relatively little phylogenetic diversity in the studied populations. Indeed, up to

90% of the intestinal gut microbiota is dominated by Firmicutes and Bacteroidetes [9], although the ratio of these phyla in a particular individual is highly variable [10]. Other phyla often found as minor constituents include Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia [10]. Despite the consistency of these main phyla between individuals, there is a considerable variation in their relative proportions and in the species present within each individual phylum. This suggests that it is highly unlikely that a core set of species form the gut microbiota in healthy individuals. It has been suggested that there are in fact three enterotypes of the gut microbiota, which can be identified based on changes in the levels of one of three genera, *Prevotella, Bacteroides* and *Ruminococcus* [11]. Despite the taxonomic diversity in the gut microbiota between individuals, the gut microbiome maintains a core functionality, which includes the genes necessary for carbohydrate and amino-acid metabolism [12]. Importantly, as many genes are only expressed under specific conditions, metagenome studies have started to be complemented with meta-transcriptomic and proteomic techniques, which will provide real-time information about gene expression and protein production by the bacteria in the gut.

Factors that influence the gut microbiota

One of the current major challenges in the field of gut microbiota research is to try to understand the factors that influence its colonization and stability in healthy, and what causes dysbiosis in patients with SLE (Figure 2). Furthermore, we need to understand what causes variability in the gut microbiota between individuals, or in the same individual over time before we can even consider how manipulating the gut microbiota can be used to treat disease.

Age

Marked changes in the gut microbiota take place with age, with the greatest variability seen early and late in life. Following the initial colonisation event at birth, the communities of gastro-intestinal bacteria undergo compositional changes during the first three years of life to form a relatively stable gut microbiota during adulthood. During infancy, the gut microbiota is relatively unstable; there is greater variation amongst children than adults, albeit less diversity, and it is much more susceptible to environmental changes [13]. At birth, the sterile neonatal gastrointestinal tract is colonized by facultative aerobes, with bacteria appearing in the faeces within a few hours of birth [14]. Gradually consumption of oxygen by these bacteria changes the intestinal environment allowing the colonisation of strict anaerobes, which make up the majority of the adult gut microbiota [15]. Many outside factors can influence the colonisation event at birth, with one of the first major determinants being the mode of delivery (e.g. vaginal birth or caesarian section) [16]. Other factors that can affect the neonatal gut microbiota include the environment during birth (e.g. at home or in the hospital), prematurity, breastfeeding and antibiotic-treatment of the mother or baby [17]. Although recent studies have commented that changes to the gut microbiota during early-life cannot be detected in adulthood [18], it is has been suggested that this is a crucial developmental period or “window of opportunity” in which changes to the gut microbiota can have long-lasting effects on the immune system [19]. Indeed, early-life antibiotic use has been identified as a predisposing risk factor for both peadiatric-onset inflammatory disorders such as Juvenile Idiopathic Arthritis (JIA) [20,21], type 1 diabetes [22] and asthma [23]. Understanding exactly how the colonisation event influences the development of the immune system may therefore be important for the prevention of systemic autoimmunity in both children and adults.

Similarly to infants, there is more microbial variation amongst elderly individuals. The most marked change in the gut microbiota of elderly individuals is an increase in the proportion of Bacteriodetes and a decrease of Firmicutes. However it is difficult to reach a definitive conclusion regarding the potential impact that this unbalance has on the immune system due to a high variation amongst different individuals [24]. Despite this, it has been suggested that a decrease in microbial diversity correlates with increased frailty and the levels of inflammatory markers during age [25]. As the world-wide population ages and given the importance of age-related onset of autoimmunity, more information is needed to understand why/how the ecological make-up of the gut microbiota changes over time and how this impacts healthy aging.

Genetics

Similarities amongst familial pairs (e.g. mother-daughter, twin-pairs) indicate that gut microbiota may be influenced by genetics [26,27]. However, as there is similar co-variation amongst monozygotic and dizygotic twins, other factors are also likely to influence familial resemblances in the gut microbiota [27].

It is known that one genetic factor that influences the gut microbiota is sex. The gut microbiota of female and male mice is significantly different after puberty [28] and studies utilising non-obese diabetic (NOD) mice have shown that germ-free mice no longer display a gender-bias in disease development [28]. More recently, studies in humans have shown that changes to the gut microbiota associated with enthesitis-related arthritis (where inflammation occurs at the sites where tendons attach to bones) and obesity can be segregated based on sex [29,30]. Notably, as women are more likely to develop autoimmunity than men, it has been proposed that sex has a two-step effect on autoimmune disease development, influencing both the production of hormones and colonization of microbes [28]. Other genetic factors may also influence the gut microbiota. For instance, HLA haplotype may support the growth of particular bacteria. One study has shown that the gut microbiota of HLA-transgenic mice expressing the rheumatoid arthritis (RA)-susceptible DRB1*0401 gene is not defined by age or sex when compared to HLA-transgenic mice expressing the RA-resistant DRB1*0402 gene, but rather an increased abundance of *Allobaculum Sp.* [31]. More work is needed to understand how autoimmune disease risk-associated alleles and sex interact to affect the composition of the gut microbiota and the development of autoimmune disease.

Environment

Environmental factors that influence microbial diversity in the gut are extremely varied. Geographically defined populations can be segregated based on characteristic differences in the gut microbiota. For example, individuals living in the USA have a distinctly different faecal gut microbiota compared to individuals living in Malawi or South America [15]. These changes in the gut microbiota are likely to be due to multiple factors including genetics and hygiene practices. However, dietary information collected from these cohorts indicates that whereas the diet of individuals living in Malawi and South America is rich in plant-derived polysaccharides, adults in the USA have a more protein-rich diet [15]. Two of the main genera of the gut microbiota used to segregate enterotype [11], namely *Prevotella* and *Bacteroides* seem to correlate with location and diet. *Prevotella* predominates in individuals who have a diet that is high in plant-derived polysaccharides or fibre, whilst *Bacteroides* is increased in diets rich in protein and saturated fats [32]. Direct evidence concerning the impact of the diet on the gut microbiota has also been collected; changing carbohydrate intake over four weeks leads to a profound and rapid change in the gut microbiota and its metabolic output [33,34,32]. Similarly to changes in enterotype, the incidence and prevalence of autoimmune disease varies based on geography, indicating that dietary differences and changes to hygiene practices that affect the gut microbiota may affect autoimmune disease development. Importantly, diet also directly influences the production of micronutrients produced by the gut microbiota. These products, such as short-chain fatty acids (SCFAs), and polyamines can directly influence host physiology (reviewed in [35]), which may in turn affect the development of autoimmunity. For example, SCFAs, such as butyrate, acetate and propionate, which are the end products of carbohydrate fermentation by the gut microbiota, support barrier function in the intestine by acting as a nutrient source for colonocytes [36] and can induce the differentiation of colonic [37] and peripheral Tregs [38].

Gut microbiota and immune system – a reciprocal relationship

In the healthy gut, the gut microbiota is constantly monitored by the mucosal immune system. Further to the physical barrier between the gut-epithelium and the bacteria in the lumen, formed by of a thick mucus layer, antimicrobial peptides, and secretory IgAs, lamina-propria resident macrophages and dendritic cells (DCs) survey the mucosal surfaces for unwanted or pathogenic bacteria [39]. Intestinal macrophages are involved in direct killing of bacteria via phagocytosis, whilst DCs, by presenting antigens to T and B cells, prime B and T cells to add another layer of protection at the mucosal surface [39]. In return, the gut microbiota directly affects the development of the immune system.

Animal models have been critical for understanding how the gut microbiota influences immune system development. Germ-free mice, which are reared in a sterile environment and are devoid of commensal gut microbiota, have smaller isolated lymphoid follicles [40] and Peyer's patches compared to specific-pathogen free (SPF) mice [41], as well as numerous deficiencies in the function and number of both lymphoid and myeloid-derived cells [41]. Perhaps the most striking differences between germ-free and SPF mice are the reduction of both the levels of secretory IgA and plasma cells in the intestine, demonstrating that commensal microbes are the driving force behind the development of the mucosal immune system [42]. T cell subsets in the gut are also abnormal in germ-free mice; there are reductions in T helper 17 (Th17) cells in the lamina propria of the small intestine [43] and in Tregs in the colonic lamina propria [44]. Changes to the peripheral immune system are also observed in germ-free mice, in particular there is a reduction in serum levels of IgG [45], perhaps due to fewer germinal centres in the spleen and mesenteric lymph nodes [46]. Mice in which the gut microbiota has been manipulated by antibiotic-treatment have also been used to provide important evidence concerning how the gut microbiota influences systemic immune homeostasis. For example, antibiotic-treatment of adult mice affects cytokine production by splenic macrophages, which has been associated with problems in the antiviral response to LCMV [47] and the differentiation of regulatory B cells [48]. Importantly, germ-free mice and antibiotic-treated mice have also been used to demonstrate that the gut microbiota directly influences the development of autoimmunity.

Evidence that the gut microbiota influences systemic autoimmunity; lessons from animal models

One of the first mouse models used to demonstrate that commensal bacteria influences peripheral autoimmunity was the K/BxN T cell receptor transgenic spontaneous mouse model of arthritis. K/BxN mice do not develop arthritis if housed in a germ-free environment, which is paralleled by a decrease in the production of autoantibodies, germinal centre formation, and Th17 cells compared to conventionally housed K/BxN (CNV) mice [49]. In this model, monocolonisation with segmentous filamentous bacteria (SFB) alone is sufficient to restore arthritis by inducing the differentiation of Th17 cells in the lamina propria, which can recirculate to the joint to cause arthritic inflammation [49]. It has also been reported that SFB-induced Th17 cells in the lamina propria can also recirculate to the brain causing inflammation in experimental autoimmune encephalitis (EAE) [50]. Interestingly, monocolonisation with SFB has also been associated with the production of antinuclear antibodies in mice treated with LTβR-Fc during and after gestation [51]. LTβR-Fc treated mice do not develop normal secondary lymphoid organs, and a proportion spontaneously produces antinuclear antibodies in an IL-17-dependent manner [51]. A caveat to this data is that no human equivalent of SFB has been identified making the clinical relevance of these studies unclear. Importantly, the gut microbiota does not only influence the differentiation of autoreactive T cells. In a spontaneous model of multiple sclerosis, demyelination is initiated in germ-free mice following colonisation with faeces isolated from SPF mice and is caused following entry of auto-antigen specific B cells into the germinal center of cervical lymph nodes [52]. In addition, we have recently demonstrated that changes to the gut microbiota caused by antibiotic-treatment or by changing the sterility of housing conditions reduces not only the severity of antigen-induced arthritis but also the differentiation of IL-10 producing regulatory B cells [48]. Alterations in the gut microbiota due to antibiotic-treatment or changes in housing environment led to a significant decrease in the production of IL-1β and IL-6, which in combination directly promote the differentiation of regulatory B cells [48].

In mouse models, a role for the intestinal gut microbiota in the development of SLE is less clear than for the development of arthritis and multiple sclerosis – the onset and severity of lupus-like disease is not profoundly altered in germ-free mice [53-55]. However, there is emerging evidence that there is dysbiosis and gut-pathology in many models of lupus. Female lupus-prone MRL/Mp-*Fas*^{lpr} (MRL/lpr) mice display changes in faecal bacteria; there is an increase the family *Lachnospiraceae* and a decrease in *Lactobacillaceae* [56]. Notably, unlike many other disorders where a decrease in diversity has been associated with disease, MRL/lpr mice have increased microbial diversity in the faeces compared to non-disease controls [56]. In this study the authors observed that there were sex-related changes in the gut microbiota of lupus-prone mice compared to healthy mice; females were more likely to have an increase in *Lachnospiraceae,* which correlated with the development of more severe disease [56]. This observation is particularly relevant to human disease as women are approximately 10 times more likely to develop SLE that men. Another study also commented that gender-related differences could be observed in the gut of lupus-prone mice. Female SWR x $NZBF_1$ (SNF_1), which develop more severe nephritis than their male counterparts, have more plasma cells and $\alpha4\beta7$-expressing T cells in the Peyer's patches than male SNF_1 mice [57]. Furthermore, analysis of RNA expression in the distal ileum showed that female SNF_1 mice express much higher levels of pro-inflammatory mediators including IL-6, IL-9, IL-17, IL-22, IFN-α and IFN-β than male mice [57]. Although the gut microbiota was not analysed in this study, it does further support a role for the gut microbiota in lupus development and the gender bias of this disease. The important role of gut microbiota in driving a gender bias in autoimmunity more generally is supported by a study showing that diabetes in female mice can be suppressed by transferring gut microbiota from male mice into immature females [58].

Further evidence reinforcing a role for the gut microbiota in the development of lupus has been demonstrated by studies showing that dietary manipulations can affect the severity and progression of disease. Feeding SNF_1 mice acidified water (AW) delays the onset of nephritis compared to mice fed neutral water (NW); this is mirrored by a decrease in circulating anti-nuclear antibodies and plasma cells [59]. AW-mice displayed gross differences in the gut microbiota compared to NW-mice, including a reduction in β-diversity. Interestingly, in contrast to the K/BxN arthritis model, although the development of disease in NW-mice was associated with an increase in Th17 associated cytokines and genes in the distal ileum, no differences in SFB were observed [59]. This suggests that one bacterium alone is unlikely to drive autoimmunity but that multiple species may support Th17 differentiation.

Evidence that the gut microbiota influences systemic autoimmunity; human sequencing studies.

NGS-studies of human faeces have demonstrated that dysbiosis can be detected in several autoimmune diseases including rheumatoid arthritis [60,61], type 1 diabetes [22], multiple sclerosis [62] and inflammatory bowel disease [63]. Varying phenotypes have been described, including the observation that the gut microbiota in patients at disease onset, during chronicity, and following treatment is different [60,61]. This suggests that, while gut microbiota may alter disease development, inflammation and medication may both also affect the gut microbiota, a significant consideration when using enterotype to predict disease or when designing future therapeutic strategies.

Studies analyzing the gut microbiota in patients with SLE have only been carried out in the last couple years [2,3,64]. Initial studies have observed that there is a reduction in the ratio of Firmicutes to Bacteroidetes in the faeces of SLE patients compared to healthy controls [2]. Preliminary metagenome analysis has also been performed that demonstrates a shift in metabolic pathways in SLE, such as an overrepresentation of genes associated with glycan metabolism and oxidative phosphorylation [2]. The case for a metabolic shift in the gut microbiome of patients with SLE has been supported by another study where metabolomics was used to analyse faecal metabolites of SLE patients, revealing that there a deficiency in metabolites associated with pyrimidine, purine, and amino acid metabolism [3]. More recently, studies have started to try to understand whether changes in particular bacterial species in the gut are associated with particularly immune abnormalities observed in SLE, such as the increase in Th17 cells or natural IgM antibodies [64]. However, these studies remain purely correlative and further work is needed to understand exactly how the gut microbiota influences immune dysfunction in patients with SLE. Importantly, it is unlikely that only the bacterial components of our gut microbiome influence the development of SLE. The common “interferon alpha signature” in the peripheral blood of SLE patients suggests a potential viral trigger for disease development [65,66]. The human body is inhabited by a multitude of viruses, known as the virome, which can have a direct influence on the host and the gut microbiome. To date, there is little information analyzing how the virome may contribute to the development of autoimmunity. However, analysis of the virome as a potential trigger for SLE represents an exciting novel avenue that will hopefully be explored in future research. A summary of key findings supporting a role for dysbiosis in the development of SLE can be found in Table 2.

Possible therapeutic strategies for modulation of the gut microbiota

The ultimate goal of therapies targeting the gut microbiota is to facilitate health by supporting the growth of anti-inflammatory commensal bacteria whilst eliminating potential pathobionts. At present, proposed therapeutic strategies can be divided into two broad categories (Figure 3). The first approach involves direct elimination or modification of the gut microbiota through antibiotics treatment or faecal transplant. Whilst targeted antibiotic strategies have been successfully used to treat disorders associated with a known pathobiont, such as peptic ulcers caused by *Helicobacter pylori [67]*, limitations of the use of antibiotics for the treatment of autoimmune disease are caused by the lack of a known pathogen. Thus, the long-term use of broad-spectrum antibiotics that could be currently used for therapy may lead to the depletion of both pathogenic and beneficial bacteria, and lead to an increase in antibiotic resistance. Notably, animal studies have also shown that whilst treatment with antibiotics suppresses inflammation, it can also lead to reduction in regulatory populations of cells, suggesting that non-specific elimination of the gut microbiota perturbs immune homeostasis [48]. Interestingly, treatment of RA patients with minocycline, a tetracycline-derived antibiotic, is efficacious in reducing symptoms [68]. However, the anti-rheumatic activity of minocycline is currently attributed to its ability to inhibit matrix metalloproteinases, not it's broad-spectrum antibiotic activity [69]. Importantly, minocycline has a high toxicity profile, leading to gastro-intestinal problems [68], and adverse reactions include the development of lupus-like disease [70,71].

Restoring microbial health by transplanting a foreign gut microbiota may represent a more attractive treatment option than antibiotic centric therapies, and faecal transplant has been very effectively used to treat recurrent *Clostridium difficile* infections. There are also case reports showing promising results for faecal transplant in the inflammatory bowel diseases, with one publication describing complete remission in patients with refractory ulcerative colitis up to 13 years later following fecal transplant [72]. There have even been studies describing an improvement in the neurological symptoms of three multiple sclerosis patients [73] after being administered faecal transplants for chronic constipation, and in a child with myoclonus dystonia following faecal transplant for chronic diarrhea [74]. More work is needed to understand whether this treatment will work for other disorders such as lupus. Indeed, the best approach may be a short course of antibiotics followed by a faecal transplant from a healthy donor.

The second therapeutic approach involves dietary modification by the use of prebiotics/probiotics to support the growth of immune-regulatory bacteria. Probiotics consist of live cultures of bacteria that confer beneficial effects to the host when properly administered. Probiotics strains that have been identified include *Bifidobacteria, Lactobacilli* and *Streptococci.* For example, administration of *Lactobacillus casei* has been shown to improve the symptoms of diabetes in KK-Ay diabetic mice [75], although these data are yet to be translated to humans. Interestingly, the idea of administering beneficial bacteria in the diet is not a new concept. There are papers from the early nineteen hundreds describing an improvement in autoimmune arthritis after patients ingested sterilized milk supplemented with live cultures of *Streptococcus lacticus* and *Bacillus bulgaricus* [76]. Prebiotics are non-digestible substances that may improve host health by promoting the growth of probiotic gut bacteria. Fructo-oligosaccharides and inulin are amongst the prebiotic compounds that are thought to promote bacteria such as *Bifidobacteria* and *Lactobacilli* [77]. Of note, inulin and fructo-oligosaccharides are broken down into short-chain fatty acids, which have been shown to promote the differentiation of Tregs [37,38], and improve barrier function in the gut [36]. Both prebiotics and probiotics have been shown to prevent antibiotic-induced diarrhea or food allergies [78]. Importantly, this represents a non-invasive treatment for suppression of autoimmunity. However, more studies in animals and in humans are needed to understand the best way to manipulate the gut microbiota to suppress inflammation and restore health. In addition, the composition of gut microbiota may also be viewed as a risk factor for disease development, like smoking for instance, and will therefore be important in predicting disease development/progression.

Conclusions

Over the last decade, much progress has been made in understanding of how the gut microbiota during health and disease. It is now know that the gut microbiota is a dynamic “forgotten organ”, which affects host physiology at both mucosal sites and peripheral sites. Advancements in sequencing technologies have been critical for the increase in information concerning gut microbiota stability during health in humans, whilst animal models have provided mechanistic information concerning exactly how the gut microbiota influences host immunity. More information is needed to understand how the gut microbiota interacts with host genetics to cause autoimmune disease. Prospective studies are now necessary to establish causation of the gut microbiota in the development of autoimmunity, as well as a greater understanding of how current therapeutics change the gut microbiota, which is likely to inform whether an “autoimmune” gut microbiota can be manipulated to restore microbial health. In conclusion, greater understanding into the diverse contributions of the gut microbiota to systemic autoimmunity will open new possibilities for diagnostic, preventative, and therapeutic approaches.

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Term	Meaning
Dysbiosis	Microbial imbalance on or inside of the host
Enterotype	A way to stratify individuals based on their gut microbiota
Metabolomics	The study of the metabolites found within a given environmental sample
Metagenomics	The study of the genetic material within a given environmental given sample
Metatrascriptomics	The study of the function and activity of transcripts found within a given environmental sample
Microbiome	The collective genomes of the microorganisms found on the host
Microbiota	The ecological community of microorganisms that live on the host
Pathobiont	A commensal bacteria that has the potential to cause pathology
Prebiotic	A non-digestible food ingredient that promotes the growth of beneficial bacteria
Probiotic	A dietary supplement containing live beneficial bacteria
Proteomics	The study of proteins found within a given environmental sample
Virome	The collection of viruses on the host
β-diversity	The ratio between local and regional diversity

Table 1. A glossary of terms commonly used in the study of the gut microbiota and gut microbiome.

Year	Experimental system	Findings	Ref
2014	***MRL-lpr mice*** Readout: disease severity, analysis of stool by 16S rRNA sequencing	Faecal samples from female mice with severe lupus-like disease have an increase in *Lachnospiraceae* compared to samples from male mice with mild disease	**[56]**
2015	***SNF$_1$ mice*** Readout: disease severity, analysis of stool by 16S rRNA sequencing	Feeding mice acidified water delays onset of lupus-like disease; delayed disease onset associated with decrease in β-diversity of faecal bacteria compared to control mice	**[59]**
2015	***SLE patients*** Readouts: analysis of stool by 16S rRNA sequencing	Lower Firmicutes/Bacteriodetes ratio in stool of SLE patients compared to healthy controls	**[2]**
2015	***SLE patients***; Readout: analysis of stool by liquid chromatography and mass spectrometry	Reduction in metabolites associated with purine, pyrimidine and amino acid metabolism in faecal samples from SLE patients compared to healthy controls.	**[3]**

Table 2. Summary of key findings supporting a role for dysbiosis in the development of SLE.

Figure legends

Figure 1. Development of autoimmune disease is dependent upon a complex interplay between genetic and environmental factors.

Although the exact aetiopathogenesis of autoimmune disease remains unknown, it is hypothesized that a combination of both genetic and environmental factors are needed for disease development. The relative importance of genetics versus environmental factors in the development of autoimmunity is yet to be understood, although it is currently under active investigation. Although several environmental factors have been linked to disease development in genetically predisposed individuals, recent research has suggested that changes in the composition of the gut microbiota may play an important role.

Figure 2. Proposed causes of dysbiosis.

Pathological changes in the gut-microbiota, or dysbiosis, has been implicated as a potential risk factor for the development of autoimmune diseases such as SLE. Many factors could contribute to dysbiosis, including host-genetics, age, diet and other environmental factors. Genetic factors that have been associated with changes in the microbiota include sex and certain disease associated risk alleles such as HLA haplotype. Changes with age have also been associated with immune pathology, such as changes to microbial exposure at birth or due to aging. Environmental factors that may cause dysbiosis and have also been associated with increasing incidences in autoimmune disease include a so-called “western diet” which is high in fat and protein, and the widespread introduction of antibiotics.

Figure 3. Proposed microbiota-targeted therapies.

Proposed microbiota targeted therapies can be split into two categories: a) Direct targeting of the microbiota by administration of selective antibiotics, which aim to target known pathobionts, or faecal transplant, where microbiota from healthy individuals is transferred into an individual with dysbiosis. b) Altering diet regime (i.e. from high fat to plant based), administration of prebiotic and/or pro-biotics; these will directly/indirectly change the composition of the gut microbiota and their metabolic product. All therapies aim to restore microbial health in individuals with dysbiosis to treat inflammation.