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INVITED MEDICAL REVIEW

The microbiome–systemic diseases connection

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The human microbiome consists of all microorganisms occupying the skin, mucous membranes and intestinal tract of the human body. The contact of the mucosal immune system with the human microbiome is a balanced interplay between defence mechanisms of the immune system and symbiotic or pathogenic microbial factors, such as microbial antigens and metabolites. In systemic autoimmune diseases (SADs) such as rheumatoid arthritis, systemic lupus erythematosus and Sjögren's syndrome, the immune system is deranged to a chronic inflammatory state and autoantibodies are an important hallmark. Specific bacteria and/or a dysbiosis in the human microbiome can lead to local mucosal inflammation and increased intestinal permeability. Proinflammatory lymphocytes and cytokines can spread to the systemic circulation and increase the risk of inflammation at distant anatomical sites, such as the joints or salivary glands. Increased intestinal permeability increases antigen exposure and the risk of autoantibody production. If the human microbiome indeed plays such a critical role in SADs, this finding holds a great promise for new therapeutic strategies, such as diet interventions and probiotics and prebiotics. This review provides a background on the human microbiome and mucosal immunity in the gut and oral cavity and gives a summary of the current knowledge on the microbiome–SADs connection.

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Keywords: autoinflammatory diseases; inflammatory diseases; bacteria; microbiology; immunopathology; pathogenesis; rheumatology; immunology; autoimmune disease

Introduction

Despite the enormous effort from investigators over the world, the etiopathogenesis of systemic autoimmune diseases (SADs) such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), systemic sclerosis and vasculitis is only partially understood. These SADs have a multifactorial etiopathogenesis, meaning that a genetic background, environmental factors, hormones and a deranged immune system are all involved to a more or lesser extent.

The genetic contribution to RA and SLE has been well studied, but is yet understudied in SS (Lessard *et al*, 2012). Concordance rates for RA and SLE in monozygotic twins are 15% and 24%, respectively (Deapen *et al*, 1992; Silman *et al*, 1993). Thus, siblings with identical genomes often do not share a systemic disease phenotype. However, the heritability – which estimates the extent to which variation in liability to disease in a population can be explained by genetic variation – is estimated to be 60% and 44% for RA and SLE, respectively (MacGregor *et al*, 2000; Kuo *et al*, 2015). No data on twin concordance or heritability in SS are yet available (Bogdanos *et al*, 2012; Lessard *et al*, 2012).

Genomewide association studies (GWAS) have revealed that single nucleotide polymorphisms (SNPs) in the human leucocyte antigen (HLA) gene are the major genetic risk factor to develop a SAD. SNPs in the HLA gene locus account for maximum odds ratios (ORs) of 3.7 in RA, 2.9 in SLE and 3.5 in SS (Castaño-Rodríguez *et al*, 2008; Raychaudhuri *et al*, 2012; Lessard *et al*, 2013), but these ORs are low compared to other autoimmune diseases such as ankylosing spondylitis and type 1 diabetes (T1D) with HLA-related ORs of 41 and 11, respectively (Lin *et al*, 2011; Noble and Erlich, 2012). Many non-HLA encoding genes related to suspected pathogenic pathways are associated with RA, SLE and SS, but these ORs are seldom higher than 1.5 (Harley *et al*, 2008; Lessard *et al*, 2013; Okada *et al*, 2014). To summarize, although variations in the human genome explain only a small part of the aetiology of SADs, a relatively strong heritability and familial aggregation of SADs is noted (Cárdenas-Roldán *et al*, 2013). This apparent discrepancy in the role of genetics in

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the aetiology of SADs might be explained by the fact that not only the human genome is inherited, but also microorganisms that colonize the human body (Frankenfeld *et al*, 2004; Li *et al*, 2007; Goodrich *et al*, 2014).

Environmental factors are considered a key factor in the development of systemic diseases (Wahren-Herlenius and Dörner, 2013). In particular, (infections with) microorganisms are thought to play a causative role in the initiation of RA, SLE and SS, although mechanisms are poorly understood. Examples of microorganisms that are associated with systemic diseases are the periodontal pathogen *Porphyromonas gingivalis* with RA and Epstein–Barr virus (EBV) with SLE and SS (Croia *et al*, 2014; Hanlon *et al*, 2014; Quirke *et al*, 2014).

An important argument of viral involvement in the etiopathogenesis is the demonstration of an increased production of type 1 interferon (type 1 IFN) in patients with RA, SLE, SS and other SADs (Gottenberg *et al*, 2006; Higgs *et al*, 2011). Type 1 IFN is upregulated upon cellular encounter with a virus and induces a cell-intrinsic antimicrobial state of infected and neighbouring cells, limiting the spread of viral pathogens. Furthermore, type 1 IFN promotes antigen exposure, natural killer cell function and activates adaptive immunity to develop high-affinity antigen-specific T and B cells (Ivashkiv and Donlin, 2014). Chronic type 1 IFN production is also associated with the development of autoreactivity, ultimately leading to autoimmunity (Ivashkiv and Donlin, 2014). Although microorganisms are suspected to play an initiating role in SADs, most autoantibodies present in the serum of SS, SLE and RA are yet not directly related to associated microbial infections. An exception is the anti-citrullinated protein antibody (ACPA) in RA patients with periodontal disease and *P. gingivalis* colonization (see section on ‘The microbiome–RA connection’) (Montgomery *et al*, 2015).

The knowledge of the role of microorganism in the development of SADs is still inconclusive. We propose three possible explanations for this gap in understanding the role of microorganisms in systemic disease development. First, investigators might have overlooked possible pathogenic bacteria or viruses because they were limited to culture-dependent and targeted DNA detection of microorganisms, revealing only one or several microorganisms. Currently, less than half of the bacteria present in the oral cavity and only 20% of bacteria in the gut are cultivated, but techniques aimed to cultivate ‘unculturable’ bacteria are still developing (Eckburg *et al*, 2005; Dewhirst *et al*, 2010; Vartoukian *et al*, 2016). Secondly, investigators have possibly searched in the wrong anatomical location for pathogenic microorganisms causing an autoimmune response. Although the human gut harbours the majority of microorganisms present in our body, investigators have mainly focused on detecting microorganisms and their associated antibodies in the blood and the affected organ or tissue (Gill *et al*, 2006; Martinez-Martinez *et al*, 2009; Croia *et al*, 2014; Hanlon *et al*, 2014). Finally, the microorganisms found to be associated with systemic diseases may very well be the ‘second hit’ in patients already predisposed to autoimmunity because of a chronic pro-inflammatory state of the immune system. For example, EBV infection is the microorganism strongest

related to SS and SLE (Croia *et al*, 2014; Hanlon *et al*, 2014). However, 95% of the general adult population has been infected with EBV during life (Luzuriaga and Sullivan, 2011). An underlying – not clinically evident – chronic pro-inflammatory state of the immune system (‘first hit’) might be present in future SLE or SS patients before they are infected with EBV (‘second hit’). This pro-inflammatory state causes an exaggerated and self-perpetuating immune response to a common viral infection such as EBV.

Next-generation sequencing (NGS) is a great tool to explore the role of microorganisms in the development of SADs, because with NGS the complete composition and functions of a microbial community can be defined. First, NGS can detect previously unknown and uncultured microorganisms. Second, NGS can be applied to many human microbial habitats including the oral cavity or gut. Third, NGS has revealed that the gut microbial composition affects local and systemic immunity (Hooper *et al*, 2012). In this review, we summarize the current general knowledge of the human microbiome in relation to the development and pathogenesis of three SADs: RA, SLE and SS.

Defining the human microbiome

In scientific literature, the term microbiome is approached from two directions. From a biologist point of view, the human microbiome is defined as ‘a characteristic microbial community occupying a reasonably well defined habitat which has distinct physiochemical properties’. This definition not only refers to the microorganisms involved, but also encompasses their ‘theatre of activity’ (Whipps *et al*, 1988), and emphasizes on *-biome* (as in community). As research on microorganisms has partly moved from biology towards genetics, the term microbiome is also defined from a genetic point of view with the emphasis on *-ome* (as in ‘-omics’ research, i.e. genomics). Herewith, the human microbiome is defined as ‘the collective of genomes of the microbes that live with us’ or as a ‘second genome of the host’. The microorganisms themselves are defined as ‘microbiota’ (Turnbaugh *et al*, 2007). In this review, we will use the latter mentioned definitions for microbiome and microbiota. The term metagenome will be used to refer to the collective set of all genomes of a microbial community (Petrosino *et al*, 2009). The human microbiome includes the collective of genomes from viruses, bacteria, archaea and fungi, but the focus of this review was on the bacterial composition in the gut and oral cavity, referred to as the gut and oral microbiome. The role of viruses in systemic diseases is extensively reviewed by others (Triantafyllopoulou and Moutsopoulos, 2007; Hanlon *et al*, 2014; Ball *et al*, 2015) and will only briefly be discussed here.

NGS and ‘meta-omics’

Sampling a complete microbial community has become possible with the culture-independent method NGS. NGS is a DNA-sequencing technique which allows massively parallel sequencing, during which millions of fragments of

DNA from a single sample are sequenced in unison (Grada and Weinbrecht, 2013). NGS facilitates high-throughput sequencing, which allows an entire microbial community to be sequenced, herewith providing much more information than culture or targeted DNA studies. With this greatly increased amount of microbial data, the range of possible statistical calculations in microbial research has also increased. This has led to new associations between the microbial composition and the health status of the human host (Turnbaugh *et al*, 2009; Scher *et al*, 2013).

In microbiome research, the two major NGS approaches are 16S ribosomal RNA (rRNA) gene sequencing and metagenomic shotgun sequencing. Both methodologies have been thoroughly reviewed by Zarco *et al* (2012) and will be summarized here briefly. The 16S rRNA gene approach is used to identify and classify bacteria that are present in a community based on sequence reads of variable regions within the 16S rRNA gene (Yarza *et al*, 2014). With metagenomics shotgun sequencing, the functional characteristics ('what can the community do?') are studied (Gill *et al*, 2006; Zhang *et al*, 2015a,b). Metagenomic shotgun sequencing is more complex, time-consuming and requires more computational power than 16S rRNA sequencing especially in processing the massive amount of reads to useful information about putative functional pathways (Thomas *et al*, 2012; Bikel *et al*, 2015). Metagenomics gives an answer to the *potential function* of a microbial population, but has a limited role in revealing the *microbial activity* measured by gene expression (Bikel *et al*, 2015). The whole process from DNA, to messenger RNA (mRNA), to proteins and finally metabolites can be studied with a variety of 'omics' techniques. Metatranscriptomic shotgun sequencing, or in short RNAseq, answers the question 'which metabolic pathways are currently active in a certain microbial population'? With RNAseq, all RNA present in a sample is sequenced and the mRNA reads are analysed. For example, comparing the relative abundance of mRNA reads of a certain gene or pathway with the relative abundance of the equivalent DNA gives insight into the relation of the functional activity to the functional potential (Franzosa *et al*, 2015a).

Sequencing microbial DNA gives insight into the functional potential of a microbial community, but measuring protein abundance (metaproteomics) provides a more direct measure of the functional activity of a microbial community. Proteomic methods rely on mass spectrometry-based shotgun quantification of peptide mass and abundance (Franzosa *et al*, 2015a). Finally, the study of metabolomics aims to identify and quantify all the small-molecule, microbial-produced metabolites in order to unravel the dynamic nature of the metabolic function of a microbial community and understand how it influences its human host (C nit *et al*, 2014).

In the future, multiple 'omics' approaches (multi-omics) will be integrated to take the next step forward in understanding the biology of microbial communities and a better understanding of the complex mechanisms of host-microbiome interactions (Franzosa *et al*, 2015a). The multi-omic approach has a great potential in unravelling the microbiome-systemic disease connection, but considerable

work needs to be carried out, especially in the investigative tools and integrate the massive amount of data in bioinformatic pipelines.

The human superorganism

Ever since Antony van Leeuwenhoek discovered his 'animalcules' in the 17th century, we know that the human body has always been inhabited by many different microorganisms. In one of his letters, van Leeuwenhoek wrote as: '...the people living in our United Netherlands are not as many as the living animals that I carry in my own mouth...'. Taking a step of 400 years to the 21st century, we now know that each human being is colonized with trillions of bacteria and that the human body can therefore be addressed as a 'superorganism' (Gill *et al*, 2006). A superorganism is an organism made out of many smaller organisms, acting in concert to produce phenomena governed by the collective.

Each human being is colonized with roughly the same amount of bacteria (4×10^{13}) as human cells (3×10^{13}) (Sender *et al*, 2016). The collective of genes in these microorganisms outnumbers the human genome by a factor of more than 100 (Qin *et al*, 2010). The potential metabolic functions of all these bacterial genes also exceed that of the human body (Maccaferri *et al*, 2011). Thus, humans can be described as superorganisms whose metabolism is a concert of microbial and human instruments.

Each human being is born almost sterile, but directly after birth, the human body becomes colonized with microorganisms from its environment. The complex and critical assembly of the host with its microorganisms starts at birth and takes several years to form a stable composition (Koenig *et al*, 2011; Yatsunenko *et al*, 2012). Delivery mode (vaginal birth *vs* Caesarean section), breastfeeding, diet, use of antibiotics, use of proton pump inhibitors, home country and the host genome are all known to influence the composition of the bacterial community in the gut (Dominguez-Bello *et al*, 2010; Koenig *et al*, 2011; Yatsunenko *et al*, 2012; David *et al*, 2014; Goodrich *et al*, 2014; Imhann *et al*, 2015; Zaura *et al*, 2015). Thus, each adult 'human superorganism' has evolved through a series of events and the individual composition of this superorganism appears to be so unique that individuals can be distinguished based on human-associated microbial communities (Franzosa *et al*, 2015b).

The human microbiome in healthy people – is there a 'core' human microbiome?

In 2007, the Human Microbiome Project (HMP) was announced as a logical conceptual and experimental extension of the Human Genome Project (Turnbaugh *et al*, 2007). One of the main goals of the project was to investigate the concept of a 'core' human microbiome, which is defined as the set of microbial genes present in a given habitat in all or the vast majority of humans (Turnbaugh *et al*, 2007). Shortly after the start of the HMP in 2008, this concept was largely discarded, because the variability of the human microbiome between individuals appears to

be very high (Hamady and Knight, 2009; Huttenhower *et al.*, 2012). As a consequence of the enormous variability in the human microbiome, it is very difficult (or even impossible) to use the presence or abundance of specific microorganisms as biomarkers for disease. When looking at higher order taxonomic levels (i.e. the genus or phylum level), human microbiome communities begin to resemble one another more, although variation in the relative abundance in the shared genera or phyla is still large (Hamady and Knight, 2009; Zaura *et al.*, 2009; Zhang *et al.*, 2015a). Although the concept of a core human microbiome, defined by a set of abundant microorganisms, is largely discarded, it appears that a core microbiome does exist in the gastrointestinal tract (gut) microbiome at the level of shared genes, especially those involved in metabolic functions (Turnbaugh *et al.*, 2009; Zhang *et al.*, 2015a). Thus, although the bacterial composition shows a large variability between individuals, the metabolic functions executed by these microorganisms are more similar in a healthy population (Lozupone *et al.*, 2012).

Observational and experimental study designs in microbiome research

Several studies have demonstrated associations of the microbiome with RA, SLE, SS and systemic sclerosis in humans and in mice (Scher *et al.*, 2013; Arron *et al.*, 2014; Hevia *et al.*, 2014; Szymula *et al.*, 2014). Up to now, human studies investigating the microbiome–systemic disease connection are observational case–control studies in which the human microbiome (or metagenome or metabolome) of mainly the gut, oral cavity and/or skin is compared between patients with systemic diseases and controls (Scher *et al.*, 2013; Arron *et al.*, 2014; Hevia *et al.*, 2014; Zhang *et al.*, 2015b). Because of the observational design with single time point measurements, these studies are unable to answer the question whether changes in the microbiome are a cause or effect of the disease. Furthermore, because of the exploratory nature and relatively low number of subjects in human microbiome studies (usually dozen to a hundred) compared to human genome studies (usually thousands), there is a considerable risk of finding false-positive associations.

In GWAS, a genetic difference found between two populations needs to exceed the threshold of $P = 5 \times 10^{-8}$ to be considered significant (Lessard *et al.*, 2013). Data in microbiome studies differ greatly from genome studies, and specific biostatistic pipelines (multiple statistical analyses performed successively) have been developed to find associations between microbiome data and metadata, such as disease parameters (Morgan *et al.*, 2012). These biostatistic pipelines often include a calculation to correct for the false discovery rate (Benjamini and Hochberg method) or for multiple testing (Bonferroni correction) (Morgan *et al.*, 2012; Scher *et al.*, 2013). Applying these methods aids in finding significant associations in the immense amount of microbial sequence data and diseases or clinical metadata.

To investigate the effect of microorganisms on the initiation of systemic diseases, animal studies are very helpful. In these studies, germ-free (GF), specific pathogen-free

(SPF) and gnotobiotic animals (usually mice) are compared with mice grown under conventional conditions. GF mice are born and raised in sterile conditions and SPF mice are mice that are free of specific pathogens (and commensals) through the administration of antibiotics. Gnotobiotic (Greek *gnōstos* = ‘known’) mice are GF mice that are exposed to one or several known microorganisms at a certain point in life. For example, autoimmune arthritis was strongly attenuated in a K/BxN mouse model under GF conditions and the introduction of segmented filamentous bacteria (SFB) into GF animals re-established arthritis rapidly (Wu *et al.*, 2010).

Mucosal immunity in the gut

The microbiome–host immunity connection involves the bidirectional relationship between microorganisms and the host innate and adaptive immune system. The microbiome–host immunity connection is mainly investigated in the gut, both in humans and mice (Wen *et al.*, 2008; Cerf-Bensussan and Gaboriau-Routhiau, 2010; Vossenkämper *et al.*, 2013). The high density of bacteria ($\geq 10^{11}$ per cm^3 intestinal content) and the large surface area of the gut (30–40 m^2) emphasize the major exposure of the gut epithelium to bacteria and the potential effect of the gut microbiome on host immunity (Helander and Fändriks, 2014).

The mucus layer residing on the epithelial lining of the intestinal tract forms a physical barrier which minimizes direct contact between bacteria and epithelial cells (van der Waaij *et al.*, 2005). A firmly adherent (inner) mucus layer is attached to the intestinal mucosa, and a more loose (outer) mucus layer covers the adherent layer and has direct contact with the luminal contents of the gut (Atuma *et al.*, 2001). The mucus layer contains a large amount of secretory immunoglobulin A (SIgA) which blocks pathogens from binding to epithelial cells and traps bacteria in the mucus layer (Mantis *et al.*, 2011). Physical clearance of entrapped bacteria is facilitated by the peristaltic movement of the bowel. Bacterial contact with the intestinal epithelium is also restricted by the antibacterial lectin RegIII γ , which limits bacterial penetration of the mucus layer in the small intestine (Vaishnava *et al.*, 2011). Antimicrobial peptides (AMPs), such as α -defensin and the human cathelicidin LL37, are produced by Paneth cells in the small intestine (Mukherjee and Hooper, 2014). Via granule exocytosis, AMPs are brought into the intestinal lumen, where they can kill bacteria through membrane disruption (Schauber *et al.*, 2003; Mukherjee and Hooper, 2014). The epithelium itself is also a central component of the intestinal immune system. It serves not only as a physical barrier, but also shares immunologic functions, by expressing pattern recognition receptors (PRRs) that recognize microbial-associated molecular patterns (MAMPs, also named pathogen-associated molecular patterns). When a MAMP binds to a PRR (such as a Toll-like receptor, TLR), an intracellular signalling cascade in the epithelial cell activates the cell to stimulate the transcription of antibacterial proteins, pro-inflammatory cytokines and chemokines (Cerf-Bensussan and Gaboriau-Routhiau, 2010). Microfold cells (M cells) are specialized cells

located between enterocytes and in close proximity of mucosa-associated lymphoid tissue (MALT, in the gut also called, gut-associated lymphoid tissue, GALT) beneath the epithelial layer. M cells are highly specialized for the phagocytosis, receptor-mediated endocytosis and transcytosis of gut lumen antigens and microorganisms across the intestinal epithelium (Mabbott *et al*, 2013). Herewith, luminal antigens are transported to the organized lymphoid tissue of the MALT located directly beneath the gut epithelium. Therefore, M cells fulfil an important immunosurveillance post in the intestinal epithelium (Mabbott *et al*, 2013). Intraepithelial lymphocytes (IELs) are another immune component of the intestinal epithelium. IELs are a heterogeneous group of antigen-experienced T cells that have selectively migrated to the intestinal epithelium (Cheroutre *et al*, 2011). They comprise both thymus-induced and peripheral-induced T cells, many of them expressing the $\gamma\delta$ T-cell receptor (up to 60%). IELs are located in the epithelial layer, in direct contact with enterocytes, which is mediated by CD103 on the surface of IELs. IELs are thus in close proximity of intestinal microorganisms, thereby fulfilling their role in the front line of immune defence against invading pathogens. IELs protect and restore the integrity of the epithelium and maintain local immune quiescence by secreting a wide range of cytokines. For example, transforming growth factor beta (TGF β) has a role in protecting the integrity of the epithelium and tumour necrosis factor (TNF) and IFN γ function as protective cytokines (Cheroutre *et al*, 2011).

Along the intestinal tract, dendritic cells (DCs) reside in the lamina propria (LP), in MALT and in the mesenteric lymph nodes (MLNs) (Coombes and Powrie, 2008). DCs in the LP and MALT have cellular extensions passing the epithelial cells into the lumen of the gut, where they can phagocytose bacteria, viruses and food peptides. MALT-associated DCs present antigen to T cells in the MALT and in MLNs, and DCs from the LP migrate directly to the MLNs (Liu and MacPherson, 1995; Iwasaki, 2007). B cells that are activated in the MALT or MLNs enter the circulation and home to the LP where they become IgA-secreting plasma cells (Hooper *et al*, 2012). The human gut contains almost 80% of all plasma cells and produces the largest amount of IgA in the body (Brandtzaeg and Johansen, 2005). The vast majority (80–90%) of the immunoglobulins produced by plasma cells in the LP is IgA (Brandtzaeg *et al*, 1999). The polymeric immunoglobulin receptor located on the basolateral surface of epithelial cells mediates transcytosis of locally produced IgA into the gut lumen where it is secreted as SIgA (Kaetzel, 2014). Besides the entrapment of bacteria in the mucus, SIgA reduces bacterial virulence factors (Forbes *et al*, 2008, 2011), prevents toxin attachment to epithelial receptors (Dallas and Rolfe, 1998) and aids in the phagocytosis of antigen by M cells to Peyer's patches (Rey *et al*, 2004). Vice versa, gut microbiota and butyrate can modulate SIgA transport by influencing the expression of the polymeric immunoglobulin receptor (Kvale and Brandtzaeg, 1995; Bruno *et al*, 2010). How the gut microbiome affects local and systemic immunity is currently a major topic of research and is discussed in more detail in the

section on the microbiome–systemic immunity connection below.

Mucosal immunity in the oral cavity

The oral mucosa is also exposed to a very high diversity of microorganisms (≈ 700 different bacterial species) and food peptides (Dewhirst *et al*, 2010). Despite this high antigen load, the oral mucosa remains in a relative state of health due to the pro-tolerogenic nature of the mucosal immune system and its antimicrobial defence mechanisms (Dewhirst *et al*, 2010; Hovav, 2014).

The epithelium of the oral cavity is a stratified layer of non-keratinized (except for the keratinized gingiva) squamous cells and has a much less adsorptive and permeable nature than the gut epithelium which is composed of a single cell layer. Cell shedding from the surface layer of the oral epithelium minimizes the colonization of bacteria (Hovav, 2014).

Saliva is one of the major factors responsible for microbial homeostasis in the oral cavity which is illustrated by the fact that reduced salivary secretion in patients with SS leads to an increase in microbial-related diseases, such as dental caries and oral candidiasis (Arendorf and Walker, 1980; Christensen *et al*, 2001). Homeostasis of the oral microbiome is maintained by saliva through modulation of bacterial attachment, modulation of bacterial growth and inhibition of bacterial growth (van't Hof *et al*, 2014). Salivary agglutinin and SIgA both bind to bacteria and prevent bacterial attachment to the oral mucosa. Bacterial modulation involves mucin 5B (MUC5B), the largest molecule in saliva. MUC5B is a peptide that is heavily glycosylated with an extremely heterogeneous set of oligosaccharides. MUC5B is the major carbohydrate source for microorganisms when external supply is absent (van't Hof *et al*, 2014). Inhibition of bacterial growth is controlled by salivary antimicrobial components, such as lysozyme, histatins, β -defensins and the human cathelicidin LL37. These antimicrobial components exert their antibacterial function by affecting cell wall integrity and pore formation and indirectly by immune signalling (van't Hof *et al*, 2014). Considering the many functions of saliva in the homeostasis of the oral microbiome, it is not surprising that hyposalivation may disturb this homeostasis.

All of the above-mentioned antimicrobial components of saliva are also present in the gingival crevicular fluid (GCF) (Fábián *et al*, 2012). The composition of GCF is very similar to serum transudate and therefore contains proteins that are also present in blood such as the complement system and IgG (Fábián *et al*, 2012). Leucocytes are also present in the GCF, with the majority being neutrophils (>90%) and the remaining fraction lymphocytes and monocytes (Delima and Van Dyke, 2003).

Dendritic cells present in the oral mucosa are mainly Langerhans cells and are capable of antigen capture, migration to lymph nodes (LNs) and antigen presentation to T cells (Hovav, 2014). However, because of the non-adsorptive nature of the oral epithelium and because the extensions of these DCs do not protrude into the oral cavity, the mucosa must be penetrated or damaged first, before antigen capture by DCs can take place at these

sites. The sublingual mucosa is an exception to this, because antigen is easily adsorbed and captured by DCs (Kweon, 2011). After capture and antigen presentation in the peripheral LNs, the DCs of the sublingual mucosa are capable of initiating broad systemic and antigen-specific protective immune responses (Kweon, 2011). Currently, this route is used in sublingual immunotherapy for type 1 respiratory allergies and may be used as a route for vaccinations in the future (Kweon, 2011; Moingeon, 2013). Furthermore, immunization with an orally administered cholera vaccine has been shown to induce strong intestinal antibody responses with local immunologic memory (Quiding, 1991).

The palatine tonsils are important lymphoepithelial organs in the oral cavity, located strategically at the surface of the digestive and respiratory tracts (Nave *et al*, 2001). The adenoids, palatine tonsils and other smaller lymphoid structures of Waldeyer's pharyngeal ring are collectively called the nasopharynx-associated lymphoid tissue (NALT) (Brandtzaeg *et al*, 2008). M cells are also present in the epithelium of the palatine tonsils (as in GALT) (Nave *et al*, 2001). T cells recognize presented antigen in the palatine tonsils (and other lymphoid tissues of NALT) and can activate B cells to become plasma cells. Plasma cells originating from both the NALT and the GALT can home to the salivary glands to become SIgA-secreting plasma cells (Brandtzaeg, 2013).

Sublingual antigen exposure has been shown to induce tolerance to antigens in respiratory allergies and strong intestinal antibody responses (Quiding, 1991; Moingeon, 2013). In conclusion, microbial homeostasis in the oral cavity is an effect of the combined function of the oral epithelium, saliva, GCF and adaptive immune responses that take place in lymphoid organs of the NALT and GALT.

The microbiome–systemic immunity connection

The ability to investigate the whole bacterial composition and function in the gut and oral cavity with NGS has resulted in a major increase of understanding the bidirectional relationship of the immune system with the gut and oral microbiome (Hooper *et al*, 2012; Zarco *et al*, 2012; Scher *et al*, 2014). Several excellent reviews have been published about how the microbiome affects local and systemic immunity and the relation between the microbiome and autoimmunity (Wu and Wu, 2012; Belkaid and Naik, 2013; Belkaid and Hand, 2014; McLean *et al*, 2014). In this section, we explain the current knowledge of how bacteria and their metabolites in the gut can influence mucosal and systemic immunity. The connection of the gut and oral microbiome with RA, SLE and SS is discussed after this section.

The effect of microorganisms on shaping the immune system has been investigated for over 50 years. The first studies were carried out with GF mice, and collectively, they showed that LNs, spleen and Peyer's patches from these mice were underdeveloped, small and relatively inactive compared to conventional raised mice (Bauer *et al*, 1963; Pollard and Sharon, 1970). When these mice

were challenged with antigen from *Salmonella paratyphi* A by swabbing the oral cavity or intraperitoneal injection, the LNs, spleen and Peyer's patches increased in size and showed distinct germinal zones (Pollard and Sharon, 1970). This and other observations indicate that the immune system is dependent on contact with microorganisms to develop to a well-functioning state (Talham *et al*, 1999). In 1989, Strachan found that presence of hay fever (or allergic rhinitis, which is a type 1 allergic reaction) was inversely correlated with the number of children in a household, which he linked to a reduced opportunity for cross-infection in young families (Strachan, 1989). Furthermore, children living on farms are exposed to a wider range of microbes than children not living on a farm and this exposure explained a substantial fraction of the inverse relation between allergic asthma and growing up on a farm (Ege *et al*, 2011). Recently, the mechanism behind this relation was clarified in mice as it was shown that exposure to endotoxin (also called lipopolysaccharide, a cell wall component of Gram-negative bacteria) was capable of suppressing a type 2 immune reaction to house dust mite, by modifying the communication between barrier epithelial cells and DCs (Schuijs *et al*, 2015). Finally, early-life consumption of raw cow's milk reduces the risk of manifest respiratory infections and fever by about 30% in infants, implicating that bacteria or peptides present in untreated milk positively affect the immune system (Loss *et al*, 2015). Thus, it seems that the presence of certain microorganisms early in life is necessary to boost an immune system that prevents infections and will not overreact to antigens generally present in the environment (Riedler *et al*, 2001; Olszak *et al*, 2012).

Early-life (0–3 years) dynamics of the gut microbiome also affect the development and progression towards type 1 diabetes (T1D). In the study by Kostic *et al* (2015), the gut microbiome was investigated in infants genetically predisposed to T1D. A marked drop in alpha-diversity (diversity within one faecal sample) was seen in T1D progressors in the time window between seroconversion and T1D diagnosis, compared to infants who did not progress to T1D (Kostic *et al*, 2015). A decreased diversity of microorganisms in the gut bacterial composition is a hallmark of intestinal dysbiosis. Besides reduced diversity, dysbiosis can be defined by (a combination of) the loss of beneficial microorganisms (such as *Bacteroides* strains and butyrate producing bacteria) and expansion of pathobionts (such as *Proteobacteria* including *Escherichia coli*) (Petersen and Round, 2014). Intestinal dysbiosis has been observed in patients with obesity (Turnbaugh *et al*, 2009), intestinal bowel diseases (Manichanh *et al*, 2012) and SLE (Hevia *et al*, 2014).

One of the factors leading to intestinal dysbiosis is diet. A high-fat/high-sugar diet in C57BL/6 mice induces a dysbiosis in the gut microbiome, illustrated by a decreased total bacterial abundance and an increased absolute abundance of *E. coli* and *Bacteroides-Prevotella* spp. (Martinez-Medina *et al*, 2014). Furthermore, release of the proinflammatory cytokines TNF α and IL-1 β into the colonic mucosa was seen in mice treated with the high-fat/high-sugar diet. Overexpression of claudin-2 was shown by immunohistochemical staining of intestinal biopsies

(Martinez-Medina *et al*, 2014). Claudin-2 is an integral membrane protein in the epithelium and plays an important role in the regulation of epithelial permeability by creating paracellular pores. Overexpression of claudin-2 is associated with increased permeability in the gut and is expressed as a response to several inflammatory cytokines (IFN γ , TNF α , IL-1 β , IL-4, IL-6, IL-13) (Suzuki, 2013). Thus, dietary changes can lead to an increased intestinal permeability (Martinez-Medina *et al*, 2014). Increased intestinal permeability can lead to the translocation of bacteria between enterocytes into the LP of the intestine (Lewis *et al*, 2010). However, cause and effect of gut dysbiosis and mucosal inflammation are difficult to distinguish, and therefore, the relation between the gut microbiome and inflammatory bowel diseases is an important research field (Huttenhower *et al*, 2014).

Depending on which bacterial or food antigens are presented by antigen presenting cells (APCs) in the immunologic compartments in the LP, the local immunologic response is directed towards inflammation or regulation (tolerance) (Belkaid and Hand, 2014). For example, SFB in mice promote the accumulation of T helper (T_H)₁₇ and T_H₁ cells in the small intestine, both involved in the production of pro-inflammatory cytokines (Gaboriau-Routhiau *et al*, 2009; Ivanov *et al*, 2009). An anti-inflammatory response is facilitated by the induction of regulatory T cells (T_{REG} cells) as a result of the presence short-chain fatty acids (SCFA) in the intestine (Arpaia *et al*, 2013). SCFA are produced by bacteria as a result of the breakdown of indigestible dietary components such as fibre. Also vitamin A (Mucida *et al*, 2007), *Clostridium* spp. (Atarashi *et al*, 2013), *Bacteroides fragilis* (Round and Mazmanian, 2010) and *Faecalibacterium prausnitzii* (Qiu *et al*, 2013) have been identified to promote the development of T_{REG} cells. Thus, metabolites and bacteria in the gut lumen are important in maintaining immunologic balance in the mucosal immune system between inflammatory and regulatory functions. It is therefore suspected that changes in the gut microbiome concomitantly lead to changes in this balance, initially affecting the gut mucosa, but also leading to systemic immune effects (Figure 1). In mouse models, it has indeed been demonstrated that changes in the gut microbiome play a significant role in the development of SADs (Scher and Abramson, 2011; Johnson *et al*, 2015; Van Praet *et al*, 2015).

The microbiome–RA connection

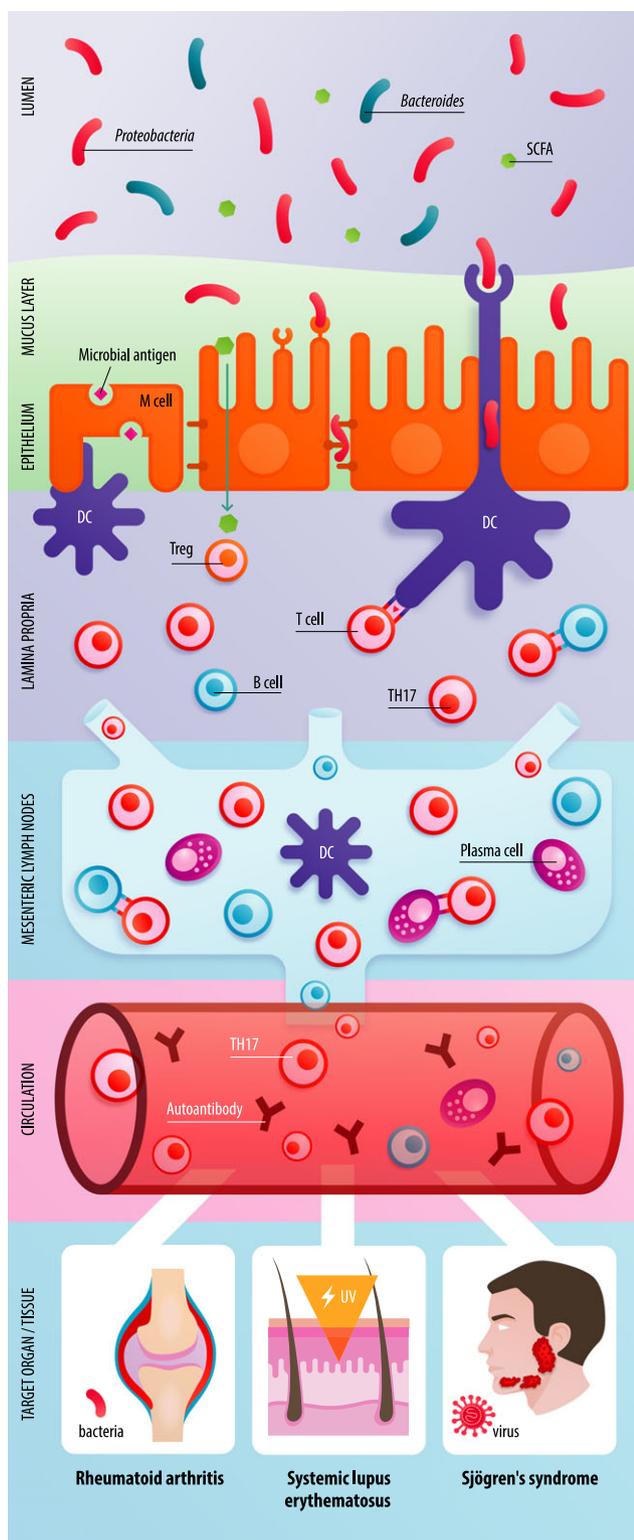
Rheumatoid arthritis is a systemic autoimmune inflammatory disease characterized by joint swelling, joint tenderness, destruction of synovial joints and the presence of autoantibodies such as rheumatoid factor (RF) and, more specifically, ACPA. The prevalence of RA in North America and Northern Europe is 500–1000 per 100 000 (Shapira *et al*, 2010). The 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA include the presence of synovitis and the number of joints involved, serology (presence of RF or ACPA), acute-phase reactants for inflammation (C-reactive protein and erythrocyte

sedimentation rate) and a duration of symptoms of at least 6 weeks (Aletaha *et al*, 2010).

Periodontal disease is more prevalent in RA patients than in controls and patients with periodontitis are at an increased risk of developing RA (Nesse *et al*, 2010; de Smit *et al*, 2012; Koziel *et al*, 2014). Therefore, the role of *P. gingivalis*, a key pathogen in periodontitis, has been studied in patients with RA. *Porphyromonas gingivalis* has the unique ability to convert the amino acid arginine in a protein to the amino acid citrulline, a process called citrullination (Gabarrini *et al*, 2015). However, protein citrullination is also a physiological process in the human body, and therefore, citrullinated proteins are also normally present. ACPAs are found in about 50% of early RA patients, and serum levels of ACPAs correlate with disease severity (Koziel *et al*, 2014). Furthermore, ACPAs are highly specific for RA (more specific than RF) and are included in the 2010 classification criteria for RA (Aletaha *et al*, 2010). Although the exact link between ACPAs and synovial inflammation in patients with RA is not fully understood, ACPA-producing B cells are enriched in the synovial fluid, indicating that ACPAs and/or ACPA-producing B cells play a role in synovial inflammation (Amara *et al*, 2013).

Fifty years ago, it was shown that the amount of *Clostridium perfringens* in faecal samples of patients with RA was increased compared to controls (Mansson and Colldahl, 1965; Olhagen and Mansson, 1968). Ten years later, it was hypothesized that not just one bacteria is involved in the pathogenesis of RA, but probably many bacterial species (Gullberg, 1978). Recently, Scher *et al* (2013) investigated the complete gut microbial composition in RA patients with NGS and demonstrated that the gut microbiome of new-onset patients with RA is characterized by an increase of *Prevotellae*, a decrease of *Bacteroides* and a loss of reportedly beneficial bacteria. Interestingly, the higher abundance of *Prevotella copri* in new-onset patients with RA was inversely correlated with genetic predisposition for RA. They explain this inverse correlation by stating that a certain threshold for *P. copri* abundance is necessary to develop RA (Scher *et al*, 2013). This *P. copri* threshold is possibly higher in patients who do not carry risk-alleles for RA. Thus, whether an individual develops RA might be the result of the sum of risk-alleles and *P. copri* abundance (Scher *et al*, 2013).

A recent study using metagenomic shotgun sequencing and a metagenome-wide association study of faecal, dental and salivary samples from treatment-naïve RA patients, RA patients treated with disease-modifying antirheumatic drugs and healthy controls demonstrated an altered gut, dental and salivary microbiome in patients with RA compared to controls (Zheng *et al*, 2015). With complex bioinformatical genetic analysis, the investigators also showed that the altered microbiome could be used to identify RA patients, correlated with clinical measures and could be used to stratify individuals on the basis of their response to therapy. Although this sounds very promising, it must be noted that the statistical methods were so complex that an accompanying paper was needed to explain the results (Rogers, 2015).



The ‘gut–joint axis’ has been postulated for RA as a mechanism for disease development. An increased intestinal permeability, caused by a dysbiosis in the gut microbiome, leads to immune stimulation with increased citrullination by bacteria, leading to new immunogenic epitopes in the intestine which induce autoantibody pro-

Figure 1 Role of the gut microbiome in the pathogenesis of systemic autoimmune diseases. Microbial dysbiosis in the gut lumen is communicated by enterocytes (through recognition of MAMP by TLRs), DCs (through phagocytosis and antigen presentation) and M cells (through transcytosis) to the (mucosal) immune system. Dysbiosis in the gut microbiome and a reduced availability of SCFAs can shift the T_{REG} – T_H17 balance in the gut towards inflammation through T_H17 expansion. A wide variety of proinflammatory cytokines is released in the lamina propria (LP), such as IL-6, IL-17, IL-21, IL-23 and IFN γ . The inflammatory state increases intestinal permeability by altering the expression of tight junction proteins, such as occludins and claudins. Increased intestinal permeability may lead to microbial translocation and increased antigen exposure in the LP. Activated T cells, B cells and DCs travel through the lymphatic vessels to the mesenteric lymph nodes (MLNs). In the MLNs, antigen presentation by DCs to T cells continues and B cells differentiate towards plasma cells in a T-cell-dependent and T-cell-independent manner. Activated B cells, T_H17 cells and antibody-producing plasma cells enter the circulation. If a mimicry antigen has been encountered in the LP or MLN, the B cells may be stimulated to differentiate towards autoantibody producing plasma cells. Increased amounts of T_H17 cells and the presence of autoantibodies in the circulation are the consequences of this microbiota-induced pro-inflammatory state. After the second hit, being a bacterial infection (in RA), UV radiation (in SLE) or a viral infection (in SS), the immunologic reaction is excessive and self-perpetuating. This causes damage in the organ or tissue and leads to clinical symptoms related to the disease. SCFA, short-chain fatty acid; MAMP, microbe-associated molecular pattern; TLR, Toll-like receptor; M cell, microfold cell; DC, dendritic cell; Treg, regulatory T cell; T_H17 , T helper 17 cell; IL, interleukin; IFN γ , interferon-gamma; MLNs, mesenteric lymph nodes; UV, ultraviolet radiation; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjögren’s syndrome

duction and activate pathogenic pathways (Lerner and Matthias, 2015). Many assumptions in this hypothesis have not been clarified yet, but the first steps in unravelling the role of the oral and gut microbiome in RA pathogenesis have been taken. Furthermore, it has been shown that microbiome analysis in patients with RA can function as a biomarker for response to therapy.

The microbiome–SLE connection

Systemic lupus erythematosus is a multisystemic autoimmune disease characterized by the production of numerous autoantibodies and the involvement of skin, joints, kidneys, brain, serosal surfaces, blood vessels, blood cells, lungs and heart (Lipsky, 2001; Goldblatt and O’Neill, 2013). SLE is a very heterogeneous disease, affecting individuals with a wide range of symptoms and disease courses, of which a butterfly rash in the face, photosensitivity, arthritis or arthralgia and renal symptoms are the most common (Goldblatt and O’Neill, 2013). SLE is 10 times more prevalent in women than in men, and the incidence of the disease is highest in women of childbearing age (Danченко *et al*, 2006). Autoreactive B cells produce autoantibodies against nuclear peptides, nucleosome, double-stranded DNA and Sjögren’s syndrome-related antigen A (SSA, also called ‘Ro’) and are present in 50–95% of patients with SLE (Goldblatt and O’Neill, 2013; Olsen and Karp, 2013).

Recently, it has been shown that the GALT is an important checkpoint for the removal of autoreactive B cells (Vossenkämper *et al*, 2013). In patients with SLE, immature B cells were poorly equipped to access the GALT due to a reduced expression of the gut homing

receptor $\alpha 4\beta 7$. Thus, failure of immature B cells to access the GALT efficiently in patients with SLE may contribute to the increased autoreactive B cells and autoantibody production in SLE (Yurasov *et al*, 2005; Vossenkämper *et al*, 2013). Furthermore, examples of GALT were not found in the biopsies of patients with SLE, whereas multiple examples of GALT were observed in healthy controls. A lower density of IgA-secreting plasma cells was present in intestinal histological biopsies of patients with SLE compared to healthy controls (Vossenkämper *et al*, 2013). The absence of GALT and reduced amount of IgA-secreting plasma cells may contribute to a disturbed mucosal immunity in the gut, leading to a dysbiosis in the bacterial composition (Benckert *et al*, 2011).

The first study to investigate the gut microbial composition in patients with SLE demonstrated that active SLE patients have more different biotypes of Enterobacteriaceae than healthy controls or inactive SLE patients (Apperloo-Renkema *et al*, 1994). The hypothesis behind this study was that a dysbiosis between indigenous and foreign bacteria can lead to translocation of bacteria from the lumen to the immunologic compartments of the gut, ultimately leading to cross-reactivity and antibody production. This hypothesis has gained support from a recent microbiome study confirming that intestinal dysbiosis is associated with SLE (Hevia *et al*, 2014). In this study, intestinal dysbiosis was defined as a reduced Firmicutes/Bacteroidetes ratio. The Firmicutes/Bacteroidetes ratio in patients with SLE was significantly lower than that of healthy subjects (median ratio 1.97 *vs* median ratio 4.86; $P < 0.002$), although the bacterial diversity was not significantly different (Hevia *et al*, 2014). Another study from the same group performed a metabolome-wide scan of gut microbiota in patients with SLE and healthy controls (Rojo *et al*, 2015). A difference in the gut metabolome (but not in the bacterial composition) was found between patients with SLE and controls, suggesting that SLE affects the functionality of the gut microbiome (Rojo *et al*, 2015). However, only 0.72% of the metabolic mass features were found to significantly differ between the SLE and healthy controls, which just might be a variation within a common range and raises the question whether this subtle difference has physiological implications (Rojo *et al*, 2015).

The above-mentioned human studies have demonstrated that there is a connection between the gut microbiome, gut mucosal immunity and SLE, but it remains unclear whether an altered gut microbiome causes SLE or that the altered gut microbiome is an effect of the disease. Two recent mouse studies have demonstrated, however, that an altered gut microbiome indeed might be a causal factor in the development of SLE. Comparing the gut microbiome of healthy mice (MRL/Mp) and lupus-prone mice (MRL/lpr) demonstrated that the relative abundance of Lactobacillaceae was significantly decreased and the relative abundance of Lachnospiraceae was significantly increased in the lupus-prone mice (Zhang *et al*, 2014). Vitamin A (retinoic acid) treatment restored the relative abundance of Lactobacillaceae to the level of healthy MRL/Mp mice. Furthermore, strong correlations were found between the relative abundance of Lactobacillaceae and Lachnospiraceae, and lupus disease indexes (spleen

weight, MLN weight and renal function) (Zhang *et al*, 2014). Another study showed that a change of pH of drinking water affected the gut microbiome, gut inflammation and disease progression in a different spontaneous mouse model of SLE [SWR \times NZB F₁ (SNF₁) mice] (Johnson *et al*, 2015). Mice receiving acidic water (pH 3.0–3.2) had a slower disease progression, less autoantibody (anti-dsDNA and anti-nucleohistone) production and lower immune cell infiltrates in the kidney compared to mice receiving neutral water (pH 7.0–7.2). Because the investigators expected to find the primary effect of a dietary intervention in the gut mucosa, they investigated the immune phenotype of small intestinal tissue of the SNF₁ mice. Mice receiving normal water showed higher expression levels of proinflammatory cytokines, such as IL17, IL21-23 and IFN α . Gut microbiome analysis showed that the relative abundance of many bacteria significantly differed between the acidic water-recipient and normal water-recipient mice, with an overall trend of promoted growth of Firmicutes in the acid water-recipient mice. Finally, by transferring the gut microbiota of acidic water-recipient mice into normal water-recipient mice by oral gavage, the investigators were able to suppress lupus progression in the normal water-recipient mice. This finding suggests that disease progression in lupus-prone SNF₁ mice is dependent on gut microbiota (Johnson *et al*, 2015).

In conclusion, studies on the human microbiome SLE connection suggest a connection between dysbiosis in the gut microbiome and SLE, although cause and effect remain unclear. Mouse models of SLE (lupus-prone) have demonstrated that an altered gut microbiome not only affect local intestinal inflammation, but also systemic autoimmunity illustrated by autoantibody production and lymphocyte infiltration in the kidneys. To investigate whether the gut microbiome in patients with SLE plays a role in disease activity, the experimental approach could be the transfer of human gut bacteria of healthy and patients with SLE to GF lupus-prone mice (Faith *et al*, 2014).

The microbiome–SS connection

Sjögren's syndrome is a SAD characterized by lymphocyte infiltration in the salivary and lacrimal glands, causing reduced saliva and tear production which subsequently leads to the two most prominent symptoms of SS: a sensation of a dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca) (Kalk *et al*, 2001; Cornec *et al*, 2015). Fatigue is another pronounced symptom of the disease. SS is 10 times more prevalent in women than in men (Qin *et al*, 2014). Depending on whether SS presents as a single disease or concurrently with other autoimmune disorders, such as SLE or RA, SS is divided into primary SS (pSS) and secondary SS. Systemic features of SS appear in extraglandular sites such as joints, skin, peripheral and central nerves, lungs, kidneys, liver and thyroid gland (Tincani *et al*, 2013; Moreira *et al*, 2014). Patients with pSS have a 13-fold increased risk for developing a non-Hodgkin lymphoma, occurring in 5–10% of all patients with pSS (Liang *et al*, 2014). Autoantibodies against the ribonuclear proteins SSA/Ro and SSB/La and RF are frequently present in patients with SS; these autoantibodies

can be detected many years before disease onset (Jonsson *et al*, 2013; Kyriakidis *et al*, 2013). Because the presence of anti-SSA/SSB antibodies is fairly specific for SS, it is used in the classification criteria for SS (Vitali *et al*, 2002; Shiboski *et al*, 2012; Shiboski and Shiboski, 2015).

The majority of studies on the role of the microbiome on SS focused on the effect of hyposalivation on the oral microorganisms applying culture techniques or targeted DNA methods to determine the presence of bacterial species. Hyposalivation in patients with SS has been associated with a higher number of *Candida* species on mucosal surfaces and an increase in acidogenic (acid producing) and aciduric (thriving well in relatively acidic environment) microorganisms such as *Lactobacillus* spp. on tooth surfaces (MacFarlane and Mason, 1974; Almståhl *et al*, 2003; Leung *et al*, 2007, 2008; Shinozaki *et al*, 2012). This is clinically relevant, because patients suffering from hyposalivation are at increased risk of (typically cervical) caries and oral candidiasis (Mathews *et al*, 2008; González *et al*, 2014). A Taiwanese population-based study revealed that the frequency of dental visits already increased years prior to the diagnosis of pSS because of dental caries, gingivitis and stomatitis (Lu *et al*, 2014). In all these manifestations, bacteria are involved in the pathogenesis; thus, a shift in the oral microbiome might be present before the more characteristic oral symptom of pSS, which is xerostomia. No data were available on oral candidiasis and cervical caries. In contrast to patients with RA, the prevalence of periodontitis is not increased in patients with pSS (Lugonja *et al*, 2016). The authors conclude that microbiome analysis is needed to identify a possible triggering bacterial pathogen for pSS. As periodontitis was not increased in patients with pSS, it is unlikely that the presumed bacterial trigger for SS is a pathogen currently associated with periodontitis.

The only study available on the role of bacteria in SS development has demonstrated that T cells from mice immunized with the Ro60 peptide (derived from the SSA autoantigen) could be activated *in vitro* by peptides from oral, skin and gut bacteria (Szymula *et al*, 2014). The most potent activator of the mouse Ro60-reactive T cells was a peptide from the von Willebrand factor type A domain protein (vWFA). Basic Local Alignment Search Tool (BLAST) analysis revealed that vWFA is present in (amongst others) the oral microorganism *Capnocytophaga ochracea* and the gut microorganism *Bacteroides intestinalis*. This study supports the hypothesis that T cells with a receptor for Ro60 (SSA) can be activated by DCs who present this specific peptide of vWFA as a result of interaction with for example *B. intestinalis*. SSA autoantibodies might be produced when the activated Ro60-reactive T cells in turn activate B cells to become plasma cells. If NGS of the oral and gut microbiome in patients with SS reveals an increased relative abundance of *C. ochracea* in the oral cavity or *B. intestinalis* in the gut, then, the microbiome–SS connection might be explained by the molecular mimicry theory.

Evidence of a direct connection between the presence of SS and the human microbiome is currently absent in human and scarce in animal studies. However, pathogenic features of SS, such as the presence of autoreactive B cells

and the involvement of T_H17 cells (Barrera *et al*, 2013; Kroese *et al*, 2014; Lin *et al*, 2014), have been associated directly or indirectly with the human microbiome. The mechanism behind the survival of autoreactive B cells in patients with SLE might also be present in SS as SLE and SS have a comparable pathogenesis (Vossenkömper *et al*, 2013). T_H17 cells have been demonstrated in the salivary glands of patients with SS (Sakai *et al*, 2008), and in our own cohort of patients with SS, we find elevated numbers of T_H17 cells in patients' peripheral blood (Verstappen GM, Kroese FGM, Meiners PM, Corneth OBJ, Huitema MG, Arends S, Vissink A, Bootsma H, Abdulahad WA, unpublished data). As discussed above, specific bacteria and possibly also dysbiosis in the gut microbiome can both lead to an increase in T_H17 cells in the LP of the gut. If these proinflammatory T_H17 cells enter the circulation, they may migrate to the exocrine glands in SS. Furthermore, in an experimental SS mouse model (C57BL/6 mice immunized with submandibular autoantigens) it was shown that T_H17 cells were able to drive the development of experimental SS as featured by reduced salivary secretion and tissue destruction in the salivary gland (Sakai *et al*, 2008; Lin *et al*, 2014). The question whether the T_H17 cells, found in the salivary glands of patients with SS, have migrated from the LP would be an interesting topic for future research.

Because we hypothesized that bacteria may play a more direct role in the recruitment of lymphocytes into the salivary glands of patients with pSS, we have investigated bacterial presence in parotid gland biopsies. Biopsies of five different patients with pSS were investigated with fluorescent *in situ* hybridization. However, using a bacterial kingdom-specific oligonucleotide probe (Bact338), we were unable to find bacterial DNA (and thus bacteria) in these parotid gland biopsies (van der Meulen TA and Harmsen HJM, unpublished data). Although one case report states that *Helicobacter pylori* is present in a parotid gland MALT lymphoma in a patient with SS (Nishimura *et al*, 2000), we were again unable to find bacterial DNA in a similar SS-related MALT lymphoma of the parotid gland (van der Meulen TA and Harmsen HJM, unpublished data).

To conclude, indirect evidence and evidence from related systemic diseases suggest a role for the gut and/or oral microbiome in the development of SS. Future studies will first need to find associations between the oral and/or gut microbiome and the presence of SS in patients and investigate the effect of raising a SS mouse model in a GF environment.

Concluding remarks

From birth on, the human body is inhabited with an enormous amount and diversity of microorganisms. The human microbiome acts in close concert with the mucosal surfaces of the body and is capable of inducing inflammatory and regulatory immunologic effects locally and at distant sites. NGS, combined with other 'omics' methods and established methods such as flow cytometry, has increased the knowledge concerning the mechanism behind the microbiome–immunity connection, but there

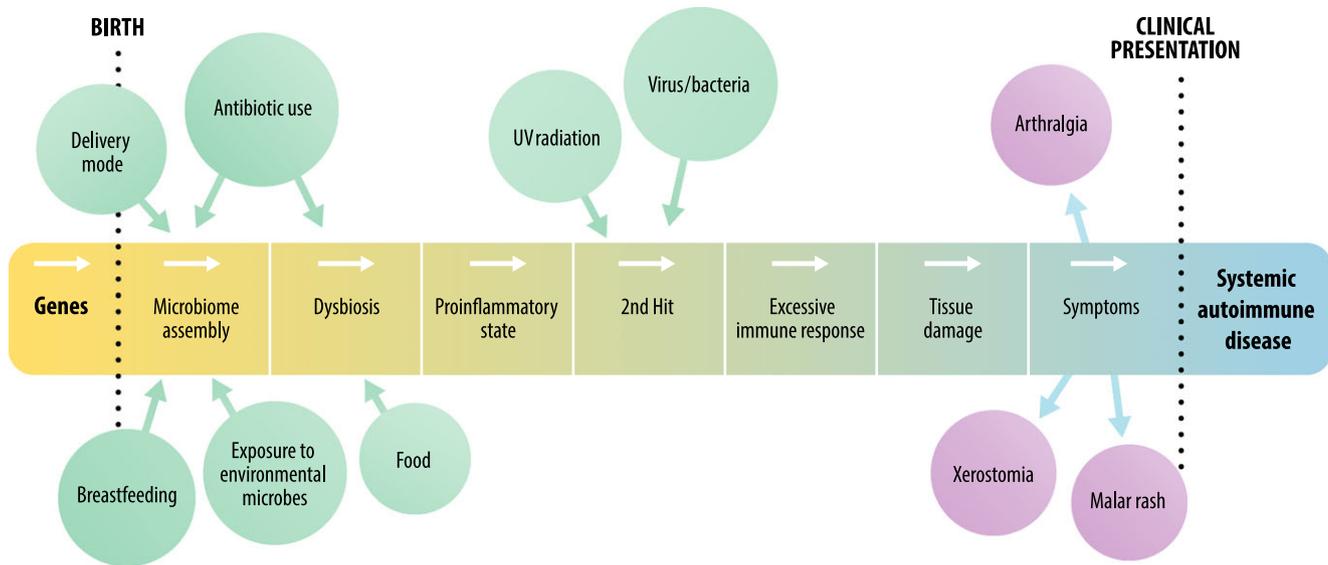


Figure 2 The development of systemic autoimmune diseases (SADs) in time. A genetic predisposition for SAD (mostly HLA related) is present before birth. The first 3 years of life are crucial for the assembly of a healthy human microbiome. Dysbiosis in the gut microbiome can ensue as a result of a Caesarean section (instead of vaginal birth), lack of breastfeeding, use of antibiotics and lack of exposure to environmental microbes (too clean). Later in life, gut dysbiosis continues or is established through a low fibre, high-fat diet and/or use of antibiotics. Dysbiosis in the gut microbiome leads to a systemic proinflammatory state (see Figure 1), which predisposes to an excessive and self-perpetuating immune response after a second hit in a joint, the skin or salivary glands. The excessive immune response becomes chronic and causes tissue damage, leading to, respectively, arthritis, dermal inflammatory infiltrates or sialoadenitis. After a certain time, the inflammatory damage is irreversible and the function of the inflamed organ starts to fail, which is experienced as, for example arthralgia (RA), malar rash (SLE) or xerostomia (SS). After symptoms occur, it can still take several years before the patient presents to a medical professional for the first time or the underlying disease is recognized by a physician and the diagnosis of a SAD is drawn. HLA, human leukocyte antigen; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome

are still many gaps. NGS of the human microbiome has, however, not yet been widely used to study the role of microbiota in the development and pathogenesis of SADs.

In patients with SLE and RA, an altered gut microbiome has been found, but the number of patients in these studies are too small for definite conclusions. Thus, it remains unknown whether the observed differences in the oral and gut microbiome of patients with SLE and RA are a cause or effect of the disease. Animal studies have provided evidence for a causal role of the microbiome in the development of SADs, but this is still to be proven in humans. Based on the available evidence, we postulate the following hypothesis on the role for the gut microbiome in the etiopathogenesis of SADs (Figures 1 and 2).

A variety of environmental factors during life – from delivery mode to antibiotic use and diet – affects the human microbiome. Unfavourable environmental factors, such as birth by C-section (because the baby is not colonized with bacteria from the urogenital and anal area of the mother), frequent antibiotic use (disturbing gut homeostasis) and a high-fat/low-fibre diet (reducing SCFA producing bacteria), all may lead to dysbiosis in the gut microbiome. Long persisting dysbiosis in the gut leads to a (low-grade) inflammatory state of the mucosal immune system with increased permeability of the gut epithelium as a consequence. The increased permeability increases the translocation of bacteria and other antigens from the lumen to the LP, exacerbating the inflammatory response in the mucosal immune system of the gut. The chronic inflammatory state of the gut mucosa will ultimately affect the systemic immune system, because proin-

flammatory lymphocytes and cytokines will be released in the circulation. The increased exposure of bacterial and food antigens increases the risk of exposure to mimicry autoantigens and/or bystander activation of autoreactive cells, and herewith the break of tolerance for self-antigens. This process may take effect without specific symptoms. Local injury in a joint or the skin or a local (viral) infection in the salivary gland may function as the second hit and arouse the chronic proinflammatory immune system. Because the proinflammatory status rules over the regulatory functions of the immune system, the second hit causes a local chronic and self-perpetuating immune response directed against autoantigens. After a certain period of time, clinical symptoms begin to appear, such as joint inflammation in RA, skin involvement in SLE and reduced saliva production in SS.

Author contributions

T.A. van der Meulen has written the manuscript in close collaboration with and using the expertise of F.G.M. Kroese, H.J.M. Harmsen and A. Vissink. These authors have contributed by revising the manuscript, figures and legends. F.K.L. Spijkervet and H. Bootsma are the project supervisors and agreed with the manuscript after revision.

References

- Aletaha D, Neogi T, Silman AJ *et al* (2010). 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* **62**: 2569–2581.

- Almståhl A, Wikström M, Stenberg I, Jakobsson A, Fagerberg-Mohlin B (2003). Oral microbiota associated with hyposalivation of different origins. *Oral Microbiol Immunol* **18**: 1–8.
- Amara K, Steen J, Murray F *et al* (2013). Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition. *J Exp Med* **210**: 445–455.
- Apperloo-Renkema HZ, Bootsma H, Mulder BI, Kallenberg CG, van der Waaij D (1994). Host-microflora interaction in systemic lupus erythematosus (SLE): colonization resistance of the indigenous bacteria of the intestinal tract. *Epidemiol Infect* **112**: 367–373.
- Arendorf TM, Walker DM (1980). The prevalence and intra-oral distribution of *Candida albicans* in man. *Arch Oral Biol* **25**: 1–10.
- Arpaia N, Campbell C, Fan X *et al* (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **504**: 451–455.
- Arron ST, Dimon MT, Li Z *et al* (2014). High rhodotorula sequences in skin transcriptome of patients with diffuse systemic sclerosis. *J Invest Dermatol* **134**: 1–8.
- Atarashi K, Tanoue T, Oshima K *et al* (2013). Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* **500**: 232–236.
- Atuma C, Strugala V, Allen A, Holm L (2001). The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. *Am J Physiol Gastrointest Liver Physiol* **280**: G922–G929.
- Ball RJ, Avenell A, Aucott L, Hanlon P, Vickers MA (2015). Systematic review and meta-analysis of the sero-epidemiological association between Epstein–Barr virus and rheumatoid arthritis. *Arthritis Res Ther* **17**: 274.
- Barrera MJ, Bahamondes V, Sepúlveda D *et al* (2013). Sjögren's syndrome and the epithelial target: a comprehensive review. *J Autoimmun* **42**: 7–18.
- Bauer H, Horowitz R, Levenson S, Popper H (1963). The response of the lymphatic tissue to the microbial flora. *Am J Pathol* **42**: 471–483.
- Belkaid Y, Hand TW (2014). Role of the microbiota in immunity and inflammation. *Cell* **157**: 121–141.
- Belkaid Y, Naik S (2013). Compartmentalized and systemic control of tissue immunity by commensals. *Nat Immunol* **14**: 646–653.
- Benckert J, Schmolka N, Kreschel C *et al* (2011). The majority of intestinal IgA+ and IgG+ plasmablasts in the human gut are antigen-specific. *J Clin Invest* **121**: 1946–1955.
- Bikel S, Valdez-Lara A, Cornejo-Granados F *et al* (2015). Combining metagenomics, metatranscriptomics and viromics to explore novel microbial interactions: towards a systems-level understanding of human microbiome. *Comput Struct Biotechnol J* **13**: 390–401.
- Bogdanos DP, Smyk DS, Rigopoulou EI *et al* (2012). Twin studies in autoimmune disease: genetics, gender and environment. *J Autoimmun* **38**: J156–J169.
- Brandtzaeg P (2013). Secretory immunity with special reference to the oral cavity. *J Oral Microbiol* **5**: 1–24.
- Brandtzaeg P, Johansen F-E (2005). Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. *Immunol Rev* **206**: 32–63.
- Brandtzaeg P, Farstad IN, Johansen F-E, Morton HC, Norderhaug IN, Yamanaka T (1999). The B-cell system of human mucosae and exocrine glands. *Immunol Rev* **171**: 45–87.
- Brandtzaeg P, Kiyono H, Pabst R, Russell MW (2008). Terminology: nomenclature of mucosa-associated lymphoid tissue. *Mucosal Immunol* **1**: 31–37.
- Bruno MEC, Rogier EW, Frantz AL, Stefka AT, Thompson SN, Kaetzel CS (2010). Regulation of the polymeric immunoglobulin receptor in intestinal epithelial cells by Enterobacteriaceae: implications for mucosal homeostasis. *Immunol Invest* **39**: 356–382.
- Cárdenas-Roldán J, Rojas-Villarraga A, Anaya J (2013). How do autoimmune diseases cluster in families? A systematic review and meta-analysis. *BMC Med* **11**: 73.
- Castaño-Rodríguez N, Diaz-Gallo L-M, Pineda-Tamayo R, Rojas-Villarraga A, Anaya J-M (2008). Meta-analysis of HLA-DRB1 and HLA-DQB1 polymorphisms in Latin American patients with systemic lupus erythematosus. *Autoimmun Rev* **7**: 322–330.
- Cénit MC, Matzaraki V, Tigchelaar EF, Zhernakova A (2014). Rapidly expanding knowledge on the role of the gut microbiome in health and disease. *Biochim Biophys Acta* **1842**: 1981–1992.
- Cerf-Bensussan N, Gaboriau-Routhiau V (2010). The immune system and the gut microbiota: friends or foes? *Nat Rev Immunol* **10**: 735–744.
- Cheroutre H, Lambolez F, Mucida D (2011). The light and dark sides of intestinal intraepithelial lymphocytes. *Nat Rev Immunol* **11**: 445–456.
- Christensen LB, Petersen PE, Thorn JJ, Schiødt M (2001). Dental caries and dental health behavior of patients with primary Sjögren syndrome. *Acta Odontol Scand* **59**: 116–120.
- Coombes JL, Powrie F (2008). Dendritic cells in intestinal immune regulation. *Nat Rev Immunol* **8**: 435–446.
- Corneec D, Saraux A, Jousse-Joulin S *et al* (2015). The differential diagnosis of dry eyes, dry mouth, and parotidomegaly: a comprehensive review. *Clin Rev Allergy Immunol* **49**: 278–287.
- Croia C, Astorri E, Murray-Brown W *et al* (2014). Implication of Epstein–Barr virus infection in disease-specific autoreactive B cell activation in ectopic lymphoid structures of Sjögren's syndrome. *Arthritis Rheumatol* **66**: 2545–2557.
- Dallas SD, Rolfe RD (1998). Binding of *Clostridium difficile* toxin A to human milk secretory component. *J Med Microbiol* **47**: 879–888.
- Danchenko N, Satia J, Anthony MS (2006). Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus* **15**: 308–318.
- David LA, Maurice CF, Carmody RN *et al* (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**: 559–563.
- Deapen D, Escalante A, Weinrib L *et al* (1992). A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* **35**: 311–318.
- Delima AJ, Van Dyke TE (2003). Origin and function of the cellular components in gingival crevice fluid. *Periodontol 2000* **31**: 55–76.
- Dewhirst FE, Chen T, Izard J *et al* (2010). The human oral microbiome. *J Bacteriol* **192**: 5002–5017.
- Dominguez-Bello MG, Costello EK, Contreras M *et al* (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA* **107**: 11971–11975.
- Eckburg PB, Bik EM, Bernstein CN *et al* (2005). Diversity of the human intestinal microbial flora. *Science* **308**: 1635–1638.
- Ege MJ, Mayer M, Normand A-C *et al* (2011). Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* **364**: 701–709.
- Fábián TK, Hermann P, Beck A, Fejérdy P, Fábián G (2012). Salivary defense proteins: their network and role in innate and acquired oral immunity. *Int J Mol Sci* **13**: 4295–4320.

- Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JI (2014). Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. *Sci Transl Med* **6**: 1–13.
- Forbes SJ, Eschmann M, Mantis NJ (2008). Inhibition of *Salmonella enterica* serovar typhimurium motility and entry into epithelial cells by a protective antilipopopolysaccharide monoclonal immunoglobulin A antibody. *Infect Immun* **76**: 4137–4144.
- Forbes SJ, Bumpus T, McCarthy EA, Corthésy B, Mantis NJ (2011). Transient suppression of *Shigella flexneri* type 3 secretion by a protective O-antigen-specific monoclonal IgA. *MBio* **2**: 9–12.
- Frankenfeld CL, Atkinson C, Thomas WK *et al* (2004). Familial correlations, segregation analysis, and nongenetic correlates of soy isoflavone-metabolizing phenotypes. *Exp Biol Med (Maywood)* **229**: 902–913.
- Franzosa EA, Hsu T, Sirota-Madi A *et al* (2015a). Sequencing and beyond: integrating molecular “omics” for microbial community profiling. *Nat Rev Microbiol* **13**: 360–372.
- Franzosa EA, Huang K, Meadow JF *et al* (2015b). Identifying personal microbiomes using metagenomic codes. *Proc Natl Acad Sci* **112**: E2930–E2938.
- Gabarrini G, de Smit M, Westra J *et al* (2015). The peptidylarginine deiminase gene is a conserved feature of *Porphyromonas gingivalis*. *Sci Rep* **5**: 13936.
- Gaboriau-Routhiau V, Rakotobe S, Lécuyer E *et al* (2009). The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* **31**: 677–689.
- Gill SR, Pop M, Deboy RT *et al* (2006). Metagenomic analysis of the human distal gut microbiome. *Science* **312**: 1355–1359.
- Goldblatt F, O’Neill SG (2013). Clinical aspects of autoimmune rheumatic diseases. *Lancet* **382**: 797–808.
- González S, Sung H, Sepúlveda D, González M, Molina C (2014). Oral manifestations and their treatment in Sjögren’s syndrome. *Oral Dis* **20**: 153–161.
- Goodrich JK, Waters JL, Poole AC *et al* (2014). Human genetics shape the gut microbiome. *Cell* **159**: 789–799.
- Gottenberg J, Cagnard N, Lucchesi C *et al* (2006). Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjögren’s syndrome. *Proc Natl Acad Sci* **103**: 2770–2775.
- Grada A, Weinbrecht K (2013). Next-generation sequencing: methodology and application. *J Invest Dermatol* **133**: e11–e14.
- Gullberg R (1978). Possible role of alterations of the intestinal flora in rheumatoid arthritis. *Rheumatology (Oxford)* **XVII** (Suppl.): 5–10.
- Hamady M, Knight R (2009). Microbial community profiling for human microbiome projects: tools, techniques, and challenges. *Genome Res* **19**: 1141–1152.
- Hanlon P, Avenell A, Aucott L, Vickers MA (2014). Systematic review and meta-analysis of the sero-epidemiological association between Epstein–Barr virus and systemic lupus erythematosus. *Arthritis Res Ther* **16**: R3.
- Harley JB, Alarcón-Riquelme ME, Criswell LA *et al* (2008). Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXX, KIAA1542 and other loci. *Nat Genet* **40**: 204–210.
- Helander HF, Fändriks L (2014). Surface area of the digestive tract – revisited. *Scand J Gastroenterol* **49**: 681–689.
- Hevia A, Milani C, López P *et al* (2014). Intestinal dysbiosis associated with systemic lupus erythematosus. *MBio* **5**: e01548–14.
- Higgs BW, Liu Z, White B *et al* (2011). Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. *Ann Rheum Dis* **70**: 2029–2036.
- van’t Hof W, Veerman ECI, Nieuw Amerongen AV, Ligtenberg AJM (2014). Antimicrobial defense systems in saliva. *Monogr Oral Sci* **24**: 40–51.
- Hooper LV, Littman DR, Macpherson AJ (2012). Interactions between the microbiota and the immune system. *Science* **336**: 1268–1273.
- Hovav A-H (2014). Dendritic cells of the oral mucosa. *Mucosal Immunol* **7**: 27–37.
- Huttenhower C, Gevers D, Knight R *et al* (2012). Structure, function and diversity of the healthy human microbiome. *Nature* **486**: 207–214.
- Huttenhower C, Kostic AD, Xavier RJ (2014). Inflammatory bowel disease as a model for translating the microbiome. *Immunity* **40**: 843–854.
- Imhann F, Bonder MJ, Vich Vila A *et al* (2015). Proton pump inhibitors affect the gut microbiome. *Gut* doi: 10.1136/gutjnl-2015-310376.
- Ivanov II, Atarashi K, Manel N *et al* (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**: 485–498.
- Ivashkiv LB, Donlin LT (2014). Regulation of type I interferon responses. *Nat Rev Immunol* **14**: 36–49.
- Iwasaki A (2007). Mucosal dendritic cells. *Annu Rev Immunol* **25**: 381–418.
- Johnson BM, Gaudreau M-C, Al-Gadban MM, (2015). Impact of dietary deviation on disease progression and gut microbiome composition in lupus-prone SNF₁ mice. *Clin Exp Immunol* **181**: 323–337.
- Jonsson R, Theander E, Sjöström B, Brokstad K, Henriksson G (2013). Autoantibodies present before symptom onset in primary Sjögren syndrome. *J Am Med Assoc* **310**: 1854–1855.
- Kaetzel CS (2014). Cooperativity among secretory IgA, the polymeric immunoglobulin receptor, and the gut microbiota promotes host-microbial mutualism. *Immunol Lett* **162**: 10–21.
- Kalk WWI, Vissink A, Spijkervet FKL, Bootsma H, Kallenberg CGM, Amerongen AVN (2001). Sialometry and sialochemistry: diagnostic tools for Sjögren’s syndrome. *Ann Rheum Dis* **60**: 1110–1116.
- Koenig JE, Spor A, Scalfone N *et al* (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci USA* **108**(Suppl.): 4578–4585.
- Kostic AD, Gevers D, Siljander H *et al* (2015). The dynamics of the human infant gut microbiome in development and in progression toward type I diabetes. *Cell Host Microbe* **17**: 260–273.
- Koziel J, Mydel P, Potempa J (2014). The link between periodontal disease and rheumatoid arthritis: an updated review. *Curr Rheumatol Rep* **16**: 408.
- Kroese FGM, Abdulahad WH, Haacke E, Bos NA, Vissink A, Bootsma H (2014). B-cell hyperactivity in primary Sjögren’s syndrome. *Expert Rev Clin Immunol* **10**: 483–499.
- Kuo C-F, Grainge MJ, Valdes AM *et al* (2015). Familial aggregation of systemic lupus erythematosus and coaggregation of autoimmune diseases in affected families. *JAMA Intern Med* **175**: 1518–1526.
- Kvale D, Brandtzaeg P (1995). Constitutive and cytokine induced expression of HLA molecules, secretory component, and intercellular adhesion molecule-1 is modulated by butyrate in the colonic epithelial cell line HT-29. *Gut* **36**: 737–742.
- Kweon S (2011). Sublingual mucosa: a new vaccination route for systemic and mucosal immunity. *Cytokine* **54**: 1–5.
- Kyriakidis NC, Kapsogeorgou EK, Tzioufas AG (2013). A comprehensive review of autoantibodies in primary Sjögren’s syndrome: clinical phenotypes and regulatory mechanisms. *J Autoimmun* **51**: 67–74.

- Lerner A, Matthias T (2015). Rheumatoid arthritis–celiac disease relationship: joints get that gut feeling. *Autoimmun Rev* **14**: 1038–1047.
- Lessard CJ, Ice JA, Maier-Moore J, Montgomery CG, Scofield H, Moser KL (2012). Genetics, genomics, and proteomics of Sjögren's syndrome. In: Ramos-Casals M, Stone JH, Moutsopoulos HM eds. *Sjögren's syndrome – Diagnosis and Therapeutics*. 1st edn. Springer: London, pp. 2.11–2.31.
- Lessard CJ, Li H, Adrianto I *et al* (2013). Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjögren's syndrome. *Nat Genet* **45**: 1284–1292.
- Leung KCM, Leung WK, McMillan AS (2007). Supra-gingival microbiota in Sjögren's syndrome. *Clin Oral Investig* **11**: 415–423.
- Leung KCM, McMillan AS, Cheung BPK, Leung WK (2008). Sjögren's syndrome sufferers have increased oral yeast levels despite regular dental care. *Oral Dis* **14**: 163–173.
- Lewis K, Lutgendorff F, Phan V, Söderholm JD, Sherman PM, McKay DM (2010). Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. *Inflamm Bowel Dis* **16**: 1138–1148.
- Li Y, Ismail AI, Ge Y, Tellez M, Sohn W (2007). Similarity of bacterial populations in saliva from African-American mother-child dyads. *J Clin Microbiol* **45**: 3082–3085.
- Liang Y, Yang Z, Qin B, Zhong R (2014). Primary Sjögren's syndrome and malignancy risk: a systematic review and meta-analysis. *Ann Rheum Dis* **73**: 1151–1156.
- Lin Z, Bei J-X, Shen M *et al* (2011). A genome-wide association study in Han Chinese identifies new susceptibility loci for ankylosing spondylitis. *Nat Genet* **44**: 73–77.
- Lin X, Rui K, Deng J *et al* (2014). Th17 cells play a critical role in the development of experimental Sjögren's syndrome. *Ann Rheum Dis* **74**: 1302–1310.
- Lipsky PE (2001). Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. *Nat Immunol* **2**: 764–766.
- Liu LM, MacPherson GG (1995). Rat intestinal dendritic cells: immunostimulatory potency and phenotypic characterization. *Immunology* **85**: 88–93.
- Loss G, Depner M, Ulfman LH *et al* (2015). Consumption of unprocessed cow's milk protects infants from common respiratory infections. *J Allergy Clin Immunol* **135**: 56–62.
- Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R (2012). Diversity, stability and resilience of the human gut microbiota. *Nature* **489**: 220–230.
- Lu M-C, Jheng C-H, Tsai T-Y, Koo M, Lai N-S (2014). Increased dental visits in patients prior to diagnosis of primary Sjögren's syndrome: a population-based study in Taiwan. *Rheumatol Int* **34**: 1555–1561.
- Lugonja B, Yeo L, Milward M *et al* (2016). Periodontitis prevalence and serum antibody reactivity to periodontal bacteria in primary Sjögren's syndrome: a pilot study. *J Clin Periodontol* **43**: 26–33.
- Luzuriaga K, Sullivan JL (2011). Infectious mononucleosis. *N Engl J Med* **362**: 1993–2000.
- Mabbott NA, Donaldson DS, Ohno H, Williams IR, Mahajan A (2013). Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. *Mucosal Immunol* **6**: 666–677.
- Maccaferri S, Biagi E, Brigidi P (2011). Metagenomics: key to human gut microbiota. *Dig Dis* **29**: 525–530.
- MacFarlane TW, Mason DK (1974). Changes in the oral flora in Sjögren's syndrome. *J Clin Pathol* **27**: 416–419.
- MacGregor AJ, Snieder H, Rigby AS *et al* (2000). Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum* **43**: 30–37.
- Manichanh C, Borruel N, Casellas F, Guarner F (2012). The gut microbiota in IBD. *Nat Rev Gastroenterol Hepatol* **9**: 599–608.
- Mansson I, Colldahl H (1965). The intestinal flora in patients with bronchial asthma and rheumatoid arthritis. *Allergy* **20**: 94–104.
- Mantis NJ, Rol N, Corthésy B (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol* **4**: 603–611.
- Martinez-Martinez RE, Abud-Mendoza C, Patiño-Marin N, Rizo-Rodríguez JC, Little JW, Loyola-Rodríguez JP (2009). Detection of periodontal bacterial DNA in serum and synovial fluid in refractory rheumatoid arthritis patients. *J Clin Periodontol* **36**: 1004–1010.
- Martinez-Medina M, Denizot J, Dreux N *et al* (2014). Western diet induces dysbiosis with increased *E. coli* in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. *Gut* **63**: 116–124.
- Mathews SA, Kurien BT, Scofield RH (2008). Oral manifestations of Sjögren's syndrome. *J Dent Res* **87**: 308–318.
- McLean MH, Dieguez D, Miller LM, Young HA (2014). Does the microbiota play a role in the pathogenesis of autoimmune diseases? *Gut* **64**: 332–341.
- Moingeon P (2013). Update on immune mechanisms associated with sublingual immunotherapy: practical implications for the clinician. *J Allergy Clin Immunol Pract* **1**: 228–241.
- Montgomery AB, Kopec J, Shrestha L *et al* (2015). Crystal structure of *Porphyromonas gingivalis* peptidylarginine deiminase: implications for autoimmunity in rheumatoid arthritis. *Ann Rheum Dis* doi: 10.1136/annrheumdis-2015-207656.
- Moreira I, Teixeira F, Martins Silva A, Vasconcelos C, Farinha F, Santos E (2014). Frequent involvement of central nervous system in primary Sjögren syndrome. *Rheumatol Int* **35**: 289–294.
- Morgan XC, Tickle TL, Sokol H *et al* (2012). Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* **13**: R79.
- Mucida D, Park Y, Kim G *et al* (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* **317**: 256–260.
- Mukherjee S, Hooper LV (2014). Review antimicrobial defense of the intestine. *Immunity* **42**: 28–39.
- Nave H, Gebert A, Pabst R (2001). Morphology and immunology of the human palatine tonsil. *Anat Embryol (Berl)* **204**: 367–373.
- Nesse W, Dijkstra PU, Abbas F *et al* (2010). Increased prevalence of cardiovascular and autoimmune diseases in periodontitis patients: a cross-sectional study. *J Periodontol* **81**: 1622–1628.
- Nishimura M, Miyajima S, Okada N (2000). Salivary gland MALT lymphoma associated with *Helicobacter pylori* infection in a patient with Sjögren's syndrome. *J Dermatol* **27**: 450–452.
- Noble JA, Erlich HA (2012). Genetics of type 1 diabetes. *Cold Spring Harb Perspect Med* **2**: a007732.
- Okada Y, Wu D, Trynka G *et al* (2014). Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* **506**: 376–381.
- Olhagen B, Månsson I (1968). Intestinal *Clostridium perfringens* in rheumatoid arthritis and other collagen diseases. *Acta Med Scand* **184**: 395–402.
- Olsen NJ, Karp DR (2013). Autoantibodies and SLE—the threshold for disease. *Nat Rev Rheumatol* **10**: 181–186.
- Olszak T, An D, Zeissig S *et al* (2012). Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* **336**: 489–493.
- Petersen C, Round JL (2014). Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol* **16**: 1024–1033.

- Petrosino JF, Highlander S, Luna RA, Gibbs RA, Versalovic J (2009). Metagenomic pyrosequencing and microbial identification. *Clin Chem* **55**: 856–866.
- Pollard M, Sharon N (1970). Responses of the Peyer's patches in germ-free mice to antigenic stimulation. *Infect Immun* **2**: 96–100.
- Qin J, Li R, Raes J *et al* (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**: 59–65.
- Qin B, Wang J, Yang Z *et al* (2014). Epidemiology of primary Sjögren's syndrome: a systematic review and meta-analysis. *Ann Rheum Dis* **74**: 1983–1989.
- Qiu X, Zhang M, Yang X, Hong N, Yu C (2013). *Faecalibacterium prausnitzii* upregulates regulatory T cells and anti-inflammatory cytokines in treating TNBS-induced colitis. *J Crohns Colitis* **7**: e558–e568.
- Quiding M (1991). Intestinal immune responses in humans: oral cholera vaccination induces strong intestinal antibody responses and interferon- γ production and evokes immunological memory. *J Clin Invest* **88**: 143–148.
- Quirke A-M, Lugli EB, Wegner N *et al* (2014). Heightened immune response to autocitrullinated *Porphyromonas gingivalis* peptidylarginine deiminase: a potential mechanism for breaching immunologic tolerance in rheumatoid arthritis. *Ann Rheum Dis* **73**: 263–269.
- Raychaudhuri S, Sandor C, Stahl EA *et al* (2012). Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet* **44**: 291–296.
- Rey J, Garin N, Spertini F, Corthésy B (2004). Targeting of secretory IgA to Peyer's patch dendritic and T cells after transport by intestinal M cells. *J Immunol* **172**: 3026–3033.
- Riedler J, Braun-Fahrlander C, Eder W *et al* (2001). Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* **358**: 1129–1133.
- Rogers GB (2015). Germs and joints: the contribution of the human microbiome to rheumatoid arthritis. *Nat Med* **21**: 839–841.
- Rojo D, Hevia A, Bargiela R *et al* (2015). Ranking the impact of human health disorders on gut metabolism: systemic lupus erythematosus and obesity as study cases. *Sci Rep* **5**: 8310.
- Round JL, Mazmanian SK (2010). Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci USA* **107**: 12204–12209.
- Sakai A, Sugawara Y, Kuroishi T, Sasano T, Sugawara S (2008). Identification of IL-18 and Th17 cells in salivary glands of patients with Sjögren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. *J Immunol* **181**: 2898–2906.
- Schauber J, Svanholm C, Termén S *et al* (2003). Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways. *Gut* **52**: 735–741.
- Scher JU, Abramson SB (2011). The microbiome and rheumatoid arthritis. *Nat Rev Rheumatol* **7**: 569–578.
- Scher JU, Sczesnak A, Longman RS *et al* (2013). Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife* **2**: e01202.
- Scher JU, Bretz WA, Abramson SB (2014). Periodontal disease and subgingival microbiota as contributors for rheumatoid arthritis pathogenesis: modifiable risk factors? *Curr Opin Rheumatol* **26**: 424–429.
- Schuijs MJ, Willart MA, Vergote K *et al* (2015). Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. *Science* **349**: 1106–1110.
- Sender R, Fuchs S, Milo R (2016). Revised estimates for the number of human and bacteria cells in the body. *bioRxiv* doi: 10.1101/036103.
- Shapira Y, Agmon-Levin N, Shoenfeld Y (2010). Geoepidemiology of autoimmune rheumatic diseases. *Nat Rev Rheumatol* **6**: 468–476.
- Shiboski C, Shiboski S (2015). ACR-EULAR classification criteria for Sjögren's syndrome: development and validation. *Scand J Immunol* **81**: 330.
- Shiboski SC, Shiboski CH, Criswell LA *et al* (2012). American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance Cohort. *Arthritis Care Res (Hoboken)* **64**: 475–487.
- Shinozaki S, Moriyama M, Hayashida J-N *et al* (2012). Close association between oral *Candida* species and oral mucosal disorders in patients with xerostomia. *Oral Dis* **18**: 667–672.
- Silman AJ, MacGregor AJ, Thomson W *et al* (1993). Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br J Rheumatol* **32**: 903–907.
- de Smit M, Westra J, Vissink A, Doombos-van der Meer B, Brouwer E, Jan van Winkelhoff A (2012). Periodontitis in established rheumatoid arthritis patients: a cross-sectional clinical, microbiological and serological study. *Arthritis Res Ther* **14**: R222.
- Strachan DP (1989). Hay fever, hygiene, and household size. *BMJ* **299**: 1259–1260.
- Suzuki T (2013). Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci* **70**: 631–659.
- Szymula A, Rosenthal J, Szczerba BM, Bagavant H, Fu SM, Deshmukh US (2014). T cell epitope mimicry between Sjögren's syndrome antigen A (SSA)/Ro60 and oral, gut, skin and vaginal bacteria. *Clin Immunol* **152**: 1–9.
- Talham GL, Jiang H, Bos NA, Cebara J (1999). Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect Immun* **67**: 1992–2000.
- Thomas T, Gilbert J, Meyer F (2012). Metagenomics – a guide from sampling to data analysis. *Microb Inform Exp* **2**: 3.
- Tincani A, Andreoli L, Cavazzana I, Doria A, Favero M *et al* (2013). Novel aspects of Sjögren's syndrome in 2012. *BMC Med* **11**: 93.
- Triantafyllopoulou A, Moutsopoulos H (2007). Persistent viral infection in primary Sjögren's syndrome: review and perspectives. *Clin Rev Allergy Immunol* **32**: 210–214.
- Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI (2007). The human microbiome project. *Nature* **449**: 804–810.
- Turnbaugh PJ, Hamady M, Yatsunenko T *et al* (2009). A core gut microbiome in obese and lean twins. *Nature* **457**: 480–484.
- Vaishnava S, Yamamoto M, Severson KM *et al* (2011). The antibacterial lectin RegIII γ promotes the spatial segregation of microbiota and host in the intestine. *Science* **334**: 255–258.
- Van Praet JT, Donovan E, Vanassche I *et al* (2015). Commensal microbiota influence systemic autoimmune responses. *EMBO J* **34**: 466–475.
- Vartoukian SR, Adamowska A, Lawlor M, Moazzez R, Dewhirst FE, Wade WG (2016). In vitro cultivation of “unculturable” oral bacteria, facilitated by community culture and media supplementation with siderophores. *PLoS ONE* **11**: e0146926.
- Vitali C, Bombardieri S, Jonsson R *et al* (2002). Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* **61**: 554–558.

- Vossenkämper A, Blair PA, Safinia N *et al* (2013). A role for gut-associated lymphoid tissue in shaping the human B cell repertoire. *J Exp Med* **210**: 1665–1674.
- van der Waaij LA, Harmsen HJM, Madjipour M *et al* (2005). Bacterial population analysis of human colon and terminal ileum biopsies with 16S rRNA-based fluorescent probes: commensal bacteria live in suspension and have no direct contact with epithelial cells. *Inflamm Bowel Dis* **11**: 865–871.
- Wahren-Herlenius M, Dörner T (2013). Immunopathogenic mechanisms of systemic autoimmune disease. *Lancet* **382**: 819–831.
- Wen L, Ley RE, Volchkov PYV *et al* (2008). Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* **455**: 1109–1113.
- Whipps JM, Lewis K, Cooke RC (1988). Mycoparasitism and plant disease control. In: Burge MN, *Fungi in Biological Control Systems*. Manchester University Press, Manchester and New York, pp. 161–187.
- Wu HJ, Wu E (2012). The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* **3**: 4–14.
- Wu H, Ivanov II, Darce J *et al* (2010). Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* **32**: 815–827.
- Yarza P, Yilmaz P, Pruesse E *et al* (2014). Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* **12**: 635–645.
- Yatsunenko T, Rey FE, Manary MJ *et al* (2012). Human gut microbiome viewed across age and geography. *Nature* **486**: 222–227.
- Yurasov S, Hammersen J, Tiller T, Makoto T, Wardemann H (2005). B-cell tolerance checkpoints in healthy humans and patients with systemic lupus erythematosus. *Ann N Y Acad Sci* **1062**: 165–174.
- Zarco MF, Vess TJ, Ginsburg GS (2012). The oral microbiome in health and disease and the potential impact on personalized dental medicine. *Oral Dis* **18**: 109–120.
- Zaura E, Keijser BJJ, Huse SM, Crielaard W (2009). Defining the healthy “core microbiome” of oral microbial communities. *BMC Microbiol* **9**: 259.
- Zaura E, Brandt BW, Teixeira de Mattos MJ *et al* (2015). Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. *MBio* **6**: e01693-15.
- Zhang H, Liao X, Sparks JB, Luo XM (2014). Dynamics of gut microbiota in autoimmune lupus. *Appl Environ Microbiol* **80**: 7551–7560.
- Zhang J, Guo Z, Xue Z *et al* (2015a). A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *ISME J* **9**: 1979–1990.
- Zhang X, Zhang D, Jia H *et al* (2015b). The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med* **21**: 895–905.
- Zheng W, Zhang Z, Liu C *et al* (2015). Metagenomic sequencing reveals altered metabolic pathways in the oral microbiota of sailors during a long sea voyage. *Sci Rep* **5**: 9131.