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Review Article

Why Should We Consider Potential Roles of Oral Bacteria in the Pathogenesis of Sjögren Syndrome?

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Conflict of Interest
The authors declare no potential conflicts of interest.

ABSTRACT

Sjögren syndrome (SS) is a chronic autoimmune disorder that primarily targets the salivary and lacrimal glands. The pathology of these exocrine glands is characterized by periductal focal lymphocytic infiltrates, and both T cell-mediated tissue injury and autoantibodies that interfere with the secretion process underlie glandular hypofunction. In addition to these adaptive mechanisms, multiple innate immune pathways are dysregulated, particularly in the salivary gland epithelium. Our understanding of the pathogenetic mechanisms of SS has substantially improved during the past decade. In contrast to viral infection, bacterial infection has never been considered in the pathogenesis of SS. In this review, oral dysbiosis associated with SS and evidence for bacterial infection of the salivary glands in SS were reviewed. In addition, the potential contributions of bacterial infection to innate activation of ductal epithelial cells, plasmacytoid dendritic cells, and B cells and to the breach of tolerance via bystander activation of autoreactive T cells and molecular mimicry were discussed. The added roles of bacteria may extend our understanding of the pathogenetic mechanisms and therapeutic approaches for this autoimmune exocrinopathy.

Keywords: Sjogren syndrome; Oral; Bacteria; Dysbiosis; Salivary gland; Pathogenesis

INTRODUCTION

Primary Sjögren syndrome (SS) is a systemic autoimmune disorder of unknown etiology that primarily targets the salivary and lacrimal glands, leading to ocular and oral dryness. Together with dryness, musculoskeletal pain and fatigue constitute the classic symptom triad. Approximately 30%–40% of patients develop extraglandular manifestations involving the joints, lung, kidney, or nervous system. SS mainly affects perimenopausal women with a 9:1 female dominance (1).

The pathology of the affected exocrine glands is characterized by periductal lymphocytic infiltrates called focal lymphocytic sialadenitis (FLS), which can develop into ectopic lymphoid structures (2). Another hallmark of SS pathology is B cell hyperactivity characterized by polyclonal hypergammaglobulinemia, increased levels of free light chains, production of diverse autoantibodies, and an increased risk of developing B cell lymphoma

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Abbreviations

ANA, antinuclear antibodies; APC, activated antigen-presenting cell; AQP, aquaporin; BCR, B cell receptor; DC, dendritic cell; EBV, Epstein-Barr virus; FLS, focal lymphocytic sialadenitis; HSG, human submandibular gland tumor; HTLV-1, human T-cell leukemia virus type 1; PAMP, pathogen-associated molecular pattern; pDC, plasmacytoid dendritic cell; RF, rheumatoid factor; SGEC, salivary gland epithelial cell; SS, Sjögren syndrome; SSA, SS-related antigen A; SSB, SS-related antigen B.

Author Contributions

Conceptualization: Choi Y; Data curation: Chang SH, Choi Y; Funding acquisition: Chang SH, Choi Y; Investigation: Chang SH, Park SH, Cho ML, Choi Y; Writing - original draft: Chang SH, Choi Y; Writing - review & editing: Chang SH, Park SH, Cho ML, Choi Y.

(3). Autoantibodies detected in patients with SS include antinuclear antibodies (ANA), rheumatoid factor (RF), anti-SS-related antigen A (SSA; also known as Ro comprised with 2 ribonucleoproteins, Ro52 and Ro60) antibodies, and anti-SS-related antigen B (SSB; also known as La) antibodies that are the traditional biomarkers of SS but are also found in other systemic autoimmune diseases. Except for the RF that targets IgG, the autoantigens targeted by ANA, anti-SSA, or anti-SSB are ubiquitously expressed in all mammalian cells. Numerous autoantibodies have been additionally identified in SS, including those against molecules more specifically expressed in the target organs, such as salivary protein 1, carbonic anhydrase 6, parotid secretory protein, aquaporin 5 (AQP5), and type 3 muscarinic acetylcholine receptor (4).

Similar to other common autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematous, SS is a multifactorial disease involving both genetic and environmental factors. The most common environmental factor for autoimmune disease development is an infection. Because of the prominent type I IFN signature found in the peripheral blood of patients with SS, viral infection has long been speculated to be a triggering factor of SS.

The ducts of the salivary glands open into the oral cavity; thus, crosstalk between the salivary glands and oral microbiome is inevitable. Dysbiosis of the oral microbiota in SS has been reported, but its connection to the etiopathogenesis of SS is not fully appreciated. In this review, we discuss how the oral microbiota can contribute to the pathogenesis of SS, creating a vicious cycle. First, the current understanding of the pathogenesis of SS is briefly described. Next, studies on oral dysbiosis in SS are reviewed, and evidence for bacterial infection of the SS-affected salivary glands is provided. Finally, how bacterial infection can contribute to innate and adaptive immune activation in SS is discussed.

INTERPLAY OF INNATE AND ADAPTIVE IMMUNE RESPONSES IN THE PATHOGENESIS OF SS

There are many excellent reviews about the pathogenesis of SS (2,5,6). Here, we briefly summarize the current understanding of the initiation and perpetuation of SS (**Fig. 1**).

Genetic and environmental factors

SS is thought to occur in genetically susceptible individuals when they are exposed to environmental triggers. As genetic factors, gene loci involved in B cell follicle organization and function (*CXCR5*, *BLK*, *PRDM1*), type I IFNs (*IRF5*), T cell activation (*HLA*, *STAT4*, *IL12*, *KLRG1*, *SH2D2A*, and *NFAT5*), and control of NF-κB activation (*TNIP1* and *TNFAIP3*) have been identified from 3 large-scale genome-wide association studies (2).

Suggested environmental factors include estrogen deficiency, infection with exogenous viruses, and overexpression of endogenous retroviruses (2,6,7).

Upregulation of endogenous retrovirus RNAs has been shown in mouse B cells after B cell receptor (BCR) crosslinking by T-cell-independent type 2 antigens (8). This suggests that the overexpressed endogenous retrovirus may contribute to the development of autoimmune diseases (2). Although an increased prevalence of human endogenous retrovirus HERV-K113 in SS patients in the United Kingdom has been reported (9), overexpression of HERV in the salivary glands or peripheral blood B cells has not been shown.

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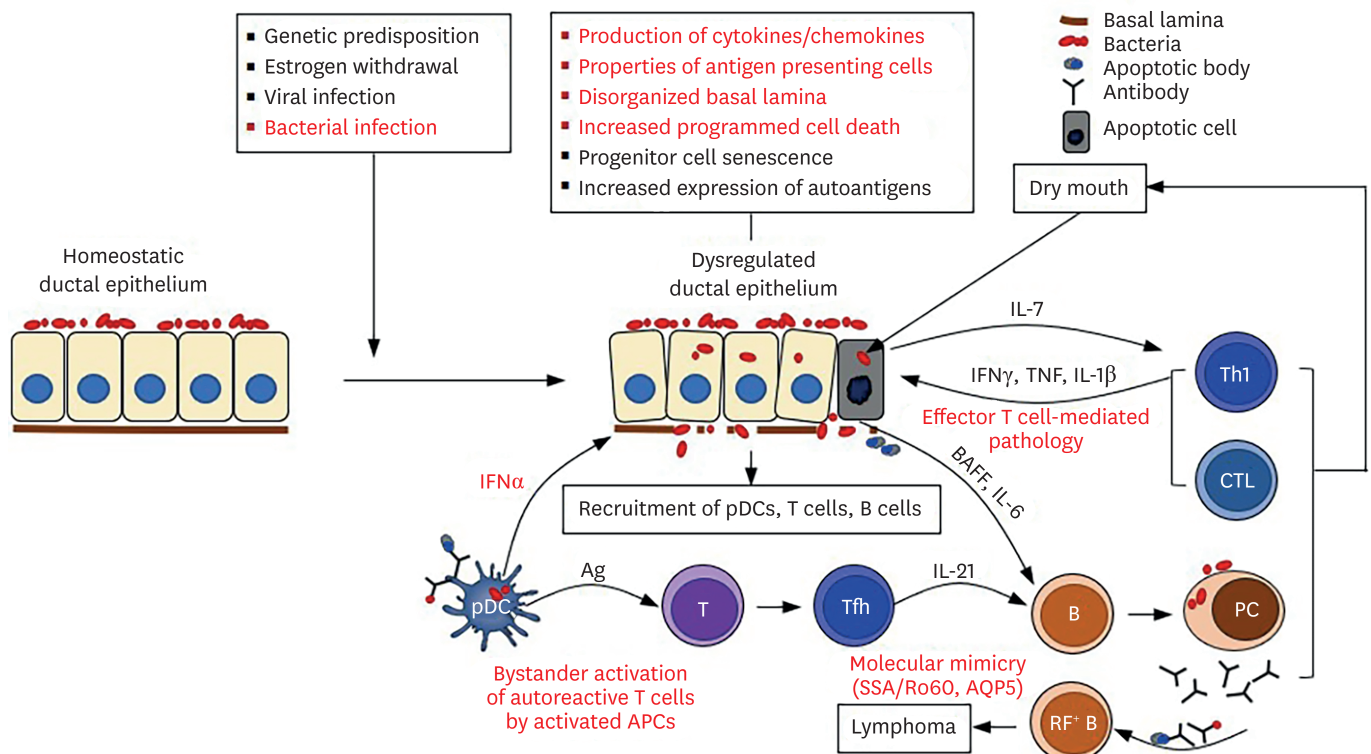

Figure 1. Current understanding and added roles of bacterial infection in the pathogenesis of SS. Genetic predisposition and environmental factors, such as estrogen withdrawal and viral infection, are believed to disrupt the homeostasis of the ductal epithelium. The production of cytokines/chemokines and disorganized basal lamina lead to the recruitment of pDCs, T cells, and B cells around the ducts with dysregulated epithelium. pDCs produce IFNα, which further activates ductal epithelial cells to produce BAFF and IL-7, and present autoantigens to the recruited T cells *in situ*. The interaction of recruited T cells with epithelial cells results in further activation of epithelial cells by cytokines or apoptosis. With the help of self-reactive T cells, aberrant activation of B cells with BAFF leads to the emergence of self-reactive B cells. CTL-mediated destruction of acinus and autoantibodies that interfere with the secretion process contribute to dryness. Stimulation of RF$^+$ B cells with anti-SSA autoantibodies complexed with apoptotic bodies through dual engagement of BCR and TLR7 seems to be crucial in lymphomagenesis. The added roles of bacterial infection in the pathogenesis of SS are depicted in red. Bacterial infection of ductal cells might contribute to dysregulation of epithelial cells, activation of pDCs, B cells, and PCs, disrupting tolerance via bystander activation of autoreactive T cells by APCs and molecular mimicry, and effector T cell-mediated pathology.
PC, plasma cell; Tfh, follicular helper T.

Human T-cell leukemia virus type 1 (HTLV-1) infects T cells and is a causative agent of adult T-cell leukemia and HTLV-1-associated myelopathy. Increased prevalence of HTLV-1 infection in SS patients compared with a general population has been repeatedly reported, together with the detection of HTLV-1 genes and proteins in the SS salivary glands (10). In addition, cultured salivary gland epithelial cells (SGECs) can be infected with HTLV-1 after coculture with an HTLV-1-infected T-cell line, which increases the expression of ICAM-1, CCL5, and CXCL10 in SGECs (11).

Epstein-Barr virus (EBV) has been extensively studied in association with SS because EBV mimics the key pathways of B-cell activation, such as BCR and CD40. EBV infects B cells and remains latent in resting memory B cells. Latent EBV can be reactivated upon BCR stimulation and undergo lytic replication in plasma cells. EBV DNA has been detected at increased levels in SS salivary glands (12). Interestingly, lytic EBV infection was observed exclusively in the SS salivary gland with ectopic lymphoid structures, and SSA/Ro52-reactive plasma cells were frequently infected with EBV (13). EBV-infected cells release EBV-encoded small RNA as a complex with SSB/La that induces TLR3 activation and the production of type I IFN (14). High titers of IgG against the EBV early antigen, a serologic marker of EBV

reactivation, were associated with anti-SSA/SSB status and B-cell activation markers, but there was no evidence of systemic EBV reactivation in SS patients (15). In addition, EBV can infect oral epithelial cells through an interaction between the EBV BMRF-2 protein and α5β1 integrins at the basolateral membranes of the polarized epithelial cells or by direct cell-to-cell contact of apical cell membranes with EBV-infected lymphocytes (16). Notably, over 90% of the general population worldwide has latent EBV infection, and EBV DNA is commonly detected in the saliva or oral washes of healthy individuals (17). Why only a small proportion of these individuals results in SS development needs to be explained.

Collectively, how viral infections might trigger SS remains unclear. All viruses implicated in SS primarily infect lymphocytes. Although HTLV-1 and EBV can infect epithelial cells, they are likely to be mediated by virus-infected T or B cells recruited to the salivary glands. Therefore, virus infection may contribute to the perpetuation of inflammation rather than triggering it in the salivary glands.

The innate arm of SS pathogenesis

Accumulating evidence indicates that epithelial cells in SS lesions are not only the target of autoimmunity but also active participants in the induction of the inflammatory process. Diverse abnormalities have been found in the salivary gland epithelium of patients with SS (6). Changes in the acinar epithelium include i) dyslocalized proteins involved in the secretory machinery (PIP2, synaptotagmin 1, and AQPs), ii) mislocalization of mucins from the apical to the basal pole, iii) production of proinflammatory cytokines, and iv) disorganized basal lamina. Changes in the ductal epithelium include i) production of cytokines (IL-1, IL-6, IL-7, IL-18, TNFα, IFNα/β, and BAFF) and chemokines (CCL3, CCL4, CCL5, CXCL9, CXCL10, CXCL11, and CXCL12), ii) upregulated expression of molecules involved in antigen presentation (HLA-ABC, HLA-DR, CD80, CD86, ICAM-1, and VCAM-1), iii) disorganized basal lamina, iv) senescence of salivary gland progenitor cells, v) increased susceptibility to programmed cell death, and vi) increased expression of SS-associated autoantigens (SSA/Ro60, SSA/Ro52, SSB/La). Importantly, the expression of ICAM-1 on epithelial cells and cytokine production were shown to precede FLS and hyposalivation in nonobese diabetic mouse model of SS, suggesting that the innate activation of salivary gland epithelium orchestrates the adaptive arm of SS pathology (18).

Chemokines expressed by ductal cells might be responsible for the periductal recruitment of T cells, B cells, and dendritic cells (DCs). In particular, infiltration of the ductal epithelium with disrupted basal lamina by $CD4^+$ T cells, $CD8^+$ T cells, and B cells and formation of lymphoepithelial lesions are unique to the salivary glands in SS (19,20). As potent producers of IFNα, plasmacytoid DCs (pDCs) detected in the SS-affected salivary glands are believed to play a central role in maintaining the well-known IFN signature of SS (21). IFNα activates ductal epithelial cells, DCs, and T cells to produce cytokines (IL-7 and BAFF) and chemokines (CXCL10).

The adaptive arm of SS pathogenesis

pDCs can also present antigens to T cells, activating the recruited T cells *in situ* (22,23). Recruited T cells interact with epithelial cells and further activate epithelial cells via cytokines (IFNγ, TNF, IL-1β), establishing a positive inflammatory loop via the IL-7/IFN axis (24). $CD8^+$ T cell-mediated apoptosis of epithelial cells might contribute to the exposure of intracellular autoantigens.

The SS-affected salivary glands provide a favorable microenvironment for the activation and survival of B cells (reviewed in 2). BAFF and IL-6 produced by epithelial cells are involved in B

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cell activation and survival. BAFF is a key cytokine that promotes the proliferation and survival of B cells. pDCs can induce plasma cell differentiation of activated B cells through IFNα and IL-6 (23). Cultured SGECs from patients with SS can support the *in vitro* differentiation of follicular Th cells through IL-6 and ICOSL (25), which play a pivotal role in ectopic germinal center formation (26). Ectopic germinal centers function as niches for autoreactive B-cells in SS. Finally, CXCL-12 and IL-6 produced by epithelial and stromal cells can support the survival of long-lived plasma cells, forming a plasma cell niche (27). The presence of anti-SSA and anti-SSB autoantibody-producing cells in the SS-affected salivary glands is correlated with the presence of autoantibodies in sera, suggesting that the salivary glands also provide a niche for autoantibody-secreting cells (28). The apoptotic bodies of epithelial cells complexed with anti-SSA autoantibodies may continuously stimulate RF-expressing B cells through dual engagement of BCR and TLR7, which is likely to be crucial in lymphomagenesis (2,29).

DYSBIOSIS OF ORAL MICROBIOTA IN SS

There are 12 studies that investigated SS-specific changes in oral microbiota using a next-generation sequencing approach (30-41). Except for one study that reported discriminatory taxa of supragingival plaque only at the species level (41), 10 studies reported significantly increased or decreased phyla and genera in the microbiota from buccal swabs, saliva, or oral washes, but one study reported no differentially distributed taxa (**Table 1**). SS-associated taxa can vary depending on the compared control group, sample size, sampling site, sequenced region of the 16S rRNA gene, threshold set for statistical significance, and geographic location. Six of 11 studies reported significant changes in the relative abundance of several phyla. An increase in Firmicutes and a decrease in Fusobacteria, Proteobacteria, and Spirochetes were reported in 2 or more studies (**Fig. 2A**). Although reduced Proteobacteria abundance in SS was reported in 3 studies, one study reported the opposite result. This finding may be attributed to the fact that Proteobacteria includes several genera increased as well as those decreased in SS (**Fig. 2B**). The genera significantly increased or decreased in ≥ 2 studies are presented in **Fig. 2C**. Changes in *Lactobacillus* (increase), *Haemophilus* (decrease), and *Neisseria* (decrease) were most frequently observed, the abundance of which is significantly correlated with stimulated whole salivary secretion (33). Clearly, reduced salivary secretion seems to contribute more to the oral dysbiosis of SS than underlying disease does in comparison with healthy controls (33). However, changes in the genera *Bifidobacterium*, *Abiotrophia*, and *Granulicatella* that were significant after adjusting for the stimulated whole salivary secretion rate were also observed in ≥ 2 studies (**Fig. 2C**). Two studies that investigated the SS and control groups with comparable levels of unstimulated whole salivary secretion rates by sequencing the V1–V3 regions of the 16S rRNA gene reported common changes, such as increases in Firmicutes and *Streptococcus* and decreases in Spirochaetes, *Moryella*, *Porphyromonas*, *Tannerella*, and *Treponema* (31,39).

Immunopathological implications of oral dysbiosis in SS

Only 2 groups further investigated the potential immunopathological sequelae of oral dysbiosis observed in SS. We tested 3 SS-associated (i.e., increased in SS) species for their ability to dysregulate human submandibular gland tumor (HSG). Two SS-associated species, *Prevotella melaninogenica* and *Rothia mucilaginosa*, efficiently invaded HSG cells. Furthermore, while *P. melaninogenica* induced upregulation of MHC molecules and CD80, *R. mucilaginosa* induced hypoxic cell death and downregulation of MHC I and CD86 (39,42). Tseng et al. (40) reported that A253 cells pretreated with *Haemophilus parainfluenzae*, a species reduced in SS

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Table 1. Dysbiosis of the oral microbiome observed in SS

Studies	Sample size	Sampling site	Sequenced region of the 16S rRNA gene	Threshold set for statistical significance	Geographical site	Phylum		Genus	
						Increased	Decreased	Increased	Decreased
Li et al., 2016 (30)	SS (n=10)/Hc (n=10)	Buccal swab	V1–V3	p<0.05	China		Proteobacteria	*Delftia* (Proteobacteria) *Leucobacter* (Actinobacteria) *Mitsuaria* (Proteobacteria) *Pseudochrobactrum* (Proteobacteria) *Ralstonia* (Proteobacteria)	*Comamona* (Proteobacteria) *Granulicatella* (Firmicutes) *Haemophilus* (Proteobacteria) *Limnohabitans* (Proteobacteria) *Neisseria* (Proteobacteria)
Siddiqui et al., 2016 (31)	SS (n=9 with normal salivation)/Hc (n=9)	Saliva	V1–V3	q threshold not reported	Norway	Firmicutes	Synergistetes Spirochaetes	*Streptococcus* (Firmicutes)	*Bacteroidaceae* (Bacteroidetes) *Catonella* (Firmicutes) *Fretibacterium* (Synergistetes) *Moryella* (Firmicutes) *Peptostreptococcaceae* (Firmicutes) *Porphyromonas* (Bacteroidetes) *Tannerella* (Bacteroidetes) *Treponema* (Spirochaetes)
van der Meulen et al., 2018 (32)	SS (n=37)/non-SS sicca (n=86)/Hc (n=24)	Buccal swab	V4	q<0.1	Netherlands		In SS (vs. Hc) Proteobacteria	In SS (vs. Hc) *Alloscardovia* (Actinobacteria) *Anaeroglobus** (Firmicutes) *Atopobium* (Actinobacteria) *Bifidobacterium** (Actinobacteria) *Dialister* (Firmicutes) *Lactobacillus* (Firmicutes) *Parvimonas** (Firmicutes) *Peptostreptococcaceae* (Firmicutes) *Scardovia** (Actinobacteria)	In SS (vs. Hc) *Abiotrophia** (Firmicutes) *Alloprevotella* (Bacteroidetes) *Bergeyella** (Bacteroidetes) *Enterococcus** (Firmicutes) *Granulicatella** (Firmicutes) *Haemophilus* (Proteobacteria) *Lautropia* (Proteobacteria) *Neisseria* (Proteobacteria) *Ruminococcaceae_G1** (Firmicutes) In pSS (vs. non-SS sicca) *Bergeyella** (Bacteroidetes) *Granulicatella** (Firmicutes)
van der Meulen et al., 2018 (33)	SS (n=36)/non-SS sicca (n=85)/Hc (n=14)	Oral wash	V4	q<0.1	Netherlands			In SS (vs. Hc) *Selenomonas* (Firmicutes) In SS (vs. non-SS sicca) *Shuttleworthia* (Firmicutes)	In SS (vs. Hc) *Streptococcus* (Firmicutes) In SS (vs. non-SS sicca) *Abiotrophia* (Firmicutes)
Zhou et al., 2018 (34)	SS (n=22)/Hc (n=23)	Oral wash	V3–V4	p<0.05 & LDA>4	China	Proteobacteria	Fusobacteria Actinobacteria	*Veillonella* (Firmicutes)	*Actinomyces* (Actinobacteria) *Haemophilus* (Proteobacteria) *Neisseria* (Proteobacteria) *Peptostreptococcus* (Firmicutes) *Porphyromonas* (Bacteroidetes) *Rothia* (Actinobacteria)
Rusthen et al., 2019 (35)	SS (n=15)/non-SS sicca (n=15)/Hc (n=15)	Saliva	16S rRNA gene V3–V5	p<0.05 with Bonferroni correction	Norway				In SS (vs. Hc) *Haemophilus* (Proteobacteria) *Neisseria* (Proteobacteria) In non-SS sicca (vs. Hc) *Haemophilus* (Proteobacteria) *Neisseria* (Proteobacteria)

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Table 1. (Continued) Dysbiosis of the oral microbiome observed in SS

Studies	Sample size	Sampling site	Sequenced region of the 16S rRNA gene	Threshold set for statistical significance	Geographical site	Phylum		Genus	
						Increased	Decreased	Increased	Decreased
Sembler-Møller et al., 2019 (36)	SS (n=24)/non-SS sicca (n=34)	Saliva	16S rRNA gene V1–V3	q threshold not reported	Denmark	None	None	None	None
van der Meulen et al., 2019 (37)	SS (n=39)/ SLE (n=30)/Hc (n=965)	Buccal swab, oral wash	V4	q<0.1	Netherlands	In SS (vs. SLE) Firmicutes	In SS (vs. SLE) Proteobacteria	In SS (vs. SLE) *Lactobacillus* (Firmicutes)	In SS (vs. SLE) *Actinomyces* (Actinobacteria) *Capnocytophaga* (Bacteroidetes) *Cardiobacterium* (Proteobacteria) *Corynebacterium* (Actinobacteria) *Granulicatella* (Firmicutes) *Leptotrichia* (Fusobacteria) *Neisseria* (Proteobacteria) *Prevotella* (Bacteroidetes) *Stomatobaculum* (Firmicutes)
Sharma et al., 2020 (38)	SS (n=37)/Hc (n=35)	Saliva	V3–V4	p<0.05 & |fold change| >2	India			*Bifidobacterium* (Actinobacteria) *Dialister* (Firmicutes) *Lactobacillus* (Firmicutes)	*Leptotrichia* (Fusobacteria)
Alam et al., 2020 (39)	SS (n=25, including 8 with normal salivation)/Con (n=25, including 11 non-SS sicca)	Oral wash	V1–V3	q<0.2	Korea	Firmicutes	Proteobacteria Fusobacteria TM7 Spirochaetes	*Atopobium* (Actinobacteria) *Lactobacillus* (Firmicutes) *Prevotella* (Bacteroidetes) *Streptococcus* (Firmicutes)	*Campylobacter* (Proteobacteria) *Capnocytophaga* (Bacteroidetes) *Cardiobacterium* (Proteobacteria) *Corynebacterium* (Actinobacteria) *Eikenella* (Proteobacteria) *Fusobacterium* (Fusobacteria) *Haemophilus* (Proteobacteria) *Johnsonella* (Firmicutes) *Kingella* (Proteobacteria) *Lachnoanaerobaculum* (Firmicutes) *Lautropia* (Proteobacteria) *Leptotrichia* (Fusobacteria) *Moryella* (Firmicutes) *Neisseria* (Proteobacteria) *Pasteurellaceae_uc* (Proteobacteria) *Porphyromonas* (Bacteroidetes) *Saccharimonas* (TM7) *Tannerella* (Bacteroidetes) *Treponema* (Spirochaetes) *Veillonellaceae_uc* (Firmicutes)
Tseng et al., 2021 (40)	SS (n=8)/Hc (n=16)	Saliva	V3–V4	p<0.05	Taiwan			*Megasphaera* (Firmicutes)	*Aggregatibacter* (Proteobacteria) *Abiotrophia* (Firmicutes) *Bifidobacterium* (Actinobacteria) *Cardiobacterium* (Proteobacteria) *Haemophilus* (Proteobacteria) *Johnsonella* (Firmicutes)

Hc, healthy controls; non-SS sicca, with dryness symptoms similar to those of primary SS patients but not fulfilling the criteria; LDA, low disease activity; SLE, systemic lupus erythematosus.
[*]Significant taking into account smoking, dental status, and stimulated whole salivary secretion rate.

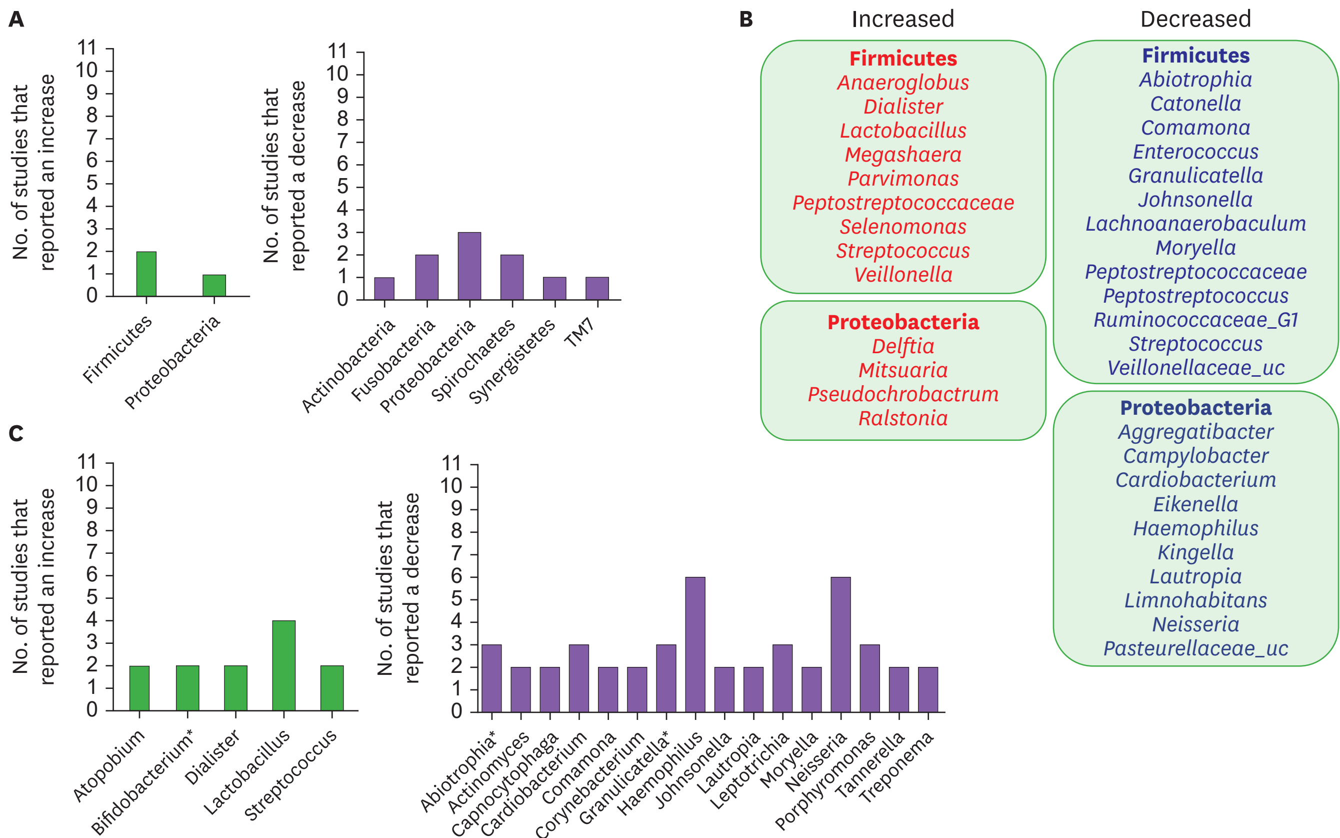

Figure 2. Significantly altered phyla and genera reported in 11 studies on the oral microbiome of patients with SS. (A) Phyla significantly increased or decreased in the indicated number of studies. (B) The list of genera significantly increased or decreased in SS that belong to either Firmicutes or Proteobacteria. (C) Genera significantly increased or decreased in the indicated number of studies (only the genera reported in 2 or more studies are shown).

with the greatest effect size, suppress CD4 T-cell proliferation partially via upregulation of PD-L1 expression. These results imply that oral commensals may confer immunomodulatory effects on SGECs, whereas SS-associated species may infect and dysregulate SGECs. However, this notion needs further verification using more oral species and SGECs.

EVIDENCE FOR BACTERIAL INFECTION OF THE SALIVARY GLANDS IN SS

Recently, we reported the presence of bacteria within the ductal epithelium and the areas of infiltration in the labial salivary glands from patients with SS (39; **Fig. 3**). The bacterial infection of the salivary glands in SS is further supported by the increased expression of bacteria-sensing TLRs. Increased *in situ* expression of the TLR2, TLR3, TLR4, TLR6, TLR7, TLR8, and TLR9 proteins in the salivary gland biopsies of SS patients has been reported (43-46). Increased expression of the TLR1, TLR2, and TLR4 genes in SS-SGECs compared to control-SGECs was also reported (47). Furthermore, stimulation of SGECs with ligands to TLR2, TLR3, and TLR4 *in vitro* significantly upregulated the expression of the respective TLR genes, suggesting that the increased TLR expression observed *in vivo* implies the triggered status of the molecule *in situ* (47). Interestingly, strong expression of TLRs (TLR2, TLR4, TLR6, TLR7, and TLR9) was observed in infiltrating mononuclear cells and ductal epithelial cells, which coincides with the pattern of bacterial infection (39,44,45).

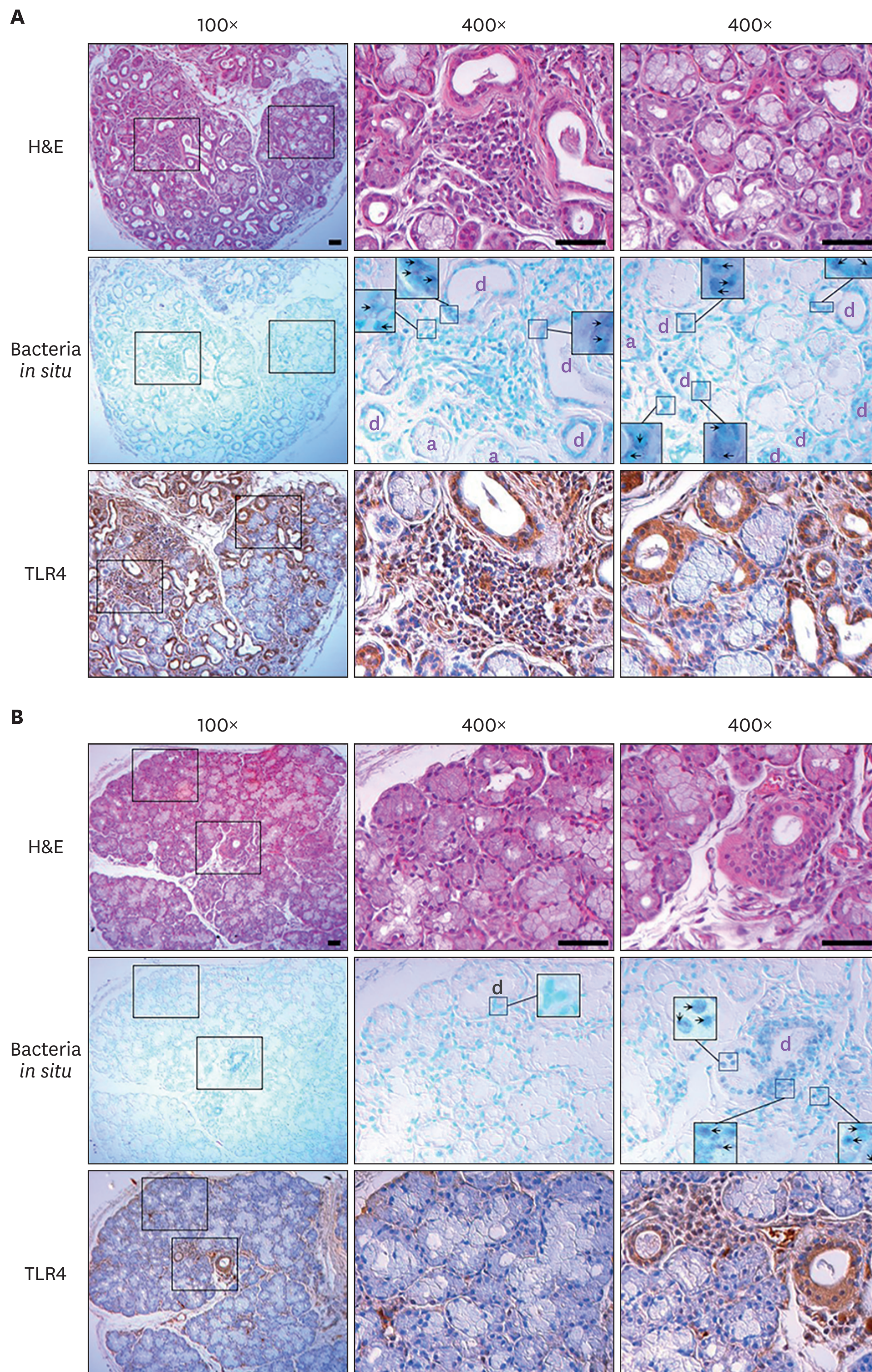

Figure 3. Bacterial infection and TLR4 expression in labial salivary gland biopsies. (A) In the labial salivary glands with focal lymphocytic sialadenitis from patients with SS, strong bacterial infection and TLR4 expression are observed not only at the area of lymphocytic infiltration and the ducts and acini nearby but also at the ducts without infiltration. (B) In labial salivary glands with nonspecific chronic inflammation from control subjects who did not meet the diagnostic criteria for SS, bacterial infection and TLR4 expression are observed only at the ducts with inflammation. Areas marked with rectangles in the image with low magnification are taken with high magnification. 'a' indicate acinus, 'd' indicate duct. Acinus and ducts infected with bacteria are marked with the letter of dark violet, while uninfected ducts are marked in dark gray. Arrows indicate representative signals of bacteria. Scale bars: 50 μm.

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To date, only viral pathogen-associated molecular patterns (PAMPs) or endogenous danger-associated molecular patterns have been considered ligands for the TLRs observed in SS-affected salivary glands (44,47,48). TLR3 and TLR7/TLR8 are known to recognize viral dsRNA and ssRNA, respectively (49). However, accumulating evidence shows that these TLRs also sense bacterial RNAs and induce type I IFN and NF-κB-dependent cytokines in monocytes, macrophages, pDCs, myeloid DCs, and keratinocytes (50-52). Therefore, bacterial PAMPs can trigger all TLRs expressed in the salivary glands and induce the activation of NF-κB, IRF3, and IRF7 (**Fig. 4**). In particular, the bacteria that can invade epithelial cells are likely to activate both surface and endosomal TLRs, resulting in the induction of proinflammatory cytokines and type I IFN (53).

Lewis et al. (54) reported the isolation of bacteria from stimulated parotid saliva collected from healthy individuals and patients with SS using modified Carlsson-Crittenden cups. In the culture of 3 consecutive 0.5 ml samples, the mean viable concentration of bacteria rapidly fell in the healthy group from 6.9×10^3 to 0.3×10^3 colony forming units (cfu)/ml, suggesting the cleansing effect of salivary secretion. Although the third sample could be collected from only 5 of 14 patients with SS due to the reduced salivary flow rate, a much higher number of bacteria (2.6×10^3 cfu/ml) was detected in the samples (54). Reduction in salivary secretion indicates loss of the antimicrobial activity of the saliva. Furthermore, the expression of human β-defensins 1 and 2 was decreased in the salivary glands of patients with SS compared with those from healthy subjects (55). Significantly higher total bacterial loads of oral

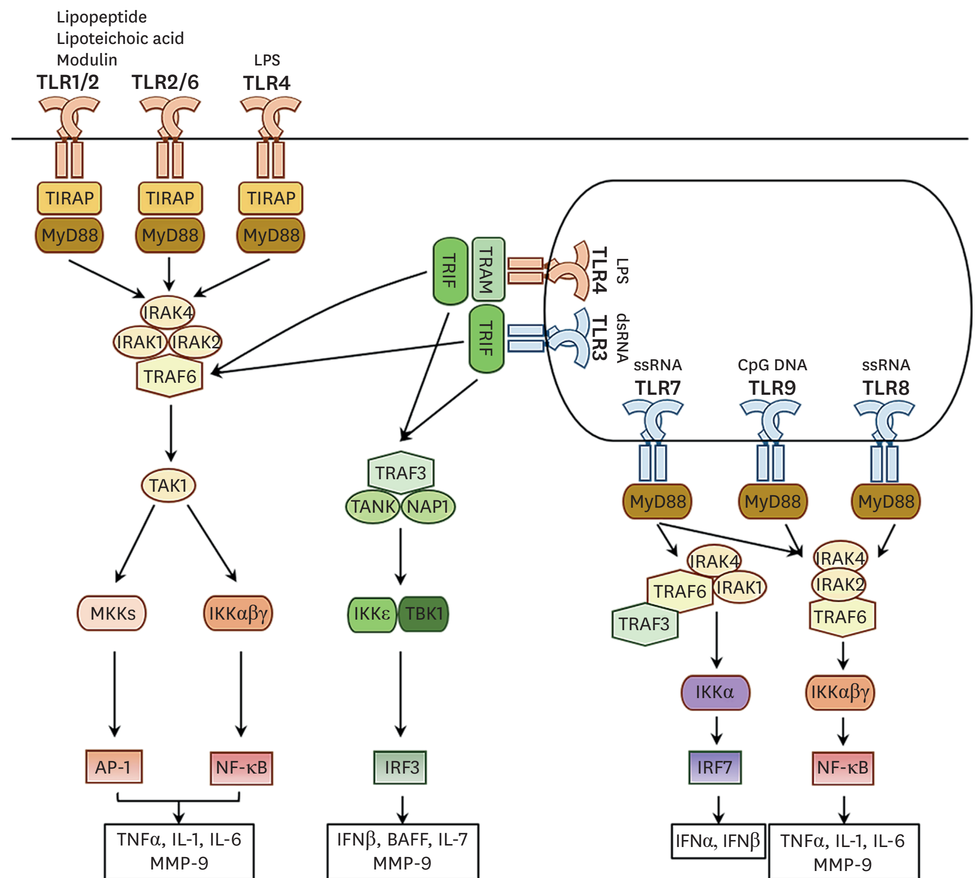

Figure 4. The ligands and signaling pathways of TLRs expressed in the salivary glands of patients with SS.

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bacterial communities in the SS group than in the control group have been reported even for the subgroup without oral dryness (39). These studies suggest that the ducts of the salivary glands normally have a microbial flora in health but the reduced mechanical flushing and salivary antimicrobial proteins/peptides in SS might provide a favorable environment for the bacterial infection of ductal epithelial cells.

THE CONTRIBUTION OF BACTERIAL INFECTION TO INNATE IMMUNE ACTIVATION

Bacterial infection of the salivary glands might contribute to the innate arm of SS pathology via interaction with 3 cell types: epithelial cells, pDCs, and B cells.

Contribution to dysregulation of epithelial cells

Stimulation of cultured SGECs with ligands for TLR2, TLR3, TLR4, or TLR7 increases the expression of HLA class I, ICAM-1, and CD40 (46,47). In addition, stimulation of SGECs with ligands for TLR2 or TLR3 induces the production of cytokines, such as IL-15, IL-7, IFNβ, and BAFF (24,56,57). The disorganized basal lamina observed in the SS-affected salivary glands is associated with increased levels of MMP-9 expression and activity in epithelial cells, and the expression of MMP-9 is dependent on NF-κB (58,59). Periodontitis-associated bacteria induce MMP-9 in gingival epithelial cells (60,61). Likewise, bacterial infection may induce MMP-9 expression in SGECs. Furthermore, a number of oral bacterial species produces cell-bound and extracellular proteinases that activate proMMP-9 and degrade laminin and collagens (62,63).

Notably, the expression of HLA-DR, CD86, ICAM-1, VCAM-1, IFNα/β, CXCL9, CXCL10, and CXCL11 is predominantly observed on ductal cells associated with heavy lymphocytic infiltration (46,64-66), while the expression of CCL3, CCL4, CCL5, and MMP-9 on ductal cells is independent of infiltration (58,64,67). Heavy bacterial infection and strong TLR4 expression were observed not only in the ducts near lymphocytic infiltration but also in those without infiltration in the SS salivary glands with FLS (39; **Fig. 3**). Gram-negative bacteria that invade ductal epithelial cells are expected to activate both NF-κB and IRFs (**Fig. 4**). Bacterial infection of ductal cells might preferentially upregulate the expression of molecules that mainly depends on NF-κB and IRF3, such as CCL3, CCL4, CCL-5 and MMP-9, contributing to the initial recruitment of T cells. The recruited T cells then contribute to the IFNγ-dependent expression of HLA-DR, CD86, CXCL9, CXCL10, and CXCL11.

The possibility that bacterial infection induces pyroptosis, apoptosis, and necroptosis in host cells should also be considered (68). Bacteria-induced cell death would contribute to not only the release of intracellular autoantigens but also barrier dysfunction.

Contribution to activation of pDCs

pDCs preferentially express TLR7 and TLR9. In the SS-affected salivary glands, pDCs might be exposed to bacterial nucleic acids by extracellular vesicles or bacteria that have crossed the epithelial barrier. Not only extracellular vesicles secreted by bacteria but also those secreted by host cells infected with bacteria contain immunostimulatory DNA, RNA, and other PAMPs (69,70). pDCs are expected to take up both bacteria (either free form or immune complex) and extracellular vesicles by micropinocytosis or endocytosis, which would contribute to the maturation of pDCs and sustained production of IFNα via TLR7 and TLR9 (**Figs. 1** and **4**).

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Contribution to innate activation of B cells and plasma cells

The SS-affected salivary glands are enriched with CD27^{+} memory B cells and fully differentiated plasma cells (71). Human memory B cells express substantial levels of TLR1, TLR6, TLR7, TLR9, and TLR10 and low levels of TLR2 (72-74). Human B cells upregulate the expression of antigen presenting molecules (HLA-DR, CD80, and CD86) and secrete cytokines (IL-1α, IL-6, IL-10) in response to stimulation of TLR1/2, TLR7, and TLR9 (73,75). In addition, TLR9 stimulation can induce strong polyclonal B cell proliferation and antibody secretion from memory B cells in the absence of BCR signaling (72). TLR7 ligands can also induce polyclonal B cell proliferation in the presence of IFNα (76). Unlike pDCs, B cells internalize exogenous materials mainly through BCRs (2). Therefore, only RF^{+} B cells that infiltrate the SS-affected salivary glands may access bacterial nucleic acids in the form of immune complexes. Whether extracellular vesicles containing bacterial nucleic acids can stimulate B cells needs to be verified. In contrast to B cells, human plasma cells express all TLRs, including TLR3 and TLR4, and stimulation of TLRs on plasma cells enhances antibody secretion (74). The endocytic ability of plasma cells has been reported (76). Therefore, bacteria are likely to stimulate plasma cells not only through the TLRs expressed on the cell surface, such as TLR2, TLR4, and TLR5, but also through endosomal TLRs. Collectively, bacterial infection of the salivary glands would contribute to the B cell hyperactivity and hypergammaglobulinemia of SS.

THE CONTRIBUTION OF BACTERIAL INFECTION TO ADAPTIVE IMMUNE ACTIVATION

Bacterial infection of the salivary glands may contribute to the adaptive arm of SS pathology via 3 mechanisms: molecular mimicry, bystander activation of autoreactive T cells by activated antigen-presenting cells (APCs), and T-cell-mediated pathology.

Molecular mimicry

The potential role of bacterial orthologs identified in human-associated bacteria in breaching tolerance to autoantigens was previously proposed (77). A recent study identified Ro60 orthologs (bacterial ribonucleoprotein) in human commensal bacterial species and clearly showed molecular mimicry between the human Ro60 and bacterial orthologs (78). Human Ro60 autoantigen-specific CD4 memory T cells from patients with systemic lupus erythematosus were activated by Ro60 ortholog-containing bacteria, and anti-Ro60-positive sera bound recombinantly expressed bacterial Ro60 orthologs. Furthermore, monocolonization of germ-free mice with a Ro60 ortholog-containing gut commensal spontaneously induced anti-human Ro60 T- and B-cell responses. Among the Ro60 orthologs, the top 3 species (*Corynebacterium amycolatum*, *Propionibacterium propionicum*, and *Actinomyces massiliensis*) closest to human Ro60 have remarkably high sequence homology with Ro60 both at the early B-cell epitope and a major T-cell epitope, 85% and 70%, respectively. *P. propionicum* and *A. massiliensis*, as oral commensals, are isolated from parotid saliva (54). *P. propionicum* is also a member of ocular commensals (79). Bacterial orthologs for AQP5, an SS-specific autoantigen, have also been identified in human oral commensal bacterial species (80,81). Repeated immunization with a peptide derived from the AQP of *P. melaninogenica*, which has 91% and 71% homology with a functional B cell epitope and an overlapping T cell epitope of human AQP5, respectively, induced anti-AQP5 autoantibodies and hyposalivation in C57BL/6 mice (82). Importantly, *P. melaninogenica* was detected within ductal cells and periductal infiltrates in the labial salivary glands of patients with SS (39). Although the

evidence for the association of *P. propionicum*, *A. massiliensis*, or *P. melaninogenica* with SS is currently insufficient, the bacterial orthologs of Ro60 or AQP5 might drive the development of autoantibodies in SS before the onset of symptoms.

Bystander activation of autoreactive T cells by activated APCs

The recruitment and maturation of DCs, including pDCs, in the salivary glands have been regarded as inappropriate. To date, viral infection or RNA-containing immune complexes have been suggested as sources that activate pDCs. However, bacterial infection can also induce the recruitment and activation of DCs. In addition, B cells are especially efficient in presenting particulate antigens, such as virus-like particles and bacteria (83). APCs activated during microbial infection can present autoantigens as well as microbial antigens, so-called "bystander activation of autoreactive T cells" (84). The breach of T-cell tolerance against autoantigens is likely to occur when T cells are repeatedly exposed to autoantigens presented by activated DCs or B cells in the setting of chronic infection.

T-cell-mediated pathology

TLR signaling by microbial components induces phagosomal delivery of MHC class I molecules from the endosomal recycling compartment and allows cross-presentation in myeloid DCs (85). Ductal epithelial cells may cross-present internalized bacterial antigens to bacteria-specific $CD8^+$ T cells, contributing to increased apoptosis. Since the ductal epithelium in SS-affected salivary glands often expresses MHC class II molecules, presentation to bacteria-specific Th1 cells is also expected. The Th1 cytokines IFNγ and TNFα disrupt an epithelial physical barrier (86). Altogether, crosstalk between bacteria-infected epithelial cells and bacteria-specific effector T cells will disrupt the epithelial barrier, facilitating continuous infection.

CONCLUSIONS AND FUTURE DIRECTION

Saliva strongly affects the ecology of the oral microbiome. Thus, the dysbiosis of the oral microbiome observed in SS patients with reduced salivary secretion is attributed more to oral dryness than to the underlying disease. Notably, correction of a dysbiotic oral microbiota in IκB-ζ-deficient mice through cohousing with wild-type mice alleviates the development of FLS, suggesting the role of oral dysbiosis in the development of SS in a mouse model (87).

Nevertheless, it is not yet clear whether oral dysbiosis causes bacterial infection of the ductal epithelium in SS-affected salivary glands. Although 2 of 3 SS-associated species efficiently invade HSG cells (39), there is a possibility that the bacteria infecting the salivary glands are not necessarily increased in the oral cavity of SS patients. A balance between epithelium-invading and noninvading taxa might be important. In addition, whether bacterial infection is the primary cause of SS or the result of oral dryness is not clear. Importantly, the reduced salivary secretion produces an environment that can facilitate bacterial infection of the ductal epithelium in the salivary glands. Therefore, bacterial infection of the salivary glands can lead to a vicious cycle of SS pathogenesis via innate and adaptive activation of epithelial cells and immune cells (**Fig. 5A**).

In conclusion, we propose that bacterial infection of the salivary glands may play an important role in the perpetuation of sialadenitis and autoantibody production in SS. Many parts of this idea need experimental verification. Characterization of the bacterial

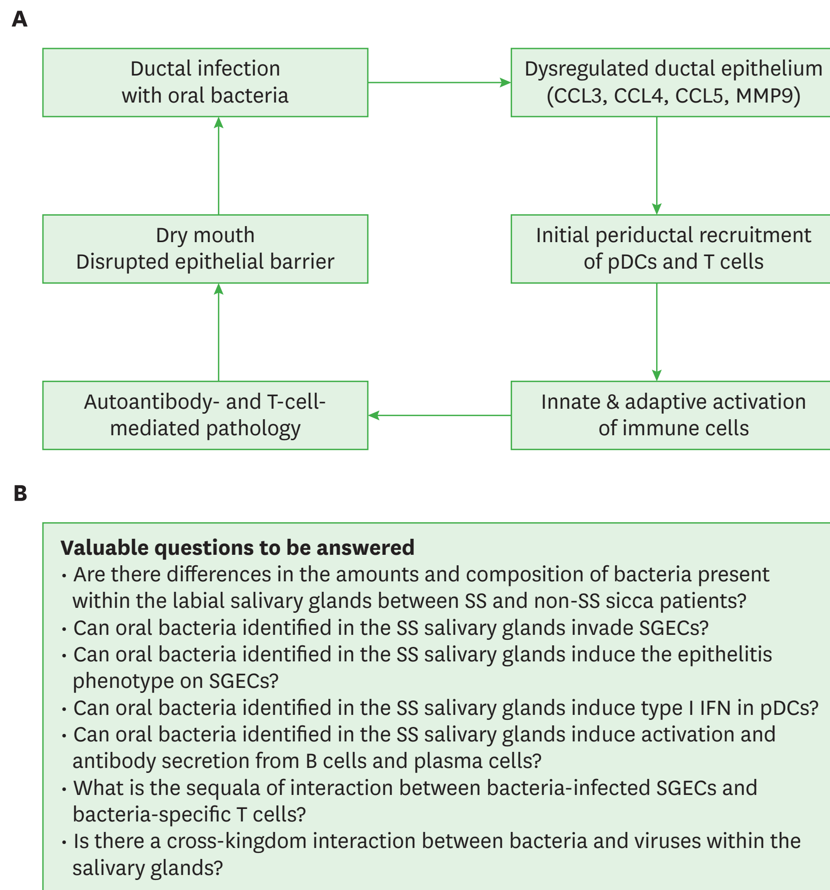

Figure 5. Contribution of the bacterial infection of the salivary glands to the perpetuation of sialadenitis in SS and future direction. (A) A vicious cycle of inflammation involving bacterial infection in the SS-affected salivary glands. (B) A list of valuable questions to be answered.

taxa present within the salivary glands of SS patients would be most important for future studies. Other valuable questions, including a cross-kingdom interaction between bacteria and viruses within the salivary glands, are listed (**Fig. 5B**). Future therapeutics must consider breaking the vicious cycle of SS pathogenesis involving bacterial infection.

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