The microbiota and autoimmunity: their role in thyroid autoimmune diseases

Hedda L. Köhling[1&2], Sue F. Plummer[2], Julian R. Marchesi[3&6], Kelly S. Davidge[4] and Marian Ludgate[5]

[1] University Hopital Essen, Institute of Medical Microbiology, Essen, Germany

[2] Cultech Ltd., Baglan, Port Talbot, United Kingdom

[3] School of Biosciences, Cardiff University, Cardiff, United Kingdom

[4] Kirkstall Ltd., Templeborough, Rotherham, United Kingdom

[5] Division of Infection & Immunity, School of Medicine, Cardiff University, Cardiff, United Kingdom.

[6] Centre for Digestive and Gut Health, Imperial College London, London, W2 1NY

Corresponding Author:

Hedda Luise Köhling, MD

University Hospital Essen

Institute of Medical Microbiology

Virchowstr. 179

45147 Essen

Germany

Phone: 0049 201 723 85429

Email: koehling.hedda-luise@uk-essen.de

Abstract

Since the 1970s, the role of infectious diseases in the pathogenesis of Graves' disease (GD) has been an object of intensive research. The last decade has witnessed many studies on *Yersinia enterocolitica*, *Helicobacter pylori* and other bacterial organisms and their potential impact on GD. Retrospective, prospective and molecular binding studies have been performed with contrary outcomes. Until now it is not clear whether bacterial infections can trigger autoimmune thyroid disease. Common risk factors for GD (gender, smoking, stress, and pregnancy) reveal profound changes in the bacterial communities of the gut compared to that of healthy controls but a pathogenetic link between GD and dysbiosis has not yet been fully elucidated. Conventional bacterial culture, *in vitro* models, next generation and high-throughput DNA sequencing are applicable methods to assess the impact of bacteria in disease onset and development. Further studies on the involvement of bacteria in GD are needed and may contribute to the understanding of pathogenetic processes. This review will examine available evidence on the subject.

Keywords: Bacteria, Graves' disease, Hashimotos's thyroiditis

Abbreviations

AITD	autoimmune thyroid disease
Anti-Tg	Antithyroglobulin
CagA	Cytotoxin-associated gene A
CD	Celiac disease
DES	Dry eye syndrome
ELISA	Enzyme-linked immunosorbent assay
GD	Graves' disease
GF	Germ free
GO	Graves' orbitopathy
HAT	Hashimoto thyroiditis
HLA	Human leukocyte antigen
HP	*Helicobacter pylori*
HPA	Hypothalamic-pituitary-adrenal axis
HUVEC	Human umbilical vein endothelial cells
IBD	Inflammatory bowel disease
IFAA	Immunofluorescent antibody assay
NOD	Non-obese diabetic
PBMC	Peripheral blood mononuclear cells
PM	Pretibial myxedema
RA	Rheumatoid arthritis
SCFA	Small chain fatty acid
SHIME	Simulator of human intestinal microbial ecosystem

69	SPF	Specific pathogen free
70	T1D	Type 1 diabetes
71	TGF-β	Transforming growth factor beta
72	TIM	TNO (gastro-) intestinal model
73	TPO	Thyroperoxidase
74	Tregs	Regulatory T cells
75	TSAb	TSH receptor stimulating antibodies
76	TSBAb	TSH-stimulation blocking antibody
77	TSHR	TSH receptor
78	rlUBT	Radiolabeled urea breath test
79	WB	Western blot
80	YE	*Yersinia enterocolitica*
81		

1. Introduction

Bacteria and bacterial antigens have long been considered as possible culprits in inducing autoimmune disease. Using the example of rheumatic fever, the link between bacteria and disease was established at the end of the 19th century by Triboulet and Coyon [1] and several decades later, experimental and clinical data indicated that autoimmunity in rheumatic fever is induced in response to group A streptococci [2]. Current mechanisms suggested to lead to autoimmune processes after a bacterial infection include molecular mimicry, epitope spreading, bystander activation and cryptic antigens [3].

In autoimmune thyroid diseases (AITD), especially Hashimoto thyroiditis (HT) and Graves' disease (GD), evidence for possible bacterial involvement in onset and progression is based solely on retrospective measures of bacterial antibodies in AITD patients (Table 1). These include the bacteria *Yersinia enterocolitica, Helicobacter pylori* and *Borrelia burgdorferi* (Figure 1). As in rheumatic fever, several tissues are targeted by the autoimmune response in GD (mainly thyroid, but also adipose tissue, skin and bone) and the whole body is affected by the hyperthyroid state. There has been limited examination of the possible connection between AITD and the microbiota using only serological methods. Other methods such as *in vitro* models, 16S rRNA gene sequencing, next generation sequencing and high throughput sequencing have already been applied to investigate the role of bacteria in other autoimmune diseases, but not in AITD. These platforms allow characterization of the microbiota from AITD affected areas (eyes) or areas adjacent to them (nose, mouth, skin) and contributing factors such as genetic predisposition and environmental factors associated with AITD. The role of microorganisms in the development of AITD is still controversial and not fully elucidated. An understanding of the precise mechanisms of interaction between

bacterial agents in inducing thyroid autoimmunity could result in the development of new strategies for prevention and treatment.

This review aims to summarize current knowledge on the role of the microbiota in thyroid autoimmunity and will focus on the bacterial component.

2. Autoimmune thyroid disease

Autoimmune disorders are a broad range of related diseases in which inappropriate immune responses of the body arise against its own cells, tissue and organs, resulting in inflammation and damage. This response may affect only a particular tissue/organ of the body (such as in autoimmune thyroiditis) or may be systemic (such as systemic lupus erythematosus). In autoimmunity, the balance between proinflammatory and regulatory mechanisms, as a requirement for sufficient tolerance of the body against its own cells, is no longer maintained. Autoimmune reactions are characterized by the appearance of autoreactive B and T cells, which can be activated via other cells and which are very specific. Several different AITDs exist, but Hashimoto´s thyroiditis (HT) and Graves' disease (GD) are the most prevalent [4]. Approximately 5% of the population is affected with HT and the disease is usually diagnosed in the fourth to sixth decade of life [5]. Graves' disease is the underlying cause of 50 to 80% of hyperthyroidism and affects approximately 0.5% of the population [6, 7]. The incidence of GD is around 0.5 per 1000 annually in England [8] and 0.77 per 1000 in women and 0.14 per 1000 in men, respectively, in Scotland [9]. AITDs are the most common organ-specific autoimmune diseases and affect more women than men, with a female-to-male ratio from 5 to 10 [10].

Hashimoto's thyroiditis, also known as autoimmune or chronic lymphocytic thyroiditis, is characterized by infiltration of the thyroid gland by inflammatory cells, subsequent atrophy of the thyroid tissue [11] and production of antithyroid antibodies, especially against thyroperoxidase (anti-TPO), antithyroglobulin (anti-Tg) and TSH-stimulation blocking antibody (TSBAb, although these are rare). The destruction and fibrous replacement of the follicle cells lead to hypothyroidism. HT is significantly more frequent in individuals suffering concurrently from other autoimmune diseases like type 1diabetes (T1D) or rheumatoid arthritis.

In 1840 Carl-Adolph von Basedow termed the three typical clinical features in Graves' disease (tachycardia, proptosis, and goiter) as the "Merseburg trias". These symptoms are due to activated thyroid autoreactive CD4+ T cells that infiltrate the thyroid and activate B cells. The latter secrete TSH receptor (TSHR) stimulating antibodies (TSAb), which in turn induce thyrocyte proliferation and secretion of excess thyroid hormones and lead to hyperthyroidism. The autoimmune response, probably to the TSHR, leads to Graves' orbitopathy (GO) which is characterized by proptosis or bulging eyes, also known as exophthalmos [12].

The pathogenesis of AITDs is multifactorial including genetic predisposition for GD (Human Leukocyte Antigen (HLA) Class I molecules C*07 and B*08 as well as HLA Class II molecules DR3 and DRB1*08 [13], CD40, CTLA-4, PTPN22, FCRL3, thyroglobulin and TSHR, reviewed in [14]), pregnancy [4] and a variety of environmental factors (iodine and selenium intake, smoking, acute psychological stress [15-19] (Figure 2). Medication can also influence disease prevalence (amiodarone, certain monoclonal antibodies, interferon alpha, and cytokines). As autoimmune disorders tend to co-exist in the same subjects, celiac disease (CD) is associated positively with orbitopathy in GD patients [20]. In both CD and GO/GD T-

helper cells 17 (Th17) play a role in pathogenesis [21-24]. Also, the cytokine IL-15 which is involved in the differentiation of Th17 cells and links innate and adaptive immune systems is increased in the sera of Hashimoto thyroiditis (HAT) patients and was detectable in 33% of thyroid-associated ophthalmopathy (another name for GO) biopsies from extraocular muscles [25, 26].

Characteristic for AITD are a Th1 pattern of immune response in HT and a predominance of T-helper cells 2 cytokines in GD, indicating a humoral pattern of immune reaction for the latter disease [27]. Furthermore follicular helper T (Tfh) cells - a relatively new subset of antigen-experienced CD4+ T cells found in B cell follicles of secondary lymphoid organs and serving as regulators in the evolution of effector and memory B-cell responses - are found to have an increased frequency in AITD. Therefore, this cell subset might also be important in the pathogenesis of AITD [28].

3. Function of commensal bacteria

In AITD, the major body sites involved, apart from the thyroid, are the eyes and the skin, but nasal and oral microbiota might also be important for GO, considering its proximity to the orbit. Likewise, the gut as the most important reservoir of bacterial metabolism for the host and as the site with the highest numbers of immune cells is discussed in the following. The bacterial communities in these sites may have potential impact on AITD and to assess this, a precise characterization of the bacterial diversity and metabolic profile of commensal bacteria from healthy persons is needed.

Due to its high vascularity, good lymphatic drainage, encapsulated position and its generation of hydrogen peroxide for the synthesis of thyroid hormone, the thyroid is remarkably resistant to infection and is rarely infected [29]. Under healthy

circumstances, the thyroid should be sterile. Apart from bacterial assessment in suppurative or non-suppurative infection, the authors are unaware of any analysis of bacteria carried out in thyroid tissue from healthy persons or AITD patients [30, 31].

When assessing thyroid disease with ocular complications it is logical to focus mainly on the microbiota of the eye. However, there is neither an agreement about a naturally existing resident microbiota on the healthy ocular surface nor about the role resident microbiota may play at this site in ocular surface physiology [32]. Because of the high antimicrobial properties of the ocular surface, the bacterial abundance is innately low and organisms found, although normally classified as commensals, may play a more important role than in other sample sites with less bacterial abundance.

The skin is the largest sensory organ of the body and harbors around 113 different bacterial phylotypes and the predominant microbiota on the skin was shown to correlate with lipid content, pH, sweat and sebum secretion [33]. The skin is predestined for complex ecological interactions with the environment and the skin microbiota perform several functions: i) inhibition of pathogen growth through antimicrobial peptides (bacteriocins (reviewed in [34]), microcins (reviewed in [35]) and phenol soluble modulins [36], ii) degradation of proteins associated with *Staphylococcus aureus* biofilm formation [37] and iii) decrease of the skin pH due to hydrolysis of sebum triglycerides by bacterial lipases and esterases [38-40]. The acidic *milieu* is unfavorable for many pathogens like *S. aureus* and *Streptococcus pyogenes* and thus the growth of coagulase-negative staphylococci and corynebacteria is supported [39, 41-43]. Furthermore, commensal bacteria tune the local cytokine production and influence regulatory T cells in the epidermis as well as mast cells [44-47].

Despite the close proximity to the skin, the nasal cavity is populated differently and is one of the main reservoirs for *S. aureus*, a commensal organism carried by 20–30% of humans. Colonization is a risk factor for nosocomial infections with this bacterium [48].

Gut commensals contribute to the host's well-being in several ways. The microbiota influences both the innate and adaptive immune system by interacting with pattern-recognition receptors such as the toll-like receptors (TLR) which are expressed on cells present in the gut wall, in particular the resident immune cells in the gut-associated lymphoid tissue (GALT). Microbial products activate the TLR and trigger the release of pro-inflammatory (TNFα, IL1 or IL6), anti- inflammatory (IL10) cytokines, or those which determine T lymphocyte phenotypes (IL17, IL23) [49, 50]. Commensal bacteria are able to actively induce regulatory responses in the gut epithelium. Regulatory T cells (Tregs), a subpopulation of T cells which maintain tolerance to self-antigens and prevent inflammatory and allergic responses, are induced via direct sensing of microbial organisms and their metabolites by dendritic cells or T cells. The luminal concentration of the bacterial metabolite butyrate positively correlates with the number of Treg cells in the colon [51] and besides other organisms, *Clostridium* spp. is able to create a transforming growth factor beta (TGF-β) rich environment and this supports Treg cell accumulation [52].They also perform a number of metabolic functions including food processing, digestion, and the synthesis of different products, e.g. vitamin B12 and short chain fatty acids (SCFA) as a main product of their metabolism [53]. SCFAs serve as an energy source for epithelial cells [54, 55], accelerate colonic transit through stimulation of the gut motility via serotonin [56, 57] and simultaneously regulate the sympathetic nervous system activity directly via the Gi/o protein-coupled receptor GPR41 at the level of the sympathetic ganglion [58]. Among the SCFAs, butyrate in particular modulates immunity and exerts an anti-inflammatory effect. This modulation is due to several effects including butyrate mediated reduction of nuclear

229 factor-κB and inhibition of histone deacetylase (HDAC) [59, 60]. HDAC prevents gene
230 transcription by keeping chromatin in a compact form and its inhibition by SCFAs alters
231 colonic gene expression and metabolic regulation [61]. Moreover, butyrate induces
232 regulatory T cells in the colonic environment [51, 62, 63].

233 The gut microbiota also protects the indigenous bacterial community against invasion
234 by new and potentially harmful bacteria (colonization resistance) [64-68]. In this context
235 secreted IgA may play a role via a process termed 'antibody mediated
236 immunoselection' (AMIS) which shapes the composition of the microbiota. It has been
237 suggested that AMIS could be exploited by using antibodies to manipulate the
238 microbiota and treat conditions caused by dysbiosis [69].

239 The gut microbiota impacts the central and enteric nervous systems [70] e.g. by
240 producing neurotransmitters such as gamma-aminobutyric acid, serotonin, dopamine,
241 noradrenaline and acetylcholine [71-75]. In turn neurotransmitters produced by the
242 host can directly influence the composition of the gut microbiota [76-78], which may be
243 relevant to the significantly higher levels of anxiety and depression reported in GD
244 patients compared with those with goiter [79].

245 It is logical that the gut microbiota will influence autoimmune conditions such as CD
246 [80-82] and inflammatory bowel disease [83-89] and indeed Di Giacinto and colleagues
247 suggested an amelioration of colitis severity using probiotic bacteria which induced an
248 immunoregulatory response involving TGF-beta-bearing regulatory cells [90]. In their
249 large multicenter study of new-onset pediatric CD, using samples from different sites,
250 Gevers and colleagues observed a correlation of specific bacteria with disease status
251 and especially the distinct microbial signature of the rectum at the disease onset offers
252 unique potential for early diagnosis [91]. In CD, Tregs are induced by gliadin in situ
253 [92]. Perhaps more surprising are the reports illustrating an impact of the gut microbiota

254 on autoimmune diseases targeting more distant sites e.g. T1D [93-99], rheumatoid
255 arthritis [100-103] and the in vivo model of multiple sclerosis [104-106]. In the NOD
256 mouse model the incidence of disease is maximal in the germ-free (GF) population
257 whereas specific pathogen free mice are protected [96, 107]. The protective microbiota
258 can be transferred from dam to pup [108] and the normally high T1D female-to-male
259 sex bias in specific pathogen free (SPF) NOD mice can be equalized through fecal
260 microbiota transplantation from male to female mice and its corresponding effects on
261 testosterone levels [109, 110]. In the development of T1D in infants, gut microbiome
262 analysis reveals a decrease in diversity once specific T1D autoantibodies were
263 detectable but before the clinical onset of disease. This is accompanied by signs of
264 intestinal inflammation through increased fecal human β-defensin 2 levels [111].
265 Interestingly, Vatanen and colleagues detected a connection between *Bacteroides*
266 species-rich microbiota and simultaneously high T1D susceptibility in a human
267 population potentially arising from a distinct microbiota-derived type of
268 lipopolysaccharide with immunoinhibitory properties [112]. For a more general review
269 on autoimmune-microbiota interactions the reader is referred to the following
270 references [113, 114].

271 When compared to the human gut and other body sites, the oral cavity ranks second
272 in total microbial load [115] and each bacterial species occupies highly specific niches
273 differing in both anatomic location (such as the lips, cheek, palate, periodontal cavity
274 and tongue) and nutrient availability [116]. The oral microbiota is regularly transferred
275 to adjacent habitats via saliva, although only 29 out of 500 microbial taxa recovered
276 from the mouth are cultivated from faecal samples [117]. Besides breaking down
277 nutrients, the function of the oral microbiota is to modify the local pH or redox potential,
278 the formation of biofilms and quorum sensing to coordinate these biofilms and gene
279 expression [118].

4. Techniques

This section discusses the methods used to assess the impact of bacteria in the onset and aggravation of autoimmune thyroid disease. For articles on mouse models (Banga and colleagues reported a model of GO based on genetic immunization using human TSHR A-subunit plasmid and close field electroporation. Induction of prolonged functional antibodies to the TSHR results in chronic disease with progression to GO-like disease [119]) and segmented filamentous bacteria in thyroid autoimmunity please read elsewhere [120-123].

4.1 Culture-dependent techniques

4.1.1 Conventional culture

Until now, it has not been possible to cultivate and isolate bacteria directly from patients with AITD either from blood or tissue. However, culturing fecal microbial communities from healthy donors, under strict anaerobic conditions, enables capture of a remarkable proportion of the gut microbiota and preserves the distinctiveness of each donor`s microbiota [124]. These efforts resulted in the discovery of new taxa [125] and far more genetic potential to form spores than previously assumed [126]. Toft and co-workers screened 107 fecal samples of GD patients for *Y. enterocolitica* (YE), but did not find an increased prevalence of YE. The isolation rate was very low (<1%) and similar to that observed in the local population with diarrheal illness [127]. A reliable animal model for GD/GO that reproduces all the aspects of the disorder has not been available, but very recently, Banga and colleagues reported a new mouse model of GO based upon immunogenic presentation of human TSHR A subunit plasmid by close field electroporation. Induction of prolonged functional antibodies to the TSHR results

in chronic disease with progression to GO [119]. In patients with dry eye syndrome (DES), a condition resulting from GO [128], Graham and colleagues performed a comparison of the bacterial community of the ocular surface in DES patients using conventional culture techniques and 16S rRNA gene PCR [129]. Coagulase negative staphylococci were found in both patients and controls, with an increase in culture positivity and mean numbers of bacteria in dry eye. The amount of identified bacterial genera and species was extended with molecular methods including potentially pathogenic bacteria such as *Klebsiella* spp. and repeated sampling and testing of a subset of patients revealed similar results.

4.2 Models (in *vitro)*

Using the example of intestinal autoimmune diseases like CD or inflammatory bowel disease (IBD) and other autoimmune diseases like primary biliary cirrhosis [130] the use of *in vitro* models in GD can generate knowledge and better understanding of the disease although all of the models have their limitations and thus do not always correlate in detail with pathophysiological conditions in a human body.

4.2.1 Cell lines

Monolayers of intestinal cell lines are composed of a single cell type and lack the variety found in the intestine, e.g. goblet cells and paneth cells and their crosstalk with other cells of the body. Nevertheless, Caco-2 cells are widely used to study CD and IBD and increase understanding of pathogenesis. In CD, Caco-2 cells exposed to gliadin proliferate, display actin rearrangements and inhibition of spontaneous differentiation [131]. In addition they have demonstrated how 1) patients' serum antibodies modulate the epithelium and 2) bifidobacteria inhibit the toxic gliadin effects [132, 133]. Other intestinal cell lines e.g. T84 and HT29 express IBD related cell

surface molecules (CD40) after treatment with cytokines [134]. Caco-2 cells have been used to investigate the beneficial effect of different commensal gut bacteria on anti-inflammatory G protein–coupled receptors expressed by intestinal cells [135] and also probiotics were co-cultured with this cell line: Mattar and co-workers showed that *Lactobacillus casei* up-regulates mucin gene expression [136].

The combination of Caco-2 cells in a transwell system with dendritic cells or THP-1 (a human monocytic cell line derived from an acute monocytic leukemia patient) provides a more physiological setting. It allows measurements of cytokine production and tight junction protein expression in response to commensal or pathogenic bacteria as well as CD triggering gliadin [137-139].

Patient-derived T-cell lines and clones from the site of inflammation or peripheral blood are widely used in CD and provide information on T cell activation in the lamina propria to specific antigens and T cell infiltration into the intestinal epithelium [140-142]. In GD, Roura-Mir and colleagues analysed lymphocytes from peripheral blood and thyroid lesions ex vivo to investigate the role of CD1-restricted T cells, which are able to present self and foreign lipid antigens to T cells. They suggested a possible effector function of CD1-restricted T cells in tissue destruction [143]. In a cell proliferation assay with peripheral blood mononuclear cells (PBMCs) cellular reactivity to Yersinia outer membrane proteins (YOP) encoded by a 72-kilobase virulence plasmid of YE was present in GD patients and controls whereas intrathyroidal lymphocytes obtained from GD patients demonstrated marked proliferation in response to the released proteins [144].

4.2.2. Mucosal biopsy organ culture

Culturing mucosal biopsies, with their histological architecture intact, allows in vivo processes to be studied in controllable conditions outside the body (ex vivo). In the past, Ussing chambers have been widely used to monitor net ion transport across living epithelium in mice and humans [145, 146]. Another approach is to study intestinal biopsies of IBD patients, in which apical to basolateral polarity is maintained by a "glued cave cylinder" to facilitate stimulation of each border in turn [147]. In CD, the importance of IL-15 was demonstrated in a culture of duodenal biopsy [148], whilst an organ culture demonstrated an impaired mucosal immune response to gliadin in T1D [149]. Ogino and colleagues showed that in Crohn's disease, CD14(+) CD163(low) cells, from the intestinal lamina propria of patients, induce the differentiation of naive T cells into Th17 cells and by doing this contribute to the pathogenesis of CD and possibly other Th17-associated diseases [150].

4.2.3 Flow models

Chambers of flow systems allow co-culture of different cell types in separate chambers; proteins and signals produced by one cell type can flow through the system to have an effect on another cell type, as would happen in the body. To date perfusion flow studies have not been applied to autoimmune disease, but given their utility it is only a matter of time until they are used to study AITD.

However, there are many examples of the use of flow in the culture of different mammalian cells [151-153].

4.2.4 3D cell culture systems

In addition to the use of flow to better mimic conditions *in vivo*, there is also a growing trend moving away from culture on 2D surfaces and into 3D scaffolds and gels. In a model for *H. pylori* infection, primary gastric glands were grown in Matrigel as a 3D

spheroid; morphological features of typical stomach tissue were evident and spheroids survived for greater than 9 months [154]. Collagen gels have been used for the 3D co-culture of rat intestinal sub-epithelial myofibroblasts with a rat intestinal epithelial cell line [155]. A simple 3D co-culture model of the gut used non-transformed human neonatal small intestinal cells and non-transformed human monocyte/macrophages for the study of the interaction of *Lactobacillus* spp. with the gut [156].

4.2.5 Intestinal models

To rebuild the intestine in a larger format than those mentioned above, *in vitro* models can include short-term batch incubators, single stage reactors through to multistage continuous systems and their evolutions (simulator of human intestinal microbial ecosystem (SHIME), EnteroMix, TNO (gastro-) Intestinal Models (TIM) and PolyfermS) have been developed [157-162]. The more complex models can mimic the microbiota and their fermentation processes in different parts of the human gut and enable evaluation of a wide range of environmental regulators of bacterial activity like substrate availability, pH and growth rates. Advantages of these systems are the lack of ethical issues surrounding sampling the human gastrointestinal tract and surrounding the use of radioactive or toxic substances. Running multi-compartment continuous systems is relatively inexpensive and microbial community development in dynamic models after inoculation with faecal microbiota is reproducible [159]; this holds true even for faecal samples of persons with high/low conversion rates of organic materials into energy sources by bacteria [163]. Obvious disadvantages of the intestinal models are the lack of physiological host environment with epithelial cells, immune cells and mucus. To help counteract this, MacFarlane and colleagues added mucin to their model [164] and combined dynamic models with cell culture systems by adding fermentor vessel effluent onto Caco-2 cells [165]. Other groups investigated

401 the effects of the culture effluent on immune cells in the macrophage cell line U937
402 [166]. Besides inoculation with healthy adult faeces diluted with phosphate buffered
403 saline, Cinquin and co-workers used immobilized infant faeces on gel beads [167].
404 Other authors inoculated their gut models with faecal samples derived from IBD
405 patients or from healthy individuals resulting in an increased production of toxic
406 metabolites by IBD microbiota [83]. Also shortened transit time, which is common in
407 irritable bowel syndrome, has been investigated [168]. By mimicking an overgrowth
408 with *Clostridium difficile* after antibiotic treatment, van Nuenen et al. observed a two-
409 fold increase of toxic proteolytic metabolites which could be neutralized by the addition
410 of different inulins, a group of naturally occurring plant polysaccharides and a
411 functional food that stimulates the growth of healthy bacteria (= prebiotics) [169].
412 Probiotics, prebiotics, their synergistic effects and other dietary components have been
413 studied in intestinal models with the aim of increasing the levels of beneficial microbes
414 [170-174]. In the SHIME model, van den Abbeele and colleagues incorporated mucin-
415 covered, simplified ecosystems (microcosms) and assessed the long term colonization
416 of lactobacilli and their stability under antibiotic treatment with tetracycline, amoxicillin
417 and ciprofloxacin [175]. More recent developments for intestinal models include the
418 Host-Microbiota Interaction module for long-term incubation [176] and the “gut-on-a-
419 chip” [177].

420 Intestinal models enable the user to perform mechanistic studies *in vitro* and to develop
421 hypotheses. Nonetheless, intestinal models will always require validation *in vivo* due
422 to the complexity of host-associated environments.

423

424 4.3 Culture-independent techniques

4.3.1 Antibody and antigen detection

Serological tests like agglutination, enzyme linked immunosorbent assay (ELISA) and Western blot (WB) as well as antigen detection tests in serum and stool or in the form of radio-labelled urea breath test for *H. pylori* detection have been used to explore the possible link between AITD and bacteria by measuring antibodies against bacterial antigens which could induce cross-reactive immune response against self-antigens. Besides genetic predisposition, 25% of the predisposition to Graves' disease is estimated to be linked to environmental factors like infections [178]. Since the 1970s and until more recently, infections with the bacterium YE have been implicated in the pathogenesis of GD caused by increased YE antibody prevalence in GD patients [179-182], but this was not reproduced by all groups [183-185] (see Table 1 and Figure 1). Also prospective studies on this field were undertaken with different outcomes: a case-control twin study and two studies in euthyroid females related to AITD patients with/without follow up revealed no causal relationship between YE infection and autoimmune thyroid disease [184-186]. However, in earlier studies with similar design a higher prevalence of antibodies against YOP was measured [187, 188]. A linear correlation between YE antibodies and antibodies against TSHR, thyroglobulin and thyroid-peroxidase has been described [189]. YE antigens not only display high-affinity binding sites for the hormone TSH and the TSHR Abs from patients with Graves' disease, but also show a sequence homology between its outer membrane porins (Omp) [190, 191] and the TSHR. In their study with mice, Luo and colleagues produced antibodies against the purified extracellular domain of human TSHR and showed that anti-TSHR antibodies reacted with the envelope antigens of YE. When mice were immunized with YE, anti-TSHR-antibodies were induced [192], supporting the concept of molecular mimicry. Hargreaves and co-workers demonstrated that a recombinant

Fab germline fragment of a monoclonal TSAb from GD mice doesn´t recognize TSHR, but does bind YE outer membrane porins [193].

The impact of antibodies to *Helicobater pylori* (HP) on GD was first observed in 1999 [194]. Similar to YE, some groups were able to show a significant increase in HP antibody prevalence and some not [195-200]. In the case of no significant difference in anti-HP-IgG a significant association between AITD and cytotoxin-associated gene A (CagA)-antibodies and between GD and CagA-antibodies was observed [200]. Interestingly, Bertalot et al. screened patients after HP eradication and found a reduction in the anti-thyroid peroxidase titre, in anti-thyroglobulin and a partially normalized anti-TSHR titre [201].

Besides these two organisms, *Borrelia burgdorferi* and the neurotoxin of *Clostridium botulinum* have also been implicated in the context of GD suggesting that antigens cross-reacting with human TSHR share multiple antigenic epitopes with other bacterial antigens [202-205].

Glycoproteins of the probiotic bacterium *Bifidobacterium bifidum* were shown to have an immunological similarity with thyroid peroxidase and thyroglobulin, pointing towards a possible role in the pathogenesis of AITD [206]. Nevertheless, several years earlier, Zhou and colleagues ruled out the induction of pathological inflammation in a mouse model of experimental autoimmune thyroiditis due to a bacterium of the same genus, namely *Bifidobacterium lactis* [207].

Viruses and their role in AITD have also been discussed and corresponding nucleic acid has been detected via PCR-based methods and immunochemistry [30, 31, 208], but viruses are beyond the scope of this review.

Overall, a large number of studies showed epidemiological, serological and molecular evidence that YE and other bacteria are potentially important in the pathogenesis of AITD and GD. None of the studies showed a direct correlation of bacterial infection to the development of AITD and most patients with one of the above mentioned bacterial infections (including those who produce anti-TSHR antibodies) do not develop GD [209]. It might be possible that the ability to produce anti-TSHR antibodies in response to YE antigens homologous to the TSHR persist only in susceptible individuals with the YE antigens acting as a trigger to the disease development. Further studies are needed to get a definite answer.

4.3.2 A possible link between microbiota and autoimmune thyroid

Over the last decade, sophisticated sequencing techniques and high throughput technologies have become affordable and allowed both characterizations of the microbes living in and on the human host as well as their metabolic functionality. The Human Microbiome Project elucidated the structure and diversity of the healthy human microbiome at almost 20 different body sites and by doing this created a large reference database [210].

Alterations in the gut microbiota have already been observed for many diseases ranging from diabetes, alcoholic liver disease and psychiatric disorders to cancer and autoimmune diseases. Numerous studies have been performed within the autoimmune sector regarding the gastrointestinal tract, joints and the neural system, but very little is available for AITD, the most frequent of the autoimmune diseases. Studies examining the microbiome of AITD patients and especially with GD/GO are not available, but many microbiome studies investigating the impact of known risk factors such as genetic risk factors (gender) and environmental risk factors (smoking, stress) have been undertaken (see Figure 2). Also studies on body sites actually or possibly

involved in GD/GO like eye, nose, throat and intestine have been performed and all are addressed in this section.

Risk factors to consider

1. Gender and genetics

   The unequal gender distribution in autoimmune disease has been covered in many publications, but only a few tried to characterize the gut microbiome of females and males to look for differences. Flow cytometry-based *in situ* hybridizations revealed higher levels in *Bacteroides* and *Prevotella* in males than in females with autoimmune disease, but no gender effects could be observed for any other bacteria [211]. Markle et al. entered the topic more deeply with the help of NOD mice [109]. Normally, the incidence of T1D is higher in female NOD SPF mice than in male. Whilst in germ free (GF) mice the incidence is equal between the two genders. Serum testosterone levels were higher in female GF mice than in SPF and higher in male SPF mice than in GF suggesting that colonization by commensal microbes elevates testosterone levels in males and may protect NOD males from developing T1D. Transplantation of the male microbiota to females resulted in altered recipient´s microbiota and consequently elevated testosterone levels and changes in metabolite production. Furthermore, the T1D diagnostic parameters islet inflammation and autoantibody production were decreased. Yurkovetskiy and colleagues obtained similar results in the same NOD mouse model before and after puberty: their 16S rRNA gene profiles indicate that the gut microbial communities depend on the gender of post-pubescent mice. After castration, female and castrated male microbiota are more similar to each other than to

non-castrated male microbiota. The microbiota differs in males and females after GF mice have been colonized with a female SPF microbiota [110].

In a large study with more than 400 twin pairs, Goodrich and colleagues characterized the gut microbiomes of monozygotic twins and found them to be more similar than those of dizygotic twins [212], Christensenellaceae belonging to the Firmicutes was the taxon with the highest heritability. Evidence from mice also suggests that the genetics of the host strongly influences the microbiome of the gastro-intestinal tract [213] and has also shown that variation in the microbiome influences disease outcomes, e.g. the occurrence of T1D in non-obese diabetic (NOD) mice or the induction of experimental autoimmune encephalomyelitis [106, 108].

The intestine is the largest immune organ in the body and is comprised of trillions of commensal organisms and is affected by treatment (antibiotics, corticosteroids) [214, 215] and diets among others.

Furthermore, genetic investigations demonstrated a connection between CD and GD [216, 217] and also T1D organ culture studies indicate an unbalanced mucosal immune response to gliadin [149].

2. Pregnancy

The prevalence of GD in pregnancy is rare and ranges between 0.1% and 1% [218]. In pregnancy, the gut microbiome changes each trimester [219] and

pregnant women have increased total bacteria and *Staphylococcus* numbers which seems to be related to increased plasma cholesterol levels. The mothers' body weights also seems to be of importance because reduced numbers of some anaerobes (*Bifidobacterium* and *Bacteroides*) and increased numbers of other anaerobic bacteria (*Staphylococcus*, *Enterobacteriaceae* and *Escherichia coli*) were detected in overweight compared to normal-weight pregnant women [220]. In pregnant mice it has been shown recently that changes in the maternal gut microbiota are dependent upon the mother's periconceptional diet but not upon increases in maternal weight gain during pregnancy [221].

3. Smoking

Smoking alters the oropharyngeal and tracheal environment in smokers compared to non-smokers, but in 2013 Biedermann and co-workers suggested an effect of smoking also on the gut microbiota [222, 223]. The group found an increase of *Firmicutes* and *Actinobacteria* and a non-significant decrease of *Bacteroides* and *Proteobacteria* with simultaneous increase in microbial diversity after smoking cessation. *Bacteroidetes* seems to be the only phylum with a significant change only after 4 weeks of smoking cessation maintained through to eight weeks. Principal component analysis separated the bacterial community composition of the smoking cessation group clearly from the control group, particularly between before and after smoking cessation.

4. Stress, anxiety

As discussed in an earlier section, stress can modify the microbiota composition and vice versa [68, 70, 77-79].

Body sites to consider

1. Nose

Despite the close proximity of the nose to the eyes, the role of the nasal microbiota in the pathogenesis of AITD has not been examined. Most of the studies compared the nasal microbiome of healthy persons [210, 224-227] with those of persons with chronic rhinosinusitis and other nasal inflammatory diseases [228-232]. Partially, these investigations included additional cultural assessment of the microbiome [225, 229, 231] and samples were taken from the depth of the sinus of patients undergoing endoscopic sinus surgery [231, 232]. The healthy nasal microbiome consists of mainly staphylococci (coagulase-negative staphylococci, *Staphylococcus aureus*), corynebacteria, propionibacteria and *Moraxella* spp., whereas between the studies of nasal inflammation, there is no apparent consensus [210]. The nasal microbiota differs seasonally and the diversity decreases within the first year of life [233]. Also Graves' disease tends to vary seasonally with more frequently relapses in spring and summer [234]. Further studies are needed to reveal possible relationships between the microbiota and disease progression.

2. Eye

Clinically recognized GO occurs in about 50% of GD patients and therefore a comparison of the eye microbiota in these patients would be helpful, but has not been done yet [12]. In a mouse model of autoimmune uveitis, it was recently shown that activation of retina-specific T cells is dependent on gut microbiota-dependent signals [235].

3. Skin

1.5% of GD patients suffer from pretibial myxedema (PM) and other GD related skin disorders [236]. Characteristic for PM are skin thickening especially in the

pre-tibial area, but the disorder can also occur in other areas. No study focused particularly on the skin microbiota in patients with GD and subsequent skin disorders, although there is evidence that the skin microbiota varies in primary immunodeficiency [237] and also in skin (affecting) disorders like psoriasis [238, 239], atopic dermatitis [240], systemic lupus erythematosus [241] and Morbus Behcet [242].

4. Throat

Several diseases except AITD have been linked to the commensal bacterial population in the human mouth. In RA, Zhang and colleagues observed a concordance between the gut and oral microbiomes in patients with RA and a dysbiosis which was partially resolved after treatment [103]. Also in Sjögren's syndrome, a systemic autoimmune disorder characterized by lymphocytic infiltrates in exocrine organs, altered bacterial communities have been noticed. Szymula and colleagues showed the ability of peptides originating from oral and gut bacteria activating Sjogren's syndrome Antigen A (SSA)/Ro60-reactive T cells [243, 244].

5. Gut

In autoimmune thyroid disease the link between microbiota and disease onset or progression has not been elucidated yet. However, possible relations should be pointed out: already in 1988, Penhale and Young found in a rat model of autoimmune thyroiditis that modulation of the gut microbiota results in a significant influence on susceptibility to thyroid autoimmunity [245]. According to them, SPF rats were markedly less susceptible to the induction of experimental autoimmune thyroiditis by thymectomy and irradiation than conventionally reared rats of the same strain. Additionally, the incidence of thyroid lesions indicating thyroiditis as well as measured autoantibodies

increased in conventional rats and the offspring of conventional reared mothers were more susceptible to develop autoimmunity. 27 years later, a PCR-denaturing gradient gel electrophoresis with universal primers targeting V3 region of the 16S rRNA gene and quantitative real-time PCR revealed a different intestinal microbiota composition in hyperthyroid patients compared to controls whereas hypothyroidism leads to bacterial overgrowth in the small intestine assessed by hydrogen glucose breath test [246, 247]. Both, hyperthyroidism and hypothyroidism often go hand in hand with thyroid autoimmunity. Not only the microbiota composition, but also its enzyme activities have to be considered: glucuronidases responsible for provision of conjugated thyroxine are mostly of bacterial origin [248, 249]. Regarding the ability to produce hormones, the gut microbiota “has the potential to produce hundreds of products. From a morphological and biochemical perspective, it is far larger and more biochemically heterogeneous than any other endocrine organ in man” [250]. T4 malabsorption can be due to diverse gut microbiota in patients with CD and lactose intolerance [251, 252]. Similar to patients with T1D, a morphological and functional damage of the intestinal barrier was found [253] [254, 255].

In 2009, Oresic and colleagues investigated the contribution of the gut microbiota to lens and retinal lipid composition. In their comprehensive lipidomic profiling of lens and retina from conventionally raised and GF mice the authors found a decrease of lens phosphatidylcholines in the presence of gut microbiota due to an increased exposure to oxidative stress than in GF mice [256].

In summary, the questions dominate the answers concerning the impact of the microbiota in AITD and there is room for future research on this topic.

5. Conclusion

Interactions between the host and the gut microbiota influence host immunity and physiology and therefore are important to maintain intestinal homeostasis. Disruption of these host–microbial interactions due to dysbiosis can alter this balance leading to disease. Currently, very little is known about the impact of bacteria and microbiota in autoimmune thyroid disease. The author and co-authors are engaged in the project "Investigation of Novel biomarkers and Definition of the role of the microbiome In Graves' Orbitopathy" (INDIGO), which is part of the Industry-Academia Partnerships and Pathways (IAPP) program in People Marie Curie Actions (FP7-PEOPLE-2013-IAPP). The project aims to identify prognostic biomarkers to facilitate early preventative intervention, to investigate the role of the microbiome on disease progression and to assess the impact of probiotics in disease reduction. Hopefully, results will answer these questions and provide insight into the influence of environmental factors on gene–microbe interactions and the potential role of intestinal bacteria in the onset and progression of Graves' disease.

Funding

This work was supported by People Marie Skłodowska-Curie Actions, Marie Curie Industry-Academia Partnerships and Pathways [grant agreement number.: 612116].

Declaration of Interest

All authors declare that they have no competing interests.

References

[1] Triboulet, Coyon. Soc med des hopitaux, 1897;14:1458.

[2] R. L. Cecil, E. E. Nicholls, W. J. Stainsby. BACTERIOLOGY OF THE BLOOD AND JOINTS IN RHEUMATIC FEVER. The Journal of experimental medicine, 1929;50:617-42.

[3] A. M. Ercolini, S. D. Miller. The role of infections in autoimmune disease. Clinical and experimental immunology, 2009;155:1-15.

[4] A. P. Weetman. Immunity, thyroid function and pregnancy: molecular mechanisms. Nature reviews Endocrinology, 2010;6:311-8.

[5] C. Cipolla, L. Sandonato, G. Graceffa, S. Fricano, A. Torcivia, S. Vieni *et al.* Hashimoto thyroiditis coexistent with papillary thyroid carcinoma. The American surgeon, 2005;71:874-8.

[6] A. P. Weetman. Graves' disease. The New England journal of medicine, 2000;343:1236-48.

[7] D. S. Cooper. Hyperthyroidism. Lancet (London, England), 2003;362:459-68.

[8] W. M. Tunbridge, D. C. Evered, R. Hall, D. Appleton, M. Brewis, F. Clark *et al.* The spectrum of thyroid disease in a community: the Whickham survey. Clinical endocrinology, 1977;7:481-93.

[9] R. W. Flynn, T. M. MacDonald, A. D. Morris, R. T. Jung, G. P. Leese. The thyroid epidemiology, audit, and research study: thyroid dysfunction in the general population. The Journal of clinical endocrinology and metabolism, 2004;89:3879-84.

[10] H. Imrie, B. Vaidya, P. Perros, W. F. Kelly, A. D. Toft, E. T. Young *et al.* Evidence for a Graves' disease susceptibility locus at chromosome Xp11 in a United Kingdom population. The Journal of clinical endocrinology and metabolism, 2001;86:626-30.

[11] E. N. Pearce, A. P. Farwell, L. E. Braverman. Thyroiditis. The New England journal of medicine, 2003;348:2646-55.

[12] L. Bartalena, M. L. Tanda. Clinical practice. Graves' ophthalmopathy. The New England journal of medicine, 2009;360:994-1001.

[13] S. C. Gough, M. J. Simmonds. The HLA Region and Autoimmune Disease: Associations and Mechanisms of Action. Curr Genomics, 2007;8:453-65.

[14] M. Marino, F. Latrofa, F. Menconi, L. Chiovato, P. Vitti. Role of genetic and non-genetic factors in the etiology of Graves' disease. Journal of endocrinological investigation, 2014.

[15] L. Chiovato, A. Pinchera. Stressful life events and Graves' disease. European journal of endocrinology / European Federation of Endocrine Societies, 1996;134:680-2.

[16] M. Bagnasco, I. Bossert, G. Pesce. Stress and autoimmune thyroid diseases. Neuroimmunomodulation, 2006;13:309-17.

[17] T. Mizokami, A. Wu Li, S. El-Kaissi, J. R. Wall. Stress and thyroid autoimmunity. Thyroid : official journal of the American Thyroid Association, 2004;14:1047-55.

[18] C. M. Dayan. Stressful life events and Graves' disease revisited. Clinical endocrinology, England; 2001, p. 13-4.

[19] M. S. Draman, M. Ludgate. Thyroid eye disease- an update. Expert Review of Ophthalmology 2016.

[20] K. A. Ponto, D. Schuppan, I. Zwiener, H. Binder, A. Mirshahi, T. Diana *et al.* Thyroid-associated orbitopathy is linked to gastrointestinal autoimmunity. Clinical and experimental immunology, 2014;178:57-64.

[21] D. Peng, B. Xu, Y. Wang, H. Guo, Y. Jiang. A high frequency of circulating th22 and th17 cells in patients with new onset graves' disease. PloS one, 2013;8:e68446.

[22] J. Shen, Z. Li, W. Li, Y. Ge, M. Xie, M. Lv *et al.* Th1, Th2, and Th17 Cytokine Involvement in Thyroid Associated Ophthalmopathy. Disease markers, 2015;2015:609593.

[23] M. Klatka, E. Grywalska, M. Partyka, M. Charytanowicz, E. Kiszczak-Bochynska, J. Rolinski. Th17 and Treg cells in adolescents with Graves' disease. Impact of treatment with methimazole on these cell subsets. Autoimmunity, 2014;47:201-11.

[24] A. Castellanos-Rubio, I. Santin, I. Irastorza, L. Castano, J. Carlos Vitoria, J. Ramon Bilbao. TH17 (and TH1) signatures of intestinal biopsies of CD patients in response to gliadin. Autoimmunity, 2009;42:69-73.

[25] G. Simsek, Y. Karter, S. Aydin, H. Uzun. Osteoporotic cytokines and bone metabolism on rats with induced hyperthyroidism; changes as a result of reversal to euthyroidism. The Chinese journal of physiology, 2003;46:181-6.
[26] A. Pappa, V. Calder, R. Ajjan, P. Fells, M. Ludgate, A. P. Weetman *et al.* Analysis of extraocular muscle-infiltrating T cells in thyroid-associated ophthalmopathy (TAO). Clinical and experimental immunology, 1997;109:362-9.
[27] C. Phenekos, A. Vryonidou, A. D. Gritzapis, C. N. Baxevanis, M. Goula, M. Papamichail. Th1 and Th2 serum cytokine profiles characterize patients with Hashimoto's thyroiditis (Th1) and Graves' disease (Th2). Neuroimmunomodulation, 2004;11:209-13.
[28] C. Zhu, J. Ma, Y. Liu, J. Tong, J. Tian, J. Chen *et al.* Increased frequency of follicular helper T cells in patients with autoimmune thyroid disease. The Journal of clinical endocrinology and metabolism, 2012;97:943-50.
[29] J. Lazarus, D. J. Hennessey. Acute and Subacute, and Riedel's Thyroiditis. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO, editors. Endotext, South Dartmouth (MA): MDText.com, Inc.; 2000.
[30] D. Thomas, V. Liakos, V. Michou, N. Kapranos, G. Kaltsas, V. Tsilivakos *et al.* Detection of herpes virus DNA in post-operative thyroid tissue specimens of patients with autoimmune thyroid disease. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association, 2008;116:35-9.
[31] K. Mori, Y. Munakata, T. Saito, J. Tani, Y. Nakagawa, S. Hoshikawa *et al.* Intrathyroidal persistence of human parvovirus B19 DNA in a patient with Hashimoto's thyroiditis. The Journal of infection, 2007;55:e29-31.
[32] M. E. Zegans, R. N. Van Gelder. Considerations in understanding the ocular surface microbiome. American journal of ophthalmology, 2014;158:420-2.
[33] E. A. Grice, H. H. Kong, S. Conlan, C. B. Deming, J. Davis, A. C. Young *et al.* Topographical and temporal diversity of the human skin microbiome. Science (New York, NY), 2009;324:1190-2.
[34] P. D. Cotter, C. Hill, R. P. Ross. Bacteriocins: developing innate immunity for food. Nature reviews Microbiology, 2005;3:777-88.
[35] S. Duquesne, V. Petit, J. Peduzzi, S. Rebuffat. Structural and functional diversity of microcins, gene-encoded antibacterial peptides from enterobacteria. Journal of molecular microbiology and biotechnology, 2007;13:200-9.
[36] A. L. Cogen, K. Yamasaki, K. M. Sanchez, R. A. Dorschner, Y. Lai, D. T. MacLeod *et al.* Selective antimicrobial action is provided by phenol-soluble modulins derived from Staphylococcus epidermidis, a normal resident of the skin. The Journal of investigative dermatology, 2010;130:192-200.
[37] S. Sugimoto, T. Iwamoto, K. Takada, K. Okuda, A. Tajima, T. Iwase *et al.* Staphylococcus epidermidis Esp degrades specific proteins associated with Staphylococcus aureus biofilm formation and host-pathogen interaction. Journal of bacteriology, 2013;195:1645-55.
[38] R. R. Roth, W. D. James. Microbial ecology of the skin. Annual review of microbiology, 1988;42:441-64.
[39] P. M. Elias. The skin barrier as an innate immune element. Seminars in immunopathology, 2007;29:3-14.
[40] H. Bruggemann, A. Henne, F. Hoster, H. Liesegang, A. Wiezer, A. Strittmatter *et al.* The complete genome sequence of Propionibacterium acnes, a commensal of human skin. Science (New York, NY), 2004;305:671-3.
[41] H. C. Korting, K. Hubner, K. Greiner, G. Hamm, O. Braun-Falco. Differences in the skin surface pH and bacterial microflora due to the long-term application of synthetic detergent preparations of pH 5.5 and pH 7.0. Results of a crossover trial in healthy volunteers. Acta dermato-venereologica, 1990;70:429-31.
[42] R. Aly, C. Shirley, B. Cunico, H. I. Maibach. Effect of prolonged occlusion on the microbial flora, pH, carbon dioxide and transepidermal water loss on human skin. The Journal of investigative dermatology, 1978;71:378-81.

[43] D. J. Hentges. The anaerobic microflora of the human body. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 1993;16 Suppl 4:S175-80.
[44] J. Seneschal, R. A. Clark, A. Gehad, C. M. Baecher-Allan, T. S. Kupper. Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells. Immunity, 2012;36:873-84.
[45] Y. Lai, A. L. Cogen, K. A. Radek, H. J. Park, D. T. Macleod, A. Leichtle *et al.* Activation of TLR2 by a small molecule produced by Staphylococcus epidermidis increases antimicrobial defense against bacterial skin infections. The Journal of investigative dermatology, 2010;130:2211-21.
[46] I. Wanke, H. Steffen, C. Christ, B. Krismer, F. Gotz, A. Peschel *et al.* Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. The Journal of investigative dermatology, 2011;131:382-90.
[47] Z. Wang, D. T. MacLeod, A. Di Nardo. Commensal bacteria lipoteichoic acid increases skin mast cell antimicrobial activity against vaccinia viruses. Journal of immunology (Baltimore, Md : 1950), 2012;189:1551-8.
[48] J. A. Kluytmans, H. F. Wertheim. Nasal carriage of Staphylococcus aureus and prevention of nosocomial infections. Infection, 2005;33:3-8.
[49] N. Kamada, S. U. Seo, G. Y. Chen, G. Nunez. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol, 2013;13:321-35.
[50] Y. Belkaid, T. W. Hand. Role of the microbiota in immunity and inflammation. Cell, 2014;157:121-41.
[51] Y. Furusawa, Y. Obata, S. Fukuda, T. A. Endo, G. Nakato, D. Takahashi *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature, England; 2013, p. 446-50.
[52] K. Atarashi, T. Tanoue, T. Shima, A. Imaoka, T. Kuwahara, Y. Momose *et al.* Induction of colonic regulatory T cells by indigenous Clostridium species. Science (New York, NY), 2011;331:337-41.
[53] V. Tremaroli, F. Backhed. Functional interactions between the gut microbiota and host metabolism. Nature, England; 2012, p. 242-9.
[54] E. N. Bergman. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev, 1990;70:567-90.
[55] D. L. Topping, P. M. Clifton. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev, 2001;81:1031-64.
[56] P. S. Kamath, M. T. Hoepfner, S. F. Phillips. Short-chain fatty acids stimulate motility of the canine ileum. Am J Physiol, 1987;253:G427-33.
[57] J. R. Grider, B. E. Piland. The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. American journal of physiology Gastrointestinal and liver physiology, United States; 2007, p. G429-37.
[58] I. Kimura, D. Inoue, T. Maeda, T. Hara, A. Ichimura, S. Miyauchi *et al.* Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). Proceedings of the National Academy of Sciences of the United States of America, United States; 2011, p. 8030-5.
[59] J. R. Davie. Inhibition of histone deacetylase activity by butyrate. The Journal of nutrition, 2003;133:2485s-93s.
[60] D. Zapolska-Downar, M. Naruszewicz. Propionate reduces the cytokine-induced VCAM-1 and ICAM-1 expression by inhibiting nuclear factor-kappa B (NF-kappaB) activation. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society, 2009;60:123-31.
[61] S. Meng, J. T. Wu, S. Y. Archer, R. A. Hodin. Short-chain fatty acids and thyroid hormone interact in regulating enterocyte gene transcription. Surgery, United States; 1999, p. 293-8.
[62] N. Arpaia, C. Campbell, X. Fan, S. Dikiy, J. van der Veeken, P. deRoos *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature, England; 2013, p. 451-5.

[63] P. M. Smith, M. R. Howitt, N. Panikov, M. Michaud, C. A. Gallini, Y. M. Bohlooly *et al.* The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science (New York, NY), United States; 2013, p. 569-73.
[64] S. L. Gorbach, M. Barza, M. Giuliano, N. V. Jacobus. Colonization resistance of the human intestinal microflora: testing the hypothesis in normal volunteers. Eur J Clin Microbiol Infect Dis, 1988;7:98-102.
[65] D. van der Waaij, J. M. Berghuis-de Vries, L.-v. Lekkerkerk. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J Hyg (Lond), 1971;69:405-11.
[66] M. Barza, M. Giuliano, N. V. Jacobus, S. L. Gorbach. Effect of broad-spectrum parenteral antibiotics on "colonization resistance" of intestinal microflora of humans. Antimicrob Agents Chemother, 1987;31:723-7.
[67] T. D. Lawley, A. W. Walker. Intestinal colonization resistance. Immunology, 2013;138:1-11.
[68] M. Bohnhoff, B. L. Drake, C. P. Miller. The effect of an antibiotic on the susceptibility of the mouse's intestinal tract to Salmonella infection. Antibiot Annu, 1955;3:453-5.
[69] R. J. Kubinak JL. Do antibodies select a healthy microbiota? Nature Reviews Immunology 2016;16:767–74
[70] D. Erny, A. L. Hrabe de Angelis, D. Jaitin, P. Wieghofer, O. Staszewski, E. David *et al.* Host microbiota constantly control maturation and function of microglia in the CNS. Nature neuroscience, 2015;18:965-77.
[71] V. A. Shishov, T. A. Kirovskaia, V. S. Kudrin, A. V. Oleskin. [Amine neuromediators, their precursors, and oxidation products in the culture of Escherichia coli K-12]. Prikl Biokhim Mikrobiol, 2009;45:550-4.
[72] E. A. Tsavkelova, I. V. Botvinko, V. S. Kudrin, A. V. Oleskin. Detection of neurotransmitter amines in microorganisms with the use of high-performance liquid chromatography. Dokl Biochem, 2000;372:115-7.
[73] Q. Lin. Submerged fermentation of Lactobacillus rhamnosus YS9 for gamma-aminobutyric acid (GABA) production. Braz J Microbiol, Brazil; 2013, p. 183-7.
[74] E. Barrett, R. P. Ross, P. W. O'Toole, G. F. Fitzgerald, C. Stanton. gamma-Aminobutyric acid production by culturable bacteria from the human intestine. J Appl Microbiol, 2012;113:411-7.
[75] P. M. Stanaszek, J. F. Snell, J. J. O'Neill. Isolation, extraction, and measurement of acetylcholine from Lactobacillus plantarum. Appl Environ Microbiol, 1977;34:237-9.
[76] S. H. Rhee, C. Pothoulakis, E. A. Mayer. Principles and clinical implications of the brain-gut-enteric microbiota axis. Nature reviews Gastroenterology & hepatology, 2009;6:306-14.
[77] E. F. Verdu, P. Bercik, M. Verma-Gandhu, X. X. Huang, P. Blennerhassett, W. Jackson *et al.* Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut, 2006;55:182-90.
[78] S. M. Collins, P. Bercik. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology, 2009;136:2003-14.
[79] K. B. Bove, T. Watt, A. Vogel, L. Hegedus, J. B. Bjoerner, M. Groenvold *et al.* Anxiety and depression are more prevalent in patients with graves' disease than in patients with nodular goitre. European thyroid journal, 2014;3:173-8.
[80] M. C. Collado, E. Donat, C. Ribes-Koninckx, M. Calabuig, Y. Sanz. Specific duodenal and faecal bacterial groups associated with paediatric coeliac disease. J Clin Pathol, England; 2009, p. 264-9.
[81] P. Wacklin, P. Laurikka, K. Lindfors, P. Collin, T. Salmi, M. L. Lahdeaho *et al.* Altered duodenal microbiota composition in celiac disease patients suffering from persistent symptoms on a long-term gluten-free diet. The American journal of gastroenterology, United States; 2014, p. 1933-41.
[82] G. Ou, M. Hedberg, P. Horstedt, V. Baranov, G. Forsberg, M. Drobni *et al.* Proximal small intestinal microbiota and identification of rod-shaped bacteria associated with childhood celiac disease. The American journal of gastroenterology, United States; 2009, p. 3058-67.

[83] M. H. van Nuenen, K. Venema, J. C. van der Woude, E. J. Kuipers. The metabolic activity of fecal microbiota from healthy individuals and patients with inflammatory bowel disease. Dig Dis Sci, 2004;49:485-91.
[84] P. Lepage, P. Seksik, M. Sutren, M. F. de la Cochetiere, R. Jian, P. Marteau *et al.* Biodiversity of the mucosa-associated microbiota is stable along the distal digestive tract in healthy individuals and patients with IBD. Inflamm Bowel Dis, United States; 2005, p. 473-80.
[85] P. Seksik, P. Lepage, M. F. de la Cochetiere, A. Bourreille, M. Sutren, J. P. Galmiche *et al.* Search for localized dysbiosis in Crohn's disease ulcerations by temporal temperature gradient gel electrophoresis of 16S rRNA. J Clin Microbiol, United States; 2005, p. 4654-8.
[86] C. Manichanh, L. Rigottier-Gois, E. Bonnaud, K. Gloux, E. Pelletier, L. Frangeul *et al.* Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut, England; 2006, p. 205-11.
[87] H. Sokol, P. Seksik, L. Rigottier-Gois, C. Lay, P. Lepage, I. Podglajen *et al.* Specificities of the fecal microbiota in inflammatory bowel disease. Inflamm Bowel Dis, United States; 2006, p. 106-11.
[88] M. P. Conte, S. Schippa, I. Zamboni, M. Penta, F. Chiarini, L. Seganti *et al.* Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease. Gut, England; 2006, p. 1760-7.
[89] E. K. Wright, M. A. Kamm, S. M. Teo, M. Inouye, J. Wagner, C. D. Kirkwood. Recent Advances in Characterizing the Gastrointestinal Microbiome in Crohn's Disease: A Systematic Review. Inflamm Bowel Dis, 2015.
[90] C. Di Giacinto, M. Marinaro, M. Sanchez, W. Strober, M. Boirivant. Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-beta-bearing regulatory cells. Journal of immunology (Baltimore, Md : 1950), 2005;174:3237-46.
[91] D. Gevers, S. Kugathasan, L. A. Denson, Y. Vazquez-Baeza, W. Van Treuren, B. Ren *et al.* The treatment-naive microbiome in new-onset Crohn's disease. Cell host & microbe, 2014;15:382-92.
[92] D. Zanzi, R. Stefanile, S. Santagata, L. Iaffaldano, G. Iaquinto, N. Giardullo *et al.* IL-15 interferes with suppressive activity of intestinal regulatory T cells expanded in Celiac disease. The American journal of gastroenterology, 2011;106:1308-17.
[93] A. Giongo, K. A. Gano, D. B. Crabb, N. Mukherjee, L. L. Novelo, G. Casella *et al.* Toward defining the autoimmune microbiome for type 1 diabetes. ISME J, England; 2011, p. 82-91.
[94] L. Wen, R. E. Ley, P. Y. Volchkov, P. B. Stranges, L. Avanesyan, A. C. Stonebraker *et al.* Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature, 2008;455:1109-13.
[95] R. Valladares, D. Sankar, N. Li, E. Williams, K. K. Lai, A. S. Abdelgeliel *et al.* Lactobacillus johnsonii N6.2 mitigates the development of type 1 diabetes in BB-DP rats. PloS one, 2010;5:e10507.
[96] M. A. Kriegel, E. Sefik, J. A. Hill, H. J. Wu, C. Benoist, D. Mathis. Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proceedings of the National Academy of Sciences of the United States of America, United States; 2011, p. 11548-53.
[97] N. Hara, A. K. Alkanani, D. Ir, C. E. Robertson, B. D. Wagner, D. N. Frank *et al.* Prevention of virus-induced type 1 diabetes with antibiotic therapy. Journal of immunology (Baltimore, Md : 1950), United States; 2012, p. 3805-14.
[98] M. Murri, I. Leiva, J. M. Gomez-Zumaquero, F. J. Tinahones, F. Cardona, F. Soriguer *et al.* Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC Med, England; 2013, p. 46.
[99] J. Peng, S. Narasimhan, J. R. Marchesi, A. Benson, F. S. Wong, L. Wen. Long term effect of gut microbiota transfer on diabetes development. J Autoimmun, 2014;53:85-94.
[100] X. Liu, Q. Zou, B. Zeng, Y. Fang, H. Wei. Analysis of fecal Lactobacillus community structure in patients with early rheumatoid arthritis. Curr Microbiol, 2013;67:170-6.

[101] J. U. Scher, A. Sczesnak, R. S. Longman, N. Segata, C. Ubeda, C. Bielski *et al.* Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife, 2013;2:e01202.
[102] J. Vaahtovuo, E. Munukka, M. Korkeamaki, R. Luukkainen, P. Toivanen. Fecal microbiota in early rheumatoid arthritis. J Rheumatol, Canada; 2008, p. 1500-5.
[103] X. Zhang, D. Zhang, H. Jia, Q. Feng, D. Wang, D. Liang *et al.* The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nature medicine, 2015;21:895-905.
[104] K. Berer, M. Mues, M. Koutrolos, Z. A. Rasbi, M. Boziki, C. Johner *et al.* Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature, England: 2011 Macmillan Publishers Limited. All rights reserved; 2011, p. 538-41.
[105] J. Ochoa-Reparaz, D. W. Mielcarz, S. Begum-Haque, L. H. Kasper. Gut, bugs, and brain: role of commensal bacteria in the control of central nervous system disease. Ann Neurol, 2011;69:240-7.
[106] Y. K. Lee, J. S. Menezes, Y. Umesaki, S. K. Mazmanian. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proceedings of the National Academy of Sciences of the United States of America, United States; 2011, p. 4615-22.
[107] P. Pozzilli, A. Signore, A. J. Williams, P. E. Beales. NOD mouse colonies around the world--recent facts and figures. Immunol Today, England; 1993, p. 193-6.
[108] L. Wen, R. E. Ley, P. Y. Volchkov, P. B. Stranges, L. Avanesyan, A. C. Stonebraker *et al.* Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature, England; 2008, p. 1109-13.
[109] J. G. Markle, D. N. Frank, S. Mortin-Toth, C. E. Robertson, L. M. Feazel, U. Rolle-Kampczyk *et al.* Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science (New York, NY), United States; 2013, p. 1084-8.
[110] L. Yurkovetskiy, M. Burrows, A. A. Khan, L. Graham, P. Volchkov, L. Becker *et al.* Gender bias in autoimmunity is influenced by microbiota. Immunity, 2013;39:400-12.
[111] A. D. Kostic, D. Gevers, H. Siljander, T. Vatanen, T. Hyotylainen, A. M. Hamalainen *et al.* The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell host & microbe, 2015;17:260-73.
[112] T. Vatanen, A. D. Kostic, E. d'Hennezel, H. Siljander, E. A. Franzosa, M. Yassour *et al.* Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. Cell, 2016;165:1551.
[113] W. E. Ruff, M. A. Kriegel. Autoimmune host-microbiota interactions at barrier sites and beyond. Trends Mol Med, 2015;21:233-44.
[114] N. W. Palm, M. R. de Zoete, R. A. Flavell. Immune-microbiota interactions in health and disease. Clinical immunology (Orlando, Fla), 2015;159:122-7.
[115] X. Ge, R. Rodriguez, M. Trinh, J. Gunsolley, P. Xu. Oral microbiome of deep and shallow dental pockets in chronic periodontitis. PloS one, 2013;8:e65520.
[116] P. D. Marsh. Microbial ecology of dental plaque and its significance in health and disease. Advances in dental research, 1994;8:263-71.
[117] W. E. Moore, L. V. Moore. The bacteria of periodontal diseases. Periodontology 2000, 1994;5:66-77.
[118] P. D. Marsh. Role of the oral microflora in health. Microbial Ecology in Health and Disease, 2000:130-7.
[119] J. P. Banga, S. Moshkelgosha, U. Berchner-Pfannschmidt, A. Eckstein. Modeling Graves' Orbitopathy in Experimental Graves' Disease. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme, 2015;47:797-803.
[120] M. Ludgate. Animal models of Graves' disease. European journal of endocrinology / European Federation of Endocrine Societies, 2000;142:1-8.
[121] M. E. Ludgate. Animal models of thyroid-associated ophthalmopathy. Thyroid : official journal of the American Thyroid Association, 2002;12:205-8.

[122] B. Wiesweg, K. T. Johnson, A. K. Eckstein, U. Berchner-Pfannschmidt. Current insights into animal models of Graves' disease and orbitopathy. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme, 2013;45:549-55.
[123] U. Berchner-Pfannschmidt, S. Moshkelgosha, S. Diaz-Cano, B. Edelmann, G. E. Gortz, M. Horstmann *et al.* Comparative Assessment of Female Mouse Model of Graves' Orbitopathy Under Different Environments, Accompanied by Proinflammatory Cytokine and T-Cell Responses to Thyrotropin Hormone Receptor Antigen. Endocrinology, 2016;157:1673-82.
[124] A. L. Goodman, G. Kallstrom, J. J. Faith, A. Reyes, A. Moore, G. Dantas *et al.* Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. Proceedings of the National Academy of Sciences of the United States of America, 2011;108:6252-7.
[125] J. C. Lagier, F. Armougom, M. Million, P. Hugon, I. Pagnier, C. Robert *et al.* Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect, 2012;18:1185-93.
[126] H. P. Browne, S. C. Forster, B. O. Anonye, N. Kumar, B. A. Neville, M. D. Stares *et al.* Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. Nature, 2016;533:543-6.
[127] A. D. Toft, C. C. Blackwell, A. T. Saadi, P. Wu, P. Lymberi, M. Soudjidelli *et al.* Secretor status and infection in patients with Graves' disease. Autoimmunity, 1990;7:279-89.
[128] G. B. Bartley, V. Fatourechi, E. F. Kadrmas, S. J. Jacobsen, D. M. Ilstrup, J. A. Garrity *et al.* Long-term follow-up of Graves ophthalmopathy in an incidence cohort. Ophthalmology, 1996;103:958-62.
[129] J. E. Graham, J. E. Moore, X. Jiru, E. A. Goodall, J. S. Dooley, V. E. Hayes *et al.* Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. Investigative ophthalmology & visual science, 2007;48:5616-23.
[130] A. Lleo, C. Selmi, P. Invernizzi, M. Podda, R. L. Coppel, I. R. Mackay *et al.* Apotopes and the biliary specificity of primary biliary cirrhosis. Hepatology (Baltimore, Md), 2009;49:871-9.
[131] K. Lindfors, T. Rauhavirta, S. Stenman, M. Maki, K. Kaukinen. In vitro models for gluten toxicity: relevance for celiac disease pathogenesis and development of novel treatment options. Experimental biology and medicine (Maywood, NJ), 2012;237:119-25.
[132] K. Lindfors, T. Blomqvist, K. Juuti-Uusitalo, S. Stenman, J. Venalainen, M. Maki *et al.* Live probiotic Bifidobacterium lactis bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. Clinical and experimental immunology, 2008;152:552-8.
[133] T. Rauhavirta, S. W. Qiao, Z. Jiang, E. Myrsky, J. Loponen, I. R. Korponay-Szabo *et al.* Epithelial transport and deamidation of gliadin peptides: a role for coeliac disease patient immunoglobulin A. Clinical and experimental immunology, 2011;164:127-36.
[134] F. Borcherding, M. Nitschke, G. Hundorfean, J. Rupp, D. von Smolinski, K. Bieber *et al.* The CD40-CD40L pathway contributes to the proinflammatory function of intestinal epithelial cells in inflammatory bowel disease. The American journal of pathology, 2010;176:1816-27.
[135] M. Fredborg, P. K. Theil, B. B. Jensen, S. Purup. G protein-coupled receptor120 (GPR120) transcription in intestinal epithelial cells is significantly affected by bacteria belonging to the Bacteroides, Proteobacteria, and Firmicutes phyla. Journal of animal science, 2012;90 Suppl 4:10-2.
[136] A. F. Mattar, D. H. Teitelbaum, R. A. Drongowski, F. Yongyi, C. M. Harmon, A. G. Coran. Probiotics up-regulate MUC-2 mucin gene expression in a Caco-2 cell-culture model. Pediatric surgery international, 2002;18:586-90.
[137] M. Bermudez-Brito, S. Munoz-Quezada, C. Gomez-Llorente, E. Matencio, F. Romero, A. Gil. Lactobacillus paracasei CNCM I-4034 and its culture supernatant modulate Salmonella-induced inflammation in a novel transwell co-culture of human intestinal-like dendritic and Caco-2 cells. BMC microbiology, 2015;15:79.
[138] D. Haller, P. Serrant, G. Peruisseau, C. Bode, W. P. Hammes, E. Schiffrin *et al.* IL-10 producing CD14low monocytes inhibit lymphocyte-dependent activation of intestinal epithelial cells by commensal bacteria. Microbiology and immunology, 2002;46:195-205.

[139] G. De Palma, J. Kamanova, J. Cinova, M. Olivares, H. Drasarova, L. Tuckova *et al.* Modulation of phenotypic and functional maturation of dendritic cells by intestinal bacteria and gliadin: relevance for celiac disease. Journal of leukocyte biology, 2012;92:1043-54.
[140] V. F. Zevallos, H. J. Ellis, T. Suligoj, L. I. Herencia, P. J. Ciclitira. Variable activation of immune response by quinoa (Chenopodium quinoa Willd.) prolamins in celiac disease. The American journal of clinical nutrition, 2012;96:337-44.
[141] C. Gianfrani, M. Maglio, V. Rotondi Aufiero, A. Camarca, I. Vocca, G. Iaquinto *et al.* Immunogenicity of monococcum wheat in celiac patients. The American journal of clinical nutrition, 2012;96:1339-45.
[142] O. Molberg, K. Kett, H. Scott, E. Thorsby, L. M. Sollid, K. E. Lundin. Gliadin specific, HLA DQ2-restricted T cells are commonly found in small intestinal biopsies from coeliac disease patients, but not from controls. Scandinavian journal of immunology, 1997;46:103-9.
[143] C. Roura-Mir, M. Catalfamo, T. Y. Cheng, E. Marqusee, G. S. Besra, D. Jaraquemada *et al.* CD1a and CD1c activate intrathyroidal T cells during Graves' disease and Hashimoto's thyroiditis. Journal of immunology (Baltimore, Md : 1950), 2005;174:3773-80.
[144] P. Arscott, E. D. Rosen, R. J. Koenig, M. M. Kaplan, T. Ellis, N. Thompson *et al.* Immunoreactivity to Yersinia enterocolitica antigens in patients with autoimmune thyroid disease. The Journal of clinical endocrinology and metabolism, 1992;75:295-300.
[145] K. W. Lomasney, N. P. Hyland. The application of Ussing chambers for determining the impact of microbes and probiotics on intestinal ion transport. Canadian journal of physiology and pharmacology, 2013;91:663-70.
[146] L. L. Clarke. A guide to Ussing chamber studies of mouse intestine. American journal of physiology Gastrointestinal and liver physiology, 2009;296:G1151-66.
[147] K. Tsilingiri, T. Barbosa, G. Penna, F. Caprioli, A. Sonzogni, G. Viale *et al.* Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. Gut, 2012;61:1007-15.
[148] L. Maiuri, C. Ciacci, S. Auricchio, V. Brown, S. Quaratino, M. Londei. Interleukin 15 mediates epithelial changes in celiac disease. Gastroenterology, 2000;119:996-1006.
[149] R. Auricchio, F. Paparo, M. Maglio, A. Franzese, F. Lombardi, G. Valerio *et al.* In vitro-deranged intestinal immune response to gliadin in type 1 diabetes. Diabetes, 2004;53:1680-3.
[150] T. Ogino, J. Nishimura, S. Barman, H. Kayama, S. Uematsu, D. Okuzaki *et al.* Increased Th17-inducing activity of CD14+ CD163 low myeloid cells in intestinal lamina propria of patients with Crohn's disease. Gastroenterology, 2013;145:1380-91.e1.
[151] N. Lee, J. W. Park, H. J. Kim, J. H. Yeon, J. Kwon, J. J. Ko *et al.* Monitoring the differentiation and migration patterns of neural cells derived from human embryonic stem cells using a microfluidic culture system. Molecules and cells, 2014;37:497-502.
[152] J. E. Nichols, J. A. Niles, S. P. Vega, J. Cortiella. Novel in vitro respiratory models to study lung development, physiology, pathology and toxicology. Stem cell research & therapy, 2013;4 Suppl 1:S7.
[153] A. Weltin, K. Slotwinski, J. Kieninger, I. Moser, G. Jobst, M. Wego *et al.* Cell culture monitoring for drug screening and cancer research: a transparent, microfluidic, multi-sensor microsystem. Lab on a chip, 2014;14:138-46.
[154] P. Schlaermann, B. Toelle, H. Berger, S. C. Schmidt, M. Glanemann, J. Ordemann *et al.* A novel human gastric primary cell culture system for modelling Helicobacter pylori infection in vitro. Gut, 2014.
[155] H. C. Huang, Y. J. Chang, W. C. Chen, H. I. Harn, M. J. Tang, C. C. Wu. Enhancement of renal epithelial cell functions through microfluidic-based coculture with adipose-derived stem cells. Tissue engineering Part A, 2013;19:2024-34.
[156] M. Trapecar, A. Goropevsek, M. Gorenjak, L. Gradisnik, M. Slak Rupnik. A co-culture model of the developing small intestine offers new insight in the early immunomodulation of enterocytes and macrophages by Lactobacillus spp. through STAT1 and NF-kB p65 translocation. PloS one, 2014;9:e86297.

[157] G. R. Gibson, J. H. Cummings, G. T. Macfarlane. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. Appl Environ Microbiol, 1988;54:2750-5.
[158] S. Possemiers, S. Bolca, E. Eeckhaut, H. Depypere, W. Verstraete. Metabolism of isoflavones, lignans and prenylflavonoids by intestinal bacteria: producer phenotyping and relation with intestinal community. FEMS microbiology ecology, 2007;61:372-83.
[159] P. Van den Abbeele, C. Grootaert, M. Marzorati, S. Possemiers, W. Verstraete, P. Gerard *et al.* Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and Clostridium cluster IX. Appl Environ Microbiol, 2010;76:5237-46.
[160] H. Makivuokko, J. Nurmi, P. Nurminen, J. Stowell, N. Rautonen. In vitro effects on polydextrose by colonic bacteria and caco-2 cell cyclooxygenase gene expression. Nutrition and cancer, 2005;52:94-104.
[161] M. Minekus, M. Smeets-Peeters, A. Bernalier, S. Marol-Bonnin, R. Havenaar, P. Marteau *et al.* A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. Applied microbiology and biotechnology, 1999;53:108-14.
[162] A. Zihler Berner, S. Fuentes, A. Dostal, A. N. Payne, P. Vazquez Gutierrez, C. Chassard *et al.* Novel Polyfermentor intestinal model (PolyFermS) for controlled ecological studies: validation and effect of pH. PloS one, 2013;8:e77772.
[163] S. Possemiers, S. Rabot, J. C. Espin, A. Bruneau, C. Philippe, A. Gonzalez-Sarrias *et al.* Eubacterium limosum activates isoxanthohumol from hops (Humulus lupulus L.) into the potent phytoestrogen 8-prenylnaringenin in vitro and in rat intestine. The Journal of nutrition, 2008;138:1310-6.
[164] S. Macfarlane, E. J. Woodmansey, G. T. Macfarlane. Colonization of mucin by human intestinal bacteria and establishment of biofilm communities in a two-stage continuous culture system. Appl Environ Microbiol, 2005;71:7483-92.
[165] B. Bahrami, M. W. Child, S. Macfarlane, G. T. Macfarlane. Adherence and cytokine induction in Caco-2 cells by bacterial populations from a three-stage continuous-culture model of the large intestine. Appl Environ Microbiol, 2011;77:2934-42.
[166] M. H. van Nuenen, R. A. de Ligt, R. P. Doornbos, J. C. van der Woude, E. J. Kuipers, K. Venema. The influence of microbial metabolites on human intestinal epithelial cells and macrophages in vitro. FEMS immunology and medical microbiology, 2005;45:183-9.
[167] C. Cinquin, G. Le Blay, I. Fliss, C. Lacroix. New three-stage in vitro model for infant colonic fermentation with immobilized fecal microbiota. FEMS microbiology ecology, 2006;57:324-36.
[168] M. W. Child, A. Kennedy, A. W. Walker, B. Bahrami, S. Macfarlane, G. T. Macfarlane. Studies on the effect of system retention time on bacterial populations colonizing a three-stage continuous culture model of the human large gut using FISH techniques. FEMS microbiology ecology, 2006;55:299-310.
[169] M. van Nuenen. The effects of various inulins and Clostridium difficile on the metabolic activity on the human colonic microbiota in vitro. Microb EcolHealth Dis, 2003:137-44.
[170] A. Maathuis, A. Hoffman, A. Evans, L. Sanders, K. Venema. The effect of the undigested fraction of maize products on the activity and composition of the microbiota determined in a dynamic in vitro model of the human proximal large intestine. Journal of the American College of Nutrition, 2009;28:657-66.
[171] E. Barroso, T. Van de Wiele, A. Jimenez-Giron, I. Munoz-Gonzalez, P. J. Martin-Alvarez, M. V. Moreno-Arribas *et al.* Lactobacillus plantarum IFPL935 impacts colonic metabolism in a simulator of the human gut microbiota during feeding with red wine polyphenols. Applied microbiology and biotechnology, 2014;98:6805-15.
[172] P. De Boever, B. Deplancke, W. Verstraete. Fermentation by gut microbiota cultured in a simulator of the human intestinal microbial ecosystem is improved by supplementing a soygerm powder. The Journal of nutrition, 2000;130:2599-606.

[173] H. Makivuokko, S. Forssten, M. Saarinen, A. Ouwehand, N. Rautonen. Synbiotic effects of lactitol and Lactobacillus acidophilus NCFM in a semi-continuous colon fermentation model. Beneficial microbes, 2010;1:131-7.
[174] C. Grootaert, P. Van den Abbeele, M. Marzorati, W. F. Broekaert, C. M. Courtin, J. A. Delcour *et al.* Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem. FEMS microbiology ecology, 2009;69:231-42.
[175] P. Van den Abbeele, S. Roos, V. Eeckhaut, D. A. MacKenzie, M. Derde, W. Verstraete *et al.* Incorporating a mucosal environment in a dynamic gut model results in a more representative colonization by lactobacilli. Microbial biotechnology, 2012;5:106-15.
[176] M. Marzorati. An in vitro technology platform to assess host-microbiota interaction in the gastrointestinal tract. Agro Food Industry Hi-tech, 2012:VIII-XI.
[177] H. J. Kim, D. Huh, G. Hamilton, D. E. Ingber. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab on a chip, 2012;12:2165-74.
[178] T. H. Brix, L. Hegedus. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease. Clinical endocrinology, 2012;76:457-64.
[179] K. Bech, J. H. Larsen, J. M. Hansen, J. Nerup. Letter: Yersinia enterocolitica infection and thyroid disorders. Lancet (London, England), 1974;2:951-2.
[180] K. Lidman, U. Eriksson, A. Fagraeus, R. Norberg. Letter: Antibodies against thyroid cells in Yersinia enterocolitica infection. Lancet (London, England), 1974;2:1449.
[181] L. Shenkman, E. J. Bottone. Antibodies to Yersinia enterocolitica in thyroid disease. Annals of internal medicine, 1976;85:735-9.
[182] M. Weiss, E. Rubinstein, E. J. Bottone, L. Shenkman, H. Bank. Yersinia enterocolitica antibodies in thyroid disorders. Israel journal of medical sciences, 1979;15:553-5.
[183] E. Resetkova, R. Notenboom, G. Arreaza, T. Mukuta, N. Yoshikawa, R. Volpe. Seroreactivity to bacterial antigens is not a unique phenomenon in patients with autoimmune thyroid diseases in Canada. Thyroid : official journal of the American Thyroid Association, 1994;4:269-74.
[184] P. S. Hansen, B. E. Wenzel, T. H. Brix, L. Hegedus. Yersinia enterocolitica infection does not confer an increased risk of thyroid antibodies: evidence from a Danish twin study. Clinical and experimental immunology, 2006;146:32-8.
[185] G. Effraimidis, J. G. Tijssen, T. G. Strieder, W. M. Wiersinga. No causal relationship between Yersinia enterocolitica infection and autoimmune thyroid disease: evidence from a prospective study. Clinical and experimental immunology, 2011;165:38-43.
[186] T. G. Strieder, J. G. Tijssen, B. E. Wenzel, E. Endert, W. M. Wiersinga. Prediction of progression to overt hypothyroidism or hyperthyroidism in female relatives of patients with autoimmune thyroid disease using the Thyroid Events Amsterdam (THEA) score. Archives of internal medicine, 2008;168:1657-63.
[187] T. G. Strieder, B. E. Wenzel, M. F. Prummel, J. G. Tijssen, W. M. Wiersinga. Increased prevalence of antibodies to enteropathogenic Yersinia enterocolitica virulence proteins in relatives of patients with autoimmune thyroid disease. Clinical and experimental immunology, 2003;132:278-82.
[188] T. H. Brix, P. S. Hansen, L. Hegedus, B. E. Wenzel. Too early to dismiss Yersinia enterocolitica infection in the aetiology of Graves' disease: evidence from a twin case-control study. Clinical endocrinology, 2008;69:491-6.
[189] D. Corapcioglu, V. Tonyukuk, M. Kiyan, A. E. Yilmaz, R. Emral, N. Kamel *et al.* Relationship between thyroid autoimmunity and Yersinia enterocolitica antibodies. Thyroid : official journal of the American Thyroid Association, 2002;12:613-7.
[190] F. Guarneri, D. Carlotta, G. Saraceno, F. Trimarchi, S. Benvenga. Bioinformatics support the possible triggering of autoimmune thyroid diseases by Yersinia enterocolitica outer membrane proteins homologous to the human thyrotropin receptor. Thyroid : official journal of the American Thyroid Association, 2011;21:1283-4.
[191] Z. Wang, Q. Zhang, J. Lu, F. Jiang, H. Zhang, L. Gao *et al.* Identification of outer membrane porin f protein of Yersinia enterocolitica recognized by antithyrotopin receptor antibodies in Graves'

disease and determination of its epitope using mass spectrometry and bioinformatics tools. The Journal of clinical endocrinology and metabolism, 2010;95:4012-20.
[192] G. Luo, J. L. Fan, G. S. Seetharamaiah, R. K. Desai, J. S. Dallas, N. Wagle *et al.* Immunization of mice with Yersinia enterocolitica leads to the induction of antithyrotropin receptor antibodies. Journal of immunology (Baltimore, Md : 1950), 1993;151:922-8.
[193] C. E. Hargreaves, M. Grasso, C. S. Hampe, A. Stenkova, S. Atkinson, G. W. Joshua *et al.* Yersinia enterocolitica provides the link between thyroid-stimulating antibodies and their germline counterparts in Graves' disease. Journal of immunology (Baltimore, Md : 1950), 2013;190:5373-81.
[194] N. Figura, G. Di Cairano, F. Lore, E. Guarino, A. Gragnoli, D. Cataldo *et al.* The infection by Helicobacter pylori strains expressing CagA is highly prevalent in women with autoimmune thyroid disorders. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society, 1999;50:817-26.
[195] D. Larizza, V. Calcaterra, M. Martinetti, R. Negrini, A. De Silvestri, M. Cisternino *et al.* Helicobacter pylori infection and autoimmune thyroid disease in young patients: the disadvantage of carrying the human leukocyte antigen-DRB1*0301 allele. The Journal of clinical endocrinology and metabolism, 2006;91:176-9.
[196] V. Bassi, C. Santinelli, A. Iengo, C. Romano. Identification of a correlation between Helicobacter pylori infection and Graves' disease. Helicobacter, 2010;15:558-62.
[197] V. Bassi, G. Marino, A. Iengo, O. Fattoruso, C. Santinelli. Autoimmune thyroid diseases and Helicobacter pylori: the correlation is present only in Graves's disease. World journal of gastroenterology : WJG, 2012;18:1093-7.
[198] J. K. Triantafillidis, D. Georgakopoulos, A. Gikas, E. Merikas, G. Peros, K. Sofroniadou *et al.* Relation between Helicobacter pylori infection, thyroid hormone levels and cardiovascular risk factors on blood donors. Hepato-gastroenterology, 2003;50 Suppl 2:cccxviii-cccxx.
[199] F. Franceschi, M. A. Satta, M. C. Mentella, R. Penland, M. Candelli, R. L. Grillo *et al.* Helicobacter pylori infection in patients with Hashimoto's thyroiditis. Helicobacter, United States; 2004, p. 369.
[200] M. Soveid, K. Hosseini Asl, G. R. Omrani. Infection by Cag A positive strains of Helicobacter pylori is associated with autoimmune thyroid disease in Iranian patients. Iranian journal of immunology : IJI, 2012;9:48-52.
[201] G. Bertalot, G. Montresor, M. Tampieri, A. Spasiano, M. Pedroni, B. Milanesi *et al.* Decrease in thyroid autoantibodies after eradication of Helicobacter pylori infection. Clinical endocrinology, England; 2004, p. 650-2.
[202] S. Benvenga, F. Guarneri, M. Vaccaro, L. Santarpia, F. Trimarchi. Homologies between proteins of Borrelia burgdorferi and thyroid autoantigens. Thyroid : official journal of the American Thyroid Association, 2004;14:964-6.
[203] S. Benvenga, L. Santarpia, F. Trimarchi, F. Guarneri. Human thyroid autoantigens and proteins of Yersinia and Borrelia share amino acid sequence homology that includes binding motifs to HLA-DR molecules and T-cell receptor. Thyroid : official journal of the American Thyroid Association, 2006;16:225-36.
[204] H. Volzke, A. Werner, L. Guertler, D. Robinson, H. Wallaschofski, U. John. Putative association between anti-Borrelia IgG and autoimmune thyroid disease? Thyroid : official journal of the American Thyroid Association, 2005;15:1273-7.
[205] E. Gregoric, J. A. Gregoric, F. Guarneri, S. Benvenga. Injections of Clostridium botulinum neurotoxin A may cause thyroid complications in predisposed persons based on molecular mimicry with thyroid autoantigens. Endocrine, 2011;39:41-7.
[206] E. P. Kiseleva, K. I. Mikhailopulo, G. I. Novik, E. Szwajcer Dey, E. L. Zdorovenko, A. S. Shashkov *et al.* Isolation and structural identification of glycopolymers of Bifidobacterium bifidum BIM B-733D as putative players in pathogenesis of autoimmune thyroid diseases. Beneficial microbes, 2013;4:375-91.
[207] J. S. Zhou, H. S. Gill. Immunostimulatory probiotic Lactobacillus rhamnosus HN001 and Bifidobacterium lactis HN019 do not induce pathological inflammation in mouse model of

experimental autoimmune thyroiditis. International journal of food microbiology, 2005;103:97-104.
[208] S. S. Hammerstad, S. Tauriainen, H. Hyoty, T. Paulsen, I. Norheim, K. Dahl-Jorgensen. Detection of enterovirus in the thyroid tissue of patients with graves' disease. Journal of medical virology, 2013;85:512-8.
[209] Y. Tomer, T. F. Davies. Infection, thyroid disease, and autoimmunity. Endocrine reviews, 1993;14:107-20.
[210] M. P. C. Human. Structure, function and diversity of the healthy human microbiome. Nature, 2012;486:207-14.
[211] S. Mueller, K. Saunier, C. Hanisch, E. Norin, L. Alm, T. Midtvedt *et al.* Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. Appl Environ Microbiol, 2006;72:1027-33.
[212] J. K. Goodrich, J. L. Waters, A. C. Poole, J. L. Sutter, O. Koren, R. Blekhman *et al.* Human genetics shape the gut microbiome. Cell, 2014;159:789-99.
[213] A. K. Benson, S. A. Kelly, R. Legge, F. Ma, S. J. Low, J. Kim *et al.* Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proceedings of the National Academy of Sciences of the United States of America, 2010;107:18933-8.
[214] C. Jernberg, S. Lofmark, C. Edlund, J. K. Jansson. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J, Unknown; 2007, p. 56-66.
[215] E. Y. Huang, T. Inoue, V. A. Leone, S. Dalal, K. Touw, Y. Wang *et al.* Using corticosteroids to reshape the gut microbiome: implications for inflammatory bowel diseases. Inflamm Bowel Dis, 2015;21:963-72.
[216] P. Holopainen, M. Arvas, P. Sistonen, K. Mustalahti, P. Collin, M. Maki *et al.* CD28/CTLA4 gene region on chromosome 2q33 confers genetic susceptibility to celiac disease. A linkage and family-based association study. Tissue antigens, 1999;53:470-5.
[217] J. M. Heward, A. Allahabadia, M. Armitage, A. Hattersley, P. M. Dodson, K. Macleod *et al.* The development of Graves' disease and the CTLA-4 gene on chromosome 2q33. The Journal of clinical endocrinology and metabolism, 1999;84:2398-401.
[218] K. Patil-Sisodia, J. H. Mestman. Graves hyperthyroidism and pregnancy: a clinical update. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists, 2010;16:118-29.
[219] O. Koren, J. K. Goodrich, T. C. Cullender, A. Spor, K. Laitinen, H. K. Backhed *et al.* Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell, 2012;150:470-80.
[220] A. Santacruz, M. C. Collado, L. Garcia-Valdes, M. T. Segura, J. A. Martin-Lagos, T. Anjos *et al.* Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. The British journal of nutrition, 2010;104:83-92.
[221] W. Gohir, F. J. Whelan, M. G. Surette, C. Moore, J. D. Schertzer, D. M. Sloboda. Pregnancy-related changes in the maternal gut microbiota are dependent upon the mother's periconceptional diet. Gut microbes, 2015;6:310-20.
[222] E. S. Charlson, J. Chen, R. Custers-Allen, K. Bittinger, H. Li, R. Sinha *et al.* Disordered microbial communities in the upper respiratory tract of cigarette smokers. PloS one, 2010;5:e15216.
[223] L. Biedermann, J. Zeitz, J. Mwinyi, E. Sutter-Minder, A. Rehman, S. J. Ott *et al.* Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. PloS one, 2013;8:e59260.
[224] C. M. Bassis, A. L. Tang, V. B. Young, M. A. Pynnonen. The nasal cavity microbiota of healthy adults. Microbiome, 2014;2:27.
[225] U. Kaspar, A. Kriegeskorte, T. Schubert, G. Peters, C. Rudack, D. H. Pieper *et al.* The culturome of the human nose habitats reveals individual bacterial fingerprint patterns. Environmental microbiology, 2015.
[226] V. R. Ramakrishnan, L. M. Feazel, S. A. Gitomer, D. Ir, C. E. Robertson, D. N. Frank. The microbiome of the middle meatus in healthy adults. PloS one, 2013;8:e85507.

[227] M. Yan, S. J. Pamp, J. Fukuyama, P. H. Hwang, D. Y. Cho, S. Holmes *et al.* Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and S. aureus carriage. Cell host & microbe, 2013;14:631-40.
[228] N. A. Abreu, N. A. Nagalingam, Y. Song, F. C. Roediger, S. D. Pletcher, A. N. Goldberg *et al.* Sinus microbiome diversity depletion and Corynebacterium tuberculostearicum enrichment mediates rhinosinusitis. Science translational medicine, 2012;4:151ra24.
[229] S. Boase, A. Foreman, E. Cleland, L. Tan, R. Melton-Kreft, H. Pant *et al.* The microbiome of chronic rhinosinusitis: culture, molecular diagnostics and biofilm detection. BMC infectious diseases, 2013;13:210.
[230] R. Aurora, D. Chatterjee, J. Hentzleman, G. Prasad, R. Sindwani, T. Sanford. Contrasting the microbiomes from healthy volunteers and patients with chronic rhinosinusitis. JAMA otolaryngology-- head & neck surgery, 2013;139:1328-38.
[231] L. M. Feazel, C. E. Robertson, V. R. Ramakrishnan, D. N. Frank. Microbiome complexity and Staphylococcus aureus in chronic rhinosinusitis. The Laryngoscope, 2012;122:467-72.
[232] K. Biswas, M. Hoggard, R. Jain, M. W. Taylor, R. G. Douglas. The nasal microbiota in health and disease: variation within and between subjects. Frontiers in microbiology, 2015;9:134.
[233] M. Mika, I. Mack, I. Korten, W. Qi, S. Aebi, U. Frey *et al.* Dynamics of the nasal microbiota in infancy: a prospective cohort study. The Journal of allergy and clinical immunology, 2015;135:905-12.e11.
[234] T. Misaki, Y. Iida, K. Kasagi, J. Konishi. Seasonal variation in relapse rate of graves' disease after thionamide drug treatment. Endocrine journal, 2003;50:669-72.
[235] R. Horai, C. R. Zarate-Blades, P. Dillenburg-Pilla, J. Chen, J. L. Kielczewski, P. B. Silver *et al.* Microbiota-Dependent Activation of an Autoreactive T Cell Receptor Provokes Autoimmunity in an Immunologically Privileged Site. Immunity, 2015;43:343-53.
[236] L. Bartalena, V. Fatourechi. Extrathyroidal manifestations of Graves' disease: a 2014 update. Journal of endocrinological investigation, 2014;37:691-700.
[237] J. Oh, A. F. Freeman, M. Park, R. Sokolic, F. Candotti, S. M. Holland *et al.* The altered landscape of the human skin microbiome in patients with primary immunodeficiencies. Genome research, 2013;23:2103-14.
[238] A. Takemoto, O. Cho, Y. Morohoshi, T. Sugita, M. Muto. Molecular characterization of the skin fungal microbiome in patients with psoriasis. The Journal of dermatology, 2015;42:166-70.
[239] A. V. Alekseyenko, G. I. Perez-Perez, A. De Souza, B. Strober, Z. Gao, M. Bihan *et al.* Community differentiation of the cutaneous microbiota in psoriasis. Microbiome, 2013;1:31.
[240] H. H. Kong, J. Oh, C. Deming, S. Conlan, E. A. Grice, M. A. Beatson *et al.* Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome research, 2012;22:850-9.
[241] A. Hevia, C. Milani, P. Lopez, A. Cuervo, S. Arboleya, S. Duranti *et al.* Intestinal dysbiosis associated with systemic lupus erythematosus. MBio, 2014;5:e01548-14.
[242] C. Consolandi, S. Turroni, G. Emmi, M. Severgnini, J. Fiori, C. Peano *et al.* Behcet's syndrome patients exhibit specific microbiome signature. Autoimmun Rev, 2015;14:269-76.
[243] K. C. Leung, W. K. Leung, A. S. McMillan. Supra-gingival microbiota in Sjogren's syndrome. Clinical oral investigations, 2007;11:415-23.
[244] A. Szymula, J. Rosenthal, B. M. Szczerba, H. Bagavant, S. M. Fu, U. S. Deshmukh. T cell epitope mimicry between Sjogren's syndrome Antigen A (SSA)/Ro60 and oral, gut, skin and vaginal bacteria. Clinical immunology (Orlando, Fla), 2014;152:1-9.
[245] W. J. Penhale, P. R. Young. The influence of the normal microbial flora on the susceptibility of rats to experimental autoimmune thyroiditis. Clinical and experimental immunology, 1988;72:288-92.
[246] E. C. Lauritano, A. L. Bilotta, M. Gabrielli, E. Scarpellini, A. Lupascu, A. Laginestra *et al.* Association between hypothyroidism and small intestinal bacterial overgrowth. The Journal of clinical endocrinology and metabolism, United States; 2007, p. 4180-4.
[247] L. Zhou, X. Li, A. Ahmed, D. Wu, L. Liu, J. Qiu *et al.* Gut microbe analysis between hyperthyroid and healthy individuals. Curr Microbiol, 2014;69:675-80.

[248] W. W. de Herder, M. P. Hazenberg, A. M. Pennock-Schroder, G. Hennemann, T. J. Visser. Rapid and bacteria-dependent in vitro hydrolysis of iodothyronine-conjugates by intestinal contents of humans and rats. Med Biol, 1986;64:31-5.
[249] M. P. Hazenberg, W. W. de Herder, T. J. Visser. Hydrolysis of iodothyronine conjugates by intestinal bacteria. FEMS Microbiol Rev, 1988;4:9-16.
[250] G. Clarke, R. M. Stilling, P. J. Kennedy, C. Stanton, J. F. Cryan, T. G. Dinan. Minireview: Gut microbiota: the neglected endocrine organ. Mol Endocrinol, 2014;28:1221-38.
[251] C. Virili, G. Bassotti, M. G. Santaguida, R. Iuorio, S. C. Del Duca, V. Mercuri *et al.* Atypical celiac disease as cause of increased need for thyroxine: a systematic study. The Journal of clinical endocrinology and metabolism, 2012;97:E419-22.
[252] M. Cellini, M. G. Santaguida, I. Gatto, C. Virili, S. C. Del Duca, N. Brusca *et al.* Systematic appraisal of lactose intolerance as cause of increased need for oral thyroxine. The Journal of clinical endocrinology and metabolism, 2014;99:E1454-8.
[253] C. Virili, M. Centanni. Does microbiota composition affect thyroid homeostasis? Endocrine, 2014.
[254] E. Bosi, L. Molteni, M. G. Radaelli, L. Folini, I. Fermo, E. Bazzigaluppi *et al.* Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia, 2006;49:2824-7.
[255] F. C. Sasso, O. Carbonara, R. Torella, A. Mezzogiorno, V. Esposito, L. Demagistris *et al.* Ultrastructural changes in enterocytes in subjects with Hashimoto's thyroiditis. Gut, England; 2004, p. 1878-80.
[256] M. Oresic, T. Seppanen-Laakso, L. Yetukuri, F. Backhed, V. Hanninen. Gut microbiota affects lens and retinal lipid composition. Exp Eye Res, England; 2009, p. 604-7.

1372 Table 1

Bacterium	Cohort	Antibodies	Method	Antibody prevalence in patients in comparison to controls	Reference
H. pylori	GD and HAT	anti- HP (IgG)	ELISA, rlUBT	no sign. Difference	[257]
H. pylori	AITD	anti- HP, anti-CagA	WB	increase in AITD (p=0.006)	[194]
H. pylori	HAT	anti-CagA (IgG)	ELISA, rlUBT	No sign. difference	[199]
H. pylori	AITD	anti- HP	ELISA	increase (p=0.032)	[195]
H. pylori	GD	anti-CagA (IgG), HP-antigens (stool)	ELISA	increase (p=<0.001) with positive AEIA; increase in anti-CagA (p=<.005)	[196]
H. pylori	GD and HAT	anti-CagA (IgG), HP-antigens (stool)	ELISA	correlation between HP (p≤0.0001) and Cag-A (p≤0.005) in GD, not in HAT	[197]
H. pylori	AITD	anti- HP (IgG), anti-CagA	WB	no sign. difference in anti-HP-IgG, association between CagA-antibodies and AITD	[200]
H. pylori	Blood donors	anti-HP (not specified)	EIA	increase in donors with thyroid autoantibodies (p=0.018)	[198]
Y. enterocolitica	GD	anti-YE	agglutination (Gruber-Widal)	increase (p<0.005)	[179]
Y. enterocolitica	Thyroid disease	anti-YE	agglutination (Gruber-Widal)	present in 42% of 36 patients with thyroid disease and in none of 77 controls	[182]
Y. enterocolitica	AITD	anti-YE 0:3 and 0:9 (IgM, IgA, IgG), YP	ELISA	anti-YE IgA 0:3 increased (p<0.01), no difference in IgM and IgG	[258]
Y. enterocolitica	GD and HAT	anti-plasmid YE proteins (IgA, IgG)	WB	increase in anti-immunogenic protein, IgA and IgG in GD and HAT (p<0.01; p<0.001)	[259]
Y. enterocolitica	GD and HAT	anti-YE 0:3, 0:5, 0:6 and 0:9	micro-agglutination	anti-YE 0:3 not significantly different, anti-0:5 increase in GD and HAT (p<0.001)	[260]
Y. enterocolitica	GD and HAT	anti-YE 0:3	agglutination (Gruber-Widal)	increase in GD (p=<0.01), no significant increase in HAT	[261]
Y. enterocolitica	GD and HAT	anti-YOP2-5	WB, PBMC proliferation assay	YOP2-5 antibodies found in GD (96%), HAT (55,5%) and controls (70,8%)	[144]
Y. enterocolitica	GD and HAT	anti-YE 0:3 and 0:9	agglutination (Gruber-Widal), ELISA	no difference, thyroid therapy didn't change immunreactivity	[183]
Y. enterocolitica	GD and HAT	anti-YE 0:3, 0:5, 0:8 and 0:9	agglutination (Gruber-Widal)	increase in GD (p=<0.05)	[189]
Y. enterocolitica	AITD	anti-YOPs 0:9 (IgA and IgG)	WB	increase of IgA and IgG antibodies against YOPs (p<0.05 and p=0.002, respectively)	[187]
Y. enterocolitica	AITD	anti-YOPs 0:9 (IgA and IgG)	WB	no difference	[184]
Y. enterocolitica	GD	anti-YOPs 0:9 (IgA and IgG)	WB	increase in IgA and IgG (p=0.054 and p=0.043, respectively)	[178]
Y. enterocolitica	GD and HAT	anti-YOPs 0:9 (IgA and IgG)	WB	no difference	[186]
Y. enterocolitica	AITD	anti-YOPs 0:9 (IgA and IgG)	WB	no difference	[185]
B. burgdorferi	GD and HAT	anti-BB (IgG)	ELISA	no difference	[204]
B. henselae	HAT	anti-BH (IgG)	IFAA	increase, case report	[262]

Table 1: Evaluation of anti-bacterial antibody prevalence in patients with autoimmune thyroid disease (AITD), Graves' disease (GD) and Hashimoto thyroiditis (HAT) with different test methods. Abbreviations: cytotoxin-associated gene A (CagA), Helicobacter pylori (HP), Yersinia enterocolitica (YE), Yersinia outer proteins (YOP), enzyme-linked immunosorbent assay (ELISA), western blot (WB), radiolabeled urea breath test (rlUBT), immunofluorescent antibody assay (IFAA), peripheral blood mononuclear cells (PBMC).

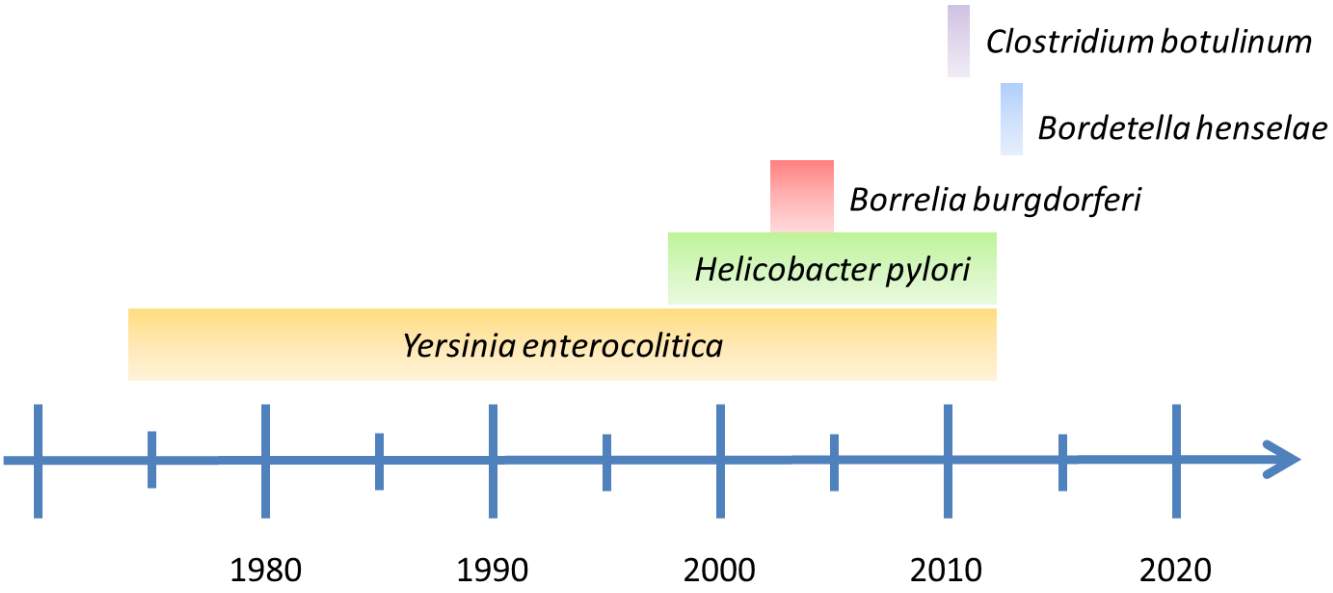

Figure 1

Figure 1: Role of bacteria in autoimmune thyroid disease: publications on this topic focusing on the five most cited bacterial organisms.

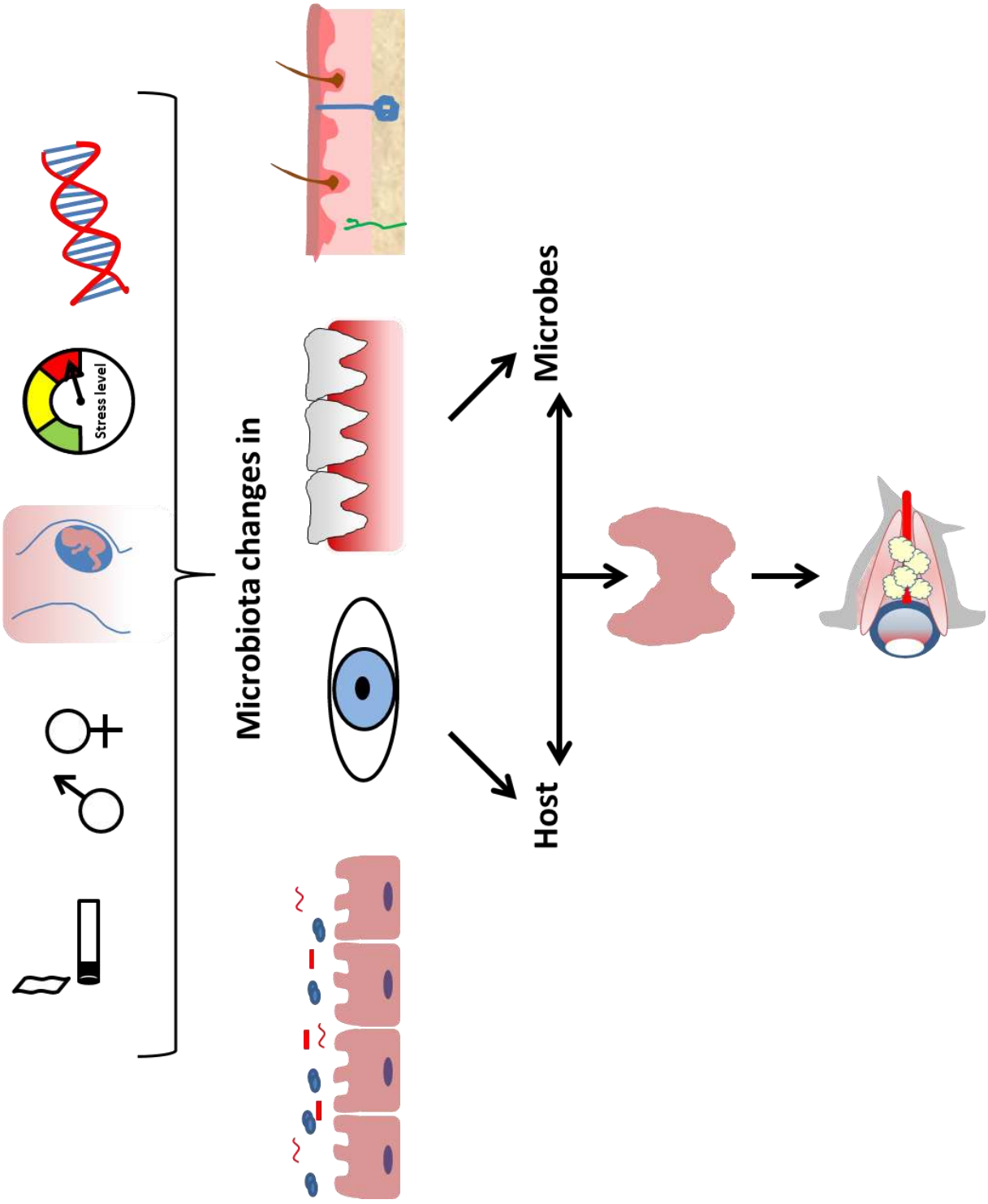

Figure 2

Figure 2: Overview of the main environmental factors influencing Graves`disease. Environmental factors affect bacterial populations at different body sites and therefore potentially contribute to the development of Graves' disease and orbitopathy.