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Does the microbiota play a role in the pathogenesis of autoimmune diseases?

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Abstract

The microbiota of the human metaorganism is not a mere bystander. These microbes have coevolved with us and are pivotal to normal development and homoeostasis. Dysbiosis of the GI microbiota is associated with many disease susceptibilities, including obesity, malignancy, liver disease and GI pathology such as IBD. It is clear that there is direct and indirect crosstalk between this microbial community and host immune response. However, the precise mechanism of this microbial influence in disease pathogenesis remains elusive and is now a major research focus. There is emerging literature on the role of the microbiota in the pathogenesis of autoimmune disease, with clear and increasing evidence that changes in the microbiota are associated with some of these diseases. Examples include type 1 diabetes, coeliac disease and rheumatoid arthritis, and these contribute significantly to global morbidity and mortality. Understanding the role of the microbiota in autoimmune diseases may offer novel insight into factors that initiate and drive disease progression, stratify patient risk for complications and ultimately deliver new therapeutic strategies. This review summarises the current status on the role of the microbiota in autoimmune diseases.

INTRODUCTION

The concept of the human metaorganism arose with the realisation that we harbour many trillions of microbes on and within the human body.[1] These microbes are located at the host-environmental interface, such as the skin, the GI tract, the genital tract and respiratory mucosal barrier. All genes of our microbial cohabitants constitute the micro-biome, and this microbiome outweighs the genetic contribution of the host by 10-fold.[1] Our personal microbial world is rich in diversity and many thousands of species survive and thrive within us. The sheer enormity of this microbial community has become apparent over the last decade as technology, such as sophisticated sequencing techniques and high throughput

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technology, has allowed for the identification of the microbial community and analysis of its function. It is clear that our micro-biota is not a mere bystander; they have coevolved with us and are pivotal to normal development and homoeostasis, from a metabolic, trophic and protective capacity.[23]

The intestinal microbiota interacts with the adjacent mucosal environment directly, impacts intestinal permeability, and influences local and systemic inflammatory activity.[4] There is also an indirect crosstalk between the microbial community and the host via their metabolites; for example, digestion of plant polysaccharides by gut bacteria yields short chain fatty acids and these in turn modulate host mucosal immune response by various mechanisms, including promotion of regulatory T cells.[5] The composition of our microbiota is not static, but changes with age, geography and is influenced by many external factors, such as diet and medication.[6–8]

Large global consortia such as the US Human Microbiome Project seek to provide knowledge on microbial composition in health and disease and it is clear that an appreciable interindividual and intraindividual variation exists, influenced by many external factors.[9] It is now known that alteration in the balance of intestinal microbial species leading to a dysbiosis is associated with many disease susceptibilities. Examples of this include obesity,[4] multiple sclerosis (MS),[10] malignancy,[11–13] liver disease,[1415] and GI pathology such as IBD, [16] but the precise mechanism of this microbial influence in disease pathogenesis remains elusive and is now becoming a major research focus.

Within this exploration of the relationship between the gut microbiota and disease, there has been interest in the role of the microbiota in the pathogenesis of systemic and organ targeted auto-immune disease. Autoimmune diseases are characterised by serological evidence of autoantibodies, pronouncing lack of tolerance and self-directed immune response. Several autoimmune diseases such as type 1 diabetes (T1D) and rheumatoid arthritis (RA) contribute significantly to global morbidity and mortality. Understanding the role of the microbiota in these diseases may offer novel insight into factors that initiate and drive disease progression, stratify patient risk for complications and ultimately could deliver new therapeutic strategies.

Reflecting the importance of this topic, the role of the microbiota in autoimmunity was the subject of a 2014 National Institutes of Health (NIH) symposium, cosponsored by the Society for Women's Health Research. This review aims to summarise current data on the role of the microbiota on auto-immunity, and concludes by summarising points raised within the closing discussion at the NIH symposium.

TYPE 1 DIABETES

T1D is a chronic, proinflammatory autoimmune disorder characterised by immune-mediated destruction of the pancreatic β cells, resulting in insulin deficiency and hyperglycaemia. Clinical manifestation of T1D usually presents in childhood and adolescence and incidence of this disease continues to increase globally.[1718] There is clear evidence of a genetic susceptibility to T1D.[19–21] However, given the 50% concordance rate in monozygotic twins[22] and the fact that immigrants exhibit risk associated with place of residence rather

than origin[23] there clearly is a central role for environmental factors in T1D pathogenesis. This has been explored extensively and the main aspects of environmental risk focus on diet, including neonatal exposure to bovine derived milk products, age at weaning and early exposure to gluten (reviewed in ref.[24]) as well as a potential infective component such as childhood viral infection, particularly Enterovirus given the seasonal timing of clinical presentation.[25]

In light of the emerging evidence that the gut microbiome has a strong and broad impact on health and disease, the question of whether the gut microflora could impact T1D has arisen. Indeed, a growing evidence base from animal and human studies suggests that changes in the gut microbiome may precede the onset of T1D and are associated with progression from detectable autoantibody levels in high-risk asymptomatic individuals through to those with clinical disease and this has been reviewed elsewhere.[26–30]

Several animal models of T1D exist.[31] Of these, the non-obese diabetic (NOD) mouse and the biobreeding diabetes-prone rodent model exhibit similar genetic predisposition and pathological disease progression to human T1D and have been used to explore the relationship between the gut microbiome and T1D.

Manipulation of the gut microbiota by different approaches, such as treatment with antibiotics,[32–35] exposure to acidified water,[3637] exposure to pathogenic and non-pathogenic bacterial strains,[3638–40] housing in germ-free conditions,[3241] along with temporal analysis of faecal microbiota preceding and during disease development has revealed the importance of the colonic microbial community in the pathogenesis of T1D. However, many conflicting reports exist in the literature and highlight the complexity of this association. It is clear from the evidence that the gut microbial community influences host immunity and this could ultimately aid emergence of disease. For example, Wen *et al*[32] revealed the importance of crosstalk between host and gut microbiota in T1D pathogenesis. MyD88 (myeloid differentiation primary response protein 88) is pivotal to bacterial sensing and downstream signalling host innate immune response, and genetic silencing of this bacterial sensor in specific pathogen-free NOD mice interrupted development of T1D as compared with wild type controls. In contrast, the same genetically altered mice, either raised in germ-free conditions or treated with antibiotics to disrupt gut commensal bacteria, developed disease. This indicates protection was mediated by a constituent of their gut microbial community although the underlying mechanisms are not fully understood. Certainly, changes in the intestinal microbiota composition were demonstrated in conjunction with loss of the ability to sense microbes via MyD88. A follow-on study showed that faecal bacterial transplant from the MyD88 null protected mice conferred protection to the wild type diabetes-prone strain, and altered mucosal immunity and faecal microbial composition of the recipient.[40] The importance of innate pattern recognition receptors in microbial sensing of the gut microbiome in directing the downstream host immune response and development of T1D has been validated elsewhere.[3442] Alkanani *et al*[34] identified a crucial role of an additional innate microbial sensor upstream of MyD88, Toll like receptor (TLR)3, but not TLR9 in this capacity to modulate the emergence of T1D.

The pH of drinking water, particularly low acidity, influences the composition of the gut microbiome and incidence of T1D.[3637] Exposure of female NOD mice to acidified water resulted in a differential induction speed and severity of insulitis and hyperglycaemia, associated with intestinal dysbiosis, along with increased gut and systemic proinflammatory status.[36] Conversely, Wolf *et al*[37] reported an increased incidence of T1D in mice exposed to neutral water, with a protective role seen along with exposure to acidified water. The differences in study design suggest that the timing of microbial disruption, in this case through exposure to acidified water, is a key consideration.[37] Alteration of the microbiome at a very early age, such as in the newborn period when the gut microbiota is being established and thus long before onset of disease, may impact subsequent disease induction in genetically susceptible individuals.

Exposure to vancomycin, an antibiotic to specifically target Gram-positive bacteria, in infant and adult NOD mice was associated with a decreased incidence of T1D, and lower levels of blood glucose and insulitis scores, respectively.[33] Notably, a single species, namely *Akkermansia muciniphila* emerged as a dominant potentially protective species in this context. This protective effect of antibiotic treatment in diabetes-prone rodents has been long recognised.[4344]

Autoimmune disease tends to be prevalent in female mice preferentially. Could the gut microbiota influence this gender-specific preponderance? Markle *et al*[39] reported that microbial exposures early in life impacts sex hormone levels and alters progression to T1D in the NOD mouse. Indeed, transplant of faeces from adult NOD mice into immature female mice led to increase in testosterone and protection from T1D.

How does this data from preclinical models translate to human disease? There have been several human studies that have confirmed the association between the gut microbiota and risk of T1D.[45] The main outcomes from these studies are presented in table 1. Children with T1D have a low abundance of butyrate-producing bacteria.[4647] Gut bacterial diversity lacks stability, confers differential changes over time in islet autoantibody-positive children as compared with nonautoimmune matched controls and children with T1D have more variation between individuals.[48] As such, children with propensity to autoimmune diabetes yield an increase in faecal Bacteriodetes and reduction in Firmicutes over time from their early childhood years, potentially even before T1D clinically develops, representing a composition that is opposite to that seen in control subjects.[464849]

Interestingly, akin to that found in the preclinical models, Brown *et al* reported a reduction in *Akkermansia* spp in those with early disease.[47] As such, a greater proportion of these mucin-degrading bacteria species, as well as butyrate secreting bacteria, were observed among healthy controls when compared with a small number of T1D cases at time of clinical presentation. In comparison, bacteria capable of producing non-butyrate short chain fatty acids were higher among T1D cases.[47] From a mechanistic perspective, metabolic focused gene expression analysis of the microbiome revealed that gut bacteria display different metabolic functional capabilities between the two groups.

However, this association is far from clear as a recent prospective stool collection study on young children did not confirm these findings of change in microbial diversity in those who developed anti-islet autoimmunity. However, despite this, the interactions between gut microbes were distorted in this group.[50] In another study, although newly diagnosed children were identified as carrying an increase in *Bacteroides*, this dysbiosis had returned to the status of control subjects in those who had received 2 years of glucose normalising treatment.[51]

Overall, most would agree that T1D is associated with a change in gut microbial composition. Certainly, it seems that no single species from the gut microbial community has emerged as a causative agent. Rather, in genetically susceptible individuals, there is emerging evidence that dysbiosis within the gut micro-biota and interruption of microbial colonisation in early life, maybe even as early as birth or the neonatal period, is associated with emergence and progression of T1D. In line with this, babies born by caesarean section have a >20% increased risk of developing T1D.[5253] It has been reported that birth mode impacts infant intestinal colonisation.[54] The speculation is that caesarean delivery is associated with a lack of exposure to maternal microbiota and impacts infant intestinal colonisation conferring risk of future autoimmunity in genetically susceptible individuals. In fact, it may be that environmental influences on the intestinal microbiota can even extend to in utero exposure. NOD mice given a broad spectrum antibiotic cocktail during gestation bore offspring with a lower gut microbial diversity and a modulation of T cell phenotype in the mesenteric lymph nodes (increased CD3+CD8+ T cells) and Peyer's patches of the intestine (reduced CD4+CD25+, but not Foxp3+ Treg subgroup). However, this only impacted emergence of hyperglycaemia to a minor level at 20 weeks of age and this risk did not persist into later life.[35]

The next consideration is whether a particular gut microbial community is linked to cause or effect in disease pathogenesis. Is the gut microbial dysbiosis an initiator of T1D, a perpetrator of increasing progression or a consequence of other pathological features? This remains unanswered. The immune mechanisms involved in islet cell destruction have been extensively studied and include pathogenic T cells, shift in B cell phenotype, features of antigen presentation, and distorted immunoregulatory mechanisms.[55] All the human studies reporting an association between altered gut microbiota and T1D have not explored this aspect of disease pathogenesis, likely a consequence of easy access to faecal sampling, offset against the invasive nature of mucosal biopsy that would not normally be pursued as part of diagnosis and management per se. From preclinical animal models, it is clear that changes in gut micro-biota or GI microbial exposures are associated with differential host immune response, including change in splenic or GI mucosa T cell phenotype,[36–384056] for example, modulation of T helper cell (Th)17 response. Whether this Th17 association is pathogenic or protective, remains under debate.[57] Additionally, it has been shown that breakdown of the GI epithelial barrier integrity is present in T1D, with increased gut permeability.[58–61]

It is inherently difficult to assess causality in human studies for several reasons; T1D is an early onset disease, with clinical presentation after destruction of islets has occurred. The preclinical phase of early islet autoimmunity is asymptomatic, and there is no biomarker that will predict disease in the general population. The aetiology is multifactorial and it is

difficult to fully account for confounding factors. In addition, all human studies to date have used faecal samples for analysis and the question of whether this, as opposed to mucosal biopsy derived analysis, reflects the true status of the microbiome in T1D remains unanswered.

Several large global collection consortia are ongoing that will yield powerful data from prospectively collected data. There are several of these, for example, The Environmental Determinants of Diabetes in the Young study, and Diabetes Prediction and prevention Project study. The ultimate question is whether the colonic microbiota can be manipulated to therapeutic advantage for T1D. More likely, this strategy may be of greater benefit to prevent the onset of T1D in high-risk individuals, such as those receiving antibiotics or other treatments in the neonatal period that may alter gut microbial acquisition and increase risk of T1D in later life.

COELIAC DISEASE

Coeliac disease, like other autoimmune conditions, requires genetic susceptibility and environmental influences.[6263] This autoimmune disease is unique in that the main environmental factor is known, well characterised and therapeutically targeted. This environmental trigger in question is dietary gluten, derived from wheat and other related grains. Gluten, composed of gliadin peptides and glutenin, evokes a predominantly T cell mediated mucosal response in the proximal small bowel,[64] with the cytokine interleukin (IL) 15 playing a pivotal role in the immunopathogenesis.[65] This results in the characteristic pathological characteristics of progressive villous atrophy, distorted crypt architecture and increase in intraepithelial cells, leading to a reduction in absorptive capacity and emergence of GI and extraintestinal symptoms.[626366] However, there is often a lag of many years after gluten exposure until disease manifests serologically or clinically. Indeed, adult onset coeliac is not uncommon, and therefore this suggests that additional environmental influences are required in coeliac disease pathogenesis.

Concordance rates amongst monozygotic twins are high at more than 80%, compared with 10% in dizygotic twins,[6768] highlighting the importance of genetic susceptibility in this disease pathogenesis. HLA class II haplotype DQ2 or DQ8 are the most characterised genetic determinant.[6263] Carriage of these haplotypes plays a pivotal role in the presentation of the gliadin peptides to CD4+ T cells. This is not the whole story, with genome wide association studies and high throughput technology identifying many other susceptibility genes.[69–71]

Coeliac disease is thought to affect 1% of the global population, and has been increasing in prevalence at a striking rate; doubling over 20 years in a Finnish population[72] and increasing fourfold in a US population.[73]

There are well-characterised autoantibodies available for serological diagnosis and screening, namely tissue transglutaminase IgA antibody and anti-endomysial IgA antibody. Both of these display high specificity and sensitivity.[626366]

As a disease of the GI tract, there has been florid interest over several years as to whether the gut microbiota could be implicated in the pathogenesis of coeliac disease.[6274] Given the proximal location of pathology in coeliac disease, the microbiota of the duodenum has been the focus of investigation in this context. Despite the hostile conditions of the proximal small bowel with fluctuating pH, digestive enzymes and bile, and robust peristalsis, a distinctive collection of bacteria appear to survive in this environment, dominated by *Streptococci*, Bacteroidetes, Proteobacteria and clusters of *Clostridium* sp.[75–78] Initial analysis employed conventional culturing or limited molecular techniques and reported differences in duodenal mucosal biopsy or faecal stream bacteria associated with coeliac disease.[7479–84] There has been no unifying pattern to identify a distinct bacterial composition or diversity that marks presence of coeliac disease, nor successful treatment. Overall, there appears to be a trend for abundance in Firmicutes and Bacteroidetes over several studies, in adults and children, respectively. However, there are other reports with opposing observations. The inconsistency of the findings in these studies is a reflection of several issues; geographical difference with undoubted impact on diet (dictated by culture) and genetic susceptibilities, differences in experimental methodology including culture versus culture-independent, the low number of patients included in analysis, and the origin of the material to be tested, that is, faecal versus mucosal biopsy.

With the advent of increasingly sophisticated technology, most recent analyses have used sequencing and other high-throughput molecular analysis and yielded conflicting results with no dramatic or distinctive dysbiosis of the duodenal micro-biota at either the phylum or genus level in children with coeliac disease compared with healthy controls.[76–78]

Therefore the debate on whether the microbiota is associated with disease pathogenesis is ongoing. If a component of the intestinal microbiota was a driving causative factor for initiation and progression of coeliac disease, one may expect an obvious candidate to emerge from analysis on adults and children, and to revert to that seen in an individual without coeliac disease, with successful treatment. This has not as yet emerged to date, but this is still an active field and therefore the debate goes on.

Nevertheless, there have been some published reports of how changes in the intestinal microbiome may influence underlying mucosal immune response. Sanchez *et al*[85] used an in vitro system to show that exposure of Caco-2 cells to digested gliadin and specific *Bacteriodes* sp resulted in increased proinflammatory cytokine profile and disruption of permeability. Exposure of dendritic cells to intestinal bacterial species, such as Enterobacteria or Bifidobacteria, led to altered phenotype and function. When these cells were subsequently cocultured with Caco-2 epithelial cells, an altered expression of proteins involved in intestinal permeability was identified.[86] When considering animal models of coeliac disease, there is no spontaneous model in small rodents. An induced rat model has been widely used (germ-free Wistar rats exposed to gliadin immediately after birth), along with transgenic mice exhibiting HLA genetic susceptibilities akin to human disease.[87] These in vivo models have been employed to understand the underlying immune activity in coeliac disease initiation and progression. With regard to the role of the microbiota in this process, exposure to specific bacterial strains in vivo does impact epithelial permeability and underlying mucosal immunity. For example, administration of the intestinal commensal

Bifidobacterium longum to the induced rat model is protective to emergence of disease and associated with increased mucosal anti-inflammatory activity such as increased IL-10.[8889] However, this strategy has been directed at exploring the effect of targeted exposure of single agents, rather than an assessment of total micro-biome composition dysbiosis in its entirety. There are additional models of coeliac disease emerging in the literature;[87] for example transfer of gliadin presensitised CD4+CD25−CD45RB^{low} T cells into a Rag-deficient murine host,[90] and these novel models may be able to shed some new light on the role of the microbiota in coeliac pathogenesis. To date, the vast majority of studies assessing the microbiota in coeliac disease has used human faecal and/or biopsy tissue specimens, as discussed below, rather than employ animal models. The main findings from the assessment of the microbiota in coeliac disease are presented in table 2.

Olivares *et al*[91] provided evidence that underlying genetic status can influence composition of the developing microbiota. Faecal stream pyrosequencing analysis from infants deemed high or low genetic risk of coeliac disease (HLA-DQ2 carriers or non-HLA-DQ2/8, respectively) was assessed for intestinal microbial composition. Interestingly, HLA status was associated with differential faecal bacterial composition, with those deemed high risk carrying increased Firmicutes and Proteobacteria, and reduced Actinobacteria, suggesting genetic status may impact the composition of the evolving intestinal microbiota. Whether this association leads to emergence of disease remains unclear and is under investigation. Similarly, Sellitto *et al*[92] performed dynamic stool sequencing analysis from birth to age 2 years, in infants genetically at high risk of coeliac disease. They reported the temporal evolution of the intestinal microbiota in this cohort, and asked whether this changed in accordance with timing of dietary gluten exposure. As expected, faecal bacterial composition changed over time. However, regardless of timing to gluten exposure, the microbiota did not reach that expected of a healthy adult by 24 months. In particular, this high-risk group carried much less Bacteroidetes. Close monitoring of these children for a longer term may give clues as to whether this dysbiosis in infancy and early childhood impacts disease emergence.

Nistal *et al*[76] report a change in bacterial richness between adults and children with coeliac disease, and provide evidence of a dysbiosis between treated and untreated adults, especially when considering unknown bacterial composites. Similarly, Schippa *et al*[93] assessed the duodenal microbiota in children before and after introduction of a gluten-free diet in the same individuals and identified around 65% similarity, with increased diversity in the active state compared with after treatment. It has been suggested that these observations may indicate that the duodenal microbiome can be modulated by exposure to dietary gluten. An alternative explanation is that it may be modulated by differences in mucosal inflammatory activity with withdrawal of the dietary stimulant.

Coeliac disease can present as a variety of symptoms, including classical GI or extraintestinal symptoms, such as the characteristic skin lesion, dermatitis herpetiformis. The factors that dictate how an individual will manifest their disease clinically are unknown. Could the microbiota be involved in this process? Wacklin *et al*[93] assessed this and found that patients presenting with GI symptoms or anaemia clustered separately on principle coordinate analysis than those with skin presentation, had a reduced duodenal mucosal

bacterial diversity and differential bacterial population characterised by an increase in Proteobacteria, and a reduction in Bacteroidetes and Firmicutes.

It has recently been shown that bacteria with enzymatic ability to degrade gluten-derived peptides are present in the oral cavity of healthy individuals.[94] It is unknown whether there is an altered abundance or functional ability of these bacteria in those with coeliac disease. Treatment strategy currently rests on adherence to a gluten-free diet, but this can be difficult to rigorously achieve, and exclusivity is a challenge. Therefore alternative and adjunct therapies are under development. One of these adjunct strategies uses oral recombinant glutenase and has reported a successful outcome in a Phase II trial.[95] It may be that lessons from endogenous oral bacteria can assist this effort. There has been some interest in whether the oral microbiome differs in those with coeliac disease. Francavilla *et al*[96] showed that children treated for coeliac disease do have an altered oral microbiome, characterised by reduction in diversity and a change in abundance of various bacterial species. Specifically, there was an increase in Bacteroidetes and a reduction in Actinobacteria with representative changes in the oral metabo-lome. The authors suggest that this parameter could in turn be developed as a non-invasive screening tool for coeliac disease in the future.

RHEUMATOID ARTHRITIS

RA is a chronic, systemic, polyarthritic disease characterised by synovial inflammation and erosion of bone and cartilage, progressing to functional disability.[9798] Longitudinal studies indicate that autoimmune aspects of RA are initiated years before clinical manifestations of the disease are evident,[99] with circulating anticyclic citrullinated peptide antibodies and rheumatoid factor (RF) evident up to a decade prior to emergence of clinical disease.[100] ACPAs are specific biomarkers for RA, present in 70–80% of patients with RA, and are typically associated with worse outcomes.[97] RA affects up to 1% of adults worldwide[9798] and is multifactorial in aetiology, requiring interaction between genetic and environmental factors for its onset.[97–99]

As with T1D, genetic factors are important[97101] but account for only a proportion of risk susceptibility for RA, and genetic predisposition does not guarantee the development of RA. [99] Although twin studies show a higher concordance in monozygotic twins (12–15%) than in dizygotic twins (3.5%), the overall concordance is low and indicative of a pivotal role for environmental influences.[102103]

There is ongoing debate on whether RA may be initiated by an infectious microorganism, [98104] and many bacteria have been proposed in this capacity, such as *Mycoplasma fermentans*,[105] *Escherichia coli*[106] and *Proteus mirabilis*.[107108] This idea of ‘molecular mimicry’ has existed for at least a century, but has never been definitively proven.[104] As part of this assessment, the oral microbiota has been explored in RA pathogenesis. Belief in the so-called ‘oral sepsis hypothesis’ resulted in tooth extraction as a common treatment for RA —a practice that dates back to the early 1900s,[109] which continued for several decades. Current literature continues to support associations between RA and the microbiota. The main findings from this assessment are presented in table 3. The periodontal microbiota has been a particular focus. Animal models indicate that the periodontal pathogens

Porphyromonas gingivalis and *Porphyromonas nigres-cans* significantly aggravate the severity of collagen-induced arthritis (CIA), with bacterially induced IL-17 directly correlated with intensity of arthritic bone erosion.[110] Moreover, in humans, patients with new-onset RA have a higher prevalence of severe periodontitis at RA disease onset despite their young age and paucity of smoking history and normal oral hygiene routine.[111] Patients with RA have more tooth loss and greater periodontal friability despite oral hygiene comparable to that in healthy controls[112] and the severity of periodontal disease is correlated with RA disease activity.[113] After controlling for a variety of confounding factors, including RA status, age, gender, education,[114] smoking,[111115] alcohol consumption and body mass index (BMI), only RA status and age predict periodontal disease.[114] In addition, patients with RA who receive treatment for periodontal disease show improvements in RA with concomitant decreases in APCAs, anti-*P. gingivalis* antibodies,[116] and proinflammatory cytokines such as TNF-α.[117]

The presence of antibodies to *P. gingivalis* is associated with the presence of RA-related autoantibodies in patients with RA,[118] as well as individuals at risk for, but who have not yet developed, RA.[115] Levels of antibodies to *P. gingivalis* correlate with levels of APCAs and RF, which are indicative of RA disease activity.[115] The question is whether this association between the oral microbiota and RA directly impacts pathogenesis. DNA of *P. gingivalis*[119] and *P. nigrescens*[120] are found in serum and synovial fluid of patients with RA. Similarly, *P. gingivalis*[111] and *P. nigrescens*[119120] are present in subgingival dental plaque and synovial fluid of patients with RA. Thus, it has been speculated that a particular species of *Porphyromonas*, perhaps working in concert with oral bacteria from other genera (including *Anaeroglobus, Prevotella* and *Leptotrichia*) may potentially serve as an environmental trigger for RA in genetically susceptible individuals.[111] However, it remains to be definitely determined whether local periodontal disease precedes the development of RA, or whether periodontitis could be an extra-articular feature of RA, in which case periodontal tissue and joints are preferential targets of the same auto-immune processes.[111] To explore this relationship, Marchesan *et al*[121] infected a CIA mouse model with *P. gingivalis*, and reported increased severity of joint disease, associated with systemic proinflammatory cytokine profiles representative of activation of the Th17 pathway.

P. gingivalis is the only known prokaryote carrying a gene capable of expressing the endogenous peptidylarginine deiminase enzyme, required for the conversion of arginine residues to citrulline. Thus, *P. gingivalis* could be involved in the pathogenesis of autoimmunity by facilitating the generation of citrullinated proteins that can foster loss of immune tolerance and production of APCAs. It has been hypothesised that individuals who possess a genetic predisposition (or other susceptibility factors) together with *P. gingivalis* within their oral microbiota are more likely to develop immune responses to citrullinated antigens. As an example, patients with RA can be positive for antibodies to citrullinated α-enolase peptide-1 (CEP-1) that cross react with bacterial enolase and there is a correlation between the presence of APCA and CEP-1, perhaps due to a shared epitope.[122]

Recently, there has been interest in the role of the respiratory tract microbiota in RA. It is suggested that, by virtue of their constant exposure to bacterial antigens, the lungs may be a potential site of early events that facilitate the initiation and, or progression of RA. While

there have not been any studies that directly examined the role of the lungs and their microbiota in patients with RA, several studies suggest that the lungs may be susceptible to proinflammatory microbiota originating from periodontal tissue.[123] First off, the respiratory mucosa houses their own unique set of microbiota that can be come perturbed in disease states.[124125] In addition, the lungs are a site of local citrullination, which can be accelerated by smoking in the absence of RA.[126] Moreover, patients with early RA and at-risk, seropositive individuals without RA, show signs of inflammatory associated airway injury, such as bronchial wall thickening, and air trapping.[127]

Could the intestinal microbiota play a pivotal role in the pathogenesis of RA?[128–130] Animal models of RA can be rescued or exacerbated by elimination or exposure to gut-residing bacteria, respectively.[131132] For example, the K/BxN T cell receptor transgenic mouse model of spontaneous inflammatory arthritis is attenuated by germ-free rearing or modulation of gut micro-biota with antibiotic treatment. In contrast, segmented filamentous bacteria related to *Clostridium* can induce proinflammatory small bowel lamina propria responses in this mouse model of arthritis, via an increase in Th17 cells and subsequent exacerbation in arthritic pathology.[132] Another spontaneous murine model of inflammatory arthritis, namely the IL-1 receptor antagonist-knockout mouse (IL-1RA$^{-/-}$), also showed no development of arthritis in germ-free conditions.[131] Crucially, exposure of these germ-free mice to *Lactobacillus bifidus*, a Gram-positive anaerobic commensal of the GI tract, exacerbated disease. Elegant use of further TLR genetic knockout in this model revealed that TLR signalling is intimately linked to arthritis pathogenesis; IL-1RA$^{-/-}$TLR2$^{-/-}$ mice displayed exacerbated arthritis through reduction in regulatory T cell response. In comparison, IL-1RA$^{-/-}$TLR4$^{-/-}$ mice were protected from arthritis through reduction in Th17 T cell response. These results suggest that innate receptor sensing, potentially of gut microbiota, may be a crucial step in disease pathogenesis and provides insight into a gut to joint mechanism in disease pathogenesis.

Mice carrying arthritis susceptibility genes (HLA DRB*0401) have a different composition of gut microbiota compared with genetically resistant counterparts (HLA DRB*0402), rich in *Clostridium*-like bacteria. This was associated with differential Th17 gene transcripts in the gut, altered mucosal immune function and increased gut permeability.[133] Dorożyńska *et al*[134] showed that modulation of the gut flora with antibiotic treatment reduced disease severity in the CIA animal model of RA, along with differential cytokine response in mesenteric lymph nodes.

How does this translate to human disease? Vaahtovuo *et al*[135] identified a dysbiosis of faecal microbiota in patients with newly diagnosed RA compared with fibromyalgic controls, characterised by a decrease in Bifidobacteria and Bacteroidetes. Similarly, faecal 16sRNA sequencing has shown that patients with new onset RA carry a distinctive enterotype of gut micro-biota characterised by an abundance of *Prevotella copri* and a relative lack of *Bacteroides*.[136] *P. copri* robustly correlates with disease severity in patients with new-onset RA although whether this impacts the initiation or progression of autoimmunity is unclear. However, this species of bacteria is capable of expanding to dominate the commensal microbiota and exacerbates experimental colitis when delivered to mice by gavage.[136]

IS THERE ANY EVIDENCE FOR THE ROLE OF THE MICROBIOME IN OTHER AUTOIMMUNE DISEASES?

Autoimmune diseases can occur at any site in the body, and indeed there is a long list of diagnoses in this category. Again these occur due to a prescribed genetic susceptibility and largely unknown environmental influences, and manifest serologically with evidence of autoantibody production. Examples include autoimmune thyroiditis, autoimmune pancreatitis, Sjogren's syndrome, systemic lupus erythematosus (SLE) and autoimmune liver diseases such as autoimmune hepatitis and primary biliary cirrhosis. Given the evidence for the role of the microbiota in the pathogenesis of T1D, RA and coeliac disease as discussed previously, there is an emerging interest in whether the micro-biota may be implicated in other, often rarer autoimmune conditions. This is certainly in its infancy, but there are a few publications appearing in the literature to this effect.

Zhou *et al*[137] recently analysed the faecal microbiota from patients with hyperthyroidism compared with healthy controls. Denaturing gradient gel electrophoresis analysis revealed an increased bacterial diversity in those with hyperthyroidism, with a reduction in *Lactobacillus* and *Bifidobacteria*. To date, this appears to be the only study of this nature in thyroid disease. However, a clue that the intestinal microbiota may be an important environmental factor appeared over a decade ago in the literature; disease susceptibility of a rat model of auto-immune thyroiditis could be affected through modulation of the gut microbiota.[138] Animals raised in specific pathogen-free conditions were less susceptible to disease. In contrast, treatment with oral antibiotics and stool transplantation from conventionally reared animals into specific pathogen-free rats, resulted in exacerbated disease. Furthermore, this effect was seen in off-spring when this modulation was given to mothers during gestation.

Sjogren's syndrome and SLE are characterised by the emergence of anti-Ro66/Sjögren's syndrome antigen A antibodies, but the initiating event leading to this is unclear. As loss of tolerance by T cells is known to be necessary in this process, Szymula *et al*[139] explored whether these T cells could be activated through recognition of gut derived bacterial antigens. They created Ro60 reactive T cell hybidomas from mice transgenic for the genetic susceptibility for Sjogren's syndrome and SLE, and tested their ability to react to different bacteria-derived peptides. They found reactivity to three peptides derived from oral commensal bacteria, and also four peptides from gut-derived commensal bacteria; three of the latter belonged to *Bacteroides* spp. This suggests that autoreactive T cells responsible for these autoimmune disorders may be primed in the gut by exposure to commensal microbiota. However, as yet, there are no reports of intestinal microbiota analysis in SLE and Sjogren's syndrome. Indeed, from animal studies using the NZB mouse model that spontaneously develops autoimmune features likened to human SLE, the potential role of the microbiota is less clear, in that there is little difference in disease emergence and autoantibody formation between germ-free and conventionally raised litters.[140] However, disease characteristics can be modified by dietary change. The mechanism of this is unclear, but it has been hypothesised that this may reflect modulation of the gut commensal bacteria.[140]

Ankylosing spondylitis (AS) is associated with a clear genetic susceptibility of HLA-B27 positivity and is characterised clinically by spinal and large joint arthropathy, enthesopathy and other systemic manifestations. There is a strong association between AS and microscopic or overt IBD.[141142] Serological evidence of anti-cBir antibodies in patients with AS have implicated flagellated bacteria in this disease.[143] Animal models of AS with HLA-B27 genotype do not develop disease in germ-free facilities.[144] These findings have fuelled interest in whether the gut microbiota could be involved in this disease pathogenesis. A small human study in 2002 using denaturing gradient gel electrophoresis techniques assessed this question and did not find any clear dysbiosis.[145] The same group went on to show that circulating T cells from patients with AS evoked a diminished IL-10 cytokine response after exposure to autologous faecal *Bacteriodes* sp.[146] Recently, Lin *et al*[147] have shown that carriage of the human transgene HLA-B27 in rats itself alters the caecal microbiota, although the mechanism by which this then crosstalks to the host immune system and impacts phenotype remains unclear. To date, there are no additional reports of human studies assessing the gut microbiota in AS compared with controls that employ high sensitivity sequencing techniques.

MS is a chronic demyelinating disorder of the central nervous system (CNS), mediated by a predominant T cell driven myelin directed autoimmunity. The initiating factor is unknown, although genetic and environmental factors play an important role. The concept of the microbiota-gut-brain axis has emerged given that the enteric microbial community has the ability to crosstalk with our nervous system,[148] for example, gut microbes can secrete various molecules that can directly impact enteric neuronal signalling, such as serotonin, melatonin or acetylcho-line, and enteric neurons express TLRs and so are able to sense and react to the microbial community directly.[149] There is a growing body of evidence to show that the intestinal microbiota may be implicated in the pathogenesis of this disease (reviewed in refs.[10149150]). The quintessential animal model of MS is the experimental allergic encephalomyelitis (EAE) mouse; progressive demyelinating neurological disease is precipitated by pathogenic autoreactive T cells induced by simultaneous injection of a myelin antigen and bacterial adjuvants. In this model, manipulation of the gut microbiota by germ-free rearing[151] or antibiotics[152–154] confers resistance to disease onset and diminishes severity. Re-establishing intestinal colonisation in germ-free resistant mice, for example monocolonisation with segmented filamentous bacteria reinstates the disease susceptibility.[151] This protection is mediated by an altered adaptive immune response, characterised by an increase in regulatory T cell[151153] and IL-10 producing regulatory B cell populations,[154] and reduction in proinflammatory Th1 and Th17 cells.[151152] Similarly, a spontaneous murine model of CNS demyelination (SJL/J mice expressing T cell receptor towards myelin peptide antigen) is also protected by germ-free rearing, with emergence of disease with gut microbial recolonisation,[155] and disease pathogenesis implicating autoreactive pathogenic T cells and autoantibody producing B cells. Restitution of germ-free EAE mice with intact *Bacteroides fragilis* conferred protection, dependant on capsular polysaccharide A, whose presence attenuated disease from a therapeutic and preventative strategy, through promotion of IL-10 producing regulatory T cells via TLR2 signalling.[156–159] As yet, there is no reported assessment of the gut microbiota in human patients with MS and this data is eagerly awaited and may yield novel therapeutic targets.

CONCLUSION

It is clear that the human microbiota holds a pivotal position in health and disease. The enormity of this relationship is just beginning to become apparent as technology allows more in-depth analysis of the microbial community we harbour, in composition and functionality. In autoimmune disease, there is a clear strong genetic predisposition. The role of environmental influences is appreciated but not fully understood for many of these diseases and the initiating factor often remains elusive. Autoimmune disease is a complex interplay between genetics, environmental exposures and immune function, and there are several ongoing unanswered questions; what dictates the spectrum of disease severity? What dictates why some individuals with appropriate genotype remain disease-free lifelong, while others harbour latent disease or overt clinical pathology? Why do autoimmune diseases present in patients of differing ages despite the same environmental exposures? Could the micro-biota be responsible for this disparity?

There is clear and increasing evidence that changes in the microbiota are associated with some autoimmune diseases as discussed in this review. This dysbiosis in the microbiota is associated with several autoimmune diseases, involving the GI mucosa that lies in close contact with luminal contents as exemplified by coeliac disease, and also autoimmunity targeted towards distant sites, such as the pancreas in T1D and joints in RA. However, for now and for the most part, the relationship between the microbiota and autoimmune diseases remains an association. The question of ‘cause or effect?’ retains prominent status. Is dysbiosis of the microbiota an initiator of autoimmune disease, a perpetrator of increasing progression or a consequence of other pathological features? This remains unanswered. It appears that the large, global, longitudinal, prospective consortium efforts that are now in place, aim to address this point and this is certainly a Herculean task. A strength of these efforts is the detail of the design and use of cutting edge technology to maximise and thoroughly analyse the data generated. This approach has the power to revolutionise our understanding of these diseases and ultimately offer insight into novel preventative or therapeutic strategies.

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Table 1

The main outcomes from studies assessing the human gut microbiome in T1D (faecal stream analysis)

Association with T1D compared with control	Reference
↓ Butyrate producing bacteria	46, 47
↓ Mucin degrading bacteria (*Akkermansia* sp)	47
Bacterial metabolic functional capabilities differ	47
Bacterial diversity lacks stability	48
↑ Bacteroidetes, ↓ Firmicutes preceding disease onset	46, 48, 49, 51
Interactions between bacteria distorted	50
Change in microbiome may revert with glucose normalizing treatment	51
No single causative agent identified	

TID, type 1 diabetes.

Table 2

The main outcomes from studies assessing the microbiome in coeliac disease (upper small bowl mucosal biopsies and faecal stream analysis)

Association with coeliac disease compared with control	Reference
Dysbiosis and ↑ diversity in adults and children	76, 81–84
No unifying pattern or distinct composition or diversity	
↑ Firmicutes in adults	76, 83
↑ Bacteroidetes in children	80, 81, 83, 84
↑ *Bifidobacterium* in children	82, 86
No difference in diversity by microarray HITChip/16S sequencing	77, 78
High genetic risk—↑ Firmicutes	91
High genetic risk—↓ Bacteroidetes	92
High genetic risk—altered diversity	91, 92
No single causative agent identified	
Altered oral microbiome	96

Table 3

The main outcomes from studies assessing the microbiome in RA

Microbiome site	Association with RA compared with control	Reference
Oral	*Porphyromonas Gingivalis and Porphyromonas nigrescans* aggravate animal models of arthritis	110, 121
	↑ Prevalence of periodontitis in patients with RA	111–115
	Evidence of periodontal pathogens in synovial fluid of patients with RA	111, 119, 120
Intestinal	Animal models of arthritis exacerbated or rescued by changes in gut microbiome	131, 132, 134
	Humans—↓ Bacteroidetes and *Bifidobacterium*	135, 136

RA, Rheumatoid arthritis.