The microbiome in primary Sjögren's syndrome

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CHAPTER 8

Summary

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease, characterized by chronic inflammation of the salivary and lacrimal glands, resulting in complaints of oral dryness (xerostomia) and ocular dryness (keratoconjunctivitis sicca). Systemic manifestation of pSS include, amongst others, fatigue, arthritis and neuropathy. The prevalence of pSS ranges from 0.02 – 0.1% and women are affected ten times more frequently than man.

Despite recent advancement in the role of genetics and immunologic pathways in the etiopathogenesis of pSS, relatively little is known about the environmental factors, which are also suspected to play a role in pSS etiology. In this respect, the role of microbiota that live in and on the human body (i.e., the human microbiome) has only scarcely been studied (**chapter 1**). Therefore, the overall aim of the research described in this PhD thesis was to assess whether pSS is associated with a disease-specific composition of microbiota in the oral cavity, gut and vagina.

In **chapter 2** we reviewed the knowledge of the connection between the human microbiome and systemic diseases, with emphasis on rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and pSS. The results of experimental and clinical studies discussed in this review suggest that the human microbiome may be involved in the etiopathogenesis of these systemic autoimmune diseases. Based upon the available evidence, at the time the literature was reviewed, we postulated a model in which the gut (and oral) microbiome may play a role in the etiology of systemic autoimmune diseases. Several clinical studies had been performed in RA and SLE patients, but the connection between gut/oral microbiota and pSS was largely unknown as microbiome studies in pSS patients had not yet been performed.

The goal of the study described in **chapter 3** was to assess whether changes in the oral microbiome of pSS patients are specific for the disease. 16S rRNA gene sequencing was performed on oral washing samples from pSS patients (n=36), disease controls (i.e. non-SS sicca patients, n=85) and healthy controls (HCs, n=14). From all patients and controls, the unstimulated and stimulated salivary flow rate were measured (UWS and SWS, respectively). Five percent of the overall oral microbiota composition was explained by disease phenotype (i.e., pSS vs. non-SS sicca vs. healthy controls), whereas nine percent was explained by SWS flow rate (in mL/min). The relative abundance of genera *Haemophilus* and *Neisseria* was positively correlated with SWS flow rate, whereas *Lactobacillus* relative abundance was negatively correlated with SWS flow rate. Interestingly, the relative abundance of *Streptococcus* was not correlated to SWS flow rate, despite both *Streptococcus* relative abundance and SWS flow rate being significantly lower in pSS patients than in healthy controls and non-SS sicca patients. Thus, on basis of these results, it was concluded that

salivary flow rate had a stronger influence on the microbiome in oral washings than the underlying disease, whereas low oral *Streptococcus* relative abundance remained associated to pSS independent of salivary flow rate.

Oral washing samples provided a general overview of the oral microbiome, but do not capture the oral mucosa microbiome specifically. Therefore, in the study described in **chapter 4**, it was assessed whether the microbiome of the buccal mucosa is specific for pSS compared with non-SS sicca patients and HCs. The bacterial composition of buccal swab samples from 37 pSS patients, 86 non-SS sicca patients and 24 HCs was determined with 16S rRNA gene sequencing. Furthermore, 16S sequencing data were used from buccal swabs collected in a general population cohort of 103 individuals to replicate microbiota associations with pSS. We observed a similar dysbiosis of the buccal mucosa microbiome in pSS and non-SS sicca patients. SWS flow rate explained a very similar percentage of variation in bacterial composition between individuals as disease phenotype (3.8 and 4.3%, respectively). Multivariate analysis showed that, when salivary flow rate was taken into account, the relative abundance of bacterial taxa did not significantly differ between pSS and non-SS sicca patients. Moreover, a microbiome-based prediction model could not distinguish pSS from non-SS sicca patients. PSS was associated with a significant difference in the relative abundance of 19 bacterial taxa compared with HCs, but the abundance of twelve of these taxa also significantly correlated with SWS flow rate. Thus, the buccal mucosa microbiome in pSS patients is determined by a combination of reduced salivary flow rate and – currently unknown – disease-specific factors.

The objective of the study described in **chapter 5** was to assess whether gut and oral microbiota compositions are specific for pSS patients, in comparison with the gut and oral microbiome of SLE patients and the gut microbiome of general population controls. 16S ribosomal RNA gene sequencing was performed on fecal samples from 39 pSS patients, 30 SLE patients and 965 individuals from the general population as well as on buccal swab and oral washing samples from the same pSS and SLE patients. The fecal microbiota composition from pSS and SLE patients differed significantly from that of population controls, but not between pSS and SLE patients. Lower bacterial richness, a lower Firmicutes/Bacteroidetes ratio and a higher relative abundance of *Bacteroides* species characterized the gut microbiome of pSS and SLE patients. Previous studies suggested that several *Bacteroides* species may be related to anti-Ro/SSA autoantibody presence, but we did not observe significant associations between fecal microbiota and anti-Ro/SSA serum positivity.

Oral microbiota composition differed significantly between pSS patients and SLE patients. Disease phenotype (pSS vs SLE) explained 8-9% of the variation in oral microbiota

composition. Furthermore, we showed that the relative abundance of two oral genera (i.e., *Actinomyces* and *Lactobacillus*) correlates between oral and fecal samples, suggesting a direct connection between the oral and gut microbiome.

From the results described in **chapter 5**, it was concluded that pSS and SLE patients share similar alterations in gut microbiota composition compared with the gut microbiome of general population controls. This finding suggests that the gut microbiome is involved in a common systemic inflammatory pathway in the pathogenesis of pSS and SLE. However, the altered gut microbiome in pSS and SLE patients may also be caused by shared disease-related factors.

In **chapter 6**, a case-control study is described in which the vaginal microbiome – assessed in cervicovaginal lavages and endocervical swabs – of nine premenopausal women with pSS was compared with that of eight age-matched premenopausal women without pSS. All samples were subjected to 16S rRNA gene sequencing. Women with pSS scored higher on symptoms of vaginal dryness, while the vaginal pH was similar to that of controls. PSS did not significantly affect the overall vaginal microbiota composition in both cervicovaginal lavage and endocervical swab samples. Despite the small size of our cohort, we were able to identify, in both pSS patients and controls, four of the five vaginal community state types that were previously identified. The distribution of the four community state types, and that of the three most prevalent genera in the vaginal microbiome (i.e., *Lactobacillus*, *Gardnerella* and *Streptococcus*), showed similar patterns in women with pSS as in controls. The results of this study indicate that the unique vaginal microbiome – dominated by acid producing lactobacilli – is less dependent on dryness than the oral microbiome. In the vagina, presence of lactobacilli are indicative for a healthy microbiome and are essential for the low pH in the vagina, thereby inhibiting the growth of other bacteria. The results of this study suggest that pSS-associated vaginal dryness in premenopausal women does not negatively influence homeostasis of the vaginal ecosystem.

In **chapter 7**, the results of the studies described in **chapter 3-6** are discussed in a broader perspective and suggestions for future studies are given.