Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Intestinal dysbiosis and probiotic applications in autoimmune diseases

Gislane Lelis Vilela de Oliveira,[1] Aline Zazeri Leite,[1] Bruna Stevanato Higuchi,[1] Marina Ignácio Gonzaga[1,2] and Vânia Sammartino Mariano[2]

[1]*Microbiome Study Group, School of Health Sciences Dr Paulo Prata, Barretos, São Paulo, and* [2]*Barretos Cancer Hospital, Barretos, São Paulo, Brazil*

doi:10.1111/imm.12765
Received 24 February 2017; revised 19 May 2017; accepted 24 May 2017.
Correspondence: Gislane Lelis Vilela de Oliveira, Faculdade de Ciências da Saúde Dr Paulo Prata (FACISB), Avenida Loja Maçônica Renovadora 68, no. 100, 14075-002, Barretos, São Paulo, Brazil. Email: glelisvilela@gmail.com
Senior author: Gislane Lelis Vilela de Oliveira

Summary

In humans, a complex interaction between the host immune system and commensal microbiota is required to maintain gut homeostasis. In this symbiotic relationship, the microbiota provides carbohydrate fermentation and digestion, vitamin synthesis and gut-associated lymphoid tissue development, as well as preventing colonization by pathobionts, whereas the host offers a niche and nutrients for the survival of the microbiota. However, when this mutualistic relationship is compromised and an altered interaction between immune cells and microorganisms occurs, the gut microbiota may cause or contribute to the establishment of infectious diseases and trigger autoimmune diseases. Researchers have made efforts to clarify the role of the microbiota in autoimmune disease development and find new therapeutic approaches to treat immune-mediated diseases. However, the exact mechanisms involved in the dysbiosis and breakdown of the gut epithelial barrier are currently unknown. Here, we provide a general overview of studies describing gut microbiota perturbations in animal models of autoimmune diseases, such as type 1 diabetes, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus. Moreover, we include the main studies concerning dysbiosis in humans and a critical discussion of the existing data on the use of probiotics in these autoimmune diseases.

Keywords: autoimmunity; dysbiosis; gut barrier; inflammation; probiotics.

Introduction

The skin surface and mucous membranes in vertebrates are colonized by a high number of microorganisms, mainly bacteria, representing commensal microbiota.[1–3] In humans, approximately 30–400 trillion microorganisms (3×10^{13} to 40×10^{13}) colonize the healthy human intestinal tract, and the vast majority reside in the distal portion.[4–6] The gut bacteria have co-evolved in a symbiotic relationship with the human host, and their composition depends on immunogenetic and environmental factors.[7,8]

From birth, gut microbiota colonization depends on several factors, including age, mode of delivery (vaginal or caesarean section), mothers' microbiota composition, early use of antibiotics and feeding regimen (breastfeeding or formula).[9,10] The mode of delivery and mode of feeding in the first years of life strongly influence the establishment of gut microbiota composition and may affect the development of autoimmune diseases.[10] Additionally, caesarean delivery and formula use have been associated with higher incidence of infectious diseases and increased susceptibility to allergic diseases.[10,11]

In humans, the intestinal microbiota tends to stabilize and reaches greater diversity at approximately 3 years of age, and these microorganisms collaborate with many host physiological processes; in turn, the host contributes

Abbreviations: CIA, collagen-induced arthritic mice; CNS, central nervous system; DAS28, disease activity score calculator for rheumatoid arthritis; EAE, experimental autoimmune encephalomyelitis; GF, germ-free mice; IFN-γ, interferon-γ; IL-17, interleukin-17; MS, multiple sclerosis; NOD, non-obese diabetic mice; NZB, New Zealand black mice; RA, rheumatoid arthritis; RRMS, relapsing–remitting multiple sclerosis; SFB, segmented filamentous bacteria; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; Th1, T helper type 1; TLR, Toll-like receptors; Treg, regulatory T

© 2017 John Wiley & Sons Ltd,

to their growth and survival by offering a niche, substrates and nutrients.[10–13] The main contributions of the microbiota to the host include carbohydrate fermentation and digestion, vitamin synthesis, gut-associated lymphoid tissue development, the polarization of specific immune responses, and the prevention of colonization by pathobionts.[14–18]

The two predominant bacterial phyla in the healthy human gut are Firmicutes and Bacteroidetes. Also present are Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia phyla but to a lesser extent.[19,20] The most prevalent genera in the adult human gut are represented by Gram-positive bacteria, such as *Clostridium*, *Bifidobacterium*, *Lactobacillus*, *Ruminococcus*, *Streptococcus*, and Gram-negative bacteria, such as *Bacteroides* and *Escherichia*.[21]

A complex interaction between the host immune system and the microbiota is required to maintain gut homeostasis.[14–18] However, when this mutualistic relationship is compromised, with alterations in bacterial function and diversity, a process called dysbiosis, the gut microbiota may cause or contribute to the establishment of infectious diseases and to autoimmune disease development.[22]

For several decades, researchers interested in enteroinfections and gut-associated diseases have studied the gut microbiota. It has been evident that the microbiota plays an important role in immune homeostasis in the gut mucosa, and researchers are extensively investigating microbiota–immune system interactions and their role in the susceptibility or resistance to gut infections and in the triggering of autoimmune, allergic and chronic inflammatory diseases.[23]

Gut microbiota and immune system

Studies in germ-free (GF) mice showed that the gut microbiota is required for normal immune system maturation, including gut-associated lymphoid tissue development, which plays important roles in tolerance induction to autoantigens in the gut mucosa.[24–26] These GF mice showed decreased numbers of $CD4^+$ T cells, secreting IgA plasma cells and antimicrobial peptides, a thinner mucus layer and Peyer's patches.[23] The spleen and lymph nodes are abnormally developed in GF mice, with decreased numbers of B and T cells in the germinal centres and parafollicular region, respectively.[27,28]

In mice and humans, during equilibrium, intestinal dendritic cells and macrophages are hyporesponsive to pathogen-associated molecular patterns.[1,29] However, when epithelial barrier breakdown occurs, the pattern recognition receptors, which are present in innate immune cells, recognize gut microbiota or pathobionts through toll-like receptors, NOD-like receptors, soluble retinoic acid-inducible gene I, or melanoma differentiation-associated protein 5, which triggers an inflammatory cascade, pro-inflammatory cytokine secretion, and the activation of adaptive immune responses.[29,30]

Gut microbiota and T cells

The resident microbiota regulates the development of specific subsets of lymphocytes in the gut. T helper type 17 (Th17) lymphocytes are essential in defence against bacterial and fungal infections and play roles in autoimmune disease development by producing pro-inflammatory cytokines, such as interleukin-17 (IL-17) and IL-22, and by the recruitment of neutrophils.[31] Th17 cells accumulate in the intestine, suggesting that intrinsic processes in the gut mucosa could regulate the development of these cells. Moreover, studies have shown that the number of Th17 cells are reduced in GF mice or antibiotic-treated adult mice, and it was observed that some particular species of Clostridia, called segmented filamentous bacteria (SFB), promote the generation of Th17 cells in the gut[32–36] (Fig. 1).

Regulatory T (Treg) cells aggregate in the intestine and help with the maintenance of homeostasis.[37,38] Treg cell depletion induces an abnormal expansion of $CD4^+$ T cells expressing T-cell receptors against commensal microbiota, resulting in gut inflammation.[1] Studies have shown that the Treg cell numbers were reduced in the lamina propria of GF mice and particular species of *Clostridium* are involved in the Treg induction in the gut.[38,39] In addition, the polysaccharide A, which is secreted by *Bacteroides fragilis*, can induce $CD4^+$ T cells into $Foxp3^+$ Treg cells, which secretes IL-10, an anti-inflammatory cytokine, and mediates mucosal tolerance[40] (Fig. 1).

Gut microbiota and B cells

An important relationship exists between intestinal microbiota and the humoral immune responses. Secreted IgA protects us against enteroinfections and coats commensal bacteria by inhibiting their binding to the intestinal epithelium and the lamina propria invasion.[41] Secreted IgA has barrier functions and shapes the microbiota composition through antibody-mediated immunoselection.[27]

B cells differentiate into plasma cells by T-helper-dependent or T-helper-independent mechanisms. In Peyer's patches, T-helper-dependent responses occur with isotype switching, somatic hypermutation and affinity maturation, which induce the differentiation of IgA-secreting plasma cells with a high affinity for antigens.[41] T-helper-independent responses are mainly mediated by B1 cells that differentiate into polyclonal IgA-secreting plasma cells with a low affinity for antigens.[27] The T-helper-dependent and T-helper-independent immune responses in the gut have been extensively reviewed in the literature.[41–46]

© 2017 John Wiley & Sons Ltd,

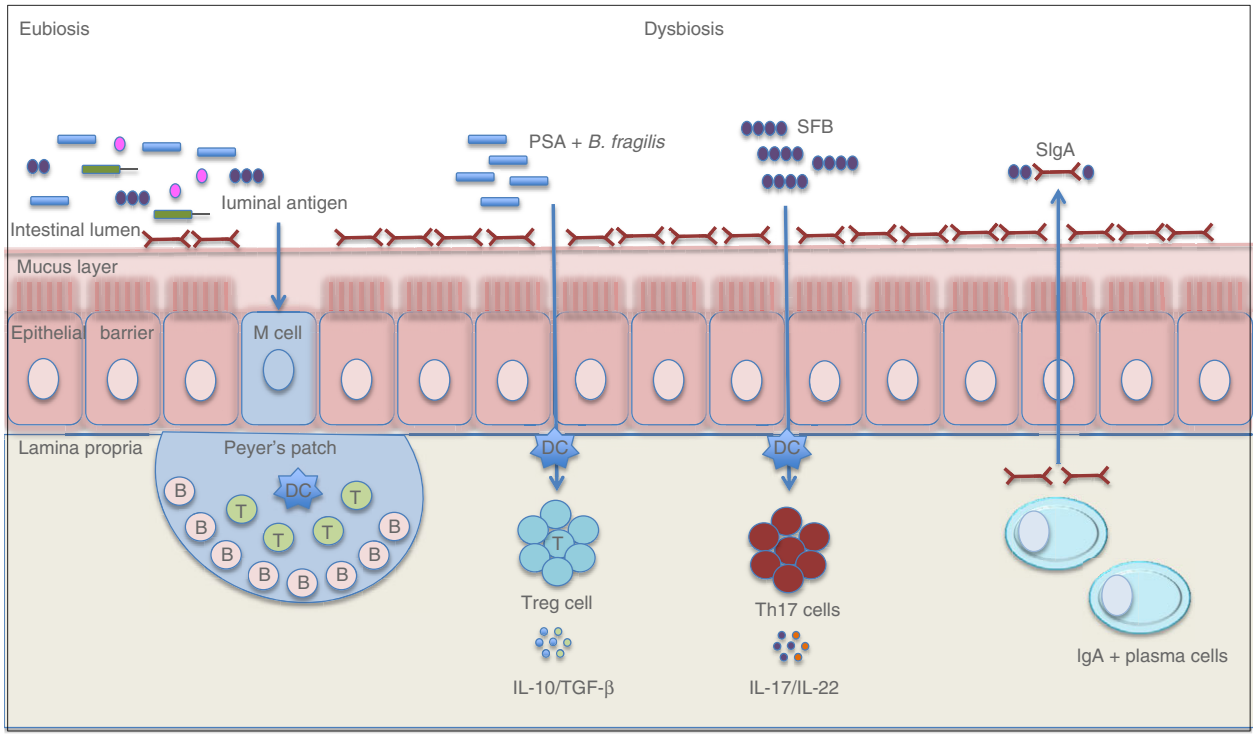

Figure 1. A schematic representation of the interaction between commensal microbiota and the immune system. Under eubiosis, there are microbiota diversity and immune homeostasis in the gut mucosa. Commensal microorganisms instruct dendritic cells to induce IgA-secreting cell differentiation, and in turn, IgA regulates the composition of the gut microbiota. During dysbiosis, there are decreased diversity in commensal microbiota and deregulated interactions between immune cells and these microorganisms. Some specific bacteria, such as *Bacteroides fragilis*, induce regulatory T cell differentiation and the secretion of anti-inflammatory cytokines, whereas segmented filamentous bacteria (SFB) promote T helper type 17 (Th17) cell differentiation and the secretion of pro-inflammatory cytokines, which play roles in several autoimmune diseases.

The gut microbiota also induces the expression of factors involved in the induction of IgA^+ B cells, such as B-cell activating factor and a proliferation-inducing ligand (known as APRIL) in dendritic cells in the lamina propria.[46] Therefore, the microbiota instructs dendritic cells and follicular dendritic cells to induce IgA-secreting plasma cells, and in turn, IgA regulates the composition and function of the gut microbiota (Fig. 1).[46] To confirm the essential role of this mutualistic relationship, studies have shown that GF mice and mice deficient in activation-induced cytidine deaminase have decreased plasma cells and alterations in the gut microbiota composition, respectively.[42–46]

Gut microbiota and autoimmune diseases

Evidence from animal models has implied the direct involvement of gut microbiota in disease development, and some intestinal microbiota are associated with autoimmune diseases.[24–26]

Intestinal dysbiosis observed in autoimmune diseases is associated with decreased bacterial function and diversity, impaired gut barrier function, increased inflammation and decreased Treg cells in the gut.[47,48] Additionally, the hypotheses proposed to link intestinal dysbiosis with autoimmune diseases include molecular mimicry, bystander T-cell activation, and the amplification of autoimmunity by pro-inflammatory milieu, which is elicited by altered gut microbiota.[49] Finally, a more recent hypothesis proposed by Lerner *et al.* implicated the post-translational modification of luminal proteins, promoted by enzymes from dysbiotic microbiota, which modify substrates in a different way from that performed under eubiotic conditions.[49] The defective post-translational modification of luminal proteins may induce neo-epitope generation that could become immunogenic and may induce systemic autoimmunity and trigger autoimmune diseases.[49]

Here, we describe several reports concerning alterations in the intestinal microbiota in some autoimmune diseases, such as type 1 diabetes, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus. Additionally, we provide a critical discussion concerning the data from the use of probiotics in autoimmune diseases.

Type 1 diabetes

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the immune destruction of insulin-

secreting pancreatic β-cells, resulting in exogenous insulin dependence to control blood glucose levels.[50] The aetiopathogenesis may involve the interaction of predisposing HLA genes and environmental factors, such as viral enteroinfections and intestinal dysbiosis.[51] According to the International Diabetes Federation, 79 100 children under the age of 15 will develop T1D annually worldwide.[52]

The role of the gut microbiota in T1D aetiology has been the subject of research over the last decade to clarify its role in disease development and determine preventive approaches, such as diet manipulation and probiotic administration.[51]

One of the first studies in non-obese diabetic mice (NOD) showed that the composition of the intestinal microbiota modulates innate and adaptive immune functions and enhances the disease in MyD88$^{-/-}$ NOD mice. However, protection against diabetes in these mice is abrogated by the administration of antibiotics and GF conditions, suggesting that commensal bacteria may be important to reduce disease susceptibility in these mice.[53] Additionally, Emani *et al.* reported that NOD mice treated with a conventional diet presented impaired tolerance to gut microbes, an altered barrier permeability, an increased number of peritoneum macrophages and a decreased abundance of Firmicutes.[54]

An imbalance among Th1, Th17 and Treg cell differentiation in the gut was reported in GF NOD mice and is associated with insulitis and pancreas inflammation.[55] Interestingly, the SFB, which are associated with the exacerbation of the Th17 responses in other autoimmune diseases, are involved in protection against diabetes in NOD mice.[56]

In biobreeding diabetes-prone rats, increased percentages of *Bacteroides*, *Ruminococcus* and *Eubacterium* reads in stool samples were observed, and there was a higher abundance of *Bifidobacterium* and *Lactobacillus* in stool samples from biobreeding diabetes-resistant rats.[57] The altered gut microbiota, such as the increased abundance of Bacteroidetes, could promote increased intestinal permeability and precede the clinical onset of T1D in animal models and pre-diabetic and diabetic patients.[58–61] In fact, the role of the gut microbiota in T1D has been suggested since 1987, and the first study in humans was performed in Finland by using stool samples from four T1D children and four matched controls.[62] They observed that children with T1D had decreased microbiota diversity compared with controls and had a reduction in the Firmicutes : Bacteroidetes ratio[62,63] (Fig. 2). Additionally, recent studies showed that the composition of the intestinal microbiota is altered in children with pre-diabetes with genetic susceptibility and autoantibodies against β-cells.[64–66]

Studies from the De Goffau group showed that children with autoantibodies against β-cells exhibit an increased number of Bacteroidetes and decreased abundance of lactate and butyrate-producing bacteria in faeces.[63,65] In agreement with this study, Brown *et al.* showed a decreased number of mucin-degrading and butyrate-producing bacteria in T1D patients compared with healthy controls.[67] Butyrate has anti-inflammatory activity, induces Treg cell differentiation in the gut and enhances the gut barrier via tight junctions.[64]

Endesfelder *et al.* did not observe significant differences in gut microbiota diversity in 22 children with positive-islet autoantibodies compared with 22 children with negative ones.[68] Additionally, children with T1D have decreased numbers of lactate-producing bacteria, such as *Bifidobacterium longum*, subspecies *infantis*. Bifidobacteria members promote carbohydrate fermentation, generate acetate and lactate, release polyphenols and linoleic acids, and have antioxidant activities.[68] They also play a role in gut-associated lymphoid tissue development maturation during early life and guarantee protection against pathobionts by bacteriocin release, causing decreases in the luminal pH and the inhibition of adhesion to epithelial cells.[69] In addition, Bifidobacteria species synthesize B-group vitamins, which can induce Treg cells in the gut mucosa[70,71] (Fig. 2).

Murri *et al.* evaluated the gut microbiota in a cohort of 16 children with T1D and 16 controls and showed an increased abundance of *Clostridium*, *Bacteroides* and *Veillonella* in patients compared with controls. Furthermore, the Firmicutes : Bacteroidetes ratio and levels of *Lactobacillus*, *Bifidobacterium* and *Prevotella* were decreased in these patients.[72]

Davis-Richardson *et al.* evaluated stool samples isolated from 29 patients with seroconverted T1D disease and 47 healthy controls and observed a high abundance of *Bacteroides dorei* and *Bacteroides vulgatus* in seroconverted patients with T1D 8 months before β-cell autoimmunity. These data suggest that early dysbiosis may be relevant to predict T1D in genetically predisposed individuals.[73]

Kostic *et al.* evaluated stool samples from 33 children genetically predisposed to T1D and reported alterations in microbiota α-diversity between and within the children over time. They also observed a decrease in the number of Gram-negative members and an inverse correlation between Lachnospiraceae and Ruminococcaceae with Enterobacteriaceae, a Gram-negative aerobe. In addition, they reported that early commensals are aerobic, whereas later microbes appear to be anaerobic.[74]

A more complex study with 35 patients newly diagnosed with T1D, 21 first-degree relatives with β-cell autoimmunity, 32 relatives with negative autoantibodies, and 23 controls showed that the numbers of *Lactobacillus* and *Staphylococcus* reads were decreased in patients and in first-degree relatives when compared with controls. Furthermore, the gut microbiota from subjects with negative and positive autoantibodies grouped together but in

© 2017 John Wiley & Sons Ltd,

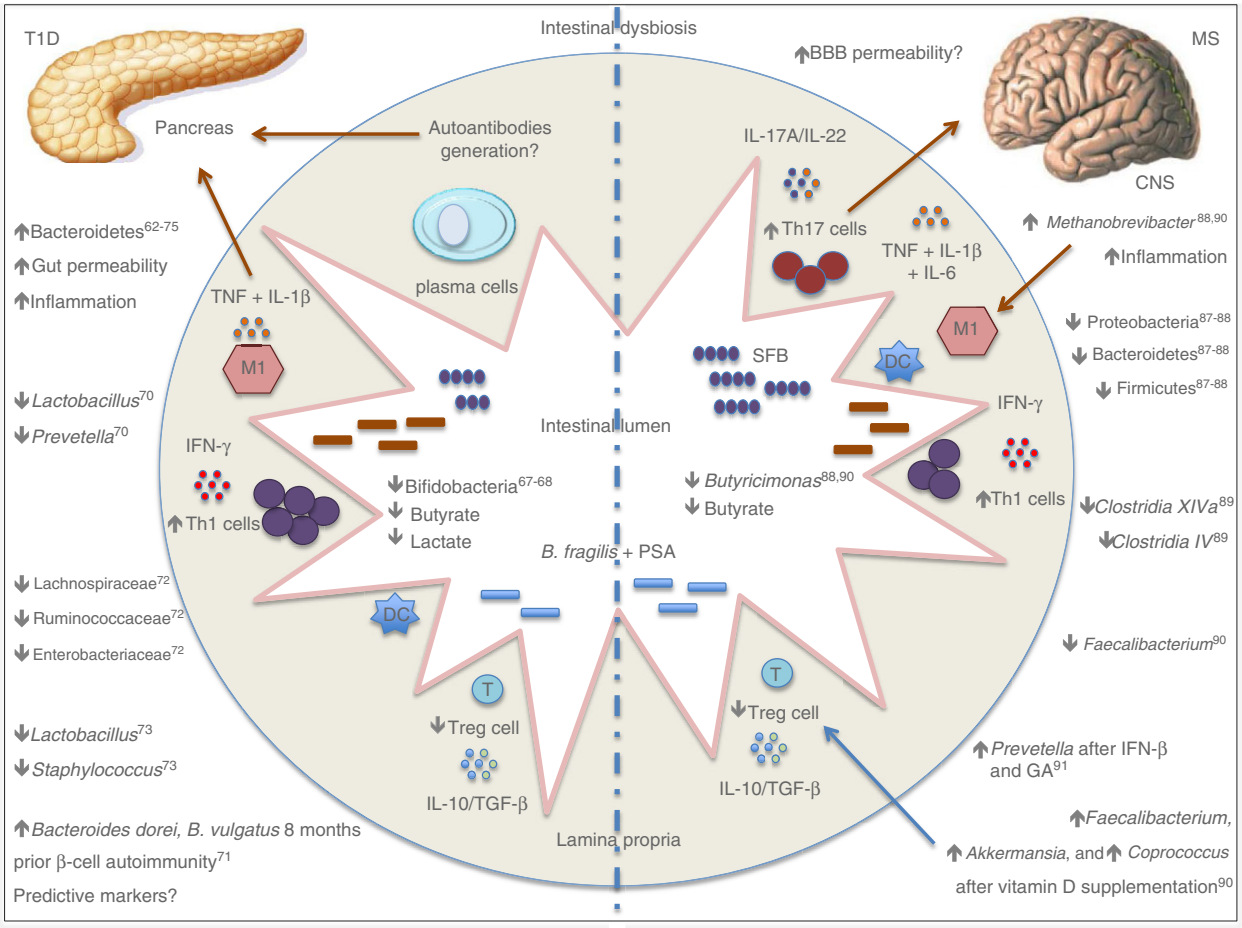

Figure 2. A schematic representation of the intestinal dysbiosis in organ-specific autoimmune diseases in humans.

a different cluster from newly diagnosed patients.[75] This study and the work performed by Meiía-León *et al.* support the hypothesis that there is an intestinal microbiota signature associated with T1D development in seropositive children.[76]

Even though studies have shown that intestinal dysbiosis can affect gut permeability via their metabolites and play a role in T1D development, there is no evidence for the real role of intestinal microbiota in the development of autoimmunity to β-cells and in tissue damage in humans. Additional studies are needed to find the specific microbial ligands that signal through immune cells in the gut and might be involved in the autoreactivity to β-cells.[77]

Multiple sclerosis

Multiple sclerosis (MS) is a chronic and inflammatory disease that affects the central nervous system (CNS) and is characterized by autoimmune reactions against myelin proteins. Susceptible HLA alleles and environmental factors, such as virus infection, a hypercaloric diet, vitamin D deficiency and dysbiosis, have been implicated in triggering MS.[78] MS promotes disability in young adults and affects twice as many women as men. According to the Multiple Sclerosis International Federation and World Health Organization (WHO), the prevalence of MS increased from 2·1 million in 2008 to 2·3 million in 2013.[79]

Studies have shown that gut microbiota can affect the development of MS, and these works implicated intestinal dysbiosis as one of the possible causes of extraintestinal disease development.[80] The colonization of GF mice with SFB promotes an increase in the number of Th17 cells in the lamina propria and CNS, worsening disease severity in experimental autoimmune encephalomyelitis (EAE), an MS animal model.[81] Likewise, the colonization of the same mice with *Bacteroides fragilis* and polysaccharide A, which induces Foxp3$^+$ Treg cell differentiation, decreases symptoms in EAE mice.[82]

The immune response in EAE is mediated mainly by Th1 and Th17 cells. The gut SFB induces Th17 cells in EAE mice. To validate the influence of the intestinal microbiota in the development of EAE, mice were treated

with antibiotics, and they exhibited a reduction in clinical score, suggesting the role of the microbiota in the induction of inflammatory cells in these models. The attenuation of the clinical score was accompanied by decreased interferon-γ (IFN-γ), macrophage inflammatory protein 1α, monocyte chemoattractant protein 1, IL-17 and IL-6 and increased IL-10 and IL-13 secretion.[83]

The colonization of EAE mice with *Bacteroides fragilis* and polysaccharide S resulted in decreased clinical scores and the protection of mice from disease.[84] This effect was achieved because of the induction of Treg cells, increased IL-10 and reduced IL-17, suggesting the use of these microorganisms as probiotics in humans.[85] Some of the tested probiotics seemed to have an effect on the autoimmunity of EAE mice. Oral administration of *Lactobacillus* spp. and *Bifidobacterium bifidum* showed a significant decrease in the clinical score and increase in the Treg cell number in treated mice.[86]

Recently, it was also demonstrated that the gut microbiota could affect the permeability of the blood–brain barrier.[86] The defective tight-junctions at the blood–brain barrier of GF mice are restored after they are colonized with conventional microbiota.[86] The use of GF mice also demonstrated the importance of intestinal microbiota in the development of EAE. The induction of EAE in GF mice resulted in the reduction of IFN-γ and IL-17A in the CNS, accompanied by an increase in the number of Treg cells in the gut.[81]

In patients, recent studies have evaluated seven individuals with relapsing–remitting MS (RRMS) and found a reduction in Firmicutes, Bacteroidetes and Proteobacteria members.[87,88] Jhangi *et al.* evaluated 22 untreated MS patients and observed an increase in *Methanobrevibacter smithii* and reduction in Firmicutes, as well as *Butyricimonas*, which are butyrate-producing members of the microbiota.[88] A Japanese study found altered intestinal microbiota in RRMS patients, with decreased *Clostridia* XIVa and IV groups and Bacteroidetes members.[89] Another study, including 15 RRMS patients with an Expanded Disability Status Score $\leq 3{\cdot}0$ showed a decreased amount of *Faecalibacterium* and increased amounts of *Akkermansia*, *Coprococcus* and *Faecalibacterium* after vitamin D supplementation[90] (Fig. 2).

A recent study investigated 60 RRMS patients, 28 untreated patients, and 43 healthy controls and showed increased amounts of *Methanobrevibacter* and *Akkermansia* and decreased amounts of *Butyricimonas* in untreated patients.[90] *Methanobrevibacter* is involved in inflammatory conditions by recruiting macrophages and activating dendritic cells.[91] *Akkermansia* species have immunoregulatory effects by converting mucin into short-chain fatty acids; however, they could play a role in degrading the mucus layer and promoting inflammation.[92,93] *Butyricimonas* species are butyrate-producing bacteria and have immunomodulatory properties by inducing Treg cells in the gut.[71] In treated patients (IFN-β and glatiramer acetate), there was an increased number of *Prevotella* compared with untreated patients.[94] This genus is associated with high-fibre ingestion and has regulatory roles via butyrate generation[95] (Fig. 2).

Chen *et al.* also reported dysbiosis in RRMS patients after comparing stool samples from 31 patients and 36 controls. They observed an increased abundance of the *Pseudomonas*, *Mycoplasma*, *Haemophilus*, *Blautia* and *Dorei* genera in patients compared with healthy counterparts, which showed a prevalence of *Prevotella* and *Parabacteroides*.[96]

Available findings on dysbiosis in animal models and patients with MS point to the gut–brain axis connection. The relationship between immunity in the gastrointestinal mucosa and commensal bacteria seems to promote important physiological homeostasis for the host.[97] However, future studies are required to determine the real role of the gut microbiota in CNS demyelinating diseases.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by chronic inflammation of multiple joints, bone erosion and cartilage destruction. Moreover, RA can affect internal organs such as the lungs, heart and kidneys. Anti-cyclic citrullinated peptide and/or rheumatoid factor are the most important autoantibodies in RA and can be found before disease onset.[98] The disease is three times more common in women, and according to WHO, the worldwide prevalence, which is between 0·3 and 1%, ranks the disease among the most common autoimmune disorders. The triggering of RA involves the interaction of HLA genes and environmental factors, such as smoking and infections.[99] Among environmental factors, dysbiosis has been identified as a possible trigger factor for autoimmunity and RA development.[100]

Experiments in animal models suggest that the gut microbiota influences local and systemic immunity and might trigger joint inflammation.[100,101] Studies in mice with collagen-induced arthritis (CIA) showed that the administration of antibiotics exacerbates the disease and increases the level of IL-6, IFN-γ and IL-17 pro-inflammatory cytokines.[102] Further study showed differences in the gut microbiota composition between CIA-susceptible and CIA-resistant mice, with a prevalence of *Desulfovibrio*, *Prevotella*, *Parabacteroides*, *Odoribacter*, *Acetatifactor*, *Blautia*, *Coprococcus* and *Ruminococcus* genera in arthritic mice, in addition to increased levels of serum IL-17 and CD4 Th17 cells in the spleen.[102]

Mice deficient in IL-1RA signalling spontaneously develop arthritis; however, under GF conditions, RA is attenuated because of decreases in IL-17 and IL-1β secretion and decreased Toll-like receptor 2 and Toll-like receptor 4 stimulation.[103–105] On the other hand, when

these IL-RA$^{-/-}$ GF mice were colonized with *Bifidobacterium bifidum*, there was an increase in clinical scores compared with mice that were conventionally housed.[104]

Recent studies have investigated the gut microbiota of the genetically arthritis-susceptible transgenic mice *0401 and in the genetically resistant transgenic mice *0402. Clostridia were prevalent in susceptible mice, whereas the Porphyromonadaceae and Bifidobacteriaceae families were dominant in resistant mice. Moreover, the authors observed increased intestinal permeability and a Th17 profile in susceptible mice, suggesting that genetic background influences the individual's microbiota profile.[106]

Similar to that observed in EAE mice, Th17 cells induced by SFB in the lamina propria induced autoantibodies involved in RA development in animal models.[107] The correlation between intestinal dysbiosis and RA aetiology is not a new concept. The 'toxaemic factor' hypothesis was proposed in the twentieth century when it was suggested that the increase in the level of Gram-negative bacteria in the intestinal lumen could induce an increase in toxic molecules/metabolites that enter the bloodstream, promoting systemic inflammation.[100] Recent studies have shown that the intestinal microbiota of newly diagnosed RA patients was dominated by Gram-negative *Prevotella* members, especially *Prevotella copri*, compared with healthy individuals.[108,109] In the work of Maeda *et al.*, the gut microbiota transplant from RA patients to GF arthritis-prone SKG mice induced an increase in the number of Th17 cells in the gut and severe arthritis. Additionally, the co-culture of SKG dendritic cells with *Prevotella copri* increased IL-17 secretion in response to RA autoantigens, suggesting that RA gut microbiota may induce autoreactive cells in the gut and promote joint inflammation[109] (Fig. 3).

Another study, which was performed by Liu *et al.*, investigated the *Lactobacillus* community by quantitative real-time PCR in faecal samples from 15 patients with RA and 15 healthy controls and reported increased absolute copy numbers of *Lactobacillus salivarius*, *Lactobacillus iners* and *Lactobacillus ruminis* in untreated RA patients that were recently diagnosed.[110]

Chen *et al.* identified the gut microbiota profile in patients with RA and found decreased species richness (α-diversity) that positively correlated with increased rheumatoid factor levels and disease progression.[111] The authors evaluated 40 patients with RA and 32 healthy controls and found increased *Eggerthella*, *Actinomyces*, *Turibacter*, *Streptococcus* and *Collinsela* reads in the gut microbiota of people with RA, with positive correlations with the pro-inflammatory cytokine IL-17. The rheumatoid factor, C-reactive protein, disease progression and methotrexate treatment correlated with β-diversity found in the gut microbiota of patients with RA, suggesting that these clinical data might a play role in gut microbiota modulation[111] (Fig. 3).

Increasing evidence suggests an association between intestinal dysbiosis and rheumatic diseases and their role in disease progression and the inflammatory microenvironment. Future studies should demonstrate the role of gut inflammation as a trigger for the development of autoimmunity and RA. The determination of an intestinal dysbiotic signature will provide us with the means to develop therapeutic tools for the adjuvant treatment of immune-mediated diseases.[112,113]

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune and heterogeneous disease characterized by damage to the skin, kidneys, lungs, joints, heart and brain.[114] The disease affects mainly females, and its worldwide prevalence varies from 30 to 60 per 100 000 in the UK and the USA.[115] The pathogenesis of SLE may involve genetic and environmental factors, such as viral infections, defective apoptosis and solar exposure to ultraviolet-B waves. Regarding immune response, it is known that autoantibodies bind mainly with nuclear and cytoplasmic antigens.[116] Moreover, increased evidence has emerged that suggests the role of intestinal dysbiosis in SLE development.[117]

In female lupus-prone mice, Zhang *et al.* reported a decrease in the relative abundance of *Lactobacillus* spp. and an increase in Lachnospiraceae members when compared with controls. Early disease onset and severe symptoms correlated with increased Lachnospiraceae reads in female lupus-prone mice. Additionally, the number of Clostridiaceae and Lachnospiraceae reads increased at specific time-points during disease progression.[118] Another study reported that dietary intervention, such as caloric restriction, in NZB/W F_1 mice promoted changes in the gut microbiota and avoided disease progression in this animal model.[119]

Similarly, Johnson *et al.* reported that (SWR $\times$ NZB) F_1 mice given drinking water with a low pH have altered gut microbiota and decreased antinuclear antibodies, and develop nephritis more slowly, suggesting that gut microbiota modulation might influence disease progression.[120] In addition, when examining GF lymphotoxin-deficient mice, Van Praet *et al.* reported that the intestinal microbiota could play a role in antinuclear antibody induction and could be associated with SFB colonization and IL-17 receptor signalling.[121]

Hevia *et al.* analysed human stool samples from 20 patients with SLE and 20 healthy controls, and the results showed decreased Firmicutes : Bacteroidetes ratios in the patients with SLE. Additionally, *in silico* analysis suggested that the intestinal dysbiosis observed in patients with SLE could be linked to an increase in oxidative phosphorylation and the glycan metabolism pathways induced by patients' intestinal microbiota.[122] In this manner, another

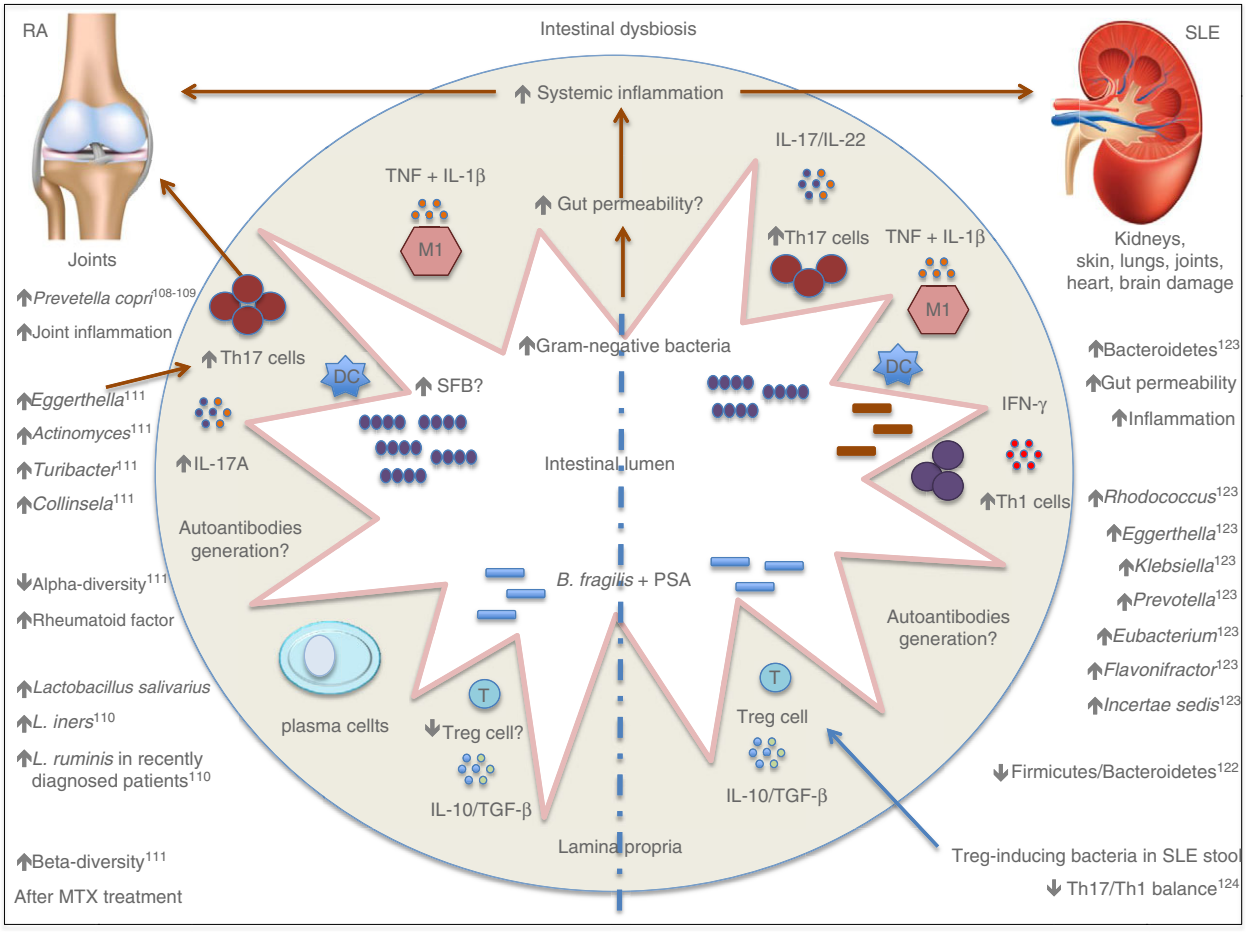

Figure 3. A schematic representation of intestinal dysbiosis in rheumatic autoimmune diseases in humans.

study evaluated stool samples from 45 patients with SLE and 48 control subjects and reported a decreased amount of Firmicutes members, an increased level of Bacteroidetes members, and a prevalence of *Rhodococcus*, *Eggerthella*, *Klebsiella*, *Prevotella*, *Eubacterium*, *Flavonifractor* and *Incertae sedis* genera in patients with SLE, suggesting a gut microbiota profile for patients with SLE[123] (Fig. 3).

Based on these reports showing intestinal dysbiosis in patients with SLE, López *et al.* evaluated the role of microbes derived from stool samples from patients with SLE and controls in the *in vitro* differentiation of Treg, Th1 and Th17 cells. The authors showed that patients' samples induced Th17 differentiation and supplementation with Treg-inducing bacteria significantly decreased the Th17/Th1 balance, supporting the use of these strains as therapeutic probiotics for autoimmune diseases.[124]

Probiotic applications in autoimmune diseases

According to the WHO, a probiotic is 'a live organism, which provides a benefit to the host when provided in adequate quantities'.[125] Studies suggest that probiotics influence systemic immune responses, ensure the homeostasis of the healthy microbiota in the intestinal mucosa and could, therefore, be used as adjuvant therapy to treat immune-mediated diseases.[125] The mechanisms proposed to achieve this include mucus secretion, antimicrobial peptide production, the maintenance of the function of the gastrointestinal–epithelial barrier, ensuring adequate interactions between the gut microbiota and the mucosal immune cells, and finally, helping the activation of the host immune system in response to pathobionts.[126] Here, we reported the results of the main clinical trials concerning the applicability of probiotics in autoimmune diseases.

In a recent study in NOD mice, the oral administration of a Lactobacillaceae-enriched probiotic protects mice from T1D by suppressing IL-1β expression and the release of immunomodulatory indoleamine 2,3-dioxygenase and by promoting the differentiation of $CD103^+$ tolerogenic dendritic cells in the gut.[127]

In humans, a TEDDY study group evaluated probiotic supplementation for children with genetic risk for T1D during their first year of life. This multicentre prospective

© 2017 John Wiley & Sons Ltd,

cohort study (USA, Finland, Germany and Sweden) investigated 7473 children ranging from 4 to 10 years in age. Early probiotic administration was correlated with a decreased risk of islet autoimmunity when compared with the group that received probiotics after 27 days of life or no supplementation.[128]

Several studies in EAE mice demonstrated the immunoregulatory functions of probiotic administration. Treatment with *Lactobacillus* spp., *Pediococcus acidolactici*, *Bifidobacterium bifidum*, *Bifidobacterium animalis* and *Bacteroides fragilis* improved CNS inflammation through the induction of Treg cells in the gut mucosa by promoting the secretion of IL-10 and transforming growth factor-β,and inducing decreased Th1/Th17 inflammatory subsets.[86,129–133]

In humans, Kouchaki *et al*. reported improved Expanded Disability Status Score, insulin resistance and a decrease in inflammatory markers in MS patients treated with probiotic supplementation containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus fermentum* and *Bifidobacterium bifidum*. This randomized double-blind placebo-controlled clinical trial analysed probiotic intake for 12 weeks in 60 MS patients.[134]

In a recent study, the oral administration of *Bacillus coagulans*, which has an anti-inflammatory effect, promoted a decrease in the level of serum amyloid A protein and decreased tumour necrosis factor serum levels in rat models of RA.[134]

Some performed studies evaluating the effect of probiotics as an adjuvant therapy for RA treatment have shown no significant results.[135] Some of these conducted studies have smaller numbers of patients and a short period of treatment.[136] In a double-blind, placebo-controlled trial, the oral administration of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* for 3 months to 29 RA patients did not improve the disease, measured by the American College of Rheumatology criteria (ACR20). However, the authors reported functional improvement within the probiotic supplementation group compared with the placebo group.[136]

Vaghef-Mehrabany *et al*. investigated the role of *Lactobacillus casei* intake in 46 RA patients for 8 weeks. This randomized, double-blind placebo-controlled trial showed improvement in disease activity score, increased levels of serum IL-10, and decreased levels of tumour necrosis factor, IL-6 and IL-12 in treated patients.[137] Another clinical trial with the same study design evaluated the administration of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum* for 8 weeks in 60 RA patients. Probiotic intake improved DAS28, decreased the level of serum C-reactive protein, and promoted a decrease in the insulin levels.[138]

In a lupus-like animal model, the administration of retinoic acid restored *Lactobacillus* spp. and improved lupus symptoms, suggesting the use of these species as a probiotic to diminish inflammation in patients with SLE.[118] Some *Lactobacillus* species have been demonstrated to have immunomodulatory properties in the host gut mucosa, such as inhibiting neutrophil extracellular trap formation, improving antioxidant status and increasing the expression of adhesion molecules in the gut.[139,140] However, currently, there are no clinical trials reported at clinicaltrials.gov investigating the role of probiotics as an adjuvant therapy in the treatment of patients with SLE.

Future studies with higher numbers of patients and longer evaluation times are necessary to corroborate these results. Such confirmation may lead to the routine use of probiotics as an adjunctive therapy in the treatment of immune-mediated diseases. However, these studies should take into consideration the patient's immunological status before probiotic introduction.

Conclusions

Emerging findings associate intestinal dysbiosis with autoimmune disease pathogenesis. Mucosal surfaces with impaired microbiota function and diversity, such as in the gut, could represent a trigger site of autoimmunity by neo-antigen generation under dysbiotic conditions. If this hypothesis is validated, all the generated data could collaborate in the discovery of new probiotics, predictive biomarkers and therapeutic approaches for use in clinical settings for the adjuvant treatment of autoimmune diseases.

Disclosures

The authors report no conflict of interest.

References

1 Kamada N, Seo SU, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. *Nat Rev Immunol* 2013; **13**:321–35.

2 Karczewski J, Poniedzialek B, Adamski Z, Rzymski P. The effects of the microbiota on the host immune system. *Autoimmunity* 2014; **47**:494–504.

3 Peterson CT, Sharma V, Elmén L, Peterson SN. Immune homeostasis, dysbiosis and therapeutic modulation of the gut microbiota. *Clin Exp Immunol* 2015; **179**:363–77.

4 Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD *et al.* Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl Environ Microbiol* 1999; **65**:4799–807.

5 Perez-Lopez A, Behnsen J, Nuccio SP, Raffatellu M. Mucosal immunity to pathogenic intestinal bacteria. *Nat Rev Immunol* 2016; **16**:135–48.

6 Webb CR, Koboziev I, Furr KL, Grisham MB. Protective and pro-inflammatory roles of intestinal bacteria. *Pathophysiology* 2016; **23**:67–80.

7 Marietta E, Rishi A, Taneja V. Immunogenetic control of the intestinal microbiota. *Immunology* 2015; **145**:313–22.

8 Sonnenbrug JL, Bäckhed F. Diet–microbiota interactions as moderators of human metabolism. *Nature* 2016; **535**:56–64.

9 Martin R, Nauta AJ, Ben Amor K, Knippels LM, Knol J, Garssen J. Early life: gut microbiota and immune development in infancy. *Benef Microbes* 2010; **1**:367–82.

10 Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA* 2010; **107**:11971–5.

11 Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M *et al.* Human gut microbiome viewed across age and geography. *Nature* 2012; **486**:222–7.

12 Dore J, Blotière H. The influence of diet on the gut microbiota and its consequences for health. *Curr Opin Biotechnol* 2015; **32**:195–9.

13 Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol* 2010; **10**:159–69.

14 Littman DR, Pamer EG. Role of the commensal microbiota in normal and pathogenic host immune responses. *Cell Host Microbe* 2011; **10**:311–23.

15 Renz H, Brandtzaeg P, Hornef M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. *Nat Rev Immunol* 2011; **12**:9–23.

16 Allin KH, Nielsen T, Pedersen O. Mechanisms in endocrinology: gut microbiota in patients with type 2 diabetes mellitus. *Eur J Endocrinol* 2015; **172**:R167–77.

17 Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. *Nature* 2011; **474**:327–36.

18 Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* 2012; **3**:289–306.

19 Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M *et al.* Diversity of the human intestinal microbial flora. *Science* 2005; **308**:1635–8.

20 Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* 2016; **14**:20–32.

21 Conlon MA, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* 2014; **7**:17–44.

22 Honda K, Littman DR. The microbiome in infectious disease and inflammation. *Annu Rev Immunol* 2012; **30**:759–95.

23 Gill N, Finlay BB. The gut microbiota: challenging immunology. *Nat Rev Immunol* 2011; **11**:636–7.

24 Hamada H, Hiroi T, Nishiyama Y, Takahashi H, Masunaga Y, Hachimura S *et al.* Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. *J Immunol* 2002; **168**:57–64.

25 Fukata M, Arditi M. The role of pattern recognition receptors in intestinal inflammation. *Mucosal Immunol* 2013; **6**:451–63.

26 Shi Y, Mu L. An expanding stage for commensal microbes in host immune regulation. *Cell Mol Immunol* 2017; **14**:339–48.

27 Kubinak JL, Round JL. Do antibodies select a healthy microbiota? *Nat Rev Immunol* 2016; **26**:767–74.

28 Tomkovich S, Jobin C. Microbiota and host immune responses: a love–hate relationship. *Immunology* 2016; **147**:1–10.

29 Kamada N, Múñez G. Role of the gut microbiota in the development and function of lymphoid cells. *J Immunol* 2013; **190**:1389–95.

30 Bevins CL, Salzman NH. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat Rev Microbiol* 2011; **9**:356–68.

31 Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. *Cell* 2010; **140**:845–58.

32 Ivanov II, Frutos RL, Manel N, Yoshinaga K, Rifkin D, Sartor RB *et al.* Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 2008; **4**:337–49.

33 Gaboriau-Routhiau V, Rakotobe S, Lécuver E, Mulder I, Lan A, Bridonneau C *et al.* The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 2009; **31**:677–89.

34 Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009; **139**:485–98.

35 Shaw MH, Kamada N, Kim YG, Núñez G. Microbiota-induced IL-1β, but not IL-6, is critical for the development of steady-state TH17 cells in the intestine. *J Exp Med* 2012; **209**:251–8.

36 Tan TG, Sefik E, Geva-Zatorsky N, Kua L, Naskar D, Teng F *et al.* Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice. *Proc Natl Acad Sci USA* 2016; **113**:E8141–50.

37 Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y *et al.* Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* 2011; **331**:337–41.

38 Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H *et al.* Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013; **500**:232–6.

39 Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S *et al.* Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* 2011; **34**:794–806.

40 Round JL, Mazmanian SK. Inducible Foxp3$^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci USA* 2010; **107**:12204–9.

41 Fagarasan S, Kawamoto S, Kanagawa O, Suzuki K. Adaptive immune regulation in the gut: T cell-dependent and T cell-independent IgA synthesis. *Annu Rev Immunol* 2010; **28**:243–73.

42 Fagarasan S, Muramatsu M, Suzuki K, Nagaoka H, Hiai H, Honio T. Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. *Science* 2002; **298**:1424–7.

43 Strugnell RA, Wijburg OL. The role of secretory antibodies in infection immunity. *Nat Rev Microbiol* 2010; **8**:656–67.

44 Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* 2004; **303**:1662–5.

45 Macpherson AJ, Geuking MB, McCoy KD. Homeland security: IgA immunity at the frontiers of the body. *Trends Immunol* 2012; **33**:160–7.

46 Tezuka H, Abe Y, Asano J, Sato T, Liu J, Iwata M *et al.* Prominent role for plasmacytoid dendritic cells in mucosal T cell-independent IgA induction. *Immunity* 2011; **34**:247–57.

47 Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* 2012; **3**:4–14.

48 Rosser EC, Mauri C. A clinical update on the significance of the gut microbiota in systemic autoimmunity. *J Autoimmun* 2016; **74**:85–93.

49 Lerner A, Aminov R, Matthias T. Dysbiosis may trigger autoimmune diseases via inappropriate post-translational modification of host proteins. *Front Microbiol* 2016; **7**:84.

50 Hara N, Alkanani AK, Ir D, Robertson CE, Wagner BD, Frank DN *et al.* The role of the intestinal microbiota in type 1 diabetes. *Clin Immunol* 2013; **146**:112–9.

51 Atkinson MA, Chervonsky A. Does the gut microbiota have a role in type 1 diabetes? Early evidence from humans and animal models of the disease. *Diabetologia* 2012; **55**:2868–77.

52 International Diabetes Federation. International Diabetes Federation Atlas, 7th edn. Karakas Print: Brussels, 2015.

53 Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesvan L, Stonebraker AC *et al.* Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 2008; **455**:1109–13.

54 Emani R, Alam C, Pekkala S, Zafar S, Emani MR, Hänninen A. Peritoneal cavity is a route for gut-derived microbial signals to promote autoimmunity in non-obese diabetic mice. *Scand J Immunol* 2015; **81**:102–9.

55 Alam C, Bittoun E, Bhagwat D, Valkonen S, Saari A, Jaakkola U *et al.* Effects of a germ-free environment on gut immune regulation and diabetes progression in non-obese diabetic (NOD) mice. *Diabetologia* 2011; **54**:1398–406.

56 Kriegel MA, Sefik E, Hill JA, Wu HJ, Benoist C, Mathis D. Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. *Proc Natl Acad Sci USA* 2011; **108**:11548–53.

57 Roesch LF, Lorca GL, Casella G, Giongo A, Naranjo A, Pionzio AM *et al.* Culture-independent identification of gut bacteria correlated with the onset of diabetes in a rat model. *ISME J* 2009; **3**:536–48.

58 Neu J, Reverte CM, Mackey AD, Liboni K, Tuhacek-Tenace LM, Hatch M *et al.* Changes in intestinal morphology and permeability in the biobreeding rat before the onset of type 1 diabetes. *J Pediatr Gastroenterol Nutr* 2005; **40**:589–95.

59 Bosei E, Molteni L, Radaelli MG, Folini L, Fermo I, Bazzigaluppi E *et al.* Increased intestinal permeability precedes clinical onset of type 1 diabetes. *Diabetologia* 2006; **49**:2824–7.

60 Sapone A, De Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F *et al.* Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes* 2006; **55**:1443–9.

61 Vaarala O. Human intestinal microbiota and type 1 diabetes. *Curr Diab Rep* 2013; **13**:601–7.

62 Hu C, Wong FS, Wen L. Type 1 diabetes and gut microbiota: friends or foe? *Pharmacol Res* 2015; **98**:9–15.

63 Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G *et al.* Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 2011; **5**:82–91.

64 De Goffau MC, Fuentes S, Van Den Bogert B, Honkanen H, de Vos WM, Welling GW *et al.* Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. *Diabetologia* 2014; **57**:1569–77.

65 Li X, Atkinson MA. The role for gut permeability in the pathogenesis of type 1 diabetes – a solid or leaky concept? *Pediatr Diabetes* 2015; **16**:485–92.

66 De Goffau MC, Luopaiärvi K, Knip M, Ilonen J, Ruohtula T, Härkönen T *et al.* Fecal microbiota composition differs between children with β-cell autoimmunity and those without. *Diabetes* 2013; **62**:1238–44.

67 Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N *et al.* Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS One* 2011; **6**:e25792.

68 Endesfelder D, Zu Castell W, Ardissone A, Davis-Richardson AG, Achenbach P, Hagen M *et al.* Compromised gut microbiota networks in children with anti-islet cell autoimmunity. *Diabetes* 2014; **63**:2006–14.

69 Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Front Microbiol* 2016; **7**:979.

70 Arpaia N, Campbell C, Fan X, Dikiv S, Van der Veeken J, De Roos P *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013; **504**:451–5.

71 Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; **504**:446–50.

72 Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriquer F *et al.* Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case–control study. *BMC Med* 2013; **21**:11–46.

73 Davis-Richardson AG, Ardissone AN, Dias R, Simell V, Leonard MT, Kemppainen KM *et al.* Bacteroides dorei dominates gut microbiome prior to autoimmunity in Finnish children at high risk for type 1 diabetes. *Front Microbiol* 2014; **5**:678.

74 Kostic AD, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen AM *et al.* The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 2015; **17**:260–73.

75 Alkanani AK, Hara N, Gottlieb PA, Ir D, Robertson CE, Wagner BD *et al.* Alterations in intestinal microbiota correlate with susceptibility to type 1 diabetes. *Diabetes* 2015; **64**:3510–20.

76 Meiía-León ME, Petrosino JF, Aiami NJ, Domínguez-Bello MG, De La Barca AM. Fecal microbiota imbalance in Mexican children with type 1 diabetes. *Sci Rep* 2014; **4**:3814.

77 Paun A, Yau C, Danska JS. The influence of the microbiome on type 1 diabetes. *J Immunol* 2017; **198**:590–5.

78 Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol* 2015; **15**:545–58.

79 Browne P, Chandraratna D, Angood C, Tremlett H, Baker C, Taylor BV *et al.* Atlas of multiple sclerosis 2013: a growing global problem with widespread inequity. *Neurology* 2014; **83**:1022–4.

80 Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C *et al.* Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* 2011; **479**:538–41.

81 Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* 2011; **108**:4615–22.

82 Ochoa-Repáraz J, Mielcarz DW, Ditrio LE, Burroughs AR, Foureau DM, Haque-Begum S *et al.* Role of gut commensal microbiota in the development of experimental autoimmune encephalomyelitis. *J Immunol* 2009; **183**:6041–50.

83 Forsythe P, Kunze WA. Voices from within: gut microbes and the CNS. *Cell Mol Life Sci* 2013; **70**:55–69.

84 Ochoa-Repáraz J, Mielcarz DW, Haque-Begum S, Kasper LH. Induction of regulatory B population in experimental allergic encephalomyelitis by alteration of the gut commensal microflora. *Gut Microbes* 2010; **1**:103–8.

85 Mielcarz DW, Kasper LH. The gut microbiome in multiple sclerosis. *Curr Treat Options Neurol* 2015; **17**:344.

86 Lavasani S, Dzhambazov B, Nouri M, Fak F, Buske S, Molin G *et al.* A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. *PLoS One* 2010; **5**:e9009.

87 Bhargava P, Mowey EM. Gut microbiome and multiple sclerosis. *Curr Neurol Neurosci Rep* 2014; **14**:492.

88 Jhangi S, Gandhi R, Glanz B, Cook S, Nejad P, Ward D *et al.* Increased Archaea species and changes with therapy in gut microbiome of multiple sclerosis subjects. *Neurology* 2014; **82**:S24.001.

89 Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T *et al.* Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to Clostridia XIVa and IV clusters. *PLoS One* 2015; **10**:e0137429.

90 Cantarel BL, Waubant E, Chehoud C, Kuczynski J, DeSantis TZ, Warrington J *et al.* Gut microbiota in multiple sclerosis: possible influence of immunomodulators. *J Invest Med* 2015; **63**:729–34.

91 Bang C, Weidenbach K, Gutsmann T, Heine H, Schmitz RA. The intestinal archaea *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* activate human dendritic cells. *PLoS One* 2014; **9**:e99411.

92 Derrien M, Van Baarlen P, Hooiveld G, Norin E, Müller M, de Vos WM. Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader *Akkermansia muciniphila*. *Front Microbiol* 2011; **2**:166.

93 Ganesh BP, Klopfleisch R, Loh G, Blaut M. Commensal *Akkermansia muciniphila* exacerbates gut inflammation in *Salmonella* Typhimurium-infected gnotobiotic mice. *PLoS One* 2013; **8**:e74963.

94 Jangi S, Gandhi R, Cox LM, Li N, Von Glehn F, Yan R *et al.* Alterations of the human microbiome in multiple sclerosis. *Nat Commun* 2016; **7**:12015.

95 Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**:105–8.

96 Chen J, Chia N, Kalari KR, Yao JZ, Novotna M, Soldan MM *et al.* Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci Rep* 2016; **6**:28484.

97 Colpitts SL, Kasper LH. Influence of the gut microbiome on autoimmunity in the central nervous system. *J Immunol* 2017; **198**:596–604.

98 Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. *Nat Rev Immunol* 2017; **17**:60–75.

99 Klareskog L, Padyukov L, Lorentzen J, Alfredsson L. Mechanisms of disease: genetic susceptibility and environmental triggers in the development of rheumatoid arthritis. *Nat Clin Pract Rheumatol* 2006; **2**:425–33.

100 Brusca SB, Abramson SB, Scher JU. Microbiome and mucosal inflammation as extra-articular triggers for rheumatoid arthritis and autoimmunity. *Curr Opin Rheumatol* 2014; **26**:101–7.

101 Dorożyńska I, Majewska-Szczepanik M, Marcińska K, Szczepanik M. Partial depletion of natural gut flora by antibiotic aggravates collagen induced arthritis (CIA) in mice. *Pharmacol Rep* 2014; **66**:250–5.

102 Liu X, Zeng B, Zhang J, Li W, Mou F, Wang H *et al.* Role of the gut microbiome in modulating arthritis progression in mice. *Sci Rep* 2016; **6**:30594.

103 Horai R, Saijo S, Tanioka H, Nakae S, Sudo K, Okahara A *et al.* Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* 2000; **191**:313–20.

104 Abdollahi-Roodsaz S, Joosten LA, Koenders MI, Devesa I, Roelofs MF, Radstake TR *et al.* Stimulation of TLR2 and TLR4 differentially skews the balance of T cells in a mouse model of arthritis. *J Clin Invest* 2008; **118**:205–16.

105 Rogier R, Koenders MI, Abdollahi-Roodsaz S. Toll-like receptor mediated modulation of T cell response by commensal intestinal microbiota as a trigger for autoimmune arthritis. *J Immunol Res* 2015; **2015**:527696.

106 Gomez A, Luckey D, Yeoman CJ, Marietta EV, Berg Miller ME, Murray JA *et al.* Loss of sex and age driven differences in the gut microbiome characterize arthritis-susceptible 0401 mice but not arthritis-resistant 0402 mice. *PLoS One* 2012; **7**:e36095.

107 Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y *et al.* Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 2010; **32**:815–27.

108 Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C *et al.* Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife* 2013; **2**:e01202.

109 Maeda Y, Kurakawa T, Umemoto E, Motooka D, Ito Y, Gotoh K *et al.* Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. *Arthritis Rheumatol* 2016; **68**:2646–61.

110 Liu X, Zou Q, Zeng B, Fang Y, Wei H. Analysis of fecal *Lactobacillus* community structure in patients with early rheumatoid arthritis. *Curr Microbiol* 2013; **67**:170–6.

111 Chen J, Wright K, Davis JM, Jeraldo O, Marietta EV, Murray J *et al.* An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. *Genoma Med* 2016; **8**:43.

112 Ciccia F, Ferrante A, Guggino G, Triolo G. The role of the gastrointestinal tract in the pathogenesis of rheumatic diseases. *Best Pract Res Clin Rheumatol* 2016; **30**:889–900.

113 Di Paola M, Cavalieri D, Albanese D, Sordo M, Pindo M, Donati C *et al.* Alteration of fecal microbiota profiles in juvenile idiopathic arthritis. Associations with HLA-B27 allele and disease status. *Front Microbiol* 2016; **7**:979.

114 Tsokos GC. Systemic lupus erythematosus. *N Engl J Med* 2011; **365**:2110–21.

115 Carter EE, Barr SG, Clarke AE. The global burden of SLE: prevalence, health disparities and socioeconomic impact. *Nat Rev Rheumatol* 2016; **12**:605–20.

116 Tsokos GC, Lo MS, Reis PC, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat Rev Rheumatol* 2016; **12**:716–30.

117 Van de Wiele TV, Van Praet JT, Marzorati M, Drennan MB, Elewaut D. How the microbiota shapes rheumatic diseases. *Nat Rev Rheumatol* 2016; **12**:398–411.

118 Zhang H, Liao X, Sparks JB, Luo XM. Dynamics of gut microbiota in autoimmune lupus. *Appl Environ Microbiol* 2014; **80**:7551–60.

119 Hsieh CC, Lin BF. Dietary factors regulate cytokines in murine models of systemic lupus erythematosus. *Autoimmun Rev* 2011; **11**:22–7.

120 Johnson BM, Gaudreau MC, Al-Gadban MM, Gudi R, Vasu C. Impact of dietary deviation on disease progression and gut microbiome composition in lupus-prone SNF1 mice. *Clin Exp Immunol* 2015; **181**:323–37.

121 Van Praet JT, Donovan E, Vanassche I, Drennan MB, Windels F, Dendooven A *et al.* Commensal microbiota influence systemic autoimmune responses. *EMBO J* 2015; **34**:466–74.

122 Hevia A, Milani C, López P, Cuervo A, Arboleva S, Duranti S *et al.* Intestinal dysbiosis associated with systemic lupus erythematosus. *MBio* 2014; **5**:e01548–14.

123 He Z, Shao T, Li H, Xie Z, Wen C. Alterations of the gut microbiome in Chinese patients with systemic lupus erythematosus. *Gut Pathog* 2016; **8**:64.

124 López P, de Paz B, Rodrígues-Carrio J, Hevia A, Sánchez B, Margolles A *et al.* Th17 responses and natural IgM antibodies are related to gut microbiota composition in systemic lupus erythematosus patients. *Sci Rep* 2016; **6**:24072.

125 Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. *Nat Rev Gastroenterol Hepatol* 2010; **7**:503–14.

126 Bron PA, Kleerebezem M, Brummer RJ, Cani PD, Mercenier A, MacDonald TT *et al.* Can probiotics modulate human disease by impacting intestinal barrier function? *Br J Nutr* 2017; **117**:93–107.

127 Dolpady J, Sorini C, Di Pietro C, Cosorich I, Ferrarese R, Saita D *et al.* Oral probiotic VSL#3 prevents autoimmune diabetes by modulating microbiota and promoting indoleamine 2,3-dioxygenase-enriched tolerogenic intestinal environment. *J Diabetes Res* 2016; **2016**:7569431.

128 Uusitalo U, Liu X, Yang J, Aronsson CA, Hummel S, Butterworth M *et al.* Association of early exposure of probiotics and islet autoimmunity in the TEDDY study. *JAMA Pediatr* 2016; **170**:20–8.

129 Ezendam J, De Klerk A, Gremmer ER, Van Loveren H. Effects of *Bifidobacterium animalis* administered during lactation on allergic and autoimmune responses in rodents. *Clin Exp Immunol* 2008; **154**:424–31.

130 Kwon HK, Kim GC, Kim Y, Hwang W, Jash A, Sahoo A *et al.* Amelioration of experimental autoimmune encephalomyelitis by probiotic mixture is mediated by a shift in T helper cell immune response. *Clin Immunol* 2013; **146**:217–27.

131 Takata K, Kinoshita M, Okuno T, Moriya M, Kohda T, Honorat JA *et al.* The lactic acid bacterium *Pediococcus acidilactici* suppresses autoimmune encephalomyelitis by inducing IL-10-producing regulatory T cells. *PLoS One* 2011; **6**:e27644.

132 Ochoa-Repáraz J, Mielcarz DW, Ditrio LE, Burroughs AR, Begum-Haque S, Dasgupta S *et al.* Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression. *J Immunol* 2010; **185**: 4101–8.

133 Ochoa-Repáraz J, Mielcarz DW, Wang Y, Begum-Haque S, Dasgupta S, Kasper DL *et al.* A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease. *Mucosal Immunol* 2010; **3**:487–95.

134 Kouchaki E, Tamtaii OR, Salami M, Bahmani F, Daneshvar Kakhaki R, Akbari E *et al.* Clinical and metabolic response to probiotic supplementation in patients with multiple sclerosis: a randomized, double-blind, placebo-controlled trial. *Clin Nutr* 2016; **16**:30214-X.

135 Abhari K, Shekarforoush SS, Hosseinzadeh S, Nazifi S, Sajedianfard J, Eskandari MH. The effects of orally administered *Bacillus coagulans* and inulin on prevention and progression of rheumatoid arthritis in rats. *Food Nutr Res* 2016; **60**:30876.

136 Pineda MA, Thompson SF, Summers K, De Leon F, Pope J, Reid G. A randomized, double-blinded, placebo-controlled pilot study of probiotics in active rheumatoid arthritis. *Med Sci Monit* 2011; **17**:CR347–54.

137 Vaghef-Mehrabany E, Alipour B, Homayouni-Rad A, Sharif SK, Asghari-Jafarabadi M, Zavvari ZS. Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis. *Nutrition* 2014; **30**:430–5.

138 Zamani B, Golkar HR, Farshbaf S, Emadi-Baygi M, Tajabadi-Ebrahimi M, Jafari P *et al.* Clinical and metabolic response to probiotic supplementation in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Int J Rheum Dis* 2016; **19**:869–79.

139 Vong L, Lorentz RJ, Assa A, Glogauer M, Sherman PM. Probiotic *Lactobacillus rhamnosus* inhibits the formation of neutrophil extracellular traps. *J Immunol* 2014; **192**:1870–7.

140 Mu Q, Zhang H, Luo XM. SLE: another autoimmune disorder influenced by microbes and diet? *Front Immunol* 2015; **6**:608.