Sjögren's Syndrome and Oral Health

Disease Characteristics and Management of Oral Manifestations

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Editor
Seunghee Cha
Center for Orphaned Autoimmune Disorders
University of Florida, College of Dentistry
Gainesville, FL
USA

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This book is dedicated to
Mio, Yoon, Alexandra, and McKayla
for their inspiration,
and to my patients for their courage.

Preface

Since the publication of the English translation of "Zur Kenntnis der Keratoconjunctivitis Sicca" in 1943, autoimmune Sjögren's syndrome (SS) has become a great interest to many clinicians and researchers worldwide. It had been 10 years after Dr. Henrik Sjögren, a Swedish ophthalmologist, presented 19 cases in his PhD dissertation that consisted of both clinical and pathological components of this unknown disease entity at the time. His tireless effort to characterize the disease was finally recognized during the 100-year anniversary of his birth, which was celebrated internationally in 1999. His prominent legacy in the field of SS has lived on ever since.

The etiology of SS is multifactorial and its pathogenesis is enigmatic. The unique involvement of the exocrine glands distinguishes SS from other medical conditions, and patients consequently suffer from severe dry mouth and dry eyes (sicca symptoms). However, chapters in this book contributed by renowned experts and pioneers cover more than sicca symptoms as SS can affect multiple internal organs, leading to various clinical presentations in patients. The heterogeneous clinical manifestations of SS and chronicity of the condition necessitate multidisciplinary patient care and collaboration among clinicians as well as support from political and community leaders. As the epitome of the latter, the Sjögren's Foundation (Sjogrens.org), an international, nonprofit organization founded in 1983, should be commended for providing excellent medical and educational information and grant resources to patients, clinicians, and researchers, and promoting initiatives and public awareness for SS over the years.

The overall format of this book consists of three parts. The vast amount of current knowledge presented in the book is undoubtedly the result of collaborative international efforts, which is important to note. The first part, with two chapters, sets the stage for this book by providing an overview of SS, recommended medical and dental management, and the importance of saliva and oral complications in SS. Chap. 1 (Stewart) describes the established diagnostic criteria and presents the gold standard of lip biopsy and serology as important clinical parameters, among others. Since the disease greatly affects the oral and dental health of patients, the importance of saliva and salivary components in SS is highly emphasized in Chap. 2 (Heller et al.).

The next five chapters present the immunopathogenesis of SS. More specifically, these include the molecular mechanisms for SS hyposalivation, roles of myoepithelial cell function in salivary gland physiology and disease, dysregulated innate immunity and adaptive immunity, and B cell expansion

and neoplasia in SS. Dissecting the mechanisms of hyposalivation in SS can be a daunting task, since multiple factors are reported to be associated with or contributed to dryness in patients. Chap. 3 (Saleh et al.) presents a comprehensive review of the molecules involved in secretory dysfunction, with a future goal of targeted therapy to restore glandular function in patients. Potential roles of myoepithelial cells in SS pathogenesis are brought into light in Chap. 4 (Maldorado et al.), thanks to the contribution of the Chirioni's group.

There has been great progress in characterizing the key immunological players in SS pathogenesis, which range from characterizations of Th1 and Th2 cells and the critical roles of autoantibodies and B cell activating factor (BAFF) in SS, to the recent interests in Th17, regulatory T cells, follicular helper and regulatory T cells, and marginal zone B cells, especially in association with a risk of non-Hodgkin's B cell lymphoma. Intensive research endeavors on innate immunity have returned with a special focus on the roles of dendritic cells and macrophages in sterile inflammation involving self-nucleic acid sensors, which is detailed in Chap. 5 by Kiripolsky and Kramer. Chap. 6 (Yu) and Chap. 7 (Anderson et al.) describe central players in SS immunopathogenesis, such as adaptive immune cells and B cells in neoplasia, respectively.

The third part of the book includes SS diagnostics and therapeutics: the differential diagnosis of glandular swelling in Chap. 8 (Bhattacharyya and Chehal), interventional sialoendoscopy for recurrent parotitis in Chap. 9 (Karagozgolu et al.), improved diagnostics by salivary gland ultrasound sonography in Chap. 10 (Thatayatikom), up-to-date pharmaceutical management of glandular and systemic manifestations of SS in Chap. 11 (Ha and Lee), recent therapeutic intervention of gene therapy in Chap. 12 (Yin and Chiorini), and clinical management of dry eye in SS in Chap. 13 (Hyon). These comprehensive chapters detailing the clinical components and management of SS will be highly informative, not only to professionals but also to patients. Chap. 10 is highly relevant to the emerging cases of SS in children and adolescents in recent years.

The highlight of this book is the set of three essays submitted by our patients, which follow this preface. Without their contributions, this book would have been unable to convey an important message to our readers of what it truly feels like to live with SS. I am immensely grateful to Diane, Suzanne, and Cameron for their willingness to share their experiences with other patients and readers by sharing their experiences through their essays. I would also like to express my sincere gratitude to Alison and Christobel on behalf of all our dedicated authors for keeping us on track and accommodating our needs in a timely and professional manner. The team at Springer has always exceeded my expectations since the beginning of my first book project with them on *Salivary Gland Development and Regeneration*.

Future progress in the SS field will certainly require continuous dialogue of innovative ideas and the exchange of knowledge and new discoveries among all those who are involved, which reminds me of the word "Consilience." This term was first coined by William Whewell in *The Philosophy of the Inductive Sciences* in 1840 and was revived by the humanist

biologist Edward O. Wilson in *Consilience: The Unity of Knowledge* in 1998. As Wilson pointed out in his book, "We can never map it all, never discover and explain everything… (But) We can connect threads into broadening webs of explanation, because we have been given the torch and the ball of thread." The exciting journey toward the unity of knowledge and novel discoveries in the field of SS and pediatric SS will continue.

Seunghee Cha
Gainesville, FL, USA

Living with Sjögren's Syndrome

What Is Sjögren's Disease to Me?

Cameron Popard

Health care professionals know the clinical definition: "Sjögren's Disease is a disorder of your immune system identified by its two most common symptoms—dry eyes and dry mouth." To me, Sjögren's is so much more than just dry eyes. To me, the definition is very different. To me, Sjögren's is having to remember to take 9 different pills every single night. It is my hands turning purple and losing feeling when the temperature drops below 60°. It is joint pain that makes it hard to move. It is fatigue and muscle pain so severe that I can't get out of bed without help. It is missing school for days at a time when I can't get out of bed at all. Sometimes it is having to stay home from school, not because *I am the one who is sick*, but because it is too risky to be in a building full of other "sick kids." Sjögren's is being hospitalized 4 times in less than 2 years with meningitis. It is monthly infusions and steroids to quiet my immune system and prevent organ damage. It is more needles, doctor's visits, and testing than most people will experience in a lifetime. It is trying to explain a disease, which I don't fully understand myself, to people who say "you don't look sick." It's wondering what my future might look like. It is worrying if it will be like this forever. Having Sjögren's is hard. It hurts. It makes me sad. I have had to miss out on fun things with my friends and family. I have had to cancel trips and events at the last second. But Sjögren's has also made me stronger, patient, more resilient, and more determined. I am only 16 years old and I have Sjögren's disease, but I refuse to let it have me.

My Journey with Sjögren's Syndrome

Diane Shurm

After numerous tests, many different doctors, and extensive weight loss (80 lbs.), I was finally diagnosed with Sjögren's syndrome, an autoimmune illness.There is a medical problem….and weight loss is not always involved.

The salivary glands cease to function, causing severe dry mouth, dental issues, and dry eye, which are also symptoms.

In my particular case, the severity of dry mouth continues to be much worse than dry eye. My ophthalmologist is very well educated in the diagnosis and treatment of Sjögren's so I am very pleased to be in her care. She

inserts very tiny plugs into my tear ducts every 3 months to help keep the eyes well lubricated.

This condition is usually treated by rheumatologists; however, the biggest hurdle for me has been finding one that treats Sjögren's….as most have minimal information on the condition.

There are just two medications available, Pilocarpine and Cevimeline; both have given me minimum success. It seems they each have many side effects. Aside from those, most of my treatment has been the "trial and error" method of OTC products that are available. The ones I have found to be most effective are Biotene products (mouth spray, mouth rinse, and most of all, the gel….I really get relief from that!). You must be very careful with everything you chose to eat or drink (no hot or spicy foods or drink for me!).

The National Sjögren's Foundation, located in Washington, DC, has many educational tools available and continues to do research to find a cure.

Until then, my prayer is REMISSION!

When Dry Mouth Was Jeopardizing My Career

Suzannah Laski

It's so easy to dismiss a dry mouth because most of the time the cause is due to dehydration. Both patients and doctors easily dismiss dry mouth without really thinking about it twice. But the thing is that some of those patients are attributing their symptom to the wrong cause. If a patient is brave enough to mention that they have dry mouth, please don't automatically dismiss it because the person who has it already did that enough. It happened to me for years. I wish my dentist, who I had been going to for decades, addressed the issue when I first said that my lips have been losing layers of skin when I wake up because they are literally glued to my front teeth. It took me about 2 years before I said anything. At that point the suction tool they used when cleaning my teeth was merely for decoration in my mouth. It no longer made the previously "dreadful" slurping noise it did years earlier and instead made the same hissing sound it does when sucking nothing but air. After I screwed up my courage I asked why has it been happening and if there's anything I can do to fix the issue. Other than adding lip balm and biotene mouthwash to my night routine and drinking more water during the day, my dentist and other doctors whom I asked all shrugged their shoulders.

It wasn't until I started pursuing voice over that I started taking my dry mouth seriously. A dry mouth with a microphone is one of the most distracting sounds imaginable. You no longer hear the meaning to any words spoken, you just hear the clicking, creaking, smacking, popping sounds of the lips, cheeks, and tongue make with scant saliva to lubricate the movements. The liquid "L"s of the American language are no longer liquid, much less employable. It took endangering my career prospects to start taking my dry mouth seriously. But what about the longer term consequences that prolonged dry mouth has on a person. What it does to the health of their teeth and gums, and what the loss of said teeth has on the person both financially and—more importantly—their quality of life. No matter your age, you see yourself

differently when you start getting more and more dental issues. You judge yourself the same as you used to judge others with bad teeth, as somehow less deserving.

I had specifically asked my doctors if there was a medication out there to help with dry mouth. I was told "no" by several doctors and others in the health field. Most voice over talent don't know that medicine is available either but some do. After trying all the other tricks of the trade (eating green apples, sucking on mint lozenges, drinking lemon water, drinking throat tea, drinking 120 oz. of water a day, using all the OTC dry mouth products on the market) and failing to get an effect that lasted more than 10 s, it was the voice over community that told me to seek a specialist. That specialist was easily able to identify and address my issue. I am now a working voice artist. But for years I wasn't and for years I had started having dental and gum issues. Although my career turned around, I'll never know if my gum issues, chipped tooth, cavities, and my new crown could have been prevented. I used to be proud of my smile and although I still get compliments, I feel like a fraud when I hear them since most of the teeth in that smile are not real. Most of the population with dry mouth are not in the voice over industry and will not be told by that community of a specialist who can save the rest of their teeth and sense of self. They will continue on a downward spiral and attribute it to bad luck in life. Now you are learning that there is something that can be done, please pass that information on. Share it along with the risks someone with prolonged dry mouth is susceptible to. Although most people can ignore the thirsty feeling, a lot won't if they knew there's more going on than that feeling.

Contents

Part I

Clinical/Laboratory Characteristics and Current Dental Management

1 Introduction, Diagnostic Criteria, Pharmacotherapy, and Dental Management

Carol M. Stewart

1.1 Introduction

1.1.1 History

A Swedish ophthalmologist, Henrik Sjögren (1899–1986), recognized a group of female patients with arthritis, extreme dryness of the eyes and mouth (sicca syndrome). In 1933, he published his thesis describing this condition and gave the term "keratoconjunctivitis sicca" to the ocular dryness. He believed that he had identified a new systemic disease, which now bears his name.

1.1.2 Definitions

SS is a chronic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands. SS is defined as primary (pSS) when oral dryness (xerostomia) and ocular dryness (keratoconjunctivitis sicca) occur alone. When pSS is associated with other rheumatologic conditions, such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE), the condition is referred to as secondary Sjögren's (sSS) [1].

1.1.3 Prevalence

After RA, SS is considered to be the most common rheumatic autoimmune disorder. In the USA, estimates are 0.2% or four million. Reported prevalence rates around the world range from 0.03% in Japan and 0.09% in Greece to 2.7% in Sweden [1]. The overall prevalence of pSS in Europe is between 0.1 and 4.8% [2]. However, due to multiple classification criteria, prevalence is difficult to determine. The commonly reported frequency ratio of women to men is 9:1, but a German report indicated a higher female prevalence [3]. In addition to middle-aged women, children, young adults, and teens are affected. As symptoms are often subtle and nonspecific, prevalence is likely higher than reported.

1.1.4 Disease Spectrum

The clinical disease spectrum is broad, and the severity of manifestations is oral, and ocular dryness may be mild and manageable in some patients, but others will deal with severe chronic

C. M. Stewart (✉)
Department of Oral and Maxillofacial Diagnostic Sciences, College of Dentistry, University of Florida, Gainesville, FL, USA
e-mail: cstewart@dental.ufl.edu

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pain, depression, major organ involvement, and lymphoma. The diminished quality of life due to oral involvement is significant [4]. Extraglandular manifestations may involve the skin, vaginal mucosa, hair, respiratory tract, gastrointestinal tract, kidneys, musculoskeletal, and neurologic systems [5–7]. Due to vague symptomology often involving multiple systems, disease recognition and diagnosis may be delayed months or years. On average, it takes 2.8 years to receive a diagnosis of Sjögren's according to the Sjögren's Syndrome Foundation, and SS has affected every racial and ethnic group [8]. Because of the complexity of this disease, manifestations often require interdisciplinary treatment. A differential diagnosis for possible SS can include other autoimmune disorders such as SLE, scleroderma, RA, and fibromyalgia, as well as HIV infection and hepatitis C. A complication of primary Sjögren's syndrome (pSS) is an elevated risk of lymphoma. It has been reported that pSS patients have a prevalence ranging from 3.4% in the first 5 years to 9.8% at 15 years after diagnosis and up to a 16-fold increased risk of developing non-Hodgkin's lymphoma (NHL) [9]. The risk of NHL in patients with pSS in Norway is increased nine times compared with the general population [10]. These are often low-grade B-cell lymphomas termed MALT or mucosa-associated lymphoid tissue lymphoma of the parotid gland. Signs may include lymphadenopathy and parotid gland enlargement.

1.2 Diagnosis

1.2.1 Signs and Symptoms

Sicca symptoms (dryness) are the most common manifestation of SS, occurring in up to 98% of cases [11]. Xerostomia and salivary hypofunction are the most common oral complications of SS [12]. Xerostomia is defined as the patient's subjective feeling of oral dryness with or without salivary gland hypofunction [13]. Salivary hypofunction is the objective measurement confirming a low salivary flow rate. Xerostomia may commonly be expressed by many patients with medical issues such as SS, RA, postradiation therapy for treatment of oral squamous cell carcinoma, and fibromyalgia. Additional factors linked to xerostomia include depression, perceived anxiety, stress, and intake of antihypertensive medications. All these factors should be considered and evaluated in patients presenting with xerostomia.

1.2.2 Diagnostic Criteria

Diagnosis is based on a review of the medical history and a complete physical examination, which includes at a minimum, a head and neck evaluation, salivary flow assessments, oral and maxillofacial imaging, and in many cases, a labial salivary gland biopsy. Appropriate ophthalmological and serological tests are performed as described in the following diagnostic criteria section. Hypertension, asthma, diabetes mellitus, hematological diseases, thyroid diseases, rheumatic diseases, gastrointestinal disorders, psychiatric disorders, and eating disorders are reviewed during the initial evaluation as these conditions can have an association with xerostomia and hyposalivation or occur concurrently with SS.

The three most commonly used criteria for the diagnosis of pSS are as follows:

1. The Copenhagen criteria proposed in 1986 included objective evidence of keratoconjunctivitis sicca, salivary gland involvement, and whole unstimulated salivary flow [14].
2. The European classification criteria was initially proposed in 1996 and was later revised in 2002 by the American-European consensus group (AECG) [15]. Criteria are found in Table 1.1.
3. The International Collaborative Clinical Alliances Cohort criteria was proposed in 2012 by the Sjogren's International Collaborative Clinical Alliances Cohort [16] and approved by the American College of Rheumatology (ACR) and Board of Directors and the European League Against Rheumatism (EULAR) Executive Committee in 2016 [17]. The final classification criteria are based on the weighted sum of five items as described in Table 1.1.

Table 1.1 Revised AECG criteria and the Sjogren's International Collaborative Clinical Alliances Cohort used for the diagnosis of primary Sjögren's syndrome

Revised AECG criteria	
Diagnosis of Sjögren's syndrome defined as the presence of four out of the six items, including positive histology or serology, or the presence of three of the four objective items	
1. Ocular symptoms: positive response to one of the following questions: (a) Have you had daily persistent trouble with dry eyes for more than 3 months? (b) Do you have a recurrent sensation of sand or gravel in the eyes? (c) Do you use tear substitutes more than three times per day?	
2. Oral symptoms: positive response to one of the following questions: (a) Have you had a daily feeling of dry mouth for more than 3 months? (b) Have you had recurrent or persistent swollen salivary glands as an adult? (c) Do you frequently drink liquids to aid swallowing dry food?	
3. Ocular signs: positive result for one of the two tests: (a) Schirmer's test performed without anesthesia (<5 mm in 5 min) (b) Rose bengal score or other ocular dye score (≥4 according to van Bijsterveld's scoring system)	
4. Histopathology: focal lymphocytic sialadenitis with a focus score ≥1 focus per 4 mm^2 of minor salivary glandular tissue	
5. Salivary gland involvement: a positive response for at least one of the following diagnostic tests: (a) Unstimulated whole salivary flow (<1.5 mL in 15 min) (b) Parotid gland sialography showing the presence of diffuse sialectasis without evidence of obstruction in the glands or (c) Salivary scintigraphy showing delayed uptake, reduced concentration, and/or delayed excretion of tracer	
6. Autoantibodies: presence in serum of antibodies to Ro (SSA) or La (SSB) antigens, or both	
2012 American College of Rheumatology classification criteria for Sjögren's syndrome [17]	
1. Positive anti-SSA (Ro) and/or anti-SSB (La) or positive RF and ANA ≥1:320	
2. Labial salivary gland biopsy with a focal lymphocytic sialadenitis with a focus score ≥ 1 focus per 4 mm^2	
3. Keratoconjunctivitis sicca with an ocular staining score ≥ 3	
2016 American College of Rheumatology/European League Against Rheumatism classification criteria for Primary Sjögren's Syndrome [18]	
1. Anti-SSA/Ro antibody positivity	Score = 3
2. Focal lymphocytic sialadenitis with a focus score of ≥1 foci/4 mm^2	Score = 3
3. Abnormal ocular staining score of ≥5 (or van Bijsterveld score of ≥4)	Score = 1
4. Schirmer's test result of ≤5 mm/5 min	Score = 1
5. Unstimulated salivary flow rate of ≤0.1 mL/min	Score = 1

Individuals with signs and/or symptoms suggestive of SS with a total score of ≥4 for the above items meet the criteria for pSS. Sensitivity and specificity against clinician-expert-derived case/non-case status in the final validation cohort were high, that is, 96% (95% CI 92% to 98%) and 95% (95% CI 92% to 97%), respectively
Table adapted from [16, 17]

1.2.3 Chairside Clinical Observations Indicative of Possible Hyposalivation

1. Lack of saliva pooling in the floor of the mouth.
2. Adherence or "sticking" of back of the mouth mirror or a gloved finger to the buccal mucosa or tongue.
3. A lobulated or deeply fissured tongue could indicate inadequate salivary flow (Fig. 1.1a).
4. Cervical caries in more than three teeth (Fig. 1.1b).
5. Lack of clear fluid flowing from Stensen's duct upon "milking" the parotid gland.
6. Salivary gland enlargement.

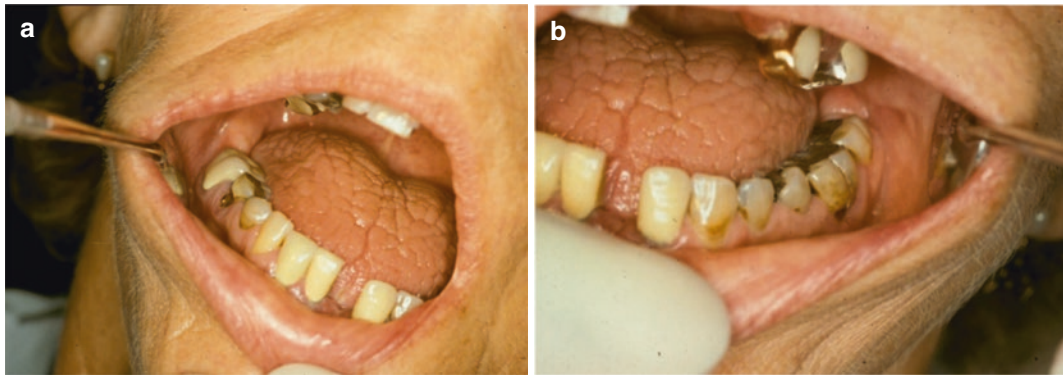

Fig. 1.1 Dry mouth complications. (**a**) Fissured tongue, (**b**) Class V decay and multiple crowns and missing teeth

1.2.4 Chairside Patient Questions to Aid the Detection of Hyposalivation and SS Diagnosis [18, 19]

- Does your mouth feel dry?
- Do you have difficulty swallowing?
- Does food stick in your mouth or throat?
- Can you eat a dry cracker without water?
- Do you have difficulty speaking?
- Has your taste sensation decreased?
- Do you keep a glass of water at your bedside at night?

If a patient answers "yes" to three or more questions, additional objective assessments should follow. These might include measurement of stimulated and unstimulated salivary flow rate to more accurately determine xerostomia from salivary hypofunction. In addition, a labial salivary gland biopsy, imaging of the salivary glands, and screening blood work for an autoimmune disorder such as SS and/or referral to a rheumatologist would follow.

1.2.5 Xerostomia, Salivary Hypofunction, and Sialometry

Xerostomia is defined as the subjective complaint of dry mouth [20]. Unfortunately, the importance of xerostomia is sometimes minimized. Xerostomia or "dry mouth" has a significant adverse impact on patients' attitudes and emotional well-being. The complaint of oral dryness requires a complete evaluation of a patient's overall health, oral motor, and sensory abilities, as well as salivary flow rates. It is often determined that patients with complaints of xerostomia do not demonstrate low flow rates based on objective sialometry measurements. Symptoms may be related to other conditions, hence the need for a comprehensive evaluation [21].

Salivary hypofunction is applied to salivary gland function below expected ranges. The normal stimulated salivary flow rate for healthy nonmedicated adults averages 1.5–2.0 mL/min, while the unstimulated salivary flow rate is approximately 0.3–0.4 mL/min [22, 23]. Flow rates will vary between individuals. Salivary hypofunction is defined as an unstimulated whole salivary flow rate (UWSFR) of <0.1 mL/min [24] and a stimulated whole salivary flow rate (SWSFR) of <0.7 mL/min [23, 25].

Salivary hypofunction may lead to oral problems such as dental caries and oral candidiasis [26–29]. Due to salivary hypofunction, the important caries preventive actions of saliva are reduced. These include flow rate which aids in a "flushing action" of organisms from the oral cavity and buffer effects which help neutralize the harmful effects of acids. It has been reported that the risk of developing dental erosions is five times more frequent in patients with UWSFRs ≤0.1 mL/min [23]. Even with good oral hygiene, individuals with Sjögren's syndrome experience

dental decay and tooth loss [30]. In Chap. 2, a thorough review of saliva, the components, and its importance will be presented.

Sialometry is an objective measurement of salivary flow rate, which will assist the health care team in the determination of the patient's caries risk assessment and hyposalivation. For the most accurate assessment of salivary flow rate, the patient should refrain from smoking, eating, or drinking for at least 1 h prior to the saliva collection. (Patients may drink water during this time.) When both the whole unstimulated and whole stimulated flow rates are planned for the same appointment, the unstimulated measurement should be performed first, before the stimulated flow rate.

To assess the patient's UWSFR, the patient is asked to sit with their head slightly tilted forward, without talking or chewing, and expectorate (spit or drool) into a preweighed collection tube for a preset time, usually 10 or 15 min. The tube is weighed following the collection and the difference is calculated. The weight difference is divided by the number of collection minutes to determine the rate of flow in mL/min. An assumption is made that saliva is similar to water, where 1 g of water/saliva at 4 °C equals 1 mL of saliva/water [31]. UWSFRs may be a more important parameter than the stimulated flow rate due to unstimulated salivary flow rates being below normal even when the stimulated flow is not affected [32]. To measure the SWSFR, the patient is asked to sit with head slightly tilted forward and chew on a small square of sterile paraffin wax for a preset time, often 10 min, while expectorating the saliva generated into a preweighed tube or container. Expectorating may be done at 30 s or 1-min intervals as appropriate. The tube is weighed again at completion of the collection period. The flow rate calculation is determined by dividing the difference between the two tube weights by the number of minutes, 10 min in this case. As previously stated, the normal stimulated salivary flow rate is greater than 0.7 mL/min.

A screening test for salivary flow has been reported using calibrated filter paper (Whatman no. 41) placed in the floor of the mouth. The length of wetness is measured after 5 min. Sometimes it is referred to as an oral Schirmer's test, due to similarities with the technique used by ophthalmologists to measure tear film wetness. The oral Schirmer's test is useful, objective, and easy to perform. The sensitivity and specificity are adequate for purposes of screening, and the procedure is well accepted [33, 34].

1.2.6 Lab/Serological Testing

Immunofluorescence testing for antinuclear antibodies (ANA) is highly relevant for the diagnosis of connective tissue disorders. In one report, up to 83% of patients with pSS tested positive for ANA. The association of positive ANA with the presence of anti-Ro/SS-A and anti-La/SS-B antibodies reached statistical significance at a titer of ANA >1/80 ($p < 0.001$) [33]. However, positive ANA titers can be found in seemingly healthy people. While assays vary, it has been reported that up to 20% or more of otherwise healthy people can express ANA. Females express this more commonly than males [35, 36]. Anti-SSA/Ro and anti-La/SSB are the hallmark antibodies in pSS. Anti-Ro/SSA and/or anti-La/SSB antibodies are found in approximately 40%–75% and 23%–52% of pSS patients, respectively [1]. Among these investigations, anti-Ro/SSA antibodies and an abnormal labial salivary gland biopsy have the highest specificity.

1.2.7 Labial Salivary Gland Biopsy

According to the 2012 criteria approved by the Sjögren's International Collaborative Clinical Alliances Cohort and Academy of Rheumatology [16], the labial salivary gland biopsy is one of the two most reliable criteria for a Sjögren's diagnosis. The second is anti-Ro/SSA antibodies. In the clinical practice, the lip biopsy is especially helpful for those patients with glandular dysfunctions and negative antibodies.

The lower lip contains a multitude of minor salivary glands that will show a characteristic

histopathologic pattern in patients with Sjögren's syndrome. The salivary glands are easily accessible lying just below the surface of the lower lip. After infiltration of local anesthesia, a small incision (5 mm) is made in the left or right side of midline in the lower lip. Approximately, 5–7 lobules of accessory salivary glands are harvested and placed in 10% formalin fixative for delivery to the specially trained pathologist (Fig. 1.2). The incision is closed with resorbable sutures. The patient should be instructed to expect some tenderness for a few days after the procedure. Approximately, 1–2% of individuals will notice some tingling and/or numbness of the lip for a period of 2–3 months. In rare instances, the altered lip sensation or partial numbness may persist.

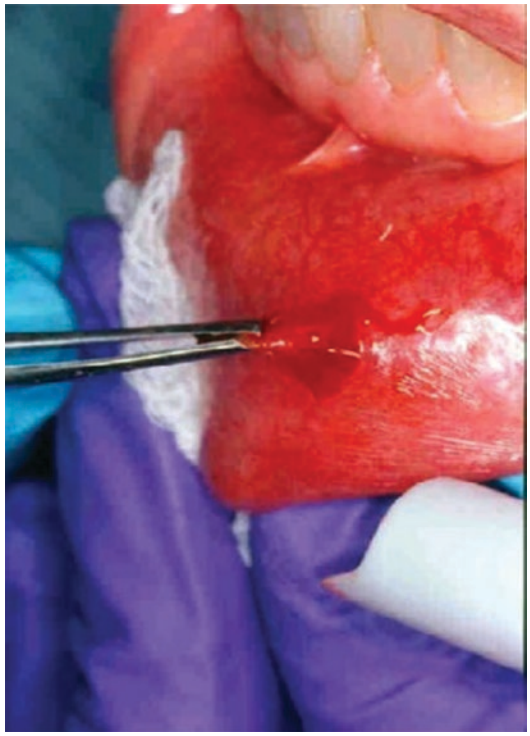

Fig. 1.2 Labial salivary gland biopsy procedure demonstrating harvesting accessory salivary gland lobule

It is essential that the labial salivary gland biopsy be interpreted by pathologists familiar with oral pathology and specifically salivary gland pathology. One of the key features found in the histopathology of the minor salivary gland in Sjögren's syndrome is "focal lymphocytic sialadenitis." It is characterized by periductal lymphocytic clusters (more than 50 lymphocytes) adjacent to normal-appearing mucous-secreting acini. The number of lymphocytic aggregates per 4 mm^2 of glandular tissue section is termed the "focus score." A focus score ≥ 1 is the validated criterion for the classification of Sjögren's syndrome from non-Sjögren's control subjects [37] (Fig. 1.3). If the diagnosis is not supportive of Sjögren's syndrome, the lip biopsy may reveal other types of glandular inflammation such as chronic sialadenitis or sarcoidosis.

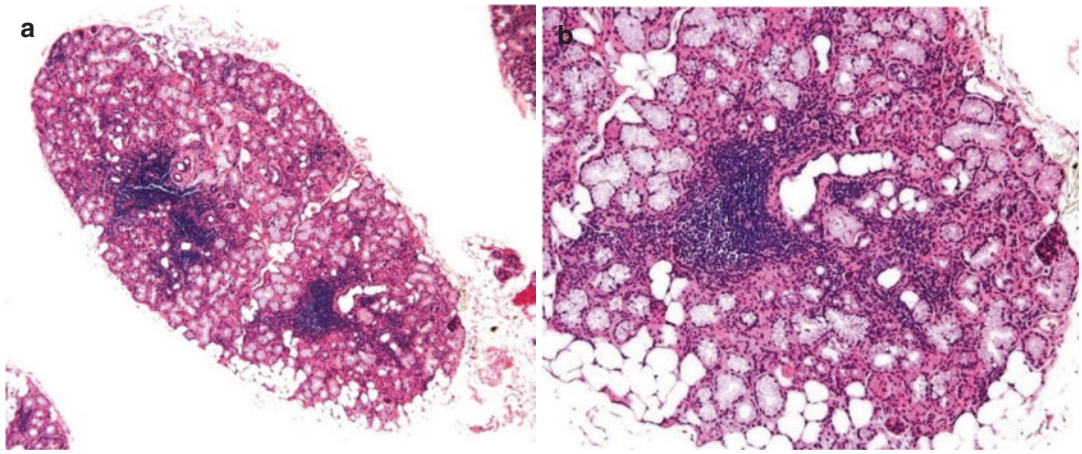

Fig. 1.3 Histopathology for labial salivary gland biopsy supportive of SS diagnosis. (**a**) Labial salivary gland biopsy demonstrating distinct focal lymphocytic clusters, H&E 10×, (**b**) lymphocytic focus surrrounding duct, with normal-appearing adjacent acini, H&E 20×. (Both photos taken by Dr. Indraneel Bhattacharyya)

1.2.8 Salivary Gland Enlargement and Lymphoma

Roughly one-third of Sjögren's patients report salivary gland enlargement. This may be episodic or chronic and may occur unilaterally or more common bilaterally [38]. Enlargement that does not respond to short-term therapy must be further assessed to rule out the possibility of NHL of B-cell type. NHL has been reported to occur in 5% of patients with pSS [9]. These are low-grade B-cell types that are usually slow-growing and respond well to treatment. Predictors for NHL in Sjogren's patients include monoclonal gammopathy, C4 hypocomplementemia, and cryoglobulinemia [39]. Additional information regarding salivary gland enlargement and diagnostic approaches will be further discussed in Part III.

1.2.9 Sialendoscopy

Sialendoscopy is a minimally invasive diagnostic and therapeutic technique for the management of obstructive salivary gland disorders [40]. The main indication for sialendoscopy is obstructive sialadenitis from sialolithiasis, mucus plugs, and anatomic ductal abnormalities [41–43]. Recent studies have shown that patients with radioiodine-induced sialadenitis and patients with salivary glands affected by autoimmune diseases, such as SS, felt relief from symptoms after sialendoscopy. A report of SS patients and controls stated that irrigation of the major salivary glands in patients with SS-enhanced salivary flow and reduced xerostomia up to 6 months after sialendoscopy [44]. The procedure is also associated with complications. In a SS cohort in Amsterdam, overall rate of complications was limited, and the sialendoscopic complications in patients with SS were regarded as minor by the authors. Most complications involved the submandibular glands in SS patients. Careful preoperative selection of patients and salivary glands was recommended to reduce the rate of complications and yield more predictable results [40].

1.3 Pharmacotherapy

1.3.1 Caries Prevention and Home Care

Nonprescription, or over the counter (OTC), agents play a role in the management of Sjögren's syndrome. Meticulous home care is essential for caries management. Patients should brush their teeth 2–3 times per day with a soft manual brush or ultrasonic device. Ideally, rinsing with water and/or brushing after each sugar intake would be beneficial. Patients could be encouraged to take a toothbrush to their place of employment, if possible. Flossing once per day will help remove dental plaque. Patients with severe hyposalivation should avoid toothpastes containing abrasive agents. Toothpastes designed for the relief of dry mouth usually do not contain sodium lauryl sulfate (SLS) and tend to be less irritating to the tissues [45]. Recall dental examinations every 4 months can serve to closely monitor caries disease activity and continue to educate and encourage the patient to assume partnership in their oral health. In a culture focused on a "beautiful white smile," the issue of whitening toothpastes and agents must be addressed. Whitening toothpastes often contain peroxide and agents harmful to gingival tissues and as well as enamel. Due to potential injury to enamel and gingival tissues, it is prudent to avoid these or consult the dentist if whitening is desired prior to special social occasions.

Salivary stimulation is widely accepted as a basic therapeutic measure for preventing caries in SS patients with dry mouth. Mechanical stimulation by chewing sugar-free gum or lozenges is often used by patients with mild salivary hypofunction [46]. A list of nonprescription agents that could be useful are listed in Table 1.2. These agents are relatively short-acting but may provide symptomatic relief. Commonly, these strategies are ineffective in providing adequate comfort and oral health. In patients without contraindications, the medical team will employ prescription anticholinergic sialogogues.

Table 1.2 Products with potential benefit for symptomatic relief for xerostomia "dry mouth"

Nonprescription topical saliva substitutes and salivary stimulants
Chewing gum
Biotene® Chewing Gum, Biotene® Dry Mouth Gum
OMNI® Thera Gum; 100% xylitol sweetened gum
Spry® Gum with xylitol
XyliChew™ 100% xylitol chewing gum
Lozenges and mints
OMNI®Thera Mints: 100% xylitol sweetened mints
OraMoist™ Dry Mouth Discs
XyliChew™ 100% xylitol mints
TheraBreath dry mouth lozenges and spray
Xylimelts dry mouth lozenges
MighTeaFlow Natural Dry Mouth Lozenge w/Xylitol (Green tea-containing product)
Oral lubricating agents (moisturizers and sprays)
Biotene® Moisturizing Mouth Spray
Moi-Stir® Oral Spray
Mouth Kote® Dry Mouth Spray
Oasis® Moisturizing Mouth Spray
Moisyn Spray
Prescription products
NeutraSal® Calcium Phosphate Rinse (packet contents must be mixed with water)
Numoisyn® lozenges
Numoisyn® liquid
Electrical stimulation (electrode-based removable appliance, Saliwell)

Note. The efficacy of nonprescription agents has not been universally validated via evidence-based scrutiny in peer review publications

Artificial saliva or saliva substitutes, available without a prescription, are often used to lubricate the mouth, but do not permanently increase salivary flow. They are available in gels, sprays, and solutions. Some common ones are Biotene®, Oral Balance® moisturizing gel and oral rinse, Mouth Kote® dry mouth spray, Oasis® mouth moisturizing spray, and many others contained in Table 1.2. Many products provide short-term relief of oral mucosal dryness by increasing the product viscosity with components as carboxymethylcellulose-type agents. Some also contain calcium, fluoride, and flavoring agents. Individual patients will determine what product provides them the greatest comfort.

1.3.2 Agents for Caries Prevention: Fluoride

The Sjögren's Syndrome Foundation Oral Working Group conducted an evidence-based examination of current research. The group reported a high level of confidence that topical fluoride should be used in Sjögren's patients with hyposalivation and rated it as a "best clinical practice" [47]. A neutral sodium fluoride (0.05%) mouth rinse at bedtime may be recommended by the dentist. More commonly, for patients with high caries risk, the dentist will prescribe a 1.1% neutral sodium fluoride-containing toothpaste (such as Prevident 5000® for dry mouth) for use once per day in place of the regular toothpaste. Alternatively, 1.1% neutral sodium fluoride gels may be administered in custom-made trays prepared by the dentist. In the evening before bedtime, the fluoride gel is placed in the tray (1/2 to 3/4 full) and left for 4–5 min. The excess gel is expectorated. Fluoride-containing products should not be swallowed. Patients should be reminded to avoid rinsing, eating, or drinking, for 30 min after the use of fluoride toothpaste and gels.

1.3.3 Agents for Caries Prevention: Non-Fluoride Remineralizing Agents

The Sjögren's Foundation Oral Working Group reviewed non-fluoride remineralizing agents such as amorphous calcium phosphate (ACP), casein phosphopeptide-ACP, calcium sodium phosphosilicate, and tricalcium phosphate**.** These had moderate support and could be considered adjunctive therapy in patients with Sjögrens disease with dry mouth and a high root caries rate. This recommendation was based on demonstrated benefit of calcium phosphate rinse [47]. These agents may deliver calcium and phosphate to the tooth surface, enhance the function of fluoride, and help treat early non-cavitated lesions.

1.3.4 Antimicrobial Agent for Caries Prevention: Chlorhexidine

Chlorhexidine gluconate rinse (0.12%) is an antimicrobial agent with some antifungal activity as well. It is used to decrease the level of *Streptococcus mutans* in the oral cavity, which plays an important role in caries development. Due to oral dryness in SS patients, the susceptibility to oral candidiasis is increased and chlorhexidine rinse provides an added benefit. Chlorhexidine varnish and gel have been used to reduce the concentration of bacteria and candida in the oral cavity. While these products may impart a brownish surface stain to the teeth, the stain can be removed with a dental prophylaxis. Chlorhexidine may be helpful for SS patients as it reduces the microbial load of *Streptococcus mutans*. However, the working group analysis concluded that support was weak as studies have not been conducted with SS patients [47].

1.3.5 Prescription Sialogogues

Results of enhanced patient comfort and changes in salivary flow with two commonly used prescription anticholinergic sialogogues, pilocarpine (Salagen®) and cevimeline (Evoxac®), have been reported. Prescription sialogogues may improve comfort and Sjögrens patient's ability to chew, swallow, taste, and speak as well as their self-esteem and ease in social settings. They may be helpful for those who wear removable dental prosthetic appliances such as conventional removable complete dentures and removable partial dentures. The effectiveness of these agents requires the presence of functional glandular tissue.

1.3.5.1 Pilocarpine

Several studies have reported that pilocarpine can improve salivary flow in Sjögren's patients [48–50]. Pilocarpine is a cholinergic parasympathomimetic agent, which will stimulate secretion of exocrine glands via muscarinic activity. The sweat, salivary, lacrimal, gastric, pancreatic, and intestinal glands and the mucous cells of the respiratory tract may be stimulated. The recommended starting dose for SS patients is 5 mg tid for adults. Dosage may be increased based on therapeutic need by the physician [48, 51]. Commonly reported adverse effects include sweating, warmth and flushing sensation, increased urinary frequency, headache, and abdominal discomfort. Pilocarpine toxicity is characterized by an exaggeration of its parasympathomimetic effects. These may include: headache, visual disturbance, lacrimation, sweating, respiratory distress, gastrointestinal spasm, nausea, vomiting, diarrhea, atrioventricular block, tachycardia, bradycardia, hypotension, hypertension, shock, mental confusion, cardiac arrhythmia, and tremors [51]. Pilocarpine is contraindicated in patients with a known hypersensitivity to pilocarpine, uncontrolled asthma, acute iritis, and narrow-angle (angle closure) glaucoma [48, 51].

1.3.5.2 Cevimeline

Cevimeline hydrochloride is a cholinergic agent with muscarinic agonist activity prominently affecting the M1 and M3 receptors prevalent in exocrine glands. It is indicated for treatment of salivary gland dysfunction in SS patients. Reports indicate the 30 mg three times per day was well tolerated and provided relief of xerostomia symptoms [52, 53]. Common side effects are sweating, excessive salivating or drooling, nausea, loss of appetite, runny or stuffy nose, flushing, frequent urge to urinate, dizziness, weakness, diarrhea, constipation, blurred vision, and muscle pain. Both pilocarpine and cevimeline are relatively contraindicated in patients with uncontrolled asthma or chronic pulmonary disease and in β-adrenergic blocker users and should be used with caution in patients with active gastric ulcers or uncontrolled hypertension [48].

Studies have compared profiles and efficacy of pilocarpine and cevimeline in secretion of saliva. They reported that the efficacy of pilocarpine (5 mg three times a day) and cevimeline

(30 mg three times a day) in secretion of saliva was similar, but cevimeline had less side effects particularly in long-lasting therapy [54, 55].

1.3.5.3 Electrostimulation

Neural electrostimulation of salivary gland function by application of electrical current through the oral mucosa on afferent nerve pathway receptors has been reported to increased production of saliva. A removable oral device for management of xerostomia consists of a custom-fitted tray upon which the electrical circuitry, stimulation electrodes, a battery, a wetness sensor, and the remote-control receiver are mounted using a sandwich technique. This device has reported to improve symptoms of xerostomia with no significant side effects [56]. A recent meta-analysis of transcutaneous nerve stimulation (TENS) identified six randomized control trials (RCTs). Results showed that the effect of TENS on salivary flow rate was not statistically significant. However, the devices provided symptomatic improvement of xerostomia [57].

1.3.6 Medication-Induced Hyposalivation

Many medications can induce salivary gland dysfunction. These include the following [58, 59]:

- Antiemetics: Block neurotransmitter dopamine D2, serotonin types 2–4, (5HT2–4), histamine type 1 (H1), and acetylcholine (muscarinic types M1–5 receptors)
- Antiobesity agents: Inhibit the neuronal uptake of norepinephrine, serotonin, and dopamine in the central nervous system
- Antiepileptics: Act centrally by inducing a decrease in the release of neurotransmitters such as glutamate, norepinephrine, serotonin, and dopamine
- Antihistamines: Central inhibitory action on histamine type 1 receptors and muscarinic receptors
- Analgesics: Inhibit the salivary reflex arc in the central nervous system by blocking noradrenaline reuptake
- Cardiovascular agents, including antihypertensive and antiarrhythmic: Block muscarinic receptors and *a*1- and *b*1-adrenergic receptors
- Gastrointestinal agents: Block muscarinic receptors
- Obstructive airway disease agents: Block muscarinic receptors M1 and M3
- Ophthalmologicals: Inhibit acetylcholinesterase inhibitors and block *a*2-adrenergic
- Muscle relaxants: Act as central *a*2-adrenergic receptors agonists
- Psycholeptics: Enhance GABA effect in the central nervous system, downgrade salivary secretory reflex, and block muscarinic and *a*1- and *a*2-adrenergic receptors
- Psychoanaleptics: Inhibit acetylcholinesterase, block serotonin, histamine, dopamine, and norepinephrine reuptake
- Obstructive airway disease agents: Block muscarinic receptors M1 and M3
- Urologicals: Block muscarinic receptors and a1-adrenergic receptors

Sometimes the physician can reduce the dose of the xerogenic medication or substitute a medication that is less xerostomia-inducing [24]. Since saliva has a diurnal variation, the mornings and especially evenings are often most problematic. When possible, patients should avoid taking medications known to produce oral dryness at bedtime.

1.3.7 Conventional Systemic Pharmacotherapy

The decision to adopt systemic treatment, and the choice of the specific treatment in pSS, is often driven by the level of disease activity and the involved organ system. The few randomized control studies evaluating the use of conventional disease-modifying antirheumatic drugs (DMARDs) inpatients with pSS did not provide conclusive evidence supporting their efficacy. Thus, treatment strategies frequently are based on experiences acquired in other autoimmune rheumatic diseases, such as SLE or RA. Following the indications given for other con-

nective disorders and in view of its favorable side effect profile, hydroxychloroquine is the drug of choice for inflammatory musculoskeletal pain, and various mild-to-moderate systemic manifestations, such as arthralgia, arthritis, and cutaneous lesions. If hydroxychloroquine is not effective, methotrexate may be considered [47]. Long-term (more than 1 month) ≥15 mg per day of corticosteroids may be useful in the management of moderate inflammatory musculoskeletal pain in primary Sjögren's, but efforts should be made to find a steroid-sparing agent as soon as possible.

1.4 Dental Management

1.4.1 Caries Prevention and Management: Patient Counseling

Prudent dietary selections and lifestyle choices can support caries prevention. Minimal sugar intake between meals should be encouraged. Sugar-free snacks which contain sweetening agents that do not promote dental caries, such as xylitol, are acceptable. Chewing gum and lozenges sweetened with xylitol promote saliva production, thereby increasing comfort during the day. While xylitol is safe for humans, patients should be advised to store xylitol-containing agents away from dogs due to toxicity and potential death from hypoglycemia [60].

Drinking water throughout the day to maintain hydration is helpful as well [61]. When taking prescription sialogogues, hydration is very important. Patients with Sjögren's should avoid alcohol consumption and alcohol-based mouth rinses, which might promote xerostomia. Other lifestyle modifications to help address oral dryness include maintaining adequate hydration by frequently sipping water or sucking on finely crushed ice. Patients should be instructed to avoid sipping on carbonated beverages throughout the day. Generally, these will have an acidic pH. SS patients lack adequate salivary buffering properties to mitigate this effect. Consequently, the sugar and low pH will promote dental caries.

Patients may express a decrease in appetite and changes in taste. They often experience intolerance to acidic food as salad dressings, citrus, and spicy foods, such as hot peppers. In addition, patients may have altered taste sensations, a diminished ability to enjoy flavors, and an overall decrease in pleasure when eating a meal. Gastroesophageal reflux disease (GERD) is a common manifestation in patients with SS. In addition to discomfort, acidic stomach contents reflux back into the mouth and can reduce salivary pH. Acidic or low salivary pH will contribute to dental erosion. Sjögrens patients should adhere to physician-prescribed medication for GERD, dietary instructions, and perhaps sleep semi upright to minimize reflux issues.

Smoking, including vaping and use of e-cigarettes, has a strong association with dry mouth and should be minimized or discontinued altogether [62]. For patients who smoke, integrating smoking-cessation counseling and associated treatment are basic components for xerostomia management [63, 64]. Excessive alcohol intake and the use of alcohol-containing mouth rinses should be avoided due to enhanced effects of mucosal dryness.

1.4.2 Management of Oral Candidiasis

Patients with SS are at higher risk of oral candidiasis than a healthy control group. In a cohort of pSS patients, 74% were found to have oral candidiasis vs. 23% in the healthy control group. The erythematous type located on the dorsal tongue and angular cheilitis was most commonly found [65]. Signs of this include angular cheilitis, painful fissures at the labial commissures, erythema of the tongue and/or palate, altered often metallic taste, and a burning sensation of the tongue or affected area. An inverse relationship between stimulated salivary flow rates and the level of *Candida* infection in SS patients has been described [28]. After confirmation of the diagnosis via smear or culture, treatment with antifungal agents, such as nystatin ointment or tabs, or clotrimazole ointment or troches could be

appropriate. Some prescription rinses may contain sucrose and sugars that can promote dental decay if used on a long-term basis. For angular candidiasis, clotrimazole ointment or triamcinolone/nystatin ointment could be applied [13]. Specially formulated troches or rinses compounded by a pharmacy using alcohol sugars as artificial sweeteners are preferred to sucrose-containing rinses. For patients who are severely compromised and/or do not respond to these agents, fluconazole (Diflucan®) would be a reasonable consideration. Before prescribing any medication, a knowledge of drug interactions and contraindications must be reviewed.

SS patients with oral candidiasis should receive instructions regarding home care procedures.

- Improve salivary flow by chewing sugar-free gum or sugar-free lozenges
- Replace their toothbrush with a new one
- Clean dental prosthesis with antifungal agents such as nystatin solution or chlorhexidine gluconate/water 50:50. Soak for 30 min and then rinse thoroughly
- Avoid wearing removable dental appliances during sleep—if possible

1.4.3 Review of Periodontal Disease in Sjögren's Syndrome Patients

Periodontal disease is an inflammatory disease that results in damage to the periodontal soft tissue and bone surrounding the tooth. Studies have reported that SS patients have 2.2 times higher risk of having adult periodontitis than healthy controls [66]. A 2015 report regarding SS patients indicated that the mean number of decayed, missing and filled teeth (DMFT) was significantly higher in SS patients than in non-SS patients. The plaque index, gingival index, and papillary bleeding index were higher in SS patients than in non-SS patients [67].

Additional reports have not supported the association of an elevated risk of periodontitis in Sjögren's patients [68–71]. Kuru reported that no significant differences could be detected in either clinical or microbiological parameters of primary or secondary SS patients compared with that of age- and gender-matched control subjects. This was a small study. A larger study using the Copenhagen criteria for determination of pSS reported periodontal pockets of 4–5 mm as well as pockets >5 mm occurred with similar prevalence among the two groups. Smoking habits did not influence the results. The authors concluded that the health status of the gingival and periodontal tissues was thus similar in SS and controls. For optimal patient oral health, measures to avoid periodontal disease, such as meticulous oral hygiene and smoking cessation, should be stressed.

1.4.4 Review of Periodontal Disease in Secondary Sjögren's (sSS) with Rheumatoid Arthritis

Some studies report that periodontal disease may be associated with rheumatoid arthritis. While a definitive relationship is yet to be determined, current thoughts are of interest to those managing sSS. Rheumatoid arthritis and periodontal disease are both chronic inflammatory diseases that share characteristics such a bone erosion/bone loss, certain immunologic features, and characteristic cytokines. Anti-citrullinated protein antibodies (Anti-CCP) are found in both diseases. *Porphyromonas gingivalis,* a gram-negative anaerobic bacterium involved in periodontal disease, is capable of citrullinating local antigens and, therefore, potentially initiating anti-CCP antibodies. Anti-CCP antibodies have been detected in joint fluid of RA patients, but the *P. gingivalis* organism has not. Periodontal local treatment only affects the clinical periodontal status and does not alter circulating levels of IL-6 tumor necrosis factor-alpha or C-reactive protein, both associated with rheumatoid arthritis [72]. A recent study of anti-CCP-positive patients without RA, anti-CCP+ with RA and healthy controls, reported that "In individuals at risk of RA,

periodontitis and *P. gingivalis* were increased before joint disease and may be a target for prevention" [73].

1.4.5 Review of Orofacial Function and TMD in Sjögren's Syndrome

Orofacial function appears to be adversely impacted in Sjogren's patients. Temporomandibular disorders seem to be more prevalent in SS patients, diagnosed with the AECG criteria, when compared with a cohort of "healthy" people**.** These include xerostomia, dysgeusia, dysphagia, and TMD symptoms to include muscle pain on chewing and difficulty in mouth opening. Based on clinical examination, myofascial pain and deviation on function were significantly higher in the SS group than controls [74]. Another recent study reported statistical significance in SS patients regarding myofunction function and jaw functional limitation when compared with controls [75]. These findings suggest that SS patients should be evaluated and monitored for orofacial concerns as they can impact quality of life and nutritional factors.

1.4.6 Restorative Considerations

A report regarding SS patients indicated that the DMFT was significantly higher in SS patients than in non-SS patients [67]. For short-term disease control, restorative agents such as silver diamine fluoride and glass ionomer cement have been suggested [76].

1.4.7 Dental Implants

Due to the high rate of caries in SS patients, many patients undergo extensive dental work including root canals and crowns. Preventing recurrent decay around crown margins is a challenge, and unfortunately, teeth must often be extracted. Due to sensitive oral mucosa, oral dryness, and inadequate denture retention, many SS patients are unable to adapt to conventional dentures. After consultation with their dentist, SS patients may select oral rehabilitation which includes dental implants. Early in the implant evolution, physicians and dentists were concerned that implants would not properly osseointegrate in SS patients due to the immune-compromised status and polypharmacy issues. Recent research has indicated that implants are a viable option for SS patients. The probability of failure was 2.8% (95% confidence interval 1.6–4.1%). A systematic analysis reported the failure rate of 4.1% at 12.9 ± 31.7 months. Implants failed at a mean time of 12.9 ± 31.7 months (range 1–160 months after implant placement). The mean marginal bone loss around implants (−2.190 ± 1.384 mm) was higher for SS patients than the general population [77].

Another cohort study, 4.8% of the implants in SS patients (5 of 104) had to be removed vs. none in the controls. The majority of SS patients (75%) were highly satisfied with the implants, and 97% would recommend implants to other SS patients [78]. A retrospective study of 50 SS patients in Netherlands concluded that SS patients seemed to perform at a comparable level with implants in healthy patients. Peri-implantitis around one or more implants was seen in 14% of the SS patients (11% of the implants) and in 12% of the healthy controls. Implant survival was 97% in SS patients (median follow-up 46 months). A total of 125 implants were inserted in the 50 matched healthy controls, and no implants were lost during a comparable follow-up period. Overall patients' satisfaction was high. Oral functioning correlated negatively with dryness, patients' satisfaction, and chewing ability in SS patients [79].

1.4.8 Quality of Life

One of the most bothersome and depressing symptoms of SS is xerostomia. Sjögren's syndrome has a significant adverse impact on the patient's quality of life. The dryness is often problematic in the morning, slightly improves

during the day, but is most severe during the evening. Evening dryness may be mitigated by using a humidifier. For some, severe oral dryness is experienced throughout the day. Swallowing finely chopped ice may be helpful or chewing sugar-free gum could be helpful. Difficulty swallowing and speaking create significant challenges in socializing, eating with friends and family, teaching, lecturing or public speaking, as well as singing [4]. Physical fitness programs and/or meditation to counter fatigue and improve energy are often helpful suggestions after approval from the multispecialty care team. An effective multipronged approach to manage xerostomia and salivary hypofunction with commonly associated depression may be needed. Combining important lifestyle changes with pharmacological interventions may be a successful approach [64, 80].

1.5 Extraglandular Manifestations

While the oral health team will not be actively involved in treating extraglandular manifestations, they should be aware of them to better communicate with the patient and their physicians. As mentioned in the Disease Spectrum section above, extraglandular manifestation is multiple and varied among patients. Treatment is decided on an individual patient basis according to disease activity and the presence and extent of extraglandular manifestations. In patients with sSS, the indication for treatment is based on the underlying disease manifestations. The most common extraglandular manifestations are arthralgia and a usually nonerosive polyarthritis, which occur in approximately 50% of patients [81]. Pulmonary involvement beyond the sicca complex typically manifests as interstitial lung disease or follicular bronchiolitis, normally after many years of disease activity [82]. About 10% of patients have cutaneous lesions, the majority in form of a vasculitis with involvement of small and medium vessels of the lower limbs. In addition, other less common skin manifestations may occur, such as annular erythema, urticarial vasculitis, or hypergammaglobulinemic purpura [81]. Renal involvement, which is found in approximately 5% of patients, is usually associated with tubulointerstitial changes. Glomerulonephritis is rare in patients with pSS [83]. The decision to intensify treatment is dependent on disease activity and the organ system involved. However, the few RCTs evaluating the use of conventional DMARDs or biologic agents in patients with Sjögren's syndrome did not provide conclusive evidence supporting their efficacy [38, 84]. The Sjögren's Syndrome Foundation clinical practice guidelines (CPGs) recommendations included a decision tree for the use of which included the use of rituximab in selected clinical settings for oral and ocular dryness and for certain extraglandular manifestation including vasculitis, severe parotid swelling, inflammatory arthritis, pulmonary disease, and mononeuritis multiplex. The CPG committee strongly discouraged the use of tumor necrosis factor inhibitors for sicca symptoms and for the majority of clinical contexts in pSS [84].

A recent systemic review of therapeutic options included seven RCTs carried out in pSS patients. The RCTs using infliximab, anakinra, and baminercept found no placebo differences for primary outcomes. The largest studies with rituximab or placebo reported no significant result in the primary outcome, but prospective studies suggested efficacy in systemic disease [85].

1.6 Outcomes Assessments

Tools to assess outcomes and patient improvement have been developed and are being validated. These are critical for research protocols, early diagnosis, specific descriptions of system involvement by system, and for appropriate clinical care. To measure treatment outcomes, determining what defines minimal clinically important improvement (MCII) and patient acceptable symptom state (PASS) are powerful measures. Two disease activity indices commonly used are the EULAR SS disease activity index (ESSDAI) and the EULAR SS patient-reported index (ESSPRI). The ESSDAI measures systemic disease activity, which is ranked via a point system,

as low, moderate, and high-activity levels [86]. Some consider the ESSDAI to be a gold standard to measure disease activity in clinical studies, and as an outcome measure, even a primary outcome measure, in current randomized clinical trials [87]. Hence, refining the domains is an important endeavor. Initial reports have indicated good construct validity and reliability in scores for both measures [88]. The ESSPRI has been used to assess a relationship between the intensity of oral dryness and other signs and symptoms commonly found in SS patients. A significant correlation was reported with quality of sleep, anxiety and depression, and a trend for anxiety and depression. No significant correlation was found between extraglandular and immunological features. In the same study, multivariate regression analysis showed that fatigue measured by ESSPRI ($p = 0.049$), sleep quality ($p = 0.008$), and hypercholesterolemia ($p = 0.008$) were independently associated with dry mouth [89]. The ESSDAI and ESSPRI support the need for a multidisciplinary therapeutic approach.

1.7 Conclusion

SS is a complex disease with multiple components and manifestations. The oral health team may be the first to suspect the condition. The overall management will certainly include the dental team who must be knowledgeable about the disease spectrum to successfully communicate with other providers. Working collaboratively, optimal patient care can be provided.

References

1. Patel R, Shahane A. The epidemiology of Sjögren's syndrome. Clin Epidemiol. 2014;6:247–55. https://doi.org/10.2147/CLEP.S47399.
2. Bowman SJ, Ibrahim GH, Holmes G, Hamburger J, Ainsworth JR. Estimating the prevalence among Caucasian women of primary Sjogren's syndrome in two general practices in Birmingham, UK. Scand J Rheumatol. 2004;33:39–43.
3. Westhoff G, Zink AZ. Epidemiology of primary Sjogren's syndrome. Z Rheumatol. 2010;69:41. https://doi.org/10.1007/s00393-009-0518-3.
4. Stewart CM, Berg KM, Cha S, Reeves WH. Salivary dysfunction and quality of life in Sjögren's syndrome—a critical oral-systemic connection. JADA. 2008;139(3):219–99.
5. Fox RI. Sjögren's syndrome. Lancet. 2005;366:321–31.
6. Bayetto K, Logan RM. Sjögren's syndrome: a review of aetiology, pathogenesis, diagnosis and management. Aust Dent J. 2010;55(Suppl 1):39–47.
7. Ienopoli S, Carsons SE. Extraglandular manifestations of primary Sjögren's syndrome. Oral Maxillofac Surg Clin North Am. 2014;26:91–9.
8. Sjogren's Syndrome Foundation. 2019. https://www.sjogrens.org/home/about-sjogrens.
9. Solans-Laque R, Lopez-Herandez A, Bosch-Gill JA, Palacios A, Campillo M, Vilardell-Tarres M. Risk, predictors, and clinical characteristics of lymphoma development in primary Sjogren's syndrome. Semin Arthritis Rheum. 2011;41(3):415–23.
10. Johnsen SJ, Brun JG, Gøransson LG, Småstuen MC, Johannesen TB, Haldorsen K, et al. Risk of non-Hodgkin's lymphoma in primary Sjögren's syndrome: a population-based study. Arthritis Care Res (Hoboken). 2013;65(5):816–21.
11. Brito-Zeron P, Theander E, Baldini C, Seror R, Retamozo S, Quartuccio L, et al. Early diagnosis of primary Sjögren's syndrome: EULAR-SS task force clinical recommendations. Expert Rev Clin Immunol. 2016;12:137–56.
12. Pinto A. Management of xerostomia and other complications of Sjögren's syndrome. Oral Maxillofac Surg Clin North Am. 2014;26(1):63–73.
13. Fox PC, Busch KA, Baum BJ. Subjective reports of xerostomia and objective measures of salivary gland performance. J Am Dent Assoc. 1987;115:581–4.
14. Manthorpe R, Oxholm P, Prause JU, Schiødt M. The Copenhagen criteria for Sjögren's syndrome. Scand J Rheumatol Suppl. 1986;61:19–21.
15. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European consensus group. Ann Rheum Dis. 2002;61:554–8.
16. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, et al. Sjögren's International Collaborative Clinical Alliance (SICCA) Research Groups. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. Arthritis Care Res (Hoboken). 2012;64(4):475–87.
17. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. International Sjögren's Syndrome Criteria Working Group. 2016 American College of Rheumatology/European league against rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient

cohorts. Ann Rheum Dis. 2017;76(1):9–16. https://doi.org/10.1136/annrheumdis-2016-210571.
18. Atkinson JC, Fox PC. Sjogren's syndrome: oral and dental considerations. J Am Dental Assoc. 1993;124(3):74–6, 78-82, 84-6.
19. Fox PC, Bowman SJ, Segal B, Vivino FB, Murukutla N, Choueiri K, et al. Oral involvement in primary Sjögren syndrome. J Am Dent Assoc. 2008;139:1592–601.
20. Hopcraft MS, Tan C. Xerostomia: an update for clinicians. Aust Dent J. 2010;55(3):238–44.
21. Fox PC, van der Ven PF, Sonies BC, Weiffenbach JM, Baum BJ. Xerostomia: evaluation of a symptom with increasing significance. J Am Dent Assoc. 1985;110(4):519–25.
22. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. 2001;85(2):162–9.
23. Pedersen AM, Bardow A, Jensen SB, Nauntofte B. Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. Oral Dis. 2002;8:117–29.
24. Screebny LM, Valdini A. Xerostomia. A neglected symptom. Arch Intern Med. 1987;147:1333–7.
25. Heintze U, Birkhed D, Björn H. Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. Swed Dent J. 1983;7:227–38.
26. Daniels TE, Silverman S Jr, Michalski JP, Greenspan JS, Sylvester RA, Talal N. The oral component of Sjogren's syndrome. Oral Surg Oral Med Oral Pathol. 1975;39:875–85.
27. Tapper-Jones L, Aldred M, Walker DM. Prevalence and intraoral distribution of Candida albicans in Sjogren's syndrome. J Clin Pathol. 1980;33:282–7.
28. Radfar L, Shea Y, Steven FH, Sankar V, Leakan RA, Baum B, et al. Fungal load and candidiasis in Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96:283–7.
29. Yan Z, Young AL, Hua H, Xu Y. Multiple oral Candida infections in patients with Sjögren syndrome—prevalence and clinical and drug susceptibility profiles. J Rheumatol. 2011;38:2428–31.
30. Mathews SA, Kurien BT, Scofield RH. Oral manifestations of Sjögren's syndrome. J Dent Res. 2008;87(4):308–18.
31. Navazesh M, Kumar SK. Measuring salivary flow: challenges and opportunities. JADA. 2008;139(5 suppl):35S–40S.
32. Inoue H, Ono K, Masuda W, Morimoto Y, Tanaka T, Yokota M, et al. Gender difference in unstimulated whole saliva flow rate and salivary gland sizes. Arch Oral Biol. 2006;51(12):1055–60. https://doi.org/10.1016/j.archoralbio.2006.06.010.
33. López-Jornet P, Camacho-Alonso F, Bermejo-Fenoll A. A simple test for salivary gland hypofunction using Oral Schirmer's test. J Oral Pathol Med. 2006;35:193–256. https://doi.org/10.1111/j.1600--0714.2006.00411.x.
34. Fontana M, Zunt S, Eckert GJ, Zero D. A screening test for unstimulated salivary flow measurement. Oper Dent. 2005;30(1):3–8.
35. Nardi N, Brito-Zeron P, Ramos-Casals M, Aguiló S, Cervera R, Ingelmo M, et al. Circulating autoantibodies against nuclear and non-nuclear antigens in primary Sjögren's syndrome: prevalence and clinical significance in 335 patients. Clin Rheumatol. 2006;25(3):341–6.
36. Pisetsky DS. Antinuclear antibodies in healthy people: the tip of autoimmunity's iceberg? Arthritis Res Ther. 2011;13(2):109. https://doi.org/10.1186/ar3282.
37. Daniels TE, Cox D, Shiboski CH, Schiødt M, Wu A, Lanfranchi H, et al. Sjögren's International Collaborative Clinical Alliance Research Groups. Associations between salivary gland histopathologic diagnoses and phenotypic features of Sjögren's syndrome among 1,726 registry participants. Arthritis Rheum. 2011;63(7):2021–30. https://doi.org/10.1002/art.30381.
38. Saraux A, Pers JO, Devauchelle-Pensec V. Treatment of primary Sjögren syndrome. Nat Rev Rheumatol. 2016;12:456–71.
39. Fragkioudaki S, Mavragani CP, Moutsopoulos HM. Predicting the risk for lymphoma development in Sjogren syndrome: an easy tool for clinical use. Medicine (Baltimore). 2016;95(25):e3766. https://doi.org/10.1097/MD.0000000000003766.
40. Karagozoglu KH, De Visscher JG, Forouzanfar T, van der Meif EH, Jager DJ. Complications of Sialendoscopy in patients with Sjögren syndrome. J Oral Maxillofac Surg. 2017;75(5):978–83. https://doi.org/10.1016/j.joms.2016.09.046.
41. Nahlieli O, Baruchin AM. Long-term experience with endoscopic diagnosis and treatment of salivary gland inflammatory diseases. Laryngoscope. 2000;110(6):988–93. https://doi.org/10.1097/00005537-200006000-00020.
42. Rasmussen ER, Lykke E, Wagner N, Nielsen T, Waersted S, Arndal H. The introduction of sialendoscopy has significantly contributed to a decreased number of excised salivary glands in Denmark. Eur Arch Otorhinolaryngol. 2016;273(8):2223–30. https://doi.org/10.1007/s00405-015-3755-x.
43. Strychowsky JE, Sommer DD, Gupta MK, Cohen N, Nahlieli O. Sialendoscopy for the management of obstructive salivary gland disease: a systematic review and meta-analysis. Arch Otolaryngol Head Neck Surg. 2012;138:541–7.
44. Karagozoglu KH, Vissink A, Forouzanfar T, Brand HS, Maarse F, Jager DJ. Sialendoscopy enhances salivary gland function in Sjögren's syndrome: a 6-month follow-up, randomised and controlled, single blind study. Ann Rheum Dis. 2018;77(7):1025–31. Epub 2018 Feb 23. https://doi.org/10.1136/annrheumdis-2017-212672.
45. Villa A, Connell CL, Abati S. Diagnosis and management of xerostomia and hyposalivation. Ther Clin Risk Manag. 2014;11:45–51.

46. Peldyak J, Makinen KK. Xylitol for caries prevention. J Dent Hyg. 2002;76(4):276–85.
47. Zero DT, Brennan MT, Daniels TE, Papas A, Stewart C, Pinto A, et al. Clinical practice guidelines for oral management of Sjögren disease: dental caries prevention. JADA. 2016;147(4):295–305.
48. Wiseman LR, Faulds D. Oral pilocarpine: a review of its pharmacological properties and clinical potential in xerostomia. Drugs. 1995;49(1):143–55.
49. Papas AS, Fernandez MM, Castano RA, Gallagher SC, Trivedi M, Shrotriya RC. Oral pilocarpine for symptomatic relief of dry mouth and dry eyes in patients with Sjögren's syndrome. Adv Exp Med Biol. 1998;438:973–8.
50. Rhodus NL, Schuh MJ. Effects of pilocarpine on salivary flow in patients with Sjögren's syndrome. Oral Surg Oral Med Oral Pathol. 1991;72(5):545–9.
51. Salagen (pilocarpine hydrochloride) [FDA prescribing information]. Woodcliffe Lake, NJ: Eisai; 2018.
52. Fife RS, Chase WF, Dore RK, Wiesenhutter CW, Lockhart PB, Tindall E, et al. Cevimeline for the treatment of xerostomia in patients with Sjögren's syndrome: a randomized trial. Arch Intern Med. 2002;162(11):1293–300.
53. Fox RI. Use of cevimeline, a muscarinic M1 and M3 agonist, in the treatment of Sjögren's syndrome. Adv Exp Med Biol. 2002;506(Pt B):1107–16.
54. Noaiseh G, Baker JF, Vivino FB. Comparison of the discontinuation rates and side-effect profiles of pilocarpine and cevimeline for xerostomia in primary Sjögren syndrome. Clin Exp Rheumatol. 2014;32:575–7.
55. Brimhall J, Jhaveri MA, Yepes JF. Efficacy of cevimeline vs pilocarpine in secretion of saliva: a pilot study. Spec Care Dentist. 2013;33:123–7.
56. Strietzel FP, Martın-Granizo R, Fedele S, Lo Russo L, Mignogna M, PA Reichart PA, Wolff A. Electrostimulating device in the management of xerostomia. Oral Dis. 2007;13:206–13. https://doi.org/10.1111/j.1601--0825.2006.01268.x.
57. Sivaramakrishnan G, Sridharan K. Electrical nerve stimulation for xerostomia: a meta-analysis of randomised controlled trials. J Tradit Complement Med. 2017;7(4):409–13. Published online 2017 Feb 14. https://doi.org/10.1016/j.jtcme.2017.01.004.
58. Villa A, Wolff A, Narayana N, Dawes C, Aframian DJ, Lynge AM, et al. World workshop on Oral medicine VI: a systematic review of medication-induced salivary gland dysfunction. Oral Dis. 2016;22:365–82. https://doi.org/10.1111/odi.12402.
59. Singh ML, Papas A. Oral implications of polypharmacy in the elderly. Dent Clin N Am. 2014;58(4):783–96.
60. Food and Drug Administration Consumer Alerts. https://www.fda.gov/consumers/consumer-updates/paws-xylitol-its-dangerous-dogs
61. Visvanathan V, Nix P. Managing the patient presenting with xerostomia: a review. Int J Clin Pract. 2010;64(3):404–7.
62. Olsson P, Turesson C, Mandl T, Jacobsson L, Theander E. Cigarette smoking and the risk of primary Sjögren's syndrome: a nested case control study. Arthritis Res Ther. 2017;19(1):50. https://doi.org/10.1186/s13075-017-1255-7.
63. Gorin SS, Heck JE. Meta-analysis of the efficacy of tobacco counseling by health care providers. Cancer Epidemiol Biomarkers Prev. 2004;13(12):2012–22.
64. Zunt SL. Xerostomia/salivary gland hypofunction: diagnosis and management. Compend Contin Educ Dent. 2018;9(6):365–9.
65. Soto-Rojas AE, Villa AR, Sifuentes-Osornio J, Alarcón-Segovia D, Kraus A. Oral candidiasis and Sjögren's syndrome. J Rheumatol. 1998;25(5):911–5.
66. Najera MP, Al-Hashimi I, Plemons JM, Rivera-Hidalgo F, Rees TD, Haghighat N, Wright JM. Prevalence of periodontal disease in patients with Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997;83:453–7.
67. Le Gall M, Cornec D, Pers JO, Saraux A, Jousse-Joulin S, Cochener B, et al. A prospective evaluation of dental and periodontal status in patients with suspected Sjögren's syndrome. Joint Bone Spine. 2016;83(2):235–6.
68. Boutsi EA, Paikos S, Dafni UG, Moutsopoulos HM, Skopouli FN. Dental and periodontal status of Sjögren's syndrome. J Clin Periodontol. 1999;27:231–5.
69. Schiødt M, Christensen LB, Petersen PE, Thorn II. Periodontal disease in primary Sjögren's syndrome. Oral Dis. 2001;7:106–8.
70. Kuru B, McCullough MJ, Yilmaz S, Porter SR. Clinical and microbiological studies of periodontal disease in Sjögren syndrome patients. J Clin Periodontol. 2002;29:92–102.
71. Jorkjend L, Johansson A, Johansson K, Bergenholtz A. Periodontitis, caries and salivary factors in Sjögren's syndrome patients compared to sex- and age-matched controls. J Oral Rehabil. 2003;30:369–78.
72. Li R, Tian C, Postlethwaite A, Jiao Y, Garcia-Godoy F, Pattanaik D, et al. Rheumatoid arthritis and periodontal disease: what are the similarities and differences? Int J Rheum Dis. 2017;20(12):1887–901.
73. Mankia K, Cheng Z, Do T, Hunt L, Meade J, Kang J, et al. Prevalence of periodontal disease and periodontopathic bacteria in anti-citrullinated protein antibody-positive at risk adults without arthritis. JAMA Netw Open. 2019;2(6):e195394. https://doi.org/10.1001/jamanetworkopen.2019.5394.
74. Crincoli V, Di Comite M, Guerrieri M, Rotolo RP, Limongelli L, Tempesta A, et al. Orofacial manifestations and temporomandibular disorders of Sjögren syndrome: an observational study. Int J Med Sci. 2018;15(5):475–83.
75. Zanin MC, Garcia DM, Rocha EM, de Felicio CM. Orofacial motor functions and temporomandibular disorders in patients with Sjogren's syndrome.

Arthritis Care Res (Hoboken). 2019;72:1057–65. [Epub ahead of print]. https://doi.org/10.1002/acr.24001.
76. Young DA, Frostad-Thomas A, Gold J, Wong A. Secondary Sjögren syndrome; a case report using silver diamine fluoride and glass ionomer cement. J Am Dent Assoc. 2018;149(8):731–41. https://doi.org/10.1016/j.adaj.2018.03.021.
77. Chrcanovic BR, Kisch J, Wennerberg A. Dental implants in patients with Sjögren's syndrome: a case series and a systematic review. Int J Oral Maxillofac Surg. 2019;48(9):1250–9. Feb 28. pii: S0901–5027(19)30069–4. https://doi.org/10.1016/j.ijom.2019.02.005.
78. Albrecht K, Callhoff J, Westhoff G, Dietrich T, Dörner T, Zink A. The prevalence of dental implants and related factors in patients with Sjögren syndrome: results from a cohort study. J Rheumatol. 2016;43(7):1380–5.
79. Korfage A, Raghoebar GM, Arends S, Meiners PM, Visser A, Kroese FG, et al. Dental implants in patients with Sjögren's syndrome. Clin Implant Dent Relat Res. 2016;18(5):937–45.
80. Wu AJ. Management of salivary hypofunction in Sjögren's syndrome. Curr Treat Options Rheumatol. 2015;1(3):255–68.
81. Ramos-Casals M, Brito-Zeron P, Solans R, Camps MT, Casanovas A, Sopeña B, et al. Systemic involvement in primary Sjögren's syndrome evaluated by the EULAR-SS disease activity index: analysis of 921 Spanish patients (GEAS-SS Registry). Rheumatology (Oxford). 2014;53(2):321–31.
82. Flament T, Bigot A, Chaigne B, Henique H, Diot E, Marchand-Adam S. Pulmonary manifestations of Sjögren's syndrome. Eur Respir Rev. 2016;25:110–23.
83. Evans R, Zdebik A, Ciurtin C, Walsh SB. Renal involvement in primary Sjögren's syndrome. Rheumatology (Oxford). 2015;54(9):1541–8.
84. Carsons SE, Vivino FB, Parke A, Carteron N, Sankar V, Brasington R, et al. Treatment guidelines for rheumatologic manifestations of Sjogren's: use of biologics, management of fatigue and inflammatory musculoskeletal pain. Arthritis Care Res (Hoboken). 2017;69(4):517–27. Epub 2017 Mar 3. Review. https://doi.org/10.1002/acr.22968.
85. Brito-Zerón P, Retamozo S, Kostov B, Baldini C, Bootsma H, De Vita S, et al. Efficacy and safety of topical and systemic medications: a systematic literature review informing the EULAR recommendations for the management of Sjögren's syndrome. RMD Open. 2019;5(2):e001064. https://doi.org/10.1136/rmdopen-2019-001064.
86. Seror R, Bootsma H, Saraux A, Bowman SJ, Theander E, Brun JG, et al. EULAR Sjögren's Task Force. Defining disease activity states and clinically meaningful improvement in primary Sjögren's syndrome with EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient-reported indexes (ESSPRI). Ann Rheum Dis. 2016;75(2):382–9. https://doi.org/10.1136/annrheumdis-2014-206008.
87. Seror R, Bowman SJ, Brito-Zeron P, Theander E, Bootsma H, Tzioufas A, et al. EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. RMD Open. 2015;1(1):e000022. https://doi.org/10.1136/rmdopen-2014-000022.
88. Seror R, Theander E, Brun JG, Ramos-Casals M, Valim V, Dörner T, et al. EULAR Sjögren's Task Force. Validation of EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient indexes (ESSPRI). Ann Rheum Dis. 2015;74(5):859–66. https://doi.org/10.1136/annrheumdis-2013-204615.
89. Gandía M, Morales-Espinoza EM, Martín-González RM, Retamozo S, Kostov B, Belenguer-Prieto R, et al. Factors influencing dry mouth in patients with primary Sjögren syndrome: usefulness of the ESSPRI index. Oral Health Dent Manag. 2014;13(2):402–7.

2 Saliva and Its Importance in Sjögren's Syndrome

Debora Heller, Ibitsam Al-Hashimi, and Valeria Bordallo Pacheco

2.1 Introduction

Oral fluid commonly referred to as "saliva" takes its origin from exocrine secretions. This mixed oral fluid is composed mainly of the salivary secretions, as well as non-exocrine components (cells, microorganisms, and gingival crevicular fluid). The presence of saliva is vital for maintaining oral health and facilitating oral functions. The lack of saliva results in a rapid deterioration in oral health and impacts patient's quality of life. A review of the complexity of this oral fluid and its role in Sjögren's syndrome (SS) is best appreciated by consideration of its many and varied components and functions.

D. Heller (✉)
Post Graduate Program in Dentistry, Cruzeiro do Sul University, Sao Paulo, Brazil

Experimental Research Center, Albert Einstein Israeli Hospital, São Paulo, São Paulo, Brazil
e-mail: debora.heller@cruzeirodosul.edu.br

I. Al-Hashimi
Division of Oral and Maxillofacial Surgery, Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX, USA

V. B. Pacheco
Post Graduate Program in Dentistry, Cruzeiro do Sul University, Sao Paulo, Brazil

2.2 The Exocrine System

The exocrine secretion is formed within the acini of the exocrine glands. The acini are a small cluster of cells at the origin of the glandular ducts. The exocrine glands are found throughout the body within several organs, and they perform various functions including food digestion, mucosal protection, thermoregulation, and lubrication. Saliva, the exocrine secretion in the oral cavity, is produced by three pairs of major salivary glands present in bilateral pairs: parotid, submandibular, and sublingual glands, as well as large number of minor salivary glands that are distributed underneath the oral mucosa, with higher density in the buccal mucosa and the lip [1–3]. Although the accumulated biofluid in the oral cavity is largely composed of glandular secretions, it is usually mixed with additional non-salivary components such as epithelial cells, blood cells, microorganisms and their products, constituents of blood and serum, gingival crevicular fluid, nasopharyngeal sputum secretions, and food debris [3, 4]. Thus, the term "whole saliva" or "oral fluid" is used to describe a mixture of glandular secretion of the salivary glands and non-exocrine components [3, 5].

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2.3 Salivary Gland Anatomy and Physiology

The salivary glands consist of secretory units that are linked to the oral cavity by an intricate network of salivary ducts [2, 6]. Each salivary gland produces a specific type of secretion directly dependent on its acinar cell type. The main types of secretory cells are called serous or mucous cells. These acinar cells form a globular structure with an opening in a single intercalated duct, and this globular structure is called the acini [2]. Serous acini release aqueous proteins with little or no glycosylation. The mucinous acini produce a viscous secretion as they contain salivary mucins. Mixed or seromucous acini contain both types of secretory cells, with a predominance of one secretory type (mucus or serous).

The parotid gland is made entirely of serous acini. The remaining major salivary glands are mixed, containing both serous and mucous acini. The submandibular gland contains more serous acini, whereas the sublingual gland contains more mucous acini. The minor salivary glands are mucous or seromucous in nature [2]. Differences in acinar cell types are reflected in the differences in proteins that each gland synthesizes. For example, parotid secretion is rich in amylase [7], proline-rich proteins [8], agglutinins (gp-340) [9] statherin [10], and histatins [11] and is mucin-free. Submandibular secretion is the major source for cystatins [12] and mucins 5B (MUC 5B; formerly known as MG1) and 7 (MUC 7; formerly known as MG2) [13]. This gland also synthesizes proline-rich proteins, statherins, and histatins. Like submandibular secretions, sublingual secretions contain high concentrations of salivary mucins [14, 15].

Serous acini produce an aqueous secretion, and mucous cells produce a viscous mucin-rich secretion. These secretions arise by the formation of interstitial fluid from the capillaries, which are then modified by the end cell to produce fluid that is secreted into the lumen. From the lumen, it passes through the ductal system where it is further modified. Most modifications occur in the striated ducts where ion exchange occurs, and secretions are changed from isotonic to hypotonic solutions. The composition of saliva is further modified in the excretory ducts prior to their final secretion in the mouth. Parotid secretion enters the oral cavity through parotid duct, known as Stensen's duct; its orifice is located in the buccal mucosa at the level of the upper first/second upper molar tooth.

Submandibular and sublingual secretions enter the oral cavity through Wharton duct, which is located in the anterior sublingual region immediately adjacent to the lingual frenulum. Several small ducts derived from the sublingual glands enter the oral cavity bilaterally in the posterior sublingual space.

Salivary secretion is under the control of the autonomic nervous system, and it is, therefore, regulated by both parasympathetic and sympathetic nervous systems. Fluid secretion and macromolecules secretion occur by separate but synergistic processes. Parasympathetic nerves release acetylcholine and stimulate fluid secretion, while sympathetic nerves release norepinephrine and stimulate protein secretion [16]. It is estimated that a person produces on average 0.5–1.5 L of saliva per day [4]. These volumes vary depending on salivary flow rates [3], which in turn are influenced by several factors [17, 18], such as the degree of hydration, circadian rhythm [19–22], circannual rhythms, exposure to light [21], type and timing of salivary stimulant [23], and medications. When salivary flow is not stimulated (resting salivary flow), the contributions of the parotid, submandibular, sublingual, and minor glands are about 25%, 60%, 7–8%, and 7–8%, respectively [3, 4]. However, when the salivary glands are stimulated usually by chewing, the contribution of the parotid glands increases to about 50% of the total salivary volume, while the submandibular glands contribute about 35%. The sublingual and smaller glands remain to be similar to resting, which is, each contributes about 7–8% [4, 17, 24]. Several large population studies of healthy individuals found the normal level of unstimulated salivary flow rate to be about 0.3 mL per minute (mL/min). However, there is great variability between normal salivary flow rates amongst individuals. Whether the flow rate is high or low appears to be

less important than whether it has changed abruptly in a particular individual [3, 4, 25]. An unstimulated salivary flow rate of less than 0.1 mL/min is considered as an indication of hyposalivation.

2.4 Salivary Components and Their Functions

Saliva is made up of more than 98% of water, carrying a multitude of dissolved proteins and inorganic components. The inorganic constituents mainly consist of electrolytes. Their concentrations depend on salivary flow and type and duration of stimulation [26]. The total ionic strength of glandular secretions and total saliva may range from 10 to 50% of the serum ionic strength [27], indicating that oral fluid is always hypotonic at all physiological flow rates. The only electrolyte that has higher saliva concentrations than that found in serum is phosphate, ranging from 4.0 to 8.0 milliequivalents per liter of saliva, while salivary and serum calcium levels are both 1.0 milliequivalents per liter. Calcium and phosphate electrolytes are important because their salivary ion product is equal to or greater than the dissociation constant of hydroxyapatite (HA), which is the major constituent of enamel mineral. This indicates that salivary secretion is always supersaturated in relation to HA. The consequence of supersaturation might result in precipitation of calcium phosphates, but such precipitation may not occur in the presence of specific salivary proteins, statherin, and proline-rich proteins that act as inhibitors of crystal growth and calcium phosphate precipitation, respectively.

2.5 Saliva Organic Constituents

Almost all organic constituents of salivary gland secretions consist of specific and unique salivary proteins and glycoprotein. In whole saliva, several of these proteins undergo proteolytic degradation [28]. Classical biochemical isolation and characterization work have led to much insights into the structure and function of the most prevalent salivary proteins (Table 2.1). One of the most interesting findings of the structural studies was the polymorphism associated with salivary proteins. About 20 major saliva protein families account for over 95% of the total salivary protein content [29]. Functional investigations in saliva indicated that most of its protective function is related to salivary macromolecules (proteins and glycoproteins). Proteomic studies employing mass spectrometry, performed in more recent years, revealed the presence of 914 proteins in the parotid and 917 proteins in the submandibular/sublingual secretion [30]. Similar studies on proteome and peptidomics of total saliva indicated the presence of more than 2000 proteins and peptides [30–35]. Other studies, including quantitative approaches, have shown that most of these components belong to low- and medium-abundance proteins and peptides. Low-abundance components are unlikely to play a significant functional role in oral defense mechanisms. These components, however, are present at concentration levels suitable for other functions, such as signaling and cell-to-cell communication. Results of proteomic studies suggested that 50% of the salivary proteome is amylase. It is interesting to note that the ten major protein families including amylase, total proline-rich proteins, cystatins, mucin 5B, S-IgA, mucin 7, statherins, carbonic anhydrase, histatins, lysozyme, albumin, secretory component of S-IgA, IgG, and IgM account for 99% of total salivary proteome. The complexity of the oral fluid and its

Table 2.1 Functions of salivary proteins

Function	Saliva component (s) involved
Lubrication and viscoelasticity	Mucins, proline-rich glycoproteins
Antimicrobial	Immunoglobulin sIgA, lysozyme, lactoferrin, lactoperoxidase, histatins, mucins, cystatins, agglutinin
Digestion	Amylase, lipase, protease, DNAse, RNAse
Buffering	Bicarbonate, phosphate, carbonic anhydrase, salivary proteins
Remineralization	Proline-rich proteins, statherins, calcium, phosphate
Inhibition of demineralization	Calcium binding proteins and phosphoproteins

rich set of components will be most appreciated if we consider its many and varied functions (Table 2.1). For the most part, they have protective functions. The presence of saliva is vital for maintaining the health of oral soft and hard tissues and essential to facilitate oral functions.

2.6 Protective Functions of Saliva

Saliva is responsible for the formation of a seromucous film known as the salivary pellicle that covers all hard and soft tissues of the oral cavity. The source of this integument has been the focus of many studies and found to be the result of a selective adsorption process of salivary proteins and peptides to the oral surfaces [36–41]. Salivary pellicle plays important roles in the oral cavity including lubrication, facilitation of oral functions, protection of oral mucosa and teeth, and modulation of the oral microbiota [42–44]. The salivary mucins play an important role in the formation of salivary pellicle and maintain tissue hydration. Salivary mucins are highly glycosylated glycoproteins, and they are produced by the submandibular, sublingual, and minor glands [45–47]. The salivary mucins possess unique properties, such as low solubility, high viscosity, high elasticity, and strong adhesiveness. Such characteristics not only allow saliva to form a layer over the oral surfaces to protect them from dryness and environmental insults, but also give saliva its "waterproof" capability [45, 48, 49]. In addition to salivary mucins, proline-rich glycoproteins are also an effective lubricant [50]. In addition, saliva can promote healing of oral wounds, which is attributed to salivary proteins as a tissue factor [51, 52], leukocyte protease inhibitor, and histatins [53]. Saliva is also essential to maintain physiologic pH of the oral cavity [4]. The major pH-regulating factor, especially during drinking or eating, is bicarbonate, and salivary phosphate, urea, and histatins have also been reported to contribute to buffering action [4, 48]. Saliva also helps maintain the integrity of the teeth by regulating demineralization and remineralization processes on the tooth surface. Salivary proline-rich phosphoproteins play a role in modulating calcium phosphate and protect the tooth surface from demineralization and calculus formation [54, 55]. Saliva also contains a spectrum of specific and nonspecific immune components that exhibit antimicrobial properties and facilitate the elimination of bacteria from the oral cavity [56]. Some salivary proteins and glycoproteins have bacteriostatic and bactericidal activity, such as lysozyme [57–59], histatins [11, 60], and cystatins [61]. Salivary histatins also possess antifungal activity against Candida albicans [11, 60, 62].

2.7 Speech, Digestion, and Related Functions

Saliva is the first-body fluid that comes into contact with food. Due to its aqueous nature, saliva facilitates mastication, bolus formation, and aids in swallowing [48]. At the same time, due to their viscoelastic properties, salivary components such as high molecular weight mucin-5B (MUC5B), mucin-7 (MUC7), and proline-rich glycoproteins (PRG) add lubricity-facilitating ingestion. Saliva also contains enzymes such as amylase, proteases, lipases, and glycosidic hydrolases that facilitate the breakdown of food. Several of these enzymes are of non-exocrine origin including gingival crevicular fluid, oral bacteria, and desquamated epithelial cells [63]. Among these enzymes is salivary α-amylase, which is produced by salivary glands, and is quantitatively the most abundant component of saliva. It is believed that taste perception is dependent on many organic and inorganic constituents of saliva. The low ionic strength of saliva, as compared to serum, is also believed to be important for taste perception of food and drinks. Finally, salivary mucins are critical for the hydration and lubrication of the oral cavity, having an important role facilitating oral functions and speech [16].

2.8 Salivary Flow and Oral Health

The presence of saliva is crucial for maintaining the health of the oral cavity, and the integrity of the teeth and oral mucosa. Reduction in salivary function not only results in deterioration of oral health,

Table 2.2 Signs and symptoms of dry mouth in SS patients

Oral mucosa	Dry erythematous mucosa Allergic mucositis Burning mouth Opportunistic infection (candidiasis) Fissured tongue Atrophic depapillated tongue Traumatic ulcers
Teeth	Enamel erosion Enamel hypocalcification Chalky enamel Tooth sensitivity Increased plaque accumulation Gingival inflammation Increased dental caries Root caries Dental attrition
Lips	Dry/cracked lip commissure Peeling Angular cheilitis
Oral functions	Difficulty chewing Difficulty swallowing (dysphagia) Difficulty talking Parafunctional habits Need for frequent sips of water Altered taste sensation (dysgeusia) Impaired phonation Halitosis Hoarse voice
Salivary glands	Swelling with/without pain or sialadenitis Discomfort/pain with eating/drinking Recurrent salivary gland infection
Quality of life	Inconvenience due to need to carry liquid everywhere Disruption of sleep Frequent chocking Social withdrawal Economic burden

but also has a detrimental impact on oral functions, nutrition, and overall health and quality of life. Clinically, reduced salivary function is manifested as dry mouth (xerostomia). Patients with dry mouth have difficulty eating, speaking, wearing a prosthesis, and present mucosal ulceration, taste alteration, burning mouth, recurrent candidal infections, and high incidence of tooth decay (Table 2.2).

2.9 Sjögren's Syndrome

SS is an inflammatory disease of the exocrine glands, which leads to exocrine gland dysfunction including the salivary and lacrimal glands. Dry mouth is one of the most frequently reported symptoms of SS [64, 65]. However, due to the abundance, high number of the salivary glands, and the subjectivity in the perception of dryness symptom, early manifestations of decreased salivary function might not be expressed as dry mouth but rather conveyed as increased tooth sensitivity, dental caries, traumatic lesions on the cheeks and tongue, oral ulcers, burning mouth, and/or intolerance to spicy and acidic food and drinks, all of which are a reflection of the impact of decreased salivary output on oral health. Furthermore, patients may not consider dry mouth as a significant symptom and do not report it unless specifically asked if their mouth feels dry or if they need frequent sips of liquid during the day or at night. Studies have reported that up to 26% of patients with SS may not report dry mouth despite having decreased salivary function [66]. Therefore, using a questionnaire addressing the impact of oral dryness and its associated symptoms might be useful for screening patients with SS [65].

Patients with SS are at high risk for dental and oral mucosal diseases. The teeth are the only mineralized tissue in the body that is exposed directly to the external environmental insults, which includes oral microorganisms, acidic food and drinks, and allergens. The continuous flow of saliva, its buffering capacity, and physiologic pH are important for neutralizing the acids produced by food, drinks, and bacterial metabolites. The continuous flow of saliva ensures continual removal of debris, bacteria, allergens, and noxious substances from the mouth. This removal is facilitated by swallowing reflex, which is stimulated by the accumulation of saliva in the mouth. Studies have shown that the accumulation of less than 1.5 mL of saliva triggers swallowing reflex. However, swallowing does not remove the entire saliva from the mouth. A small portion of the saliva remains in the mouth as a coat covering the oral mucosa and teeth, which is referred to as salivary pellicle (dental pellicle and mucosal pellicle). Dental pellicle is the salivary coat that covers the teeth, and mucosal pellicle is the salivary coat that covers the oral mucosa.

The dental pellicle plays an important role in maintaining the integrity of the teeth [48].

Reduction in salivary output is associated with reduced buffering capacity of saliva, decline in salivary pH, decreased oral clearance, and compromised the dental pellicle. These changes subject the teeth to environmental insults. Salivary proteins and glycoproteins that contribute to the formation of the dental pellicle are selectively adsorbed to the tooth surface. They possess antimicrobial activity that modulates the microbial flora around the teeth, contain proteins and glycoproteins that secure the mineral component of the enamel tooth surface, and protect the teeth from demineralization by acids produced by oral bacteria and food and drinks. The reduction in salivary function compromises the tooth structure resulting in enamel hypocalcification, which is manifested as increased tooth sensitivity and appearance of chalky areas on the tooth surface due to leakage of calcium from the enamel, as shown in Fig. 2.1. The hypocalcified areas of the teeth become a footing for bacterial growth and colonization, especially cariogenic bacteria leading to dental caries. Progressive dental caries is a widespread problem in patients with SS due to bacterial colonization [67]. Higher levels of cariogenic bacteria were reported in patients with SS as compared to healthy controls [68]. The cervical portion of the teeth is a common site for caries, which is known as "amputation caries" as it often cause fracture of the tooth at the gingival margin [69]. The decreased salivary function in SS also disrupts the balance of the microbial microbiota of the oral cavity, which allows the growth of pathogenic/cariogenic bacteria, especially areas with potential for plaque accumulation, such as the cervical region of the tooth [68, 70]. Studies have reported that the number of cervical decay lesions was negatively correlated with salivary flow rate, i.e., the lower salivary output, the higher number of teeth with cervical dental caries. Furthermore, studies have also shown that patients with SS are also at higher risk for periodontal diseases [70–72].

The reduction in salivary output and the shift in bacterial balance also foster the growth of opportunistic infections, particularly candidiasis [73]. Recurrent oral candidiasis is prevalent among patients with SS [74–76], as shown in Fig. 2.2. Previous studies have demonstrated that SS patients with severe reduction in salivary output have higher number of candida in their mouth than those with moderate loss of salivary output, and the number of oral candida microorganism correlates adversely with the level of salivary output [77]. These findings support the important role of saliva in modulating microbial flora and maintaining oral homeostasis.

The salivary tissue coat "blanket" that covers the oral mucosal surface is known as mucosal pellicle. The mucosal pellicle provides a protective physical barrier for the underlying tissue. It prevents desiccation of the oral mucosa, protects from external environmental insults, and provides lubrication of the oral tissues to facilitate oral functions. Diminished salivary flow deprives the patients of the salivary protection and subjects the oral mucosa to variety of physical and environmental insults. SS patients have higher

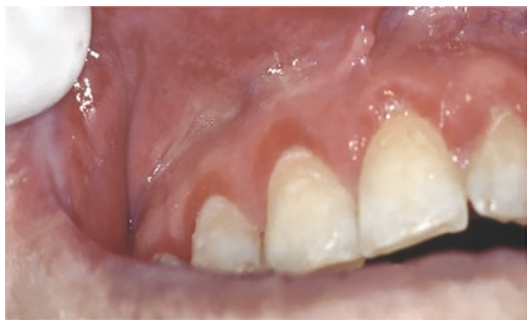

Fig. 2.1 Enamel hypocalcification, plaque accumulation, gingival inflammation, and dental attrition in a patient with Sjögren's syndrome

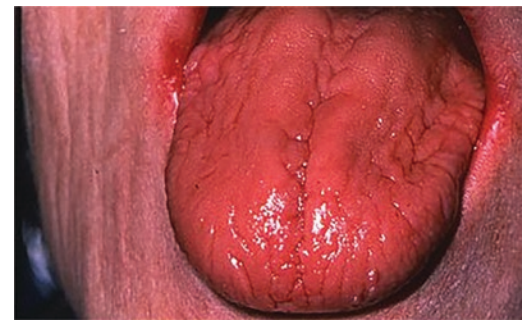

Fig. 2.2 Sjögren's syndrome patient with depapillated tongue, glossitis, and angular cheilitis due to atrophic oral candidiasis

incidence of traumatic ulcers, especially of the tongue and cheeks, and difficulty talking, eating, and swallowing due to lack of salivary lubrication. The oral mucosa also becomes more vulnerable to developing mucosal allergies (contact mucositis) and lichenoid lesions to variety of food, drinks, dental materials, and oral hygiene products [78]. Burning mouth and intolerance to acidic and spicy food and drinks is one of the most prevalent oral mucosal conditions in patients with dry mouth and SS. It occurs due to the diminished salivary function and disruption of the mucosal pellicle, which results in mucosal desiccation, atrophy of the oral mucosa, allergic mucositis, and recurrent oral candidiasis.

Salivary gland enlargement/swelling with/or without sialadenitis is another complication of SS. In a prospective cohort study of 484 SS patients, 20% of the patients had salivary gland enlargement [79]. Recurrent salivary gland infection may precede the onset of dry mouth, especially in children [80, 81].

In recent years, scientific investigations have evaluated salivary proteome to identify new biomarkers to diagnose, monitor, or prognosticate SS [82]. For instance, analysis of mucin composition has been carried out in SS patients and healthy age-matched controls, where heavily glycosylated mucins MUC5B and MUC7 showed a reduction in glycosylation in Sjögren's patients, particularly on MUC7 [83]. Furthermore, immunological proteins such as the major SS-related autoantibodies, anti-Ro/SSA and/or anti-La/SSB, and several cytokines have shown significantly increased levels in saliva of patients suffering from SS [84]. Moreover, proteome studies employing mass spectrometry carried out in more recent years revealed the presence of distinct protein patterns characteristic of SS [85, 86].

Furthermore, whole stimulated saliva collected from primary SS patients overexpressed proteins vital for immune regulation such as CD44 and beta-2 microglobulin compared to the level in non-SS patients [87]. Interestingly, microbiome diversity and relative abundance of bacterial genus or subspecies in SS saliva remains to be similar to those found in healthy subjects although more research is warranted to confirm the data [88, 89]. The observed changes on the biochemical properties of saliva that correlate with xerostomia could play a potential role in diagnosis and future therapies for this multifactorial syndrome [90, 91].

Lastly, it is important to realize that saliva is important for our social and physical well-being. The lack of saliva in SS also compromises patients' health and nutrition because reduction in saliva also diminishes taste perception and decreases patients' ability to process food, leading to alteration in food selection [92, 93]. Enjoyment of talking, eating, and drinking is a key component of social activities and interactions. The compromised oral functions in patients with SS hamper their social life and often lead to social withdrawal, which might contribute to the high frequency of depression among Sjögren's patients [78, 94, 95]. Furthermore, the deterioration of oral and dental health necessitates frequent dental visits, thereby imposing additional financial burden and psychological stress for the patients. The combined physical, social, financial, and psychological stress associated with SS has a profound impact on patients' quality of life.

References

1. Silvers AR, Som PM. Salivary glands. Radiol Clin North Am. 1998;36(5):941–66.
2. Holsinger FC, Bui DT. Anatomy, function, and evaluation of the salivary glands. In: Myers EN, Ferris RL, editors. Salivary gland disorders. Berlin: Springer; 2007. p. 1–16.
3. Dawes C. Salivary flow patterns and the health of hard and soft oral tissues. J Am Dent Assoc. 2008;139(Suppl):18S–24S.
4. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. 2001;85(2):162–9.
5. Schipper R, Loof A, de Groot J, Harthoorn L, van Heerde W, Dransfield E. Salivary protein/peptide profiling with SELDI-TOF-MS. Ann N Y Acad Sci. 2007;1098:498–503.
6. Baker OJ. Tight junctions in salivary epithelium. J Biomed Biotechnol. 2010;2010:278948.
7. Schneyer LH. Amylase content of separate salivary gland secretions of man. J Appl Physiol. 1956;9(3):453–5.
8. Oppenheim FG, Hay DI, Franzblau C. Proline-rich proteins from human parotid saliva. I. Isola-

tion and partial characterization. Biochemistry. 1971;10(23):4233–8.
9. Malamud D, Appelbaum B, Kline R, Golub EE. Bacterial aggregating activity in human saliva: comparisons of bacterial species and strains. Infect Immun. 1981;31(3):1003–6.
10. Schlesinger DH, Hay DI. Complete covalent structure of statherin, a tyrosine-rich acidic peptide which inhibits calcium phosphate precipitation from human parotid saliva. J Biol Chem. 1977;252(5):1689–95.
11. Oppenheim FG, Xu T, McMillian FM, Levitz SM, Diamond RD, Offne GD, et al. Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. J Biol Chem. 1988;263(16):7472–7.
12. Rathman WM, van Zeyl MJ, van den Keijbus PA, Veerman EC, Nieuw Amerongen AV. Comparison of a salivary 14 kD protein displaying cysteine proteinase inhibitory activity with other salivary cystatins. J Biol Buccale. 1990;18(1):9–18.
13. Reddy MS, Bobek LA, Haraszthy GG, Biesbrock AR, Levine MJ. Structural features of the low-molecular-mass human salivary mucin. Biochem J. 1992;287:639–43.
14. Loomis RE, Prakobphol A, Levine MJ, Reddy MS, Jones PC. Biochemical and biophysical comparison of two mucins from human submandibular-sublingual saliva. Arch Biochem Biophys. 1987;258(2):452–64.
15. Veerman EC, van den Keybus PA, Vissink A, Nieuw Amerongen AV. Human glandular salivas: their separate collection and analysis. Eur J Oral Sci. 1996;104(4):346–52.
16. Pedersen AM, Sørensen CE, Proctor GB, Carpenter GH. Salivary functions in mastication, taste and textural perception, swallowing and initial digestion. Oral Dis. 2018;24:1399–416.
17. Dawes C. Factors influencing salivary flow rate and composition. In: Edgar EM, Dawes C, O'Mullane DM, editors. Saliva and oral health. 3rd ed. London: British Dental Association; 2004. p. 32–49.
18. Dodds MW, Johnson DA, Yeh CK. Health benefits of saliva: a review. J Dent. 2005;33(3):223–33.
19. Dawes C. Circadian rhythms in human salivary flow rate and composition. J Physiol. 1972;220(3):529–45.
20. Ferguson DB, Fort A, Elliott AL, Potts AJ. Circadian rhythms in human parotid saliva flow rate and composition. Arch Oral Biol. 1973;18(10):1155–73.
21. Dawes C. Circadian rhythms in the flow rate and composition of unstimulated and stimulated human submandibular saliva. J Physiol. 1975;244(2):535–48.
22. Ferguson DB, Botchway CA. A comparison of circadian variation in the flow rate and composition of stimulated human parotid, submandibular and whole salivas from the same individuals. Arch Oral Biol. 1980;25(8–9):559–68.
23. Dawes C. The effects of flow rate and duration of stimulation on the concentrations of protein and the main electrolytes in human parotid saliva. Arch Oral Biol. 1969;14(3):277–94.
24. Matsuo R. Role of saliva in the maintenance of taste sensitivity. Crit Rev Oral Biol Med. 2000;11(2):216–29.
25. Ship JA, Fox PC, Baum BJ. How much saliva is enough? 'Normal' function defined. J Am Dent Assoc. 1991;122(3):63–9.
26. Dawes C, Dong C. The flow rate and electrolyte composition of whole saliva elicited by the use of sucrose-containing and sugar-free chewing-gums. Arch Oral Biol. 1995;40(8):699–705.
27. Boackle RJ. The interaction of salivary secretions with the human complement system—a model for the study of host defense systems on inflamed mucosal surfaces. Crit Rev Oral Biol Med. 1991;2(3):355–67.
28. Helmerhorst EJ. Whole saliva proteolysis: wealth of information for diagnostic exploitation. Ann N Y Acad Sci. 2007;1098:454–60.
29. Ruhl S. The scientific exploration of saliva in the post-proteomic era: from database back to basic function. Expert Rev Proteomics. 2012;9(1):85–96.
30. Denny P, Hagen FK, Hardt M, Liao L, Yan W, Arellanno M, et al. The proteomes of human parotid and submandibular/sublingual gland salivas collected as the ductal secretions. J Proteome Res. 2008;7(5):1994–2006.
31. Amado F, Lobo MJ, Domingues P, Duarte JA, Vitorino R. Salivary peptidomics. Expert Rev Proteomics. 2010;7(5):709–21.
32. Bandhakavi S, Stone MD, Onsongo G, Van Riper SK, Griffin TJ. A dynamic range compression and three-dimensional peptide fractionation analysis platform expands proteome coverage and the diagnostic potential of whole saliva. J Proteome Res. 2009;8(12):5590–600.
33. Loo JA, Yan W, Ramachandran P, Wong DT. Comparative human salivary and plasma proteomes. J Dent Res. 2010;89(10):1016–23.
34. Siqueira WL, Salih E, Wan DL, Helmerhorst EJ, Oppenheim FG. Proteome of human minor salivary gland secretion. J Dent Res. 2008;87(5):445–50.
35. Vitorino R, Lobo MJ, Ferrer-Correira AJ, Dubin JR, Tomer KB, Domingues PM, et al. Identification of human whole saliva protein components using proteomics. Proteomics. 2004;4(4):1109–15.
36. Armstrong WG. Amino-acid composition of the acquired pellicle of human tooth enamel. Nature. 1966;210(5032):197–8.
37. Leach SA, Critchley P, Kolendo AB, Saxton CA. Salivary glycoproteins as components of the enamel integuments. Caries Res. 1967;1(2):104–11.
38. Armstrong WG, Hayward AF. Acquired organic integuments of human enamel: a comparison of analytical studies with optical, phase-contrast and electron microscope examinations. Caries Res. 1968;2(4):294–305.
39. Yao Y, Grogan J, Zehnder M, Lendenmann U, Nam B, Wu Z, et al. Compositional analysis of human acquired enamel pellicle by mass spectrometry. Arch Oral Biol. 2001;46(4):293–303.

40. Siqueira WL, Custodio W, McDonald EE. New insights into the composition and functions of the acquired enamel pellicle. J Dent Res. 2012;91(12):1110–8.
41. Heller D, Helmerhorst EJ, Oppenheim FG. Saliva and serum protein exchange at the tooth enamel surface. J Dent Res. 2017;96(4):437–43.
42. Lindh L, Aroonsang W, Sotres J, Arnebrant T. Salivary pellicles. Monogr Oral Sci. 2014;24:30–9.
43. Heller D, Helmerhorst EJ, Gower AC, Siqueira WL, Paster BJ, Oppenheim FG. Microbial diversity in the early in vivo-formed dental biofilm. Appl Environ Microbiol. 2016;82(6):1881–8.
44. Pedersen AM, Belstrøm D. The role of natural salivary defences in maintaining a healthy oral microbiota. J Dent. 2019;80(Suppl 1):S3–S12.
45. Tabak LA, Levine MJ, Mandel ID, Ellison SA. Role of salivary mucins in the protection of the oral cavity. J Oral Pathol. 1982;11(1):1–17.
46. Tabak LA. Structure and function of human salivary mucins. Crit Rev Oral Biol Med. 1990;1(4):229–34.
47. Slomiany BL, Murty VL, Piotrowski J, Slomiany A. Salivary mucins in oral mucosal defense. Gen Pharmacol. 1996;27(5):761–71.
48. Mandel ID. The functions of saliva. Dent Res. 1987;66:623–7.
49. Edgar WM. Saliva and dental health. Clinical implications of saliva: report of a consensus meeting. Br Dent J. 1990;169(3–4):96–8.
50. Hatton MN, Loomis RE, Levine MJ, Tabak LA. Masticatory lubrication. The role of carbohydrate in the lubricating property of a salivary glycoprotein-albumin complex. Biochem J. 1985;230(3):817–20.
51. Hunter JB. The action of saliva and gastric juice on the clotting of blood. Br J Surg. 1928;16(62):203–7.
52. Gross PL. Salivary microvesicles clot blood. Blood. 2011;117(11):2989.
53. Oudhoff MJ, Bolsche JG, Nazmi K, Kalay H, van't Hof W, Amerongen AVN, Veerma ECI. Histatins are the major wound-closure stimulating factors in human saliva as identified in a cell culture assay. FASEB J. 2008;22(11):3805–12.
54. Bennick A. Salivary proline-rich proteins. Mol Cell Biochem. 1982;45(2):83–99.
55. Bennick A, Chau G, Goodlin R, Abrams S, Tustian D, Madapallimattam G. The role of human salivary acidic proline-rich proteins in the formation of acquired dental pellicle in vivo and their fate after adsorption to the human enamel surface. Arch Oral Biol. 1983;28(1):19–27.
56. Scannapieco FA. Saliva-bacterium interactions in oral microbial ecology. Crit Rev Oral Biol Med. 1994;5(3–4):203–48.
57. Pollock JJ, Katona LI, Goodman H, Cho MI, Iacono VJ. Bacteriolysis of Streptococcus mutans BHT by lysozyme and inorganic anions normally present in human saliva. Arch Oral Biol. 1981;26(9):711–6.
58. Laible NJ, Germaine GR. Adsorption of lysozyme from human whole saliva by Streptococcus sanguis 903 and other oral microorganisms. Infect Immun. 1982;36(1):148–59.
59. Laible NJ, Germaine GR. Bactericidal activity of human lysozyme, muramidase-inactive lysozyme, and cationic polypeptides against Streptococcus sanguis and Streptococcus faecalis: inhibition by chitin oligosaccharides. Infect Immun. 1985;48(3):720–8.
60. Edgerton M, Koshlukova SE, Lo TE, Chrzan BG, Straubinger RM, Raj PA. Candidacidal activity of salivary histatins: identification of a histatin 5-binding protein on Candida albicans. J Biol Chem. 1998;273(32):20438–47.
61. Ganeshnarayan K, Velliyagounder K, Furgang D, Fine DH. Human salivary cystatin SA exhibits antimicrobial effect against Aggregatibacter actinomycetemcomitans. J Periodontal Res. 2012;47(5):661–73.
62. Gyurko C, Lendenmann U, Helmerhorst EJ, Troxler RF, Oppenheim FG. Killing of Candida albicans by histatin 5: cellular uptake and energy requirement. Antonie Van Leeuwenhoek. 2001;79(3–4):297–309.
63. Helmerhorst EJ, Oppenheim FG. Saliva: a dynamic proteome. J Dent Res. 2007;86(8):680–93.
64. Fox PC, Bowman SJ, Segal B, Vivino FB, Murukutla N, Choueiri K, et al. Oral involvement in primary Sjögren syndrome. J Am Dent Assoc. 2008;139(12):1592–601.
65. Al-Hashimi I, Khuder S, Haghighat N, Zipp M. Frequency and predictive value of the clinical manifestations in Sjögren's syndrome. J Oral Pathol Med. 2001;30:1–6.
66. Daniels TE, Whitcher JP. Association of patterns of labial salivary gland inflammation with keratoconjunctivitis sicca. Arthritis Rheum. 1994;37:869–77.
67. Christensen LB, Petersen PE, Thorn JJ, Schiødt M. Dental caries and dental health behavior of patients with primary Sjögren syndrome. Acta Odontol Scand. 2001;59:116–20.
68. Kolavic SA, Gibson G, Al-Hashimi I. The level of cariogenic micro-organisms in patients with Sjögren's syndrome. Spec Care Dentist. 1997;17(2):65–9.
69. Karmiol M, Walsh RF. Dental caries after radiotherapy of the oral regions. J Am Dent Assoc. 1975;91:838–45.
70. Almståhl A, Wikström M. Electrolytes in stimulated whole saliva in individuals with hyposalivation of different origins. Arch Oral Biol. 2003;48(5):337–44.
71. Najera M, Al-Hashimi I, Plemons J, Rivera-Hidalgo F, Rees TD, Haghighat N. Prevalence of periodontal disease in patients with Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997;83(4):453–7.
72. Macfarlane TW, Mason DK. Changes in oral flora in Sjögren's syndrome. J Clin Pathol. 1974;27:416–9.
73. Meulen TA, Harmsen HJ, Bootsma H, Liefers SC, Vila AV, Zhernakova A, et al. Dysbiosis of the buccal mucosa microbiome in primary Sjögren's syndrome patients. Rheumatology. 2018;57:2225–34.
74. Tapper-Jones L, Aldred M, Walker DM. Prevalence and intraoral distribution of Candida albicans in Sjögren's syndrome. J Clin Pathol. 1980;33:282–7.
75. Hemandez YL, Daniels TE. Oral candidiasis in Sjögren's syndrome: prevalence, clinical correlations,

and treatment. Oral Surg Oral Med Oral Pathol. 1989;68:324–9.
76. Navazesh M, Wood GJ, Brightman VJ. Relationship between salivary flow rates and Candida albicans counts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;80:284–8.
77. Abraham CM, Al-Hashimi I, Haghighat N. Evaluation the level of oral Candida in patients with Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;86:65–8.
78. Al-Hashimi I. The management of Sjögren's syndrome in dental practice. J Am Dent Assoc. 2001;132(10):1409–17.
79. Theander E, Manthorpe R, Jacobsson LT. Mortality and causes of death in primary Sjögren's syndrome: a prospective cohort study. Arthritis Rheum. 2004;50(4):1262–9.
80. Wang SL, Zou ZJ, Yu SF, Zhu JR. Recurrent swelling of parotid glands and Sjögren's syndrome. Int J Oral Maxillofac Surg. 1993;22(6):362–5.
81. Hearth-Holmes M, Baethge BA, Abreo F, Wolf RE. Autoimmune exocrinopathy presenting as recurrent parotitis of childhood. Arch Otolaryngol Head Neck Surg. 1993;119(3):347–9.
82. Katsiougiannis S, Wong DT. The proteomics of saliva in Sjögren's syndrome. Rheum Dis Clin North Am. 2016;42(3):449–56.
83. Chaudhury NM, Proctor GB, Karlsson NG, Carpenter GH, Flowers SA. Reduced Mucin-7 (Muc7) Sialylation and altered saliva rheology in Sjögren's syndrome associated oral dryness. Mol Cell Proteomics. 2016;15(3):1048–59.
84. Ching KH, Burbelo PD, Gonzalez-Begne M, Roberts ME, Coca A, Sanz I, et al. Salivary anti-Ro60 and anti-Ro52 antibody profiles to diagnose Sjogren's syndrome. J Dent Res. 2011;90:445–9.
85. Deutsch O, Krief G, Konttinen YT, Zaks B, Wong DT, Aframian DJ, et al. Identification of Sjogren's syndrome oral fluid biomarker candidates following high-abundance protein depletion. Rheumatology. 2015;54:884–90.
86. Giusti L, Baldini C, Bazzichi L, Bombardieri S, Lucacchini A. Proteomic diagnosis of Sjogren's syndrome. Expert Rev Proteomics. 2007;4:757–67.
87. Aqrawi LA, Galtung HK, Guerreiro EM, Øvstebø R, Thiede B, Utheim TP, et al. Proteomic and histopathological characterisation of sicca subjects and primary Sjögren's syndrome patients reveals promising tear, saliva and extracellular vesicle disease biomarkers. Arthritis Res Ther. 2019;21(1):181.
88. Sharma D, Sandhya P, Vellarikkal SK, Surin AK, Jayarajan R, Verma A, et al. Saliva microbiome in primary Sjogren's syndrome reveals distinct set of disease associated microbes. Oral Dis. 2020;26(2):295–301.
89. Sembler-Møller ML, Belstrøm D, Locht H, Enevold C, Pedersen AML. Next-generation sequencing of whole saliva from patients with primary Sjögren's syndrome and non-Sjögren's sicca reveals comparable salivary microbiota. J Oral Microbiol. 2019;11(1):1660566.
90. Barrera MJ, Aguilera S, Veerman E, Quest AF, Diaz-Jimenez D, Urzua U, et al. Salivary mucins induce a toll-like receptor 4-mediated pro-inflammatory response in human submandibular salivary cells: are mucins involved in Sjogren's syndrome? Rheumatology. 2015;54(8):1518–27.
91. Fleissig Y, Deutsch O, Reichenberg E, Redlich M, Zaks B, Palmon A, Aframian DJ. Different proteomic protein patterns in saliva of Sjögren's syndrome patients. Oral Dis. 2009;15(1):61–8.
92. Dusek M, Simmons J, Buschang RH, Al-Hashimi I. Masticatory function in patients with xerostomia. Gerodontology. 1996;13(1):3–6.
93. Nesvold MB, Jensen JL, Lene H, Hove LH, Singh PB, Young A, et al. Dietary intake, body composition, and oral health parameters among female patients with primary Sjögren's syndrome. Nutrients. 2018;10:866–77.
94. Rusthen S, Young A, Herlofson BB, Aqrawi LA, Rykke M, Hove LH, et al. Oral disorders, saliva secretion, and oral health-related quality of life in patients with primary Sjögren's syndrome. Eur J Oral Sci. 2017;125:265–71.
95. Cui Y, Xia L, Li L, Zhao Q, Chen S, Gu Z. Anxiety and depression in primary Sjögren's syndrome: a cross-sectional study. BMC Psychiatry. 2018;18:131.

Part II

Immunopathogenesis of SS

3 Mechanisms of Secretory Dysfunction in Autoimmune Sjögren's Syndrome

Wafaa Saleh, Harpreet Singh, Joseph Katz, and Seunghee Cha

3.1 Introduction

3.1.1 Autoimmune Sjögren's Syndrome (SS)

Sjögren's syndrome (SS) is a chronic autoimmune disorder with unique clinical features of oral and ocular dryness due to the infiltration of immune cells in the exocrine glands, such as the salivary and lacrimal glands, respectively. The disease's features consist of, but are not limited to, female predominance, hypergammaglobulinemia, high levels of serum autoantibodies, and upregulation of type I interferon (IFN)-associated molecules [1, 2]. The fact that some of these features are also found in other systemic autoimmune disorders implies common, underlying pathogenic mechanisms present in autoimmune diseases. The overactive immune cells stimulated by the aberrant innate immune responses result in dysregulated B and T cell functions. Levels of proinflammatory cytokines are upregulated in the blood and in the target organs of SS [3, 4]. Genetic and epigenetic factors, hormonal influences, and the immunological basis for underlying pathogenesis of SS have been extensively studied over the years. As there are countless factors contributing to secretory dysfunction in SS and the degree of dryness widely varies among SS patients, this chapter will cover the roles of inflammatory mediators encompassing inflammatory cytokines, autoantibodies, microRNAs (miRNAs), reactive oxygen species (ROS), factors impairing the calcium signaling pathway, and other variables like sex hormones (Fig. 3.1).

W. Saleh
Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University, Mansoura, Dakhlya, Egypt

Division of Oral Medicine, Department of Oral and Maxillofacial Diagnostic Sciences, College of Dentistry, University of Florida, Gainesville, FL, USA

H. Singh
Division of Craniofacial Pain, Department of Diagnostic Sciences, Tufts University, School of Dental Medicine, Boston, MA, USA

Division of Oral Medicine, Department of Oral and Maxillofacial Diagnostic Sciences, College of Dentistry, University of Florida, Gainesville, FL, USA

J. Katz · S. Cha (✉)
Division of Oral Medicine, Department of Oral and Maxillofacial Diagnostic Sciences, College of Dentistry, University of Florida, Gainesville, FL, USA

Center for Orphaned Autoimmune Disorders (COAD), College of Dentistry, University of Florida, Gainesville, FL, USA
e-mail: scha@dental.ufl.edu

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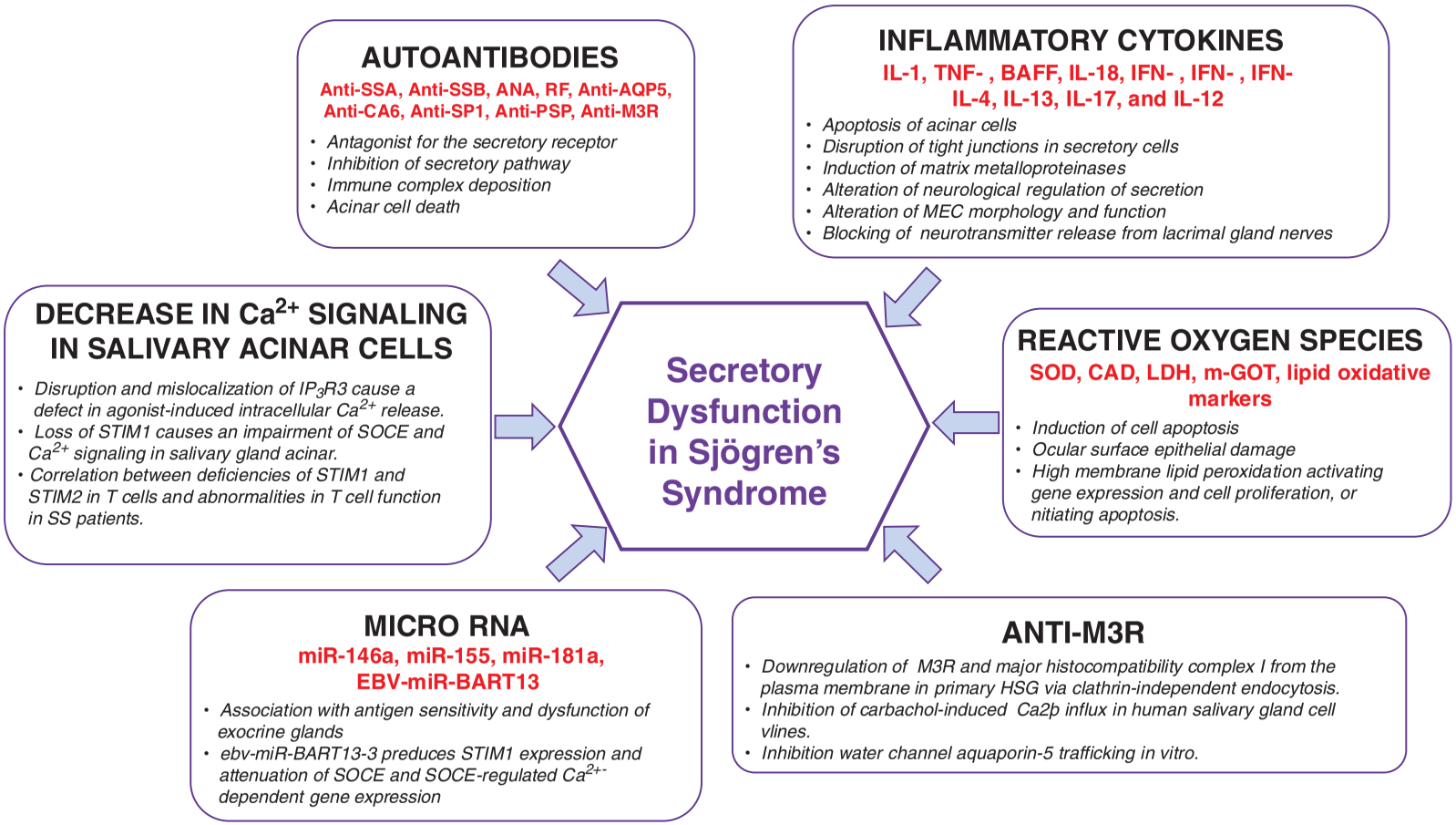

Fig. 3.1 Summary of various factors involved in secretory dysfunction in Sjögren's syndrome

3.1.2 Neural Regulation of Secretion

The regulation of exocrine gland secretion is controlled by the autonomic nervous system. Dysautonomia, due to cholinergic dysfunction, can cause xerostomia (dry mouth) and xerophthalmia (dry eyes) in primary SS (pSS) patients [5]. The secretion of saliva is controlled by sympathetic and parasympathetic stimulation. The parasympathetic stimulation of the salivary secretion is mediated by acetylcholine and substance P, which control the serous salivary secretion via muscarinic type 3 acetylcholine receptor (M3R) and substance P receptors, respectively. Norepinephrine, which is secreted by the sympathetic adrenergic nerves, regulates the secretion of mucous saliva mediated by the α- adrenergic receptor [6].

Innervation of the lacrimal gland is through sensory nerves as well as parasympathetic and sympathetic innervation. Parasympathetic nerves dominate around most acini [6]. Sympathetic innervation is less presented, and the sensory nerves regulate minimum innervation [7]. The sensory neuropeptides cause partial lacrimal gland secretion. Parasympathetic nerves release the neurotransmitters of acetylcholine and vasoactive intestinal peptide (VIP). M3R located in the basolateral membranes of lacrimal acinar cells causes tear secretion through acetylcholine stimulation [7]**.** In lacrimal gland ductal cells, M3R can also promote tear movement [8]. In addition, sympathetic nerves are activated by the sensory nerves of the ocular surface and neurotransmitters of norepinephrine and neuropeptide. Acetylcholine, VIP, and norepinephrine stimulate independent pathways although their pathways interact [9].

3.2 Factors Influencing Secretion in SS

3.2.1 Cytokines

3.2.1.1 Potential Mechanisms of Secretory Dysfunction by Inflammatory Cytokines

Induction of Apoptosis. One of the most studied mechanisms of SS is apoptosis of fluid secreting acinar cells [10]. Moreover, apoptosis of acinar cells may act as a fundamental and immunological basis for homing of inflammatory cells to target tissues, which precipitates glandular damage. Studies have been inconclusive as to whether there is increased apoptosis of glandular epithelial cells that is intrinsic to the target organs or infiltrating immune cells cause apoptosis via cytotoxic T effector cells and inflammatory cytokines. It was suggested that cell death may be activated in the cells by autocrine or paracrine manners as consequences of infiltrating Fas (CD95)-bearing T cells [11]. A study has also suggested that IgG fraction of SS can penetrate A-253 cells of human salivary glands (HSG) and initiate the apoptosis process, suggesting autoantibody-induced apoptotic cell death [12].

Disruption of Tight Junctions. Expression and organization of abnormal tight junction (TJ) proteins in SS salivary glands has been reported [13]. Downregulation of ZO-1 and occludin-G and overexpression of claudin-1 and claudin-4 were detected in minor salivary gland cells of SS patients [13]. Moreover, in Par-C10 cells (rat parotid gland cell line), IFN-γ and/or TNF-α have been shown to disrupt TJ barrier monolayers [14], causing changes in TJ morphology and decrease expression of TJ proteins [15]. It is presumed that altered TJ by inflammatory cytokines disrupts the establishment of proper osmolality between the lumen and the blood side of the secretory end units in the exocrine glands, contributing to secretory dysfunction.

Induction of Matrix Metalloproteinases (MMPs). An increased production of MMP-3 and MMP-9 by ocular surface cells in response to inflammation has been observed in SS [16]. MMP-9 lyses TJ proteins found in the apical epithelium, causing disruption of the corneal barrier function and exposing the nerve endings that terminate in the subapical cell layer [17]. MMP-3 is a physiological activator of MMP-9. IL17, produced by Th17 and T cells, was reported to stimulate MMP-3 and MMP-9 production and disrupt the corneal barrier in mouse models [18, 19]. Schenke-Layland K et al. reported that lymphocytic infiltration in NOD

mice with SS-like exocrinopathy was associated with an upregulation of MMPs [20].

Alteration of Neurological Regulation of Secretion. Inflammatory changes in lacrimal glands may lead to reduced secretion through inhibition of neurotransmitter release or action, damage to secretomotor innervation, or inflammatory cytokines and autoantibodies [21]. Inflammation of the lacrimal gland alters the epithelial phenotype, sensitizes nerve endings, and causes death of apical epithelial cells [22, 23]. The potential sources of inflammatory cytokines in damaged lacrimal glands include epithelial, endothelial, and neural cells as well as infiltrating lymphocytes [24].

Alteration of Myoepithelial Cell (MEC) Morphology and Function. Since MECs are responsible for the production of extracellular matrix and basement membrane components [25], Hawley et al. found that chronic inflammation of the SS lacrimal glands causes a harmful impact on MEC morphology and function. They reported that less contractile proteins and oxytocin receptors were expressed in chronically inflamed lacrimal glands of mice. Moreover, oxytocin stimulation failed to cause acinar contraction of diseased lacrimal glands, which highlighted the adverse effect of inflammation on contractile function of MEC and acini [26].

3.2.1.2 Proinflammatory Cytokines in SS

Knockout of cytokine genes in clinical trials as well as in animal models suggest that CD4+ T cells have a distinctive role in the initiation and progression of SS. The main repertoire of cells include Th1, Th2, and Th17 cells [27, 28]. Some researchers suggest that a Th2 response predominates in the initial manifestation of the disease [29] while others found that Th17 as well as Th1 cytokines initiate and maintain the disease [30]. Th1 cells secrete IFN-γ and IL-2. Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 [31, 32]. In addition, Th17 cells represent a principal source of IL-17A, IL-17F, and IL-22. The pathogenic roles of these inflammatory cytokines in SS are detailed below.

IL-17. Under normal conditions, Th17 cells play a potent role in immunity against extracellular bacteria and fungi by secreting cytokines and chemokines [33]. The T_H17-derived IL-17A has been implicated in a growing list of autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and SS [34]. IL-17 and other Th17-related cytokines were upregulated in salivary gland tissues as well as in saliva of SS mice and patients [35, 36]. IL-6, TNF-α, and IL-17 were elevated in SS-related dry eye [37–39]. In addition, aqueous tear deficiency is identified to be associated with elevation of proinflammatory cytokines in tear, which was detected by microarray [40].

TNF-α. Elevated TNF-α expression in serum and salivary cells of SS patients was detected in comparison to the expression in the cells derived from non-SS sicca patients [41, 42]. Multiple in vitro studies have reported the induction of apoptotic changes of HSG by TNF-α, alone or in combination with other inflammatory cytokines [14, 43]. TNF-α in salivary glands is produced by the salivary gland epithelium as well as salivary gland-infiltrating CD4+ and CD8+ T cells [44].

BAFF. B-cell activating factor (BAFF) is a member of the TNF ligand superfamily, which is known to rescue B cells from apoptosis or stimulate B-cell survival [45]. High levels of BAFF were detected in patients with SLE, SS, and RA [44]. BAFF plays a major role in the complex interactions between natural and acquired immunity. It is produced by monocytes, T cells, B cells, and epithelial cells. Significantly elevated BAFF levels were reported in SS, especially in anti-Ro/SSA and anti-La/SSB sero-positive patients. Furthermore, a correlation between higher BAFF levels and B-cell lymphoma in SS was reported [46]. An association between the clonal expansion of B cells and upregulation of BAFF was detected in the salivary gland cells of SS patients, and those were correlated with disease activity [47, 48].

Because of the important pathological roles in BAFF in SS, a BAFF inhibitor that was initially approved for SLE was applied to treat patients with SS. Belimumab is a monoclonal antibody that antagonizes BAFF, resulting in the inhibition of B-cell survival [28, 49]. It directly decreases the activation of B cells and indirectly decreases the development of IgD+CD27+ class-switched memory B cells, plasma blasts, and plasma cells in studies [50–52]. A prospective, open-label, bicentric study on belimumab revealed an improvement of the subjective symptoms and physician evaluation on systemic activity by 30% [53, 54].

IL-1β. Inflammasomes represent a group of complex polypeptide proteins of the major innate immune response. The activation of inflammasomes is induced by various pathogenic molecules as nucleic acids and can lead to proinflammatory cytokine production such as IL-1β and IL-18 [55, 56]. Studies in animal models and patients with dry eye disease (DED) revealed that inflammasomes are involved in exocrinopathy of the glands [57]. IL-1β is an inflammatory mediator, which plays a major role in keratinizing squamous metaplasia in SS. In addition, mucin glycan acidification has been accelerated with the interruption of IL-1R1 signaling [58]. IL-33, a derivative of IL-1 cytokine, appears to be increased in the SS patient's serum and acts as a danger signal since it is mostly secreted from affected cells [59].

IL-18. IL-18 belongs to the IL-1 superfamily [60]. It is a multi-target cytokine which participates in both innate and adaptive immune responses. A high correlation between IL18 and the degree of disease severity in SS patients was reported [60]. IL-18 is released subsequent to the activation of purine nucleotide ($P2X_7$) receptor. $P2X_7$ receptor stimulation by ATP has also emerged as an essential regulator of autonomic function in the salivary glands. In addition, the activation of the $P2X_7$ receptor causes the inhibition of M3R-induced fluid secretion [61], failure of salivary secretion, and salivary gland inflammation in pSS through interfering with autonomic signaling [62]. The $P2Y_2$ receptor is another nucleotide receptor that was upregulated *in vivo* after 3 days from ligation of rat salivary gland duct [63]. Salivary gland cells of the SS-prone NOD.B10 mouse model showed an upregulation of the $P2Y_2$ receptor [64]. In addition, the authors suggested that $P2Y_2$ receptors for cytokine-like nucleotides have an important role in inflammation [65].

IL-12. The principal cell type of IL-12 secretion includes dendritic cells and macrophages. IL-12 plays an important role in the differentiation of T cells that produces IFN-γ [66]. IL-12 can augment the immune system through a contribution to the proliferation and movement of NK cells and T cells [66]. Authors have reported the high level of IL-12 in the affected organs of SS patients [67, 68]. Noticeably high levels of IL-12 mRNA and protein levels were reported at the earlier stages of SS in mouse models compared to the control [69]. Besides, as detailed by Vosters et al., thyroid gland-specific IL-12-transgenic mice illustrated SS-like organ inflammation of salivary glands, anti-La/SSB autoantibody production, and secretory dysfunction [70].

IFNs. IFNs play an essential role within the pathogenesis of SS. The presence of an IFN-induced gene expression has been illustrated within the salivary organs, peripheral mononuclear cells (PBMC), monocytes, and B cells of SS patients [71–73]. Significant correlation between the type I IFN (IFN-α/IFN-β) signature with higher disease activity and higher levels of autoantibodies was reported in SS patients [74]. Recently, upregulation of type II IFN (IFN-γ)-induced gene expression within the salivary organs of pSS has been documented [75, 76]. Type I and II IFNs bind to distinctive receptors and induce overlapping gene expression patterns. Hence, it can be challenging to determine which type of IFN triggers the IFN-induced gene expression profile seen in pSS [77]. IFN-γ is the major cytokine produced by Th1 cells that directs activation of NK cells, macrophages, CD8 + T cells, and mediates immune responses [78, 79]. IFN-γ causes the cornification of corneal and conjunctival epithelial cells and cellular apoptosis [47]. In animal models with dry eyes, progressive loss of conjunctival goblet cells as well as expanded expression of IFN-γ was identified

[48]. For differentiation between SS-related aqueous tear deficiency and non-SS aqueous tear deficiency, high level of IFN-γ was detected in blood, tears, conjunctiva, and salivary glands of patients with SS-related tear deficiency [42, 80].

IL-4. IL-4 plays a principal role in SS pathogenesis. Elevated IL-4 has been detected in the biopsied minor salivary gland with lymphocytic infiltration of SS patients [29, 81, 82]. Studies performed in NOD and NOD.B10-*H2b* mice reported that the deficiency of IL-4 or STAT6 gene prevented the formation of IgG1-type anti-M3R autoantibody, which was known to function to block the secretory pathway in SS [69, 83, 84]. Likewise, while NOD.B10-*H2b* mice have the ability to induce salivary dysfunction in C57BL/6 mice after a passive transfer, those from STAT6-deficient NOD.B10-*H2b* mice did not induce loss of secretion in the C57BL/6 mouse [84]. This indicates the crucial role of IL-4 in STAT6-mediated isotype switching of anti-M3R and consequent secretory dysfunction [69, 83, 84].

IL-13. IL-13 is a Th2 cytokine and has an impact on B cells and macrophages [85, 86]. IL-13 mRNA was found in the parotid and submandibular glands of SS patients [29, 87]. It was detected in the lymph nodes that drain the salivary glands of SS mice [88]. It was discovered that blocking the function of IL-13 in mice with neutralizing antibodies improved salivary secretion [88]. These results highlight the pathogenic role of IL-13 in secretory dysfunction of SS [89].

IL-6. An increased level of IL-6 on the ocular surface has been consistently reported [90]. IL-6 is a well-known neural sensitizer, and it can reduce the stimulatory threshold of corneal nerve endings, making patients more sensitive to normal environmental stress factors such as low humidity air drafts [91]. IL-6 augments the disease phenotype by inhibiting regulatory T cells and stimulating IL-17 T cells, which are the key immune cells in the pathogenesis of SS [92–94]. IL-6 was reported to be elevated in the serum, saliva, and tears of pSS individuals [95]. IL-6 is also known to be correlated with cell hyperactivity and enhances STAT-3 activation. In Sle1.Yaa mice that develop lupus- and SS-like symptoms, the deficiency of IL-6 alleviates autoantibody production and salivary gland inflammation [96].

3.2.2 Autoantibodies

One of the major characteristics of SS is hyperactivity of B cells, shown as production of autoantibodies against many cellular autoantigens [97]. Studies have indicated that although most autoantibodies in SS do not cause clinical manifestations of dryness, autoantibodies affecting the neural regulation of secretion may contribute to glandular dysfunction, even in the absence of immune cell infiltration in the target glands or destruction of the salivary parenchyma. Since autoantibodies are critical for SS diagnosis, commonly detected autoantibodies in SS are listed in this section, regardless of their roles in affecting saliva secretion.

3.2.2.1 Anti-Ro/SSA and Anti-La/SSB

Anti-Ro/SSA antibodies react with two different proteins, Ro52/TRIM21 and Ro60/TROVE2. Ro52 is an IFN-inducible protein, which serves as a negative regulator for proinflammatory cytokine production [98, 99]. The protein localizes to the cytoplasm and functions as an E3-ubiquitin-protein ligase that plays a role in controlling cell proliferation as well as cell death [98]. Ro60/TROVE2 protein is a ring-shaped RNA-binding protein which participates in the control of nascent transcripts, including the recognition and leading of misfolded defective RNAs to degradation [100]. La/SSB is RNA-binding protein, which can bond to precursor RNA molecules protecting them from deterioration and stimulating their correct processing, folding, and expansion by particular ribonucleases [101]. Anti-Ro/SSA and anti-La/SSB are the most frequently detected autoantibodies in patients with SS. Anti-Ro52 shows the highest specificity in SS patients (66.7%). Anti-Ro60 and anti-La/SSB were reported in 52.1% and 49% of SS patients' sera, respectively [99, 102]. In mouse models of SS, such as NOD.$H2^{h4}$ mice, autoantibodies to Ro and La were identified at 8 weeks of age, preceding the follicular lymphoid

changes detection in the salivary glands [103]. The American-European Classification criteria of SS diagnostic criteria and the recent 2016 ACR/EULAR criteria emphasizes anti-Ro/SSA as the major autoantibodies for pSS diagnosis [104, 105]. The association of the presence of these autoantibodies with early disease onset, longer disease duration, parotid gland enlargement, higher frequency of extraglandular manifestations, and more intense lymphocytic infiltration of the minor salivary glands has been reported [106, 107]. Extraglandular manifestations associated with positive anti-Ro/SSA and anti-La/SSB include splenomegaly, lymphadenopathy, vasculitis, and Raynaud's phenomenon [107, 108]. The anti-Ro/SSA and anti-La/SSB antibody profile in SS patients seems to remain constant throughout the course of the disease even after the administration of B-cell depletion therapy with rituximab. The treatment did not appear to alter secretory functions in patients [109], except for one SS study that indicated improved stimulated and unstimulated whole saliva flow rates with rituximab [110].

3.2.2.2 Anti-Nuclear Antibodies (ANA)

Anti-nuclear antibodies (ANA) are a group of autoantibodies that react with various nuclear and cytoplasmic components of normal human cells [111]. The gold standard method for ANA detection is by indirect immunofluorescence on HEp-2 cells [112]. Positivity of ANA in 59–85% of pSS was reported [113]. Moreover, pSS ANA was reported to be correlated with a higher number of involved organs as well as higher levels of autoantibodies, anti-Ro/SSA, anti-La/SSB, positive RF, and antiphospholipid antibodies [113]. ANA is not SS-specific as individuals with other autoimmune conditions can show ANA positivity [114].

3.2.2.3 Rheumatoid Factor (RF)

Rheumatoid factor (RF) is an autoantibody that acts against the Fc portion of IgG immunoglobulin. Levels of RF in SS patients range between 36% and 74% [113, 115]. Higher levels of RF in SS patients were correlated with the younger age of patients and female predominance. Furthermore, it was correlated with positive minor salivary gland biopsies [113] and higher disease activity [116]. Higher levels of both IgM and IgA RF have been reported in SS patients, specially IgA RF, which have been correlated with higher levels of other autoantibodies of SS, focal scores of salivary gland biopsies, and renal diseases [117].

3.2.2.4 Autoantibodies to Aquaporins (Anti-AQP5)

Aquaporins (AQPs) is a family of 15 water channels, which plays a critical role in water secretion across the plasma membranes [118]. AQPs, especially AQP1 and AQP5, have been detected in the salivary glands and lacrimal glands [119, 120]. In salivary glands, AQP5 is expressed on acinar cells while AQP1 is expressed on endothelial and myoepithelial cells [121]. In mouse lacrimal glands, AQP5 is localized mainly in the ductal cells rather than acinar cells [122]. Detection of anti-AQP1 and anti-AQP5 was reported in sera of patients with pSS and they were correlated with low salivary flow rate during rest [123–125]. In addition, antibodies to AQP8 and AQP9 were detected in SS patient sera [125].

To clarify the mechanism by which autoantibodies to AQP5 play a role in SS, AQP5 contains 6 transmembrane α helices which are joined by 2 intracellular loops and 3 extracellular loops. Extracellular loop E and intracellular loop B aid in the formation of a water channel where 2 NPA motifs (asparagine-proline-alanine) from each loop overlap [126, 127]. Autoantibodies to AQP5 bind to extracellular loops A and C and to the pore-forming extracellular loop E of AQP5, causing the inhibition of water flow. Extracellular epitopes of loops A, C, and E were detected in 89% of the SS samples compared to only 0.5% of the control samples [126].

3.2.2.5 Autoantibodies to Carbonic Anhydrase 6 (CA6), Salivary Protein 1 (SP1), and Parotid Secretory Protein (PSP)

Unexplained dry eye or dry mouth may not show positivity to anti-Ro/SSA or anti-La/SSB. This

necessitates other markers for diagnosis of SS. Autoantibodies to carbonic anhydrase 6 (CA6), salivary protein1 (SP1), and parotid secretory protein (PSP) have been recently introduced as early autoantibodies detected in SS patients [128–130]. CA6 is an enzyme that plays a role in the buffering capacity of saliva. In parotid and submandibular glands, CA6 was detected in the cytoplasm and the secretory granules of the serous acinar cells [131]. Anti-CA6 is one of the most common autoantibodies in patients with dry eye. It is also correlated with disease severity and younger age patients [132]. PSP is a secreted protein that can bind and clear many infectious agents [133]. SP1 was initially identified to be expressed at high levels in mouse submandibular glands as well as lachrymal glands [134].

Matossian and Micucci detected autoantibodies to SP1, PSP, and CA6 in 91% of the SS patients with positive SS biomarkers [135], which highlights the role of the new biomarkers in the earlier diagnosis of SS. Autoantibodies to SP1, PSP, and CA6 showed early expression in SS mice models than anti-Ro/SSA and anti-La/SSB [136]. Furthermore, these autoantibodies were produced in SS patients and patients with idiopathic dry mouth as well as dry eye [136, 137]. They were reported to be positive in 45% of SS patients who were seronegative for anti-Ro/SSA and anti-La/SSB [136]. In a study of group of patients who had xerostomia, xerophthalmia, and at least 3 positive SS criteria, 76% of patients were positive for autoantibodies to SP1, PSP, and CA6. However, positive anti-Ro/SSA and Anti-La/SSB antibodies were only 31% [137].

One study demonstrates that 62% of SS patients had antibodies to Ro or La. Among those who are positive, 54% had antibodies to SP-1, 54% had antibodies to CA6, and 69% had antibodies to SP-1 or CA6. Only 20% of patients had antibodies to only CA6 or SP-1. In this cohort, 18% had antibodies to PSP and 38% lacked antibodies to either Ro or La. Evaluating a sex and age matched cohort of normal controls revealed that 4.3% of the controls had antibodies to Ro or La, 4.3% had antibodies to SP-1, 3% had antibodies to CA6, and 10% had antibodies to PSP [136].

3.2.2.6 Anti-Muscarinic Type 3 Receptor (M3R) Autoantibodies

Muscarinic receptors represent acetylcholine receptors, which are G protein-coupled receptor complexes in the neuronal plasma membranes. There are 5 identified types of muscarinic receptors (M1R-M5R). M3R is expressed in exocrine glands and it has an essential role in saliva secretion. M3R is stimulated by acetylcholine secreted from postganglionic fibers of parasympathetic innervation [138]. For saliva secretion, acetylcholine binds to M3R and causes increase in the level of intracellular Ca^{2+} through inositol 1, 4, 5-triphosphate (IP_3) and IP_3 receptors. After that, increased intracellular Ca^{2+} induces salivary gland secretion through activation of apical membrane Cl^- [139]. Anti-M3R autoantibodies were detected in pSS patients [140]. Studies have shown the antagonistic roles of anti-M3R in secretory dysfunction in SS [140, 141] [142, 143]. Anti-M3R autoantibodies were reported to downregulate M3R and major histocompatibility complex I from the plasma membrane in primary HSG by clathrin-independent endocytosis, which may be associated with consequent pathogenesis of salivary dysfunction in pSS patients [144].

3.2.2.6.1 Detection of Anti-M3R Autoantibodies in Patient Sera

Bacman et al. detected circulating autoantibodies against acinar cells and M3R by ELISA utilizing both rat exorbital lacrimal acinar cell membranes and synthetic 25-mer peptide of the second extracellular loop in human M3R [140]. Furthermore, the IgG fraction and anti-M3R peptide autoantibodies were able to activate nitric oxide synthase attached to lacrimal gland M3R in the same patients [145]. Inhibition of carbachol-induced intracellular calcium release was detected in IgG-treated HSG cells [142]. In addition, Gao et al. showed that 60% of pSS and 100% of sSS patients were positive for IgG when an assay with M3R expressed on transfected Chinese hamster ovarian cells was utilized while none of the healthy controls were positive for anti-M3R autoantibodies. Multiple isotypes of anti-M3R were detected in SS patients' sera and the most common types were IgG1, IgG3, and

IgA [141]. A cohort study detected anti-M3R autoantibodies in 90% of pSS patients [143]. Moreover, a higher frequency of leukopenia was reported in positive anti-M3R pSS patients compared to patients without anti-M3R autoantibodies [143]. Interestingly, Dawson et al. showed in their in vitro study that neither sera from pSS patients nor from healthy controls contained anti-M3R IgG fractions [146].

Autoantibodies to the extracellular domains (N terminal region, the first, the second, and the third extracellular loop) of M3R were examined in 42 SS and 42 healthy controls by ELISA using synthesized peptide antigens encoding these domains. Titers and positivity of anti-M3R antibodies to every extracellular domain of M3R were significantly higher in SS patients than in healthy controls [147]. Anti-M3R autoantibodies in the sera of pSS patients were detected by He et al., which reported that antibodies against cycle second extracellular domain of M3R peptide in the sera of 62.2% of their patients with pSS [148]. Nevertheless, autoantibodies against the functional epitopes of anti-M3R were reported in less than 50% of SS patients [149].

Recently, Zuo et al. reported that there was high prevalence of anti-M3R antibodies in adsorbed SS patient plasma (75%), which was significantly higher than the healthy controls (2%) [150]. In addition, anti-M3R antibodies were significantly associated with anti-Ro/SSA in their analysis Therefore, authors proposed that anti-Ro/SSA in combination with anti-M3R autoantibodies are the most sensitive and specific for SS, which potentially enables non-invasive identification of pSS patients without a lip biopsy [150].

3.2.2.6.2 Anti-M3R Autoantibodies in SS Studies with Animal Models

Because M3R is a major receptor responsible for saliva secretion in the salivary glands, animal studies involving M3R or anti-M3R are described herein. Induction of the SS mouse model by immunization of a mixture of peptides whose sequences were derived from the extracellular loops of M3R was described by Yang et al. [151]. They found the signs of inflammation in the lacrimal glands, salivary glands, and the intestines in the mouse model. Interestingly, Qian et al. reported that chronic activation of M3R in lacrimal glands stimulated downregulation of post-receptor signaling molecules without significant changes in their molecular contents [152]. Cytokines were also implicated in the inhibition of neurotransmitter release from the lacrimal gland efferent nerves [153]. Zoukhri et al. discovered in the study that IL1α and IL1β, along with TNF-α, produced by invading lymphocytes in the lacrimal gland acini cause blocking of neurotransmitter release from the lacrimal gland nerves in mice [153].

Cavill et al. reported that IgG raised in rabbits against a peptide of the second extracellular loop of human M3R was able to directly inhibit carbachol-induced smooth muscle contraction of the murine colon, confirming the antagonistic potency of anti-M3R autoantibodies [154]. Iizuka et al. transferred monoclonal antibodies against conformational epitopes of M3R into mice [155]. They described two anti-M3R IgM monoclonal antibodies capable of binding to the extracellular surface domain of M3R-expressing COS-7 cells, which decreased the pilocarpine-induced production of saliva and inhibit the membrane-associated expression of AQP-5 [155].

M2R and M3R work in concert to initiate contraction of smooth muscles [8]. Waterman et al. demonstrated that serum and purified IgG fraction from SS included antibodies that maximally inhibited response to carbachol-induced contraction of the mouse isolated bladder by 50%, while the muscarinic receptor antagonist 4-DAMP eliminated the response [156]. Park et al. demonstrated that contraction of the smooth muscle in the gastrointestinal tract and contractile motility of colon were inhibited in patients with pSS IgG and anti-M3R activity [157]. A new model with M3R-induced sialadenitis was generated by using $Rag1^{-/-}$ mice which were inoculated with splenocytes from $M3R^{-/-}$ mice immunized with M3R synthetic peptides. It was found that severe SS-like sialadenitis was developed in mice with M3R-induced sialadenitis [158]. Cell transfer experiments using $M3R^{-/-}$ x $IFN\gamma^{-/-}$ mice and $M3R^{-/-}$ x $IL\text{-}17^{-/-}$ mice indicated that IFNγ and

IL-17 are key cytokines in the pathogenesis of sialadenitis, reemphasizing the crucial roles of M3R-reactive Th1 and Th17 cells in autoimmune SS [158].

Most recently, Chen et al. investigated whether antibodies against linear epitopes of M3R are pathogenic in vivo [159]. They found that transfer of the antibodies derived from mice immunized with linear or cyclic peptides encoding for the second extracellular loop of M3R to Balb/c mice revealed no inhibitory effect on pilocarpine-induced saliva or tear production in the recipient animals. These results suggest that antibodies against linear or cyclic epitope within the second extracellular loop of M3R may not be pathogenic in regard to impairment of secretion function of exocrine glands [159]. M3R expression on the membrane of HSG cells and carbachol-induced [Ca^{2+}] transients (CICTs) were significantly decreased after preincubation of HSG cells with pSS IgG. However, control IgG preincubation had no effect on both the M3R expression level and CICTs [160].

In addition, M3R activation plays a major role in trafficking of AQP5 from the cytoplasm to the apical membrane, which accelerates water transport across the cell membrane [158]. A study demonstrated that SS-derived IgG inhibited carbachol-induced AQP-5 trafficking to the plasma membrane by using an AQP5-transfected HSG cell line expressing AQP-5 as a GFP-tagged protein with a confocal imaging system [161]. While internalization or direct suppression of M3R by anti-M3R autoantibody plays a role in secretory dysfunction, the autoantibodies were also shown to modulate submandibular gland Na+/K + -ATPase activity via prostaglandin E2 and cyclic AMP [162].

3.2.3 MicroRNAs (miRNAs)

3.2.3.1 Roles of miRNA in Dry Mouth

MicroRNAs (miRNAs) represent single-stranded RNA molecules that regulate post-transcription of gene expression in plants and animals [163]. Translation of messenger RNAs was prevented by miRNA, so it can downregulate target molecules within various genomic contexts as growth, inflammation, and regeneration [164]. miRNA has been involved in autoimmune disorders as it was utilized as a micromanager of numerous stages of immune regulations [165]. Dysregulation of miRNA was implicated in a wide variety of diseases, such as cancer, neurodegenerative diseases, cardiovascular diseases, and autoimmune conditions [166]. The reported SS-associated miRNA studies have provided valuable tools to discover the impact of miRNAs on SS disease pathogenesis [166–170]. Comparative array analysis of miRNA expression in salivary glands of SS and control subjects had revealed distinct miRNA signatures in SS patients, associated with glandular inflammation and dysfunction [171]. miRNAs in SS MSG revealed a correlation between relative expression of selected miRNAs and the minor salivary gland focus score. Furthermore, studying miRNAs from patients with preserved salivary flow in comparison to low salivary flow reported nine of differentially expressed miRNA, seven of which were upregulated in the group with decreased salivary gland function [167].

Alevizos et al. reported the presence of miRNA in SS salivary gland cells. MicroRNA expression patterns clearly separated patients with SS from non-SS control subjects. Furthermore, they showed that miRNA expression can differentiate SS patients in relation to the lymphocytic infiltration and salivary hypofunction [167]. Pauley et al. reported overexpression of miR-146a in the PBMCs of SS patients in comparison to healthy controls [165] as well as in the salivary glands of 8 weeks age mice prior to disease onset. Twofold increased level of miR-155 has been found also in cultured salivary gland epithelial cells from SS than controls [172]. Other studies supported the upregulation of miR-146a in PBMCs from SS patients [173–175]. Furthermore, miR-146a and miR-155 were overexpressed in B lymphocytes as well as purified T-lymphocytes [176]. Authors measured the levels of miR-155 in salivary gland cells of SS patients and the controls. They found the levels of miR-155 was twice in SS patients in comparison to the controls [177]. Recently, Wang et al.

reported downregulation of miR-16 and miR-181a in minor salivary glands of SS patients [169] and Peng et al. also reported that miR-181 was highly expressed in PBMCs of SS, which was correlated with ANA levels. They assumed that B cells could contribute to miR-181a expression and both can have a major role in SS pathogenesis [170]. Although miRNA deregulation was detected in PBMCs, sera, and saliva of SS, their role in the pathogenesis of SS needs more investigations.

Epstein–Barr virus (EBV) DNA was detected in salivary gland cells of SS patients [178–180]. In EBV salivary infected cells, EBV secretes miRNAs which could infect the normal salivary cells [181]. ebv-miR-BART13 is miRNA secreted by EBV and showed higher expression in salivary cells as well as B cells of pSS patients [182]. In addition, it showed downregulation of key proteins involved in Ca^{2+} signaling in the salivary glands. STIM1 and STIM2 are stromal interacting molecules 1 and 2. They can detect changes in Ca^{2+} concentrations inside the endoplasmic reticulum (ER) [183, 184]. STIM1 can detect store-operated Ca^{2} entry (SOCE) which provides influx of Ca^{2+} in salivary cells. It is an important regulator for fluid secretion in salivary glands [185]. Gallo et al. concluded in their study that ebv-miR-BART13-3p reduces STIM1 expression. They concluded that miRNAs have a pathogenic role in calcium signaling defects which results in dysfunction of saliva secretion [182].

3.2.3.2 Roles of miRNA in Dry Eyes

miRNAs were correlated with the physiological and pathological processes in the ocular surface [186]. miRNAs can act as diagnostic biomarkers for diseases as they resist degrading enzymes such as RNases. In addition, they are detected in all cells and in body fluids [187]. miRNAs have a role in several physiological events and in mediation of disease. They inhibit gene expression by blocking messenger RNA. Autoimmune diseases such as SS are known to show altered expression of specific miRNAs. These miRNAs play key roles in modulating inflammation, delaying or enhancing wound healing, cell differentiation metabolism, and survival [188]. Most recently, Kim et al. investigated the expression of miRNAs in the tears of SS patients and compared them to healthy controls. They found that the expression of 14 miRNAs were significantly different in SS patients compared to that in the controls [189].

It has been reported that samples of patient's pterygium showed that miRNA-145 is downregulated in the ocular surface disease and is inversely related to the extension, severity, and vascularity [190, 191]. Higher expression of miRNA-146a and miRNA-155 was reported to be correlated with the clinical manifestations of SS [165] [175]. In a mouse model of SS, it was reported that miRNA-146a was relatively highly expressed at 8 weeks in submandibular gland as well as lacrimal gland, and in the kidney at 20 weeks of age [165]. miRNAs in SS may be correlated with SS biomarkers, anti-Ro/SSA as well as anti-La/SSB autoantibodies. The Ro and La proteins are utilized by infective virus to replicate inside of the cells. Virus infection is one of the proposed etiologies for DED and ocular surface disorder. It can induce the production of neoantigens, plasma cells, cytotoxic T cells, chemokines, cytokines, immunoglobulins, memory lymphocytes, and tissue damage and dysfunction due to apoptosis and inflammation in the target tissues [192]. One can predict that production of disrupted miRNAs can be induced by Ro or La proteins modified by viral RNA which can deviate them from normal function as well as it can induce inflammatory changes in SS [193].

3.2.4 Reactive Oxygen Species (ROS)

3.2.4.1 Roles of ROS in Salivary Dysfunction of SS Patients

Higher levels of tissue damage are due to imbalance between ROS levels and the ability of the body to detoxify ROS [194]. ROS are highly reactive free radicals which are secreted by activated cells of the immune system. They are more reactive than molecular oxygen [195]. Protection of the body against the damaging effect of ROS is achieved through the endogenous antioxidant enzymes such as superoxide dismutase (SOD)

and catalase (CAT) that cleave ROS [196]. Oxidative stress caused by ROS has been detected in autoimmune diseases as rheumatoid arthritis, SLE, and SS [197–200]. It was also implicated in apoptosis of the living cells [201]. Stimulation of submandibular gland with anti-M3R resulted in upregulation of SOD and CAT levels in the sera of pSS patients, which was associated with detection of presence of anti-Ro/SSA, dry mouth, xerophthalmia, and xeroderma [196]. The authors concluded that increased SOD and CAT acted as a defensive reaction to the increased ROS, which can cause irreversible cellular and tissue damage and potentially contributing to salivary dysfunction.

Protein carbonyl (PC) and advanced oxidation protein products (AOPP) are two markers of protein oxidation, which can be used to measure the levels of oxidative stress in pSS [202]. Higher levels of both markers were detected in SS patients when compared to the healthy controls, but there was no associations between fatigue levels and PC or AOPP in those patients [202]. In addition, labial biopsy specimens from SS patients revealed the high levels of oxidative stress markers [203]. Cell damage markers, such as lactate dehydrogenase (LDH), hex-anoyl-lysine (HEL), 8-hydroxy-2 -deoxyguanosine (8-OHdG) and a mitochondrial glutamic-oxaloacetic transaminase (m-GOT), were used for salivary gland damage. Elevated levels of 8-OHdG and HEL were measured in SS patients' saliva. However, in normal patients and other salivary gland disorders, no changes of those markers were detected [200]. High levels of oxygen stress markers, such as oxidative DNA damage and propanoyl-lysine, were detected in saliva from SS patients and there was a link between oxidative stress and mitochondrial dysfunction in SS patients [204].

3.2.4.2 Roles of ROS in Dry Eyes of SS Patients

SS adversely affects lacrimal gland function, reducing tear secretion due to inflammation and ROS formation [205–207]. The oxidative stress caused by ROS can be involved in eye pathology in various conditions, such as DED, conjunctivochalasis, age-related macular degeneration, and ocular surface epithelial damage induced by UV light and tobacco smoke [207]. A study utilizing a murine DED model showed high correlation between excessive oxidative stress and epithelial damage of the ocular surfaces as well as lacrimal gland secretory dysfunction [208]. Inhibition of the oxidative stresses might be used for preventing cell death as a therapeutic management of DED in which the management not only aims to substitute the tear film, but also to decrease the inflammation through inhibition of oxidative stress on ocular tissues [204, 209, 210].

Evaluation of conjunctiva in dry eye patients showed high levels of oxidative stress in comparison to healthy control subjects [211]. During evaluation of the lipid oxidative stress markers in conjunctiva and tears of SS patients and healthy controls, authors found SS patients had worse tear stability and vital staining scores more than the controls. In addition, the density of conjunctival inflammatory cell was significantly higher in SS subjects [207]. The authors suggested that the increased membrane lipid peroxidation may increase the immune and inflammatory response, activate gene expression and cell proliferation, or initiate apoptosis. Therefore, the pathogenesis of ocular surface changes in SS may be related to peroxidative lipid membrane destruction, high levels of ROS, inflammatory pathologic changes, or variability of ocular surface antioxidant status [207].

Čejková et al. measured the level of antioxidant enzymes, such as SOD, CAT, and glutathione peroxidase, in the conjunctival epithelium of SS patients by IHC [212]. High expression of antioxidant enzymes was reported in normal eyes. However, in dry eyes, the level of those enzymes was much less noticeable if correlated to the symptoms of severe dry eye symptoms. So, the authors assumed that oxidative injuries of anterior eye surfaces in SS patients may be related to lower levels of antioxidant enzymes. Cytokines also are involved in causing oxidative stress in lacrimal glands as Beauregard et al. found that IL1β can induce nitrous oxide production in the lacrimal gland [213].

3.2.5 Calcium Signaling in SOCE

3.2.5.1 Overview

Stimulation of fluid secretion from cells is enhanced by increasing cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) as it regulates ion channel activities and derives secretion movement across the apical membrane using osmotic gradient [214, 215]. In salivary acinar cells, increase in $[Ca^{2+}]_i$ is initiated in response to release of Ca^{2+} from ER which is mediated by inositol 1,4,5, trisphosphate (IP_3) with the help of inositol trisphosphate receptor (IP_3R). IP_3R is an intracellular Ca^{2+} release channel. In salivary gland cells, the major subtypes of IP_3Rs found are IP_3R2 and 3, both of which are localized in the apical region of the acinar cells [216, 217]. Unregulated increases in cytosolic Ca^{2+}, due to release from either ER or other intracellular stores, or Ca^{2+} entry, can be extremely deleterious to cells [218]. Deficits in Ca^{2+} signaling have been associated with cellular and functional damage in many cell types, including neuronal cells and lymphocytes [216].

3.2.5.2 Calcium Signaling for Saliva Secretion

Increased level of $[Ca^{2+}]_i$ is accomplished through activation of SOCE, which can convert the transient high level of $[Ca^{2+}]$i to continuous elevation that can stimulate normal secretion of salivary glands [216]. The key proteins involved in generation of $[Ca^{2+}]$i signal include M1R, M3R, G-protein-coupled receptor-signaling complex, IP_3Rs, and SOCE [219, 220]. High levels of $[Ca^{2+}]$i activates the K^+ and Cl^- channels as well as the Na^+-K^+-$2Cl^-$ cotransporter and Na^+/H^+ and $Cl^-/HCO3^-$ exchangers [221]. Additionally, recruitment of AQP5 into the apical plasma membranes is triggered by the rise in $[Ca^{2+}]$i, thus resulting in increased salivary fluid secretion [222]. One of the best Ca^{2+}channels is the IP_3R in the ER [217]. IP_3R is controlled by both IP_3 and Ca^{2+}. This regulation of IP_3R by IP_3 and $[Ca^{2+}]$i ensures that the channel is relatively more active when $[Ca^{2+}]$i is low and less active when $[Ca^{2+}]_i$ is high, thus protecting ER Ca^{2+} stores and regulating $[Ca^{2+}]$i within the physiological range required for the cell function [223]. This feed-forward and feedback regulation of IP3R can also explain the spread of the Ca^{2+} wave from the apical to the basal membrane in salivary gland cells [214].

3.2.5.3 Mechanisms and Physiological Relevance of SOCE

IP_3-mediated Ca^{2+} release via IP_3R and consequent depletion of ER Ca^{2+} are the main triggering event in the activation of Ca^{2+} entry via the SOCE mechanism [224]. SOCE is achieved by the gating of plasma membrane Ca^{2+} channels, e.g., Orai1 (calcium release-activated calcuim channel protein 1) and TRPC1 (transient receptor potential canonical 1) [225, 226].

STIM1 and Orai are well-known as important components of SOCE [226]. STIM1 is an ER Ca^{2+}-binding protein and responds to diminished ER $[Ca^{2+}]$. It goes through extensive conformational changes that cause extension of its C-terminal domain, and translocation to the periphery of the cells where it clusters at specific ER-plasma membrane junctions [216, 227, 228]. Orai1 has been well-known as calcium release-activated calcium channel protein 1 and it is the foremost pore-forming component of SOCE-activated Ca^{2+} channels in the membrane [226] Orai1 function has been studied in two other exocrine glands, lacrimal and pancreatic. $Orai1^{-/-}$ mice display loss of lacrimal gland function and reduced SOCE in lacrimal gland acinar cells [229].

STIM2 is a second ER-localized Ca^{2+}-sensor protein and is correlated with SOCE and Ca^{2+} signaling. It shares significantly sequence homology with STIM1. STIM2 has a comparatively lower Ca^{2+}affinity and can detect small depletions of ER $[Ca^{2+}]$, whereas STIM1 has higher affinity for Ca^{2+}, responding only to substantial Ca^{2+}depletion in the ER [230]. Similar to STIM1, STIM2 translocates and clusters to form puncta in ER-plasma membrane junctions, where it clusters with Orai1 and STIM1 [231, 232]. In mice salivary gland cells, it was reported that STIM2 knockout caused decrease in salivary gland secretion while salivary acinar cells showed a reduction of $[Ca^{2+}]$i, which was increased by stimulation with muscarinic receptor agonists.

STIM2 recruits STIM1 and enables formation of STIM1 puncta in ER-plasma membrane junctions by low intensity of the stimulus [233]. These findings demonstrate a critical role for STIM2 in regulating SOCE-mediated Ca^{2+} signaling and cell function. However, other studies indicate that involvement of STIM2 in SOCE might vary [234]. Bird et al. have previously suggested that the oscillatory pattern of SOCE is primarily dependent on the mobilization of STIM1, not STIM2 [235].

TRPC1 is a principal channel for salivary gland function and secretion. Lack of TRPC1 caused decrease of SOCE and diminished salivary secretion [236, 237]. In addition, TRPC1 is essential for other cellular physiological functions such as protein secretion in acinar cells of pancreas [238, 239]. TRPC1 and TRPC3 channels generate relatively non-selective, Ca^{2+}-permeable channel. Reduced SOCE and fluid secretion was found in mice lacking TRPC1 or TRPC3 [39]. These findings assumed that function and activation of TRCP1 after depletion of ER-Ca^{2+}store mainly depend on Orai1 [185, 216, 240]. Another study done by Chen et al. showed that recruitment of TRPC1 to the plasma membrane was triggered through Orai1-mediated Ca2 + entry [185].

3.2.5.4 Impaired Ca^{2+}Signaling in SS

Decrease in Ca^{2+}signaling in salivary acinar cells of SS patients who had lower level of inflammatory cell infiltration in the glands was reported [216, 241]. Toes et al. performed a study on biopsy of minor salivary glands in SS patients. They reported that Ca^{2+}signaling stimulation by neurotransmitter was impaired and it was associated with diminution of salivary secretion.

We have also shown that anti-M3R autoantibodies suppress carbachol-medicated Ca^{2+}signaling pathway, as described earlier in this chapter. Furthermore, they showed that minor salivary gland acinar cells had low to relatively high lymphocytic infiltration which was associated with a decrease in carbachol-stimulated Ca^{2+} increase [241].

It was reported that disruption of IP_3Rs causes a defect in agonist-induced intracellular Ca^{2+} release. Loss of IP_3R2 and mislocalization of IP_3R3 were detected in cells situated in intact sites of the salivary glands from SS patients [216]. In addition, Zeng et al. had confirmed these findings in the NOD mouse model for pSS by showing that IP_3R insufficiency in salivary acinar cells contributes to the salivary dysregulation of pSS [216, 242]. Loss of lacrimal and salivary secretion as well as progressive eye inflammation was reported in mice lacking IP_3R2 and IP_3R. Decreased levels of IP_3R2 and intracellular Ca^{2+}release was reported in salivary acini from IL14α transgenic mice with SS [216, 243].

A study reported a targeted deletion of both STIM1 and STIM2 in T-lymphocytes of mice with SS [244]. Authors presented lower levels of SOCE as well as cytokines in T-lymphocytes. Moreover, other studies with ablation of STIM1 and STIM2 in T cells found that mice developed loss of saliva secretion and exocrinopathy. In addition, higher level of SS serum autoantibodies and lymphocytic infiltration was detected in submandibular gland cells [183, 216].

Lower levels of STIM1 and STIM2 proteins were detected in PBMCs from pSS patients in comparison to the healthy controls [216, 245]. Furthermore, SOCE is reduced in T cells from those patients. These findings may suggest that there was a correlation between deficiencies of STIM1 or STIM2 in T cells and the presence of abnormalities in T cell function. This further indicates that abnormal SOCE may be a contributing factor in the pathogenesis of salivary gland dysfunction in SS patients. Loss of STIM1 causes defects in IP_3R and an impairment of SOCE and Ca^{2+}signaling in salivary gland acinar cells of SS patients [216, 245, 246]. Feske et al. showed that STIM1 deficiency were associated with the clinical signs of lymphoproliferation and autoimmunity as well as secretory dysfunction in SS.

3.3 Other Conditions Contributory to SS Secretory Dysfunction

3.3.1 Effect of Sex Hormones in SS

SS almost affects women and is usually associated with diminished tear secretion, destruction

and dysfunction of glandular epithelium, and inflammation of lacrimal glands [21]. This female predominance and onset of SS after menopause suggest that sex hormones are involved in pathogenesis of SS [247].

Estrogen is a primary female sex hormone. It is responsible for regulation of the female reproductive system and secondary sex characteristics [248]. Androgens are steroid hormone that promotes male secondary sex characters [249]. Estrogen can activate B cells which leads to production of antibodies and autoantibodies, but androgens were found to decrease maturation of B cell which results in decrease of antibodies production [250]. Interestingly, lower dose of estrogen secreted after occurrence of menopause are still capable of enhancing B-cell activation and antibody production suggesting that estrogen in general may provide a protective role against development of SS [251, 252]. Recently, Tellefsen et al. reported that sex-related differences in gene expression of lacrimal glands in MRL/lpr and NOD mice contributed to lacrimal gland disease in SS [253].

Estrogen deficiency was studied in female SS model mice NOD.B10.H2 in which ovariectomy resulted in significantly increased inflammation of lacrimal glands while estrogen replacement of the same mice resulted in reducing inflammation through decreasing B and T cells infiltration [252]. Ishimaru et al. recommended the need of estrogen for salivary gland health. They performed ovariectomy in healthy female mice which resulted in SS-like disease with apoptosis of salivary gland epithelial cells [254]. Low circulating estrogen levels were found to be associated with ocular dryness in SS patients [255]. However, other studies have reported the implication of estrogen in the pathogenesis as well as progression of SS [256].

Increased apoptosis of lacrimal gland and elevated levels of oral dryness in SS were found to be correlated with lower levels of circulating androgens [255, 257]. In female MRL/lpr and NZB/NZW FI mouse models of SS, higher levels of androgens were able to suppress inflammation of the lacrimal glands [258, 259]. Similarly, administration of testosterones to female mice models of SS as MRL/lpr and NZB/NZW F1 were found to increase the function of lacrimal glands as well as decrease inflammation [259–261]. In addition, testosterone was found to alleviate dry eye signs and symptoms and stimulate tear secretion in SS patients [256]. Most recently, Morthen et al. reported that testosterone was able to cause significant decrease of numerous immune-related genes which were associated with inflammatory process as lymphocyes activation and cytokines production in SS mice [262].

3.3.2 Meibomian Gland Dysfunction in SS

Meibomian glands are lipid-secreting glands, located in the tarsal plate of eyelids. Each gland consists of many acini connected by common duct through the entire length of the gland [263]. The meibomian gland has sympathetic, parasympathetic, and sensory innervation, and it was reported that the parasympathetic neurotransmitters activate muscarinic acetylcholine receptors resulting in increased intracellular [Ca^{2+}] and promotion of cell proliferation [8]. Meibomian gland dysfunction (MGD) is commonly characterized by terminal duct gland obstruction and/or qualitative/quantitative changes in glandular secretion [263]. MGD is a major cause of evaporative dry eye by destabilizing the tear film, enhancing evaporation and osmolarity of the tear film, decreasing tear stability and lubrication, and damaging the ocular surface epithelia [21]. SS patients had a higher prevalence of MGD, which is associated with diminished expression and quality of meibum and higher meibomian gland dysfunction. Furthermore, it was reported that SS patients are more liable to reduced volume, stability and surface activity of tears as well as higher rate of ocular surface damage and DED [24, 264]. Villain et al. found that SS patients with dry eye have higher acinar cell reflectivity and peri-glandular inflammation of meibomian gland in comparison to normal control patients [265]. SS patients have higher meibomian gland dropout scores [266]. Chen et al. reported higher scores of dry eye symptoms, higher osmolarity of the tear film, decreased tear production, increased meibomian gland atrophy, and higher lid margin

abnormalities in pSS patients compared to the control group [267]. The meibomian gland is significantly more damaged in SS patients (57.9%) than in non-SS patients [268].

Possible explanations of MGD in SS patients include damage of the gland by inflammatory cells and cytokines, as meibomian glands represent major autoimmune target in SS. In addition, cytokines and inflammatory cells secreted locally from the conjunctiva or reached the conjunctiva through the lacrimal secretion may cause direct damage to the meibomian glands [269]. Additional possible mechanisms of meibomian gland damage in SS include lymphocytic infiltration adjacent to tarsal conjunctiva [268] and neural-meibomian gland epithelial cell disruption by cytokines [268]. Furthermore, it was assumed that hyperkeratinization of the terminal duct epithelium of meibomian glands was induced by conjunctival inflammatory cytokines secreted into the tear [21].

3.3.3 Conjunctival Disease in SS

The conjunctiva is a moist mucous membrane which consists of an epithelium covering a lamina propria with loose connective tissue [270]. It secretes a variety of products into the tears and helps as a barrier against various antigens from the outer environment [270]. However, keratoconjunctivitis sicca is one of the primary clinical and histological manifestations of SS. It is not documented if these ocular changes are due to inflammatory infiltration of conjunctiva or secondary to inflammation of lacrimal glands and DED. Histological changes of conjunctiva in SS include lymphocytic infiltration, squamous metaplasia, and decrease in goblet cell number [271]. Other changes include increased osmolarity and apoptotic cells in the conjunctiva [272]. The study reported that the conjunctival disease was not a result of decreased tear volume, but due to inflammation. You et al. studied the ocular surface disease in mice and their association with development of SS-like lacrimal gland disease. It was reported that SS-prone mice developed higher conjunctival goblet cell loss, inflammatory infiltration of the lacrimal glands as well as conjunctiva and higher expression of inflammatory cytokines [273]. Contreras-Ruiz studied keratoconjunctivitis sicca in the thrombospondin-1 deficient mouse model of SS [274]. Authors detected inflammation in the conjunctiva, which disturbs the secretory function of goblet cells and compromises the protective function of tears, leading to ocular surface damage in the mouse model.

3.4 Conclusion

SS is heterogeneous in terms of its clinical manifestations. It is a multi-factorial disease, as exemplified in the low concordance rates of SS in twin studies [275, 276]. Delineating the factors that trigger onset, precipitate progression, and damage the target organs that lead to deficient secretion can be a daunting task. The molecules described here could essentially be a small part of the bigger picture. Some patients may suffer from severe dry mouth and eyes due to more prominent apoptotic cell death and complete loss of glandular acinar cells while others may retain intact glandular architecture with a high titer of anti-M3R autoantibodies or altered SOCE that impair the normal secretory pathway. Some may have both in place, causing dryness. Therefore, the differential roles of these factors in each SS patient need to be taken into consideration when evaluating sicca symptoms. If any of these contributing factors can be quantitatively measured and monitored through the development of personalized medicine, it will certainly prevent the rapid deterioration of glandular functions in patients with SS.

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References

1. Mavragani CP, Crow MK. Activation of the type I interferon pathway in primary Sjogren's syndrome. J Autoimmun. 2010;35(3):225–31.
2. Mavragani CP, Moutsopoulos HM. Sjögren syndrome. CMAJ. 2014;186(15):E579–86.
3. Boumba D, Skopouli FN, Moutsopoulos HM. Cytokine mRNA expression in the labial salivary gland tissues from patients with primary Sjogren's syndrome. Br J Rheumatol. 1995;34(4):326–33.
4. Aqrawi LA, Kvarnström M, Brokstad KA, Jonsson R, Skarstein K, Wahren-Herlenius M. Ductal epithelial expression of Ro52 correlates with inflammation in salivary glands of patients with primary Sjögren's syndrome. Clin Exp Immunol. 2014;177(1):244–52.
5. Mandl T, Granberg V, Apelqvist J, Wollmer P, Manthorpe R, Jacobsson LT. Autonomic nervous symptoms in primary Sjogren's syndrome. Rheumatology (Oxford). 2008;47(6):914–9.
6. Dartt DA. Neural regulation of lacrimal gland secretory processes: relevance in dry eye diseases. Prog Retin Eye Res. 2009;28(3):155–77.
7. Kruse AC, Hu J, Pan AC, Arlow DH, Rosenbaum DM, Rosemond E, et al. Structure and dynamics of the M3 muscarinic acetylcholine receptor. Nature. 2012;482(7386):552–6.
8. Kam WR, Sullivan DA. Neurotransmitter influence on human meibomian gland epithelial cells. Invest Ophthalmol Vis Sci. 2011;52(12):8543–8.
9. Dartt DA. Dysfunctional neural regulation of lacrimal gland secretion and its role in the pathogenesis of dry eye syndromes. Ocul Surf. 2004;2(2):76–91.
10. Manganelli P, Fietta P. Apoptosis and Sjögren syndrome. Semin Arthritis Rheum. 2003;33(1):49–65.
11. Polihronis M, Tapinos NI, Theocharis SE, Economou A, Kittas C, Moutsopoulos HM. Modes of epithelial cell death and repair in Sjögren's syndrome (SS). Clin Exp Immunol. 1998;114(3):485–90.
12. Sisto M, Lisi S, Castellana D, Scagliusi P, D'Amore M, Caprio S, et al. Autoantibodies from Sjögren's syndrome induce activation of both the intrinsic and extrinsic apoptotic pathways in human salivary gland cell line A-253. J Autoimmun. 2006;27(1):38–49.
13. Ewert P, Aguilera S, Alliende C, Kwon YJ, Albornoz A, Molina C, et al. Disruption of tight junction structure in salivary glands from Sjögren's syndrome patients is linked to proinflammatory cytokine exposure. Arthritis Rheum. 2010;62(5):1280–9.
14. Baker OJ, Camden JM, Redman RS, Jones JE, Seye CI, Erb L, et al. Proinflammatory cytokines tumor necrosis factor-alpha and interferon-gamma alter tight junction structure and function in the rat parotid gland par-C10 cell line. Am J Physiol Cell Physiol. 2008;295(5):C1191–201.
15. Baker OJ. Current trends in salivary gland tight junctions. Tissue Barriers. 2016;4(3):e1162348.
16. Chotikavanich S, de Paiva CS, Li DQ, Chen JJ, Bian F, Farley WJ, et al. Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. Invest Ophthalmol Vis Sci. 2009;50(7):3203–9.
17. Sobrin L, Liu Z, Monroy DC, Solomon A, Selzer MG, Lokeshwar BL, et al. Regulation of MMP-9 activity in human tear fluid and corneal epithelial culture supernatant. Invest Ophthalmol Vis Sci. 2000;41(7):1703–9.
18. De Paiva CS, Chotikavanich S, Pangelinan SB, Pitcher JD, Fang B, Zheng X, et al. IL-17 disrupts corneal barrier following desiccating stress. Mucosal Immunol. 2009;2(3):243–53.
19. Chauhan SK, El Annan J, Ecoiffier T, Goyal S, Zhang Q, Saban DR, et al. Autoimmunity in dry eye is due to resistance of Th17 to Treg suppression. J Immunol. 2009;182(3):1247–52.
20. Schenke-Layland K, Xie J, Magnusson M, Angelis E, Li X, Wu K, et al. Lymphocytic infiltration leads to degradation of lacrimal gland extracellular matrix structures in NOD mice exhibiting a Sjögren's syndrome-like exocrinopathy. Exp Eye Res. 2010;90(2):223–37.
21. Bron AJ, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, et al. TFOS DEWS II pathophysiology report. Ocul Surf. 2017;15(3):438–510.
22. Pflugfelder SC. Tear dysfunction and the cornea: LXVIII Edward Jackson Memorial Lecture. Am J Ophthalmol. 2011;152(6):900–9.e1.
23. Stern ME, Schaumburg CS, Dana R, Calonge M, Niederkorn JY, Pflugfelder SC. Autoimmunity at the ocular surface: pathogenesis and regulation. Mucosal Immunol. 2010;3(5):425–42.
24. Sullivan DA, Dana R, Sullivan RM, Krenzer KL, Sahin A, Arica B, et al. Meibomian gland dysfunction in primary and secondary Sjögren syndrome. Ophthalmic Res. 2018;59(4):193–205.
25. Gudjonsson T, Rønnov-Jessen L, Villadsen R, Rank F, Bissell MJ, Petersen OW. Normal and tumor-derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. J Cell Sci. 2002;115(Pt 1):39–50.
26. Hawley D, Tang X, Zyrianova T, Shah M, Janga S, Letourneau A, et al. Myoepithelial cell-driven acini contraction in response to oxytocin receptor stimulation is impaired in lacrimal glands of Sjögren's syndrome animal models. Sci Rep. 2018;8(1):9919.
27. Jin JO, Yu Q. T cell-associated cytokines in the pathogenesis of Sjögren's syndrome. J Clin Cell Immunol. 2013;(9).
28. Furuzawa-Carballeda J, Sanchez-Guerrero J, Betanzos JL, Enriquez AB, Avila-Casado C, Llorente L, et al. Differential cytokine expression and regulatory cells in patients with primary and secondary Sjogren's syndrome. Scand J Immunol. 2014;80(6):432–40.

29. Mitsias DI, Tzioufas AG, Veiopoulou C, Zintzaras E, Tassios IK, Kogopoulou O, et al. The Th1/Th2 cytokine balance changes with the progress of the immunopathological lesion of Sjogren's syndrome. Clin Exp Immunol. 2002;128(3):562–8.
30. Moriyama M, Hayashida JN, Toyoshima T, Ohyama Y, Shinozaki S, Tanaka A, et al. Cytokine/chemokine profiles contribute to understanding the pathogenesis and diagnosis of primary Sjögren's syndrome. Clin Exp Immunol. 2012;169(1):17–26.
31. Pertovaara M, Antonen J, Hurme M. Th2 cytokine genotypes are associated with a milder form of primary Sjogren's syndrome. Ann Rheum Dis. 2006;65(5):666–70.
32. van Woerkom JM, Kruize AA, Wenting-van Wijk MJ, Knol E, Bihari IC, Jacobs JW, et al. Salivary gland and peripheral blood T helper 1 and 2 cell activity in Sjögren's syndrome compared with non-Sjögren's sicca syndrome. Ann Rheum Dis. 2005;64(10):1474–9.
33. Chizzolini C, Dufour AM, Brembilla NC. Is there a role for IL-17 in the pathogenesis of systemic sclerosis? Immunol Lett. 2018;195:61–7.
34. Lee BH, Tudares MA, Nguyen CQ. Sjögren's syndrome: an old tale with a new twist. Arch Immunol Ther Exp (Warsz). 2009;57(1):57–66.
35. Sakai A, Sugawara Y, Kuroishi T, Sasano T, Sugawara S. Identification of IL-18 and Th17 cells in salivary glands of patients with Sjögren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. J Immunol. 2008;181(4):2898–906.
36. Nguyen CQ, Hu MH, Li Y, Stewart C, Peck AB. Salivary gland tissue expression of interleukin-23 and interleukin-17 in Sjögren's syndrome: findings in humans and mice. Arthritis Rheum. 2008;58(3):734–43.
37. Lee SY, Han SJ, Nam SM, Yoon SC, Ahn JM, Kim TI, et al. Analysis of tear cytokines and clinical correlations in Sjögren syndrome dry eye patients and non-Sjögren syndrome dry eye patients. Am J Ophthalmol. 2013;156(2):247–53.e1.
38. Zhang LW, Zhou PR, Wei P, Cong X, Wu LL, Hua H. Expression of interleukin-17 in primary Sjögren's syndrome and the correlation with disease severity: a systematic review and meta-analysis. Scand J Immunol. 2018;87(4):e12649.
39. Liu R, Gao C, Chen H, Li Y, Jin Y, Qi H. Analysis of Th17-associated cytokines and clinical correlations in patients with dry eye disease. PLoS One. 2017;12(4):e0173301.
40. Boehm N, Riechardt AI, Wiegand M, Pfeiffer N, Grus FH. Proinflammatory cytokine profiling of tears from dry eye patients by means of antibody microarrays. Invest Ophthalmol Vis Sci. 2011;52(10):7725–30.
41. Howard Tripp N, Tarn J, Natasari A, Gillespie C, Mitchell S, Hackett KL, et al. Fatigue in primary Sjögren's syndrome is associated with lower levels of proinflammatory cytokines. RMD Open. 2016;2(2):e000282.
42. Kang EH, Lee YJ, Hyon JY, Yun PY, Song YW. Salivary cytokine profiles in primary Sjögren's syndrome differ from those in non-Sjögren sicca in terms of TNF-α levels and Th-1/Th-2 ratios. Clin Exp Rheumatol. 2011;29(6):970–6.
43. Pauley KM, Gauna AE, Grichtchenko II, Chan EK, Cha S. A secretagogue-small interfering RNA conjugate confers resistance to cytotoxicity in a cell model of Sjögren's syndrome. Arthritis Rheum. 2011;63(10):3116–25.
44. Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjogren's syndrome. J Immunol. 1994;152(11):5532–9.
45. Mackay F, Schneider P, Rennert P, Browning J. BAFF AND APRIL: a tutorial on B cell survival. Annu Rev Immunol. 2003;21:231–64.
46. Varin MM, Le Pottier L, Youinou P, Saulep D, Mackay F, Pers JO. B-cell tolerance breakdown in Sjögren's syndrome: focus on BAFF. Autoimmun Rev. 2010;9(9):604–8.
47. Corrales RM, de Paiva CS, Li DQ, Farley WJ, Henriksson JT, Bergmanson JP, et al. Entrapment of conjunctival goblet cells by desiccation-induced cornification. Invest Ophthalmol Vis Sci. 2011;52(6):3492–9.
48. Pflugfelder SC, De Paiva CS, Moore QL, Volpe EA, Li DQ, Gumus K, et al. Aqueous tear deficiency increases conjunctival interferon-γ (IFN-γ) expression and goblet cell loss. Invest Ophthalmol Vis Sci. 2015;56(12):7545–50.
49. Sanz I, Lee FE. B cells as therapeutic targets in SLE. Nat Rev Rheumatol. 2010;6(6):326–37.
50. Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzová D, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. Arthritis Rheum. 2011;63(12):3918–30.
51. Manzi S, Sánchez-Guerrero J, Merrill JT, Furie R, Gladman D, Navarra SV, et al. Effects of belimumab, a B lymphocyte stimulator-specific inhibitor, on disease activity across multiple organ domains in patients with systemic lupus erythematosus: combined results from two phase III trials. Ann Rheum Dis. 2012;71(11):1833–8.
52. Navarra SV, Guzmán RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. Lancet. 2011;377(9767):721–31.
53. Mariette X, Seror R, Quartuccio L, Baron G, Salvin S, Fabris M, et al. Efficacy and safety of belimumab in primary Sjögren's syndrome: results of the BELISS open-label phase II study. Ann Rheum Dis. 2015;74(3):526–31.

54. Seror R, Nocturne G, Lazure T, Hendel-Chavez H, Desmoulins F, Belkhir R, et al. Low numbers of blood and salivary natural killer cells are associated with a better response to belimumab in primary Sjögren's syndrome: results of the BELISS study. Arthritis Res Ther. 2015;17:241.
55. Kono H, Kimura Y, Latz E. Inflammasome activation in response to dead cells and their metabolites. Curr Opin Immunol. 2014;30:91–8.
56. Horvath GL, Schrum JE, De Nardo CM, Latz E. Intracellular sensing of microbes and danger signals by the inflammasomes. Immunol Rev. 2011;243(1):119–35.
57. Niu L, Zhang S, Wu J, Chen L, Wang Y. Upregulation of NLRP3 inflammasome in the tears and ocular surface of dry eye patients. PLoS One. 2015;10(5):e0126277.
58. Chen YT, Nikulina K, Lazarev S, Bahrami AF, Noble LB, Gallup M, et al. Interleukin-1 as a phenotypic immunomodulator in keratinizing squamous metaplasia of the ocular surface in Sjögren's syndrome. Am J Pathol. 2010;177(3):1333–43.
59. Theoharides TC, Petra AI, Taracanova A, Panagiotidou S, Conti P. Targeting IL-33 in autoimmunity and inflammation. J Pharmacol Exp Ther. 2015;354(1):24–31.
60. Roescher N, Tak PP, Illei GG. Cytokines in Sjögren's syndrome. Oral Dis. 2009;15(8):519–26.
61. Nakamoto T, Brown DA, Catalán MA, Gonzalez-Begne M, Romanenko VG, Melvin JE. Purinergic P2X7 receptors mediate ATP-induced saliva secretion by the mouse submandibular gland. J Biol Chem. 2009;284(8):4815–22.
62. Cai FZ, Lester S, Lu T, Keen H, Boundy K, Proudman SM, et al. Mild autonomic dysfunction in primary Sjögren's syndrome: a controlled study. Arthritis Res Ther. 2008;10(2):R31.
63. Ahn JS, Camden JM, Schrader AM, Redman RS, Turner JT. Reversible regulation of P2Y(2) nucleotide receptor expression in the duct-ligated rat submandibular gland. Am J Physiol Cell Physiol. 2000;279(2):C286–94.
64. Schrader AM, Camden JM, Weisman GA. P2Y2 nucleotide receptor up-regulation in submandibular gland cells from the NOD.B10 mouse model of Sjögren's syndrome. Arch Oral Biol. 2005;50(6):533–40.
65. Woods LT, Camden JM, Khalafalla MG, Petris MJ, Erb L, Ambrus JL, et al. P2Y. Oral Dis. 2018;24(5):761–71.
66. Vignali DA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. Nat Immunol. 2012;13(8):722–8.
67. Manoussakis MN, Boiu S, Korkolopoulou P, Kapsogeorgou EK, Kavantzas N, Ziakas P, et al. Rates of infiltration by macrophages and dendritic cells and expression of interleukin-18 and interleukin-12 in the chronic inflammatory lesions of Sjogren's syndrome: correlation with certain features of immune hyperactivity and factors associated with high risk of lymphoma development. Arthritis Rheum. 2007;56(12):3977–88.
68. Szodoray P, Alex P, Brun JG, Centola M, Jonsson R. Circulating cytokines in primary Sjögren's syndrome determined by a multiplex cytokine array system. Scand J Immunol. 2004;59(6):592–9.
69. Gao J, Killedar S, Cornelius JG, Nguyen C, Cha S, Peck AB. Sjögren's syndrome in the NOD mouse model is an interleukin-4 time-dependent, antibody isotype-specific autoimmune disease. J Autoimmun. 2006;26(2):90–103.
70. Vosters JL, Landek-Salgado MA, Yin H, Swaim WD, Kimura H, Tak PP, et al. Interleukin-12 induces salivary gland dysfunction in transgenic mice, providing a new model of Sjögren's syndrome. Arthritis Rheum. 2009;60(12):3633–41.
71. Wildenberg ME, van Helden-Meeuwsen CG, van de Merwe JP, Drexhage HA, Versnel MA. Systemic increase in type I interferon activity in Sjögren's syndrome: a putative role for plasmacytoid dendritic cells. Eur J Immunol. 2008;38(7):2024–33.
72. Emamian ES, Leon JM, Lessard CJ, Grandits M, Baechler EC, Gaffney PM, et al. Peripheral blood gene expression profiling in Sjögren's syndrome. Genes Immun. 2009;10(4):285–96.
73. Imgenberg-Kreuz J, Sandling JK, Almlöf JC, Nordlund J, Signér L, Norheim KB, et al. Genome-wide DNA methylation analysis in multiple tissues in primary Sjögren's syndrome reveals regulatory effects at interferon-induced genes. Ann Rheum Dis. 2016;75(11):2029–36.
74. Brkic Z, Maria NI, van Helden-Meeuwsen CG, van de Merwe JP, van Daele PL, Dalm VA, et al. Prevalence of interferon type I signature in CD14 monocytes of patients with Sjogren's syndrome and association with disease activity and BAFF gene expression. Ann Rheum Dis. 2013;72(5):728–35.
75. Hall JC, Casciola-Rosen L, Berger AE, Kapsogeorgou EK, Cheadle C, Tzioufas AG, et al. Precise probes of type II interferon activity define the origin of interferon signatures in target tissues in rheumatic diseases. Proc Natl Acad Sci U S A. 2012;109(43):17609–14.
76. Hall JC, Baer AN, Shah AA, Criswell LA, Shiboski CH, Rosen A, et al. Molecular subsetting of interferon pathways in Sjögren's syndrome. Arthritis Rheumatol. 2015;67(9):2437–46.
77. Kalunian KC, Merrill JT, Maciuca R, McBride JM, Townsend MJ, Wei X, et al. A phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-α) in patients with systemic lupus erythematosus (ROSE). Ann Rheum Dis. 2016;75(1):196–202.
78. Bulosan M, Pauley KM, Yo K, Chan EK, Katz J, Peck AB, et al. Inflammatory caspases are critical for enhanced cell death in the target tissue of Sjogren's syndrome before disease onset. Immunol Cell Biol. 2009;87(1):81–90.
79. Cha S, Brayer J, Gao J, Brown V, Killedar S, Yasunari U, et al. A dual role for interferon-gamma

in the pathogenesis of Sjogren's syndrome-like autoimmune exocrinopathy in the nonobese diabetic mouse. Scand J Immunol. 2004;60(6):552–65.
80. Chauhan SK, Jin Y, Goyal S, Lee HS, Fuchsluger TA, Lee HK, et al. A novel pro-lymphangiogenic function for Th17/IL-17. Blood. 2011;118(17):4630–4.
81. Reksten TR, Jonsson MV, Szyszko EA, Brun JG, Jonsson R, Brokstad KA. Cytokine and autoantibody profiling related to histopathological features in primary Sjogren's syndrome. Rheumatology (Oxford). 2009;48(9):1102–6.
82. Ohyama Y, Nakamura S, Matsuzaki G, Shinohara M, Hiroki A, Fujimura T, et al. Cytokine messenger RNA expression in the labial salivary glands of patients with Sjogren's syndrome. Arthritis Rheum. 1996;39(8):1376–84.
83. Brayer JB, Cha S, Nagashima H, Yasunari U, Lindberg A, Diggs S, et al. IL-4-dependent effector phase in autoimmune exocrinopathy as defined by the NOD.IL-4-gene knockout mouse model of Sjogren's syndrome. Scand J Immunol. 2001;54(1–2):133–40.
84. Nguyen CQ, Gao JH, Kim H, Saban DR, Cornelius JG, Peck AB. IL-4-STAT6 signal transduction-dependent induction of the clinical phase of Sjögren's syndrome-like disease of the nonobese diabetic mouse. J Immunol. 2007;179(1):382–90.
85. Wynn TA. IL-13 effector functions. Annu Rev Immunol. 2003;21:425–56.
86. Emson CL, Bell SE, Jones A, Wisden W, McKenzie AN. Interleukin (IL)-4-independent induction of immunoglobulin (Ig)E, and perturbation of T cell development in transgenic mice expressing IL-13. J Exp Med. 1998;188(2):399–404.
87. Villarreal GM, Alcocer-Varela J, Llorente L. Differential interleukin (IL)-10 and IL-13 gene expression in vivo in salivary glands and peripheral blood mononuclear cells from patients with primary Sjögren's syndrome. Immunol Lett. 1996;49(1–2):105–9.
88. Mahlios J, Zhuang Y. Contribution of IL-13 to early exocrinopathy in Id3−/− mice. Mol Immunol. 2011;49(1–2):227–33.
89. Sayed BA, Christy A, Quirion MR, Brown MA. The master switch: the role of mast cells in autoimmunity and tolerance. Annu Rev Immunol. 2008;26:705–39.
90. Lam H, Bleiden L, de Paiva CS, Farley W, Stern ME, Pflugfelder SC. Tear cytokine profiles in dysfunctional tear syndrome. Am J Ophthalmol. 2009;147(2):198–205.e1.
91. Quarta S, Vogl C, Constantin CE, Üçeyler N, Sommer C, Kress M. Genetic evidence for an essential role of neuronally expressed IL-6 signal transducer gp130 in the induction and maintenance of experimentally induced mechanical hypersensitivity in vivo and in vitro. Mol Pain. 2011;7:73.
92. Alunno A, Carubbi F, Bistoni O, Caterbi S, Bartoloni E, Mirabelli G, et al. T regulatory and T helper 17 cells in primary Sjögren's syndrome: facts and perspectives. Mediators Inflamm. 2015;2015:243723.
93. Onishi RM, Gaffen SL. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. Immunology. 2010;129(3):311–21.
94. Nguyen CQ, Yin H, Lee BH, Carcamo WC, Chiorini JA, Peck AB. Pathogenic effect of interleukin-17A in induction of Sjögren's syndrome-like disease using adenovirus-mediated gene transfer. Arthritis Res Ther. 2010;12(6):R220.
95. Tishler M, Yaron I, Geyer O, Shirazi I, Naftaliev E, Yaron M. Elevated tear interleukin-6 levels in patients with Sjögren syndrome. Ophthalmology. 1998;105(12):2327–9.
96. Maier-Moore JS, Horton CG, Mathews SA, Confer AW, Lawrence C, Pan Z, et al. Interleukin-6 deficiency corrects nephritis, lymphocyte abnormalities, and secondary Sjögren's syndrome features in lupus-prone Sle1.Yaa mice. Arthritis Rheumatol. 2014;66(9):2521–31.
97. Akpek EK, Mathews P, Hahn S, Hessen M, Kim J, Grader-Beck T, et al. Ocular and systemic morbidity in a longitudinal cohort of Sjögren's syndrome. Ophthalmology. 2015;122(1):56–61.
98. Yoshimi R, Ueda A, Ozato K, Ishigatsubo Y. Clinical and pathological roles of Ro/SSA autoantibody system. Clin Dev Immunol. 2012;2012:606195.
99. Oke V, Wahren-Herlenius M. The immunobiology of Ro52 (TRIM21) in autoimmunity: a critical review. J Autoimmun. 2012;39(1–2):77–82.
100. Yang K, Shi HX, Liu XY, Shan YF, Wei B, Chen S, et al. TRIM21 is essential to sustain IFN regulatory factor 3 activation during antiviral response. J Immunol. 2009;182(6):3782–92.
101. Wolin SL, Cedervall T. The La protein. Annu Rev Biochem. 2002;71:375–403.
102. Routsias JG, Tzioufas AG. Sjögren's syndrome—study of autoantigens and autoantibodies. Clin Rev Allergy Immunol. 2007;32(3):238–51.
103. Karnell JL, Mahmoud TI, Herbst R, Ettinger R. Discerning the kinetics of autoimmune manifestations in a model of Sjogren's syndrome. Mol Immunol. 2014;62(2):277–82.
104. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European league against rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. Ann Rheum Dis. 2017;76(1):9–16.
105. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European consensus group. Ann Rheum Dis. 2002;61(6):554–8.
106. Mavragani CP, Tzioufas AG, Moutsopoulos HM. Sjögren's syndrome: autoantibodies to cellular antigens. Clinical and molecular aspects. Int Arch Allergy Immunol. 2000;123(1):46–57.
107. Tzioufas AG, Wassmuth R, Dafni UG, Guialis A, Haga HJ, Isenberg DA, et al. Clinical, immuno-

logical, and immunogenetic aspects of autoantibody production against Ro/SSA, La/SSB and their linear epitopes in primary Sjögren's syndrome (pSS): a European multicentre study. Ann Rheum Dis. 2002;61(5):398–404.
108. Ramos-Casals M, Solans R, Rosas J, Camps MT, Gil A, Del Pino-Montes J, et al. Primary Sjögren syndrome in Spain: clinical and immunologic expression in 1010 patients. Medicine (Baltimore). 2008;87(4):210–9.
109. Seror R, Sordet C, Guillevin L, Hachulla E, Masson C, Ittah M, et al. Tolerance and efficacy of rituximab and changes in serum B cell biomarkers in patients with systemic complications of primary Sjögren's syndrome. Ann Rheum Dis. 2007;66(3):351–7.
110. Meijer JM, Meiners PM, Vissink A, Spijkervet FK, Abdulahad W, Kamminga N, et al. Effectiveness of rituximab treatment in primary Sjogren's syndrome: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 2010;62(4):960–8.
111. Rehman HU. Antinuclear antibodies: when to test and how to interpret findings. J Fam Pract. 2015;64(1):E5–8.
112. Hira-Kazal R, Shea-Simonds P, Peacock JL, Maher J. How should a district general hospital immunology service screen for anti-nuclear antibodies? An 'in-the-field' audit. Clin Exp Immunol. 2015;180(1):52–7.
113. Martel C, Gondran G, Launay D, Lalloué F, Palat S, Lambert M, et al. Active immunological profile is associated with systemic Sjögren's syndrome. J Clin Immunol. 2011;31(5):840–7.
114. Fox RI, Stern M, Michelson P. Update in Sjögren syndrome. Curr Opin Rheumatol. 2000;12(5):391–8.
115. Nardi N, Brito-Zerón P, Ramos-Casals M, Aguiló S, Cervera R, Ingelmo M, et al. Circulating autoantibodies against nuclear and non-nuclear antigens in primary Sjögren's syndrome: prevalence and clinical significance in 335 patients. Clin Rheumatol. 2006;25(3):341–6.
116. Maślińska, M., Mańczak, M. & Kwiatkowska, B. Usefulness of rheumatoid factor as an immunological and prognostic marker in PSS patients. Clin Rheumatol. 2019;38:1301–1307.
117. Peen E, Mellbye OJ, Haga HJ. IgA rheumatoid factor in primary Sjogren's syndrome. Scand J Rheumatol. 2009;38(1):46–9.
118. Verkman AS, Mitra AK. Structure and function of aquaporin water channels. Am J Physiol Renal Physiol. 2000;278(1):F13–28.
119. Aure MH, Ruus AK, Galtung HK. Aquaporins in the adult mouse submandibular and sublingual salivary glands. J Mol Histol. 2014;45(1):69–80.
120. Tsubota K, Hirai S, King LS, Agre P, Ishida N. Defective cellular trafficking of lacrimal gland aquaporin-5 in Sjogren's syndrome. Lancet. 2001;357(9257):688–9.
121. Delporte C, Steinfeld S. Distribution and roles of aquaporins in salivary glands. Biochim Biophys Acta. 2006;1758(8):1061–70.
122. Sasaki Y, Tsubota K, Kawedia JD, Menon AG, Yasui M. The difference of aquaporin 5 distribution in acinar and ductal cells in lacrimal and parotid glands. Curr Eye Res. 2007;32(11):923–9.
123. Alam J, Koh JH, Kim N, Kwok SK, Park SH, Song YW, et al. Detection of autoantibodies against aquaporin-5 in the sera of patients with primary Sjögren's syndrome. Immunol Res. 2016;64(4):848–56.
124. Alam J, Choi YS, Koh JH, Kwok SK, Park SH, Song YW, et al. Detection of autoantibodies against Aquaporin-1 in the sera of patients with primary Sjögren's syndrome. Immune Netw. 2017;17(2):103–9.
125. Tzartos JS, Stergiou C, Daoussis D, Zisimopoulou P, Andonopoulos AP, Zolota V, et al. Antibodies to aquaporins are frequent in patients with primary Sjögren's syndrome. Rheumatology (Oxford). 2017;56(12):2114–22.
126. Alam J, Koh JH, Kwok SK, Park SH, Park K, Choi Y. Functional epitopes for anti-aquaporin 5 antibodies in Sjögren syndrome. J Dent Res. 2017;96(12):1414–21.
127. Takata K, Matsuzaki T, Tajika Y. Aquaporins: water channel proteins of the cell membrane. Prog Histochem Cytochem. 2004;39(1):1–83.
128. Suresh L, Malyavantham K, Shen L, Ambrus JL Jr. Investigation of novel autoantibodies in Sjogren's syndrome utilizing Sera from the Sjogren's international collaborative clinical alliance cohort. BMC Ophthalmol. 2015;15:38.
129. Vishwanath S, Everett S, Shen L, Malyavantham K, Suresh L, Ambrus JL. Xerophthalmia of Sjogren's syndrome diagnosed with anti-salivary gland protein 1 antibodies. Case Rep Ophthalmol. 2014;5(2):186–9.
130. Beckman KA. Detection of early markers for Sjögren syndrome in dry eye patients. Cornea. 2014;33(12):1262–4.
131. Parkkila S, Kaunisto K, Rajaniemi L, Kumpulainen T, Jokinen K, Rajaniemi H. Immunohistochemical localization of carbonic anhydrase isoenzymes VI, II, and I in human parotid and submandibular glands. J Histochem Cytochem. 1990;38(7):941–7.
132. Karakus S, Baer AN, Agrawal D, Gurakar M, Massof RW, Akpek EK. Utility of novel autoantibodies in the diagnosis of Sjögren's syndrome among patients with dry eye. Cornea. 2018;37(4):405–11.
133. Khovidhunkit W, Hachem JP, Medzihradszky KF, Duchateau PN, Shigenaga JK, Moser AH, et al. Parotid secretory protein is an HDL-associated protein with anticandidal activity. Am J Physiol Regul Integr Comp Physiol. 2005;288(5):R1306–15.
134. Dickinson DP, Abel K, Near J, Taylor BA, Gross KW. Genetic and tissue-specific variation in the expression of a closely linked murine multigene family on chromosome 15 that encodes salivary and lacrimal proteins. Biochem Genet. 1989;27(9–10):613–37.
135. Matossian C, Micucci J. Characterization of the serological biomarkers associated with Sjögren's

syndrome in patients with recalcitrant dry eye disease. Clin Ophthalmol. 2016;10:1329–34.
136. Shen L, Suresh L, Lindemann M, Xuan J, Kowal P, Malyavantham K, et al. Novel autoantibodies in Sjogren's syndrome. Clin Immunol. 2012;145(3):251–5.
137. Shen L, Kapsogeorgou EK, Yu M, Suresh L, Malyavantham K, Tzioufas AG, et al. Evaluation of salivary gland protein 1 antibodies in patients with primary and secondary Sjogren's syndrome. Clin Immunol. 2014;155(1):42–6.
138. Eglen RM. Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. Auton Autacoid Pharmacol. 2006;26(3):219–33.
139. Dawson L, Tobin A, Smith P, Gordon T. Antimuscarinic antibodies in Sjögren's syndrome: where are we, and where are we going? Arthritis Rheum. 2005;52(10):2984–95.
140. Bacman S, Perez Leiros C, Sterin-Borda L, Hubscher O, Arana R, Borda E. Autoantibodies against lacrimal gland M3 muscarinic acetylcholine receptors in patients with primary Sjögren's syndrome. Invest Ophthalmol Vis Sci. 1998;39(1):151–6.
141. Gao J, Cha S, Jonsson R, Opalko J, Peck AB. Detection of anti-type 3 muscarinic acetylcholine receptor autoantibodies in the sera of Sjogren's syndrome patients by use of a transfected cell line assay. Arthritis Rheum. 2004;50(8):2615–21.
142. Li J, Ha YM, Kü NY, Choi SY, Lee SJ, Oh SB, et al. Inhibitory effects of autoantibodies on the muscarinic receptors in Sjögren's syndrome. Lab Invest. 2004;84(11):1430–8.
143. Kovacs L, Marczinovits I, Gyorgy A, Toth GK, Dorgai L, Pal J, et al. Clinical associations of autoantibodies to human muscarinic acetylcholine receptor 3(213-228) in primary Sjogren's syndrome. Rheumatology (Oxford). 2005;44(8):1021–5.
144. Kim N, Shin Y, Choi S, Namkoong E, Kim M, Lee J, et al. Effect of Antimuscarinic autoantibodies in primary Sjögren's syndrome. J Dent Res. 2015;94(5):722–8.
145. Bacman S, Berra A, Sterin-Borda L, Borda E. Muscarinic acetylcholine receptor antibodies as a new marker of dry eye Sjogren syndrome. Invest Ophthalmol Vis Sci. 2001;42(2):321–7.
146. Dawson LJ, Allison HE, Stanbury J, Fitzgerald D, Smith PM. Putative anti-muscarinic antibodies cannot be detected in patients with primary Sjogren's syndrome using conventional immunological approaches. Rheumatology (Oxford). 2004;43(12):1488–95. Epub 2004 Sep 7.
147. Tsuboi H, Matsuo N, Iizuka M, Nakamura Y, Matsumoto I, Sumida T. Analysis of epitopes and function of anti-M3 muscarinic acetylcholine receptor antibodies in patients with Sjögren's syndrome. Nihon Rinsho Meneki Gakkai Kaishi. 2010;33(4):222–8.
148. He J, Guo JP, Ding Y, Li YN, Pan SS, Liu Y, et al. Diagnostic significance of measuring antibodies to cyclic type 3 muscarinic acetylcholine receptor peptides in primary Sjogren's syndrome. Rheumatology (Oxford). 2011;50(5):879–84.
149. Deng C, Hu C, Chen S, Li J, Wen X, Wu Z, et al. Meta-analysis of anti-muscarinic receptor type 3 antibodies for the diagnosis of Sjögren syndrome. PLoS One. 2015;10(1):e0116744.
150. Zuo J, Williams AE, Park YJ, Choi K, Chan AL, Reeves WH, et al. Muscarinic type 3 receptor autoantibodies are associated with anti-SSA/Ro autoantibodies in Sjogren's syndrome. J Immunol Methods. 2016;437:28–36.
151. Yang L, Wang Y, Xing R, Bai L, Li C, Li Z, et al. Mimotope mimicking epidermal growth factor receptor alleviates mononuclear cell infiltration in exocrine glands induced by muscarinic acetylcholine 3 receptor. Clin Immunol. 2016;163:111–9.
152. Qian L, Wang Y, Xie J, Rose CM, Yang T, Nakamura T, et al. Biochemical changes contributing to functional quiescence in lacrimal gland acinar cells after chronic ex vivo exposure to a muscarinic agonist. Scand J Immunol. 2003;58(5):550–65.
153. Zoukhri D, Hodges RR, Byon D, Kublin CL. Role of proinflammatory cytokines in the impaired lacrimation associated with autoimmune xerophthalmia. Invest Ophthalmol Vis Sci. 2002;43(5):1429–36.
154. Cavill D, Waterman SA, Gordon TP. Antibodies raised against the second extracellular loop of the human muscarinic M3 receptor mimic functional autoantibodies in Sjogren's syndrome. Scand J Immunol. 2004;59(3):261–6.
155. Iizuka M, Wakamatsu E, Tsuboi H, Nakamura Y, Hayashi T, Matsui M, et al. Pathogenic role of immune response to M3 muscarinic acetylcholine receptor in Sjögren's syndrome-like sialoadenitis. J Autoimmun. 2010;35(4):383–9.
156. Waterman SA, Gordon TP, Rischmueller M. Inhibitory effects of muscarinic receptor autoantibodies on parasympathetic neurotransmission in Sjogren's syndrome. Arthritis Rheum. 2000;43(7):1647–54.
157. Park K, Haberberger RV, Gordon TP, Jackson MW. Antibodies interfering with the type 3 muscarinic receptor pathway inhibit gastrointestinal motility and cholinergic neurotransmission in Sjögren's syndrome. Arthritis Rheum. 2011;63(5):1426–34.
158. Sumida T, Tsuboi H, Iizuka M, Hirota T, Asashima H, Matsumoto I. The role of M3 muscarinic acetylcholine receptor reactive T cells in Sjögren's syndrome: a critical review. J Autoimmun. 2014;51:44–50.
159. Chen Y, Zheng J, Huang Q, Deng F, Huang R, Zhao W, et al. Autoantibodies against the second extracellular loop of M3R do neither induce nor indicate primary Sjögren's syndrome. PLoS One. 2016;11(2):e0149485.
160. Jin M, Hwang SM, Davies AJ, Shin Y, Bae JS, Lee JH, et al. Autoantibodies in primary Sjögren's syndrome patients induce internalization of muscarinic type 3 receptors. Biochim Biophys Acta. 2012;1822(2):161–7.

161. Lee BH, Gauna AE, Perez G, Park YJ, Pauley KM, Kawai T, et al. Autoantibodies against muscarinic type 3 receptor in Sjogren's syndrome inhibit aquaporin 5 trafficking. PLoS One. 2013;8(1):e53113.
162. Passafaro D, Reina S, Sterin-Borda L, Borda E. Cholinergic autoantibodies from primary Sjögren's syndrome modulate submandibular gland Na+/K+-ATPase activity via prostaglandin E2 and cyclic AMP. Eur J Oral Sci. 2010;118(2):131–8.
163. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215–33.
164. Tijsterman M, Plasterk RH. Dicers at RISC; the mechanism of RNAi. Cell. 2004;117(1):1–3.
165. Pauley KM, Stewart CM, Gauna AE, Dupre LC, Kuklani R, Chan AL, et al. Altered miR-146a expression in Sjogren's syndrome and its functional role in innate immunity. Eur J Immunol. 2011;41(7):2029–39.
166. Cha S, Mona M, Lee KE, Kim DH, Han K. MicroRNAs in autoimmune Sjogren's syndrome. Genomics Inform. 2018;16(4):e19.
167. Alevizos I, Alexander S, Turner RJ, Illei GG. MicroRNA expression profiles as biomarkers of minor salivary gland inflammation and dysfunction in Sjögren's syndrome. Arthritis Rheum. 2011;63(2):535–44.
168. Alevizos I, Illei GG. MicroRNAs in Sjögren's syndrome as a prototypic autoimmune disease. Autoimmun Rev. 2010;9(9):618–21.
169. Wang Y, Zhang G, Zhang L, Zhao M, Huang H. Decreased microRNA-181a and −16 expression levels in the labial salivary glands of Sjögren syndrome patients. Exp Ther Med. 2018;15(1):426–32.
170. Peng L, Ma W, Yi F, Yang YJ, Lin W, Chen H, et al. MicroRNA profiling in Chinese patients with primary Sjögren syndrome reveals elevated miRNA-181a in peripheral blood mononuclear cells. J Rheumatol. 2014;41(11):2208–13.
171. Kapsogeorgou EK, Gourzi VC, Manoussakis MN, Moutsopoulos HM, Tzioufas AG. Cellular microRNAs (miRNAs) and Sjogren's syndrome: candidate regulators of autoimmune response and autoantigen expression. J Autoimmun. 2011;37(2):129–35.
172. Le Dantec C, Varin MM, Brooks WH, Pers JO, Youinou P, Renaudineau Y. Epigenetics and Sjogren's syndrome. Curr Pharm Biotechnol. 2012;13(10):2046–53.
173. Zilahi E, Tarr T, Papp G, Griger Z, Sipka S, Zeher M. Increased microRNA-146a/b, TRAF6 gene and decreased IRAK1 gene expressions in the peripheral mononuclear cells of patients with Sjögren's syndrome. Immunol Lett. 2012;141(2):165–8.
174. Chen JQ, Papp G, Szodoray P, Zeher M. The role of microRNAs in the pathogenesis of autoimmune diseases. Autoimmun Rev. 2016;15(12):1171–80.
175. Shi H, Zheng LY, Zhang P, Yu CQ. miR-146a and miR-155 expression in PBMCs from patients with Sjogren's syndrome. J Oral Pathol Med. 2014;43(10):792–7.
176. Wang-Renault SF, Boudaoud S, Nocturne G, Roche E, Sigrist N, Daviaud C, et al. Deregulation of microRNA expression in purified T and B lymphocytes from patients with primary Sjogren's syndrome. Ann Rheum Dis. 2018;77(1):133–40.
177. Le Dantec C, Varin MM, Brooks WH, Pers JO, Youinou P, Renaudineau Y. Epigenetics and Sjögren's syndrome. Curr Pharm Biotechnol. 2012;13(10):2046–53.
178. Fox RI, Pearson G, Vaughan JH. Detection of Epstein-Barr virus-associated antigens and DNA in salivary gland biopsies from patients with Sjogren's syndrome. J Immunol. 1986;137(10):3162–8.
179. Mariette X, Gozlan J, Clerc D, Bisson M, Morinet F. Detection of Epstein-Barr virus DNA by in situ hybridization and polymerase chain reaction in salivary gland biopsy specimens from patients with Sjogren's syndrome. Am J Med. 1991;90(3):286–94.
180. Saito I, Servenius B, Compton T, Fox RI. Detection of Epstein-Barr virus DNA by polymerase chain reaction in blood and tissue biopsies from patients with Sjogren's syndrome. J Exp Med. 1989;169(6):2191–8.
181. Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, Lindenberg JL, et al. Functional delivery of viral miRNAs via exosomes. Proc Natl Acad Sci U S A. 2010;107(14):6328–33.
182. Gallo A, Jang SI, Ong HL, Perez P, Tandon M, Ambudkar I, et al. Targeting the ca(2+) sensor STIM1 by Exosomal transfer of Ebv-miR-BART13-3p is associated with Sjogren's syndrome. EBioMedicine. 2016;10:216–26.
183. Cheng KT, Alevizos I, Liu X, Swaim WD, Yin H, Feske S, et al. STIM1 and STIM2 protein deficiency in T lymphocytes underlies development of the exocrine gland autoimmune disease, Sjogren's syndrome. Proc Natl Acad Sci U S A. 2012;109(36):14544–9.
184. Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, et al. STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. Nature. 2005;437(7060):902–5.
185. Cheng KT, Liu X, Ong HL, Swaim W, Ambudkar IS. Local Ca^2+ entry via Orai1 regulates plasma membrane recruitment of TRPC1 and controls cytosolic Ca^2+ signals required for specific cell functions. PLoS Biol. 2011;9(3):e1001025.
186. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843–54.
187. Rassi DM, De Paiva CS, Dias LC, Módulo CM, Adriano L, Fantucci MZ, et al. Review: MicroRNAS in ocular surface and dry eye diseases. Ocul Surf. 2017;15(4):660–9.
188. Rassi DM, De Paiva CS, Dias LC, Modulo CM, Adriano L, Fantucci MZ, et al. Review: MicroRNAS in ocular surface and dry eye diseases. Ocul Surf. 2017;15(4):660–9.
189. Kim YJ, Yeon Y, Lee WJ, Shin YU, Cho H, Sung YK, et al. Comparison of MicroRNA expression in tears

of Normal subjects and Sjogren syndrome patients. Invest Ophthalmol Vis Sci. 2019;60(14):4889–95.
190. Chien KH, Chen SJ, Liu JH, Woung LC, Chen JT, Liang CM, et al. Correlation of microRNA-145 levels and clinical severity of pterygia. Ocul Surf. 2013;11(2):133–8.
191. Lee SK, Teng Y, Wong HK, Ng TK, Huang L, Lei P, et al. MicroRNA-145 regulates human corneal epithelial differentiation. PLoS One. 2011;6(6):e21249.
192. Alves M, Angerami RN, Rocha EM. Dry eye disease caused by viral infection: review. Arq Bras Oftalmol. 2013;76(2):129–32.
193. Liang C, Xiong K, Szulwach KE, Zhang Y, Wang Z, Peng J, et al. Sjogren syndrome antigen B (SSB)/La promotes global microRNA expression by binding microRNA precursors through stem-loop recognition. J Biol Chem. 2013;288(1):723–36.
194. Perricone C, De Carolis C, Perricone R. Glutathione: a key player in autoimmunity. Autoimmun Rev. 2009;8(8):697–701.
195. Raffaello A, Rizzuto R. Mitochondrial longevity pathways. Biochim Biophys Acta. 2011;1813(1):260–8.
196. Reina S, Rodríguez M, Stranieri G, Borda E. Action of anti-M3 muscarinic acetylcholine receptor IgG of primary Sjögren's syndrome on the enzymatic antioxidant system in rat submandibular gland. J Oral Pathol Med. 2015;44(10):876–83.
197. Wanchu A, Khullar M, Sud A, Bambery P. Elevated nitric oxide production in patients with primary Sjögren's syndrome. Clin Rheumatol. 2000;19(5):360–4.
198. Bohanec Grabar P, Logar D, Tomsic M, Rozman B, Dolzan V. Genetic polymorphisms modifying oxidative stress are associated with disease activity in rheumatoid arthritis patients. Dis Markers. 2009;26(1):41–8.
199. Caulfield VL, Balmer C, Dawson LJ, Smith PM. A role for nitric oxide-mediated glandular hypofunction in a non-apoptotic model for Sjogren's syndrome. Rheumatology (Oxford). 2009;48(7):727–33.
200. Ryo K, Yamada H, Nakagawa Y, Tai Y, Obara K, Inoue H, et al. Possible involvement of oxidative stress in salivary gland of patients with Sjogren's syndrome. Pathobiology. 2006;73(5):252–60.
201. Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. Immunol Today. 1994;15(1):7–10.
202. Norheim KB, Jonsson G, Harboe E, Hanasand M, Gøransson L, Omdal R. Oxidative stress, as measured by protein oxidation, is increased in primary Sjøgren's syndrome. Free Radic Res. 2012;46(2):141–6.
203. Kurimoto C, Kawano S, Tsuji G, Hatachi S, Jikimoto T, Sugiyama D, et al. Thioredoxin may exert a protective effect against tissue damage caused by oxidative stress in salivary glands of patients with Sjögren's syndrome. J Rheumatol. 2007;34(10):2035–43.
204. Pagano G, Castello G, Pallardó FV. Sjøgren's syndrome-associated oxidative stress and mitochondrial dysfunction: prospects for chemoprevention trials. Free Radic Res. 2013;47(2):71–3.
205. Augustin AJ, Spitznas M, Kaviani N, Meller D, Koch FH, Grus F, et al. Oxidative reactions in the tear fluid of patients suffering from dry eyes. Graefes Arch Clin Exp Ophthalmol. 1995;233(11):694–8.
206. Nakamura S, Shibuya M, Nakashima H, Hisamura R, Masuda N, Imagawa T, et al. Involvement of oxidative stress on corneal epithelial alterations in a blink-suppressed dry eye. Invest Ophthalmol Vis Sci. 2007;48(4):1552–8.
207. Wakamatsu TH, Dogru M, Matsumoto Y, Kojima T, Kaido M, Ibrahim OM, et al. Evaluation of lipid oxidative stress status in Sjögren syndrome patients. Invest Ophthalmol Vis Sci. 2013;54(1):201–10.
208. Uchino Y, Kawakita T, Miyazawa M, Ishii T, Onouchi H, Yasuda K, et al. Oxidative stress induced inflammation initiates functional decline of tear production. PLoS One. 2012;7(10):e45805.
209. Han X, Fan Z, Yu Y, Liu S, Hao Y, Huo R, et al. Expression and characterization of recombinant human phospholipid hydroperoxide glutathione peroxidase. IUBMB Life. 2013;65(11):951–6.
210. Augustin HG, Braun K, Telemenakis I, Modlich U, Kuhn W. Ovarian angiogenesis. Phenotypic characterization of endothelial cells in a physiological model of blood vessel growth and regression. Am J Pathol. 1995;147(2):339–51.
211. Macri A, Scanarotti C, Bassi AM, Giuffrida S, Sangalli G, Traverso CE, et al. Evaluation of oxidative stress levels in the conjunctival epithelium of patients with or without dry eye, and dry eye patients treated with preservative-free hyaluronic acid 0.15% and vitamin B12 eye drops. Graefes Arch Clin Exp Ophthalmol. 2015;253(3):425–30.
212. Cejková J, Ardan T, Simonová Z, Cejka C, Malec J, Dotrelová D, et al. Decreased expression of antioxidant enzymes in the conjunctival epithelium of dry eye (Sjögren's syndrome) and its possible contribution to the development of ocular surface oxidative injuries. Histol Histopathol. 2008;23(12):1477–83.
213. Beauregard C, Brandt PC, Chiou GC. Induction of nitric oxide synthase and over-production of nitric oxide by interleukin-1beta in cultured lacrimal gland acinar cells. Exp Eye Res. 2003;77(1):109–14.
214. Melvin JE, Yule D, Shuttleworth T, Begenisich T. Regulation of fluid and electrolyte secretion in salivary gland acinar cells. Annu Rev Physiol. 2005;67:445–69.
215. Ambudkar IS. Polarization of calcium signaling and fluid secretion in salivary gland cells. Curr Med Chem. 2012;19(34):5774–81.
216. Ambudkar I. Calcium signaling defects underlying salivary gland dysfunction. Biochim Biophys Acta Mol Cell Res. 2018;1865(11 Pt B):1771–7.
217. Mikoshiba K, Hisatsune C, Futatsugi A, Mizutani A, Nakamura T, Miyachi K. The role of Ca2+ signaling in cell function with special reference to exocrine secretion. Cornea. 2008;27(Suppl 1):S3–8.

218. Berridge MJ. Calcium signalling remodelling and disease. Biochem Soc Trans. 2012;40(2):297–309.
219. Ambudkar IS. Ca^{2+} signaling and regulation of fluid secretion in salivary gland acinar cells. Cell Calcium. 2014;55(6):297–305.
220. Ambudkar IS. Regulation of calcium in salivary gland secretion. Crit Rev Oral Biol Med. 2000;11(1):4–25.
221. Nguyen HV, Stuart-Tilley A, Alper SL, Melvin JE. Cl(−)/HCO(3)(−) exchange is acetazolamide sensitive and activated by a muscarinic receptor-induced [ca(2+)](i) increase in salivary acinar cells. Am J Physiol Gastrointest Liver Physiol. 2004;286(2):G312–20.
222. Ishikawa Y, Eguchi T, Skowronski MT, Ishida H. Acetylcholine acts on M3 muscarinic receptors and induces the translocation of aquaporin5 water channel via cytosolic Ca2+ elevation in rat parotid glands. Biochem Biophys Res Commun. 1998;245(3):835–40.
223. Mikoshiba K. IP3 receptor/Ca2+ channel: from discovery to new signaling concepts. J Neurochem. 2007;102(5):1426–46.
224. Putney JW. Capacitative calcium entry revisited. Cell Calcium. 1990;11(10):611–24.
225. Cheng KT, Ong HL, Liu X, Ambudkar IS. Contribution and regulation of TRPC channels in store-operated Ca2+ entry. Curr Top Membr. 2013;71:149–79.
226. Prakriya M. Store-operated Orai channels: structure and function. Curr Top Membr. 2013;71:1–32.
227. Zhou Y, Srinivasan P, Razavi S, Seymour S, Meraner P, Gudlur A, et al. Initial activation of STIM1, the regulator of store-operated calcium entry. Nat Struct Mol Biol. 2013;20(8):973–81.
228. Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. Nature. 2008;454(7203):538–42.
229. Xing J, Petranka JG, Davis FM, Desai PN, Putney JW, Bird GS. Role of Orai1 and store-operated calcium entry in mouse lacrimal gland signalling and function. J Physiol. 2014;592(5):927–39.
230. Stathopulos PB, Ikura M. Structural aspects of calcium-release activated calcium channel function. Channels (Austin). 2013;7(5):344–53.
231. Wang X, Wang Y, Zhou Y, Hendron E, Mancarella S, Andrake MD, et al. Distinct Orai-coupling domains in STIM1 and STIM2 define the Orai-activating site. Nat Commun. 2014;5:3183.
232. Ong HL, Ambudkar IS. Molecular determinants of TRPC1 regulation within ER-PM junctions. Cell Calcium. 2015;58(4):376–86.
233. Ong HL, de Souza LB, Zheng C, Cheng KT, Liu X, Goldsmith CM, et al. STIM2 enhances receptor-stimulated Ca^{2+} signaling by promoting recruitment of STIM1 to the endoplasmic reticulum-plasma membrane junctions. Sci Signal. 2015;8(359):ra3.
234. Kar P, Bakowski D, Di Capite J, Nelson C, Parekh AB. Different agonists recruit different stromal interaction molecule proteins to support cytoplasmic Ca2+ oscillations and gene expression. Proc Natl Acad Sci U S A. 2012;109(18):6969–74.
235. Bird GS, Hwang SY, Smyth JT, Fukushima M, Boyles RR, Putney JW. STIM1 is a calcium sensor specialized for digital signaling. Curr Biol. 2009;19(20):1724–9.
236. Liu X, Cheng KT, Bandyopadhyay BC, Pani B, Dietrich A, Paria BC, et al. Attenuation of store-operated Ca2+ current impairs salivary gland fluid secretion in TRPC1(−/−) mice. Proc Natl Acad Sci U S A. 2007;104(44):17542–7.
237. Hong JH, Li Q, Kim MS, Shin DM, Feske S, Birnbaumer L, et al. Polarized but differential localization and recruitment of STIM1, Orai1 and TRPC channels in secretory cells. Traffic. 2011;12(2):232–45.
238. Parekh AB, Putney JW. Store-operated calcium channels. Physiol Rev. 2005;85(2):757–810.
239. Ong HL, de Souza LB, Cheng KT, Ambudkar IS. Physiological functions and regulation of TRPC channels. Handb Exp Pharmacol. 2014;223:1005–34.
240. Ong HL, Cheng KT, Liu X, Bandyopadhyay BC, Paria BC, Soboloff J, et al. Dynamic assembly of TRPC1-STIM1-Orai1 ternary complex is involved in store-operated calcium influx. Evidence for similarities in store-operated and calcium release-activated calcium channel components. J Biol Chem. 2007;282(12):9105–16.
241. Teos LY, Zhang Y, Cotrim AP, Swaim W, Won JH, Ambrus J, et al. IP3R deficit underlies loss of salivary fluid secretion in Sjögren's syndrome. Sci Rep. 2015;5:13953.
242. Zeng M, Szymczak M, Ahuja M, Zheng C, Yin H, Swaim W, et al. Restoration of CFTR activity in ducts rescues acinar cell function and reduces inflammation in pancreatic and salivary glands of mice. Gastroenterology. 2017;153(4):1148–59.
243. Inaba T, Hisatsune C, Sasaki Y, Ogawa Y, Ebisui E, Ogawa N, et al. Mice lacking inositol 1,4,5-trisphosphate receptors exhibit dry eye. PLoS One. 2014;9(6):e99205.
244. Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, et al. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. Nat Immunol. 2008;9(4):432–43.
245. Feske S, Picard C, Fischer A. Immunodeficiency due to mutations in ORAI1 and STIM1. Clin Immunol. 2010;135(2):169–82.
246. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature. 2006;441(7090):179–85.
247. Mavragani CP, Fragoulis GE, Moutsopoulos HM. Endocrine alterations in primary Sjogren's syndrome: an overview. J Autoimmun. 2012;39(4):354–8.

248. Kow LM, Pfaff DW. Rapid estrogen actions on ion channels: a survey in search for mechanisms. Steroids. 2016;111:46–53.
249. Lizneva D, Gavrilova-Jordan L, Walker W, Azziz R. Androgen excess: investigations and management. Best Pract Res Clin Obstet Gynaecol. 2016;37:98–118.
250. Rubtsov AV, Rubtsova K, Kappler JW, Marrack P. Genetic and hormonal factors in female-biased autoimmunity. Autoimmun Rev. 2010;9(7):494–8.
251. Beeson PB. Age and sex associations of 40 autoimmune diseases. Am J Med. 1994;96(5):457–62.
252. Mostafa S, Seamon V, Azzarolo AM. Influence of sex hormones and genetic predisposition in Sjogren's syndrome: a new clue to the immunopathogenesis of dry eye disease. Exp Eye Res. 2012;96(1):88–97.
253. Tellefsen S, Morthen MK, Richards SM, Lieberman SM, Rahimi Darabad R, Kam WR, et al. Sex effects on gene expression in lacrimal glands of mouse models of Sjogren syndrome. Invest Ophthalmol Vis Sci. 2018;59(13):5599–614.
254. Ishimaru N, Arakaki R, Watanabe M, Kobayashi M, Miyazaki K, Hayashi Y. Development of autoimmune exocrinopathy resembling Sjogren's syndrome in estrogen-deficient mice of healthy background. Am J Pathol. 2003;163(4):1481–90.
255. Forsblad-d'Elia H, Carlsten H, Labrie F, Konttinen YT, Ohlsson C. Low serum levels of sex steroids are associated with disease characteristics in primary Sjogren's syndrome; supplementation with dehydroepiandrosterone restores the concentrations. J Clin Endocrinol Metab. 2009;94(6):2044–51.
256. Sullivan DA, Rocha EM, Aragona P, Clayton JA, Ding J, Golebiowski B, et al. TFOS DEWS II sex, gender, and hormones report. Ocul Surf. 2017;15(3):284–333.
257. Brandt JE, Priori R, Valesini G, Fairweather D. Sex differences in Sjogren's syndrome: a comprehensive review of immune mechanisms. Biol Sex Differ. 2015;6:19.
258. Vendramini AC, Soo C, Sullivan DA. Testosterone-induced suppression of autoimmune disease in lacrimal tissue of a mouse model (NZB/NZW F1) of Sjogren's syndrome. Invest Ophthalmol Vis Sci. 1991;32(11):3002–6.
259. Sato EH, Ariga H, Sullivan DA. Impact of androgen therapy in Sjogren's syndrome: hormonal influence on lymphocyte populations and Ia expression in lacrimal glands of MRL/Mp-lpr/lpr mice. Invest Ophthalmol Vis Sci. 1992;33(8):2537–45.
260. Sullivan DA, Edwards JA. Androgen stimulation of lacrimal gland function in mouse models of Sjogren's syndrome. J Steroid Biochem Mol Biol. 1997;60(3–4):237–45.
261. Rocha FJ, Sato EH, Sullivan BD, Sullivan DA. Comparative efficacy of androgen analogues in suppressing lacrimal gland inflammation in a mouse model (MRL/lpr) of Sjogren's syndrome. Adv Exp Med Biol. 1994;350:697–700.
262. Morthen MK, Tellefsen S, Richards SM, Lieberman SM, Rahimi Darabad R, Kam WR, et al. Testosterone influence on gene expression in lacrimal glands of mouse models of Sjogren syndrome. Invest Ophthalmol Vis Sci. 2019;60(6):2181–97.
263. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, Craig JP, McCulley JP, Den S, et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. Invest Ophthalmol Vis Sci. 2011;52(4):1930–7.
264. Goto E, Matsumoto Y, Kamoi M, Endo K, Ishida R, Dogru M, et al. Tear evaporation rates in Sjögren syndrome and non-Sjögren dry eye patients. Am J Ophthalmol. 2007;144(1):81–5.
265. Villani E, Beretta S, De Capitani M, Galimberti D, Viola F, Ratiglia R. In vivo confocal microscopy of meibomian glands in Sjögren's syndrome. Invest Ophthalmol Vis Sci. 2011;52(2):933–9.
266. Menzies KL, Srinivasan S, Prokopich CL, Jones L. Infrared imaging of meibomian glands and evaluation of the lipid layer in Sjögren's syndrome patients and nondry eye controls. Invest Ophthalmol Vis Sci. 2015;56(2):836–41.
267. Chen X, Utheim Ø, Xiao J, Adil MY, Stojanovic A, Tashbayev B, et al. Meibomian gland features in a Norwegian cohort of patients with primary Sjögren's syndrome. PLoS One. 2017;12(9):e0184284.
268. Shimazaki J, Goto E, Ono M, Shimmura S, Tsubota K. Meibomian gland dysfunction in patients with Sjögren syndrome. Ophthalmology. 1998;105(8):1485–8.
269. Pflugfelder SC, Jones D, Ji Z, Afonso A, Monroy D. Altered cytokine balance in the tear fluid and conjunctiva of patients with Sjögren's syndrome keratoconjunctivitis sicca. Curr Eye Res. 1999;19(3):201–11.
270. Knop N, Knop E. Ultrastructural anatomy of CALT follicles in the rabbit reveals characteristics of M-cells, germinal centres and high endothelial venules. J Anat. 2005;207(4):409–26.
271. Diebold Y, Chen LL, Tepavcevic V, Ferdman D, Hodges RR, Dartt DA. Lymphocytic infiltration and goblet cell marker alteration in the conjunctiva of the MRL/MpJ-Fas(lpr) mouse model of Sjögren's syndrome. Exp Eye Res. 2007;84(3):500–12.
272. McGinnigle S, Naroo SA, Eperjesi F. Evaluation of dry eye. Surv Ophthalmol. 2012;57(4):293–316.
273. You IC, Bian F, Volpe EA, de Paiva CS, Pflugfelder SC. Age-related conjunctival disease in the C57BL/6.NOD-Aec1Aec2 mouse model of Sjogren syndrome develops independent of lacrimal dysfunction. Invest Ophthalmol Vis Sci. 2015;56(4):2224–33.
274. Contreras-Ruiz L, Regenfuss B, Mir FA, Kearns J, Masli S. Conjunctival inflammation in thrombospondin-1 deficient mouse model of Sjögren's syndrome. PLoS One. 2013;8(9):e75937.
275. Lee WS, Yoo WH. Primary Sjogren's syndrome in monozygotic twins. Int J Rheum Dis. 2014;17(5):578–9.
276. Peri Y, Agmon-Levin N, Theodor E, Shoenfeld Y. Sjögren's syndrome, the old and the new. Best Pract Res Clin Rheumatol. 2012;26(1):105–17.

4 Myoepithelial Cell Function in Salivary Gland Physiology and Disease

José O. Maldonado, Paola Pérez Riveros, and John A. Chiorini

4.1 Introduction

In humans, there are three pairs of major salivary glands (SG): the parotid (PG), submandibular (SMG), and sublingual (SLG); as well as hundreds of minor or accessory SG (MSG) located throughout the oral cavity and upper airway immediately subjacent to the mucosa (e.g., lingual, labial, palatal, pharyngeal, buccal) [1, 2]. SG are compound exocrine tubuloacinar glands whose major function is to produce and release saliva into the oral cavity [2]. The major SG are responsible for over 90% of the total saliva and they are most active during the process of mastication/eating, while minor SG produce less than 10% of total saliva. MSG are active throughout the day providing constant lubrication and buffering to the oral mucosal tissues [3, 4]. The acini are formed by serous cells, mucous cells, and/or seromucous cells, and myoepithelial cells (MEC) that envelope the secretory acini and intercalated ducts (Fig. 4.1) [5, 6].

The proportion and distribution of these cell types vary between the different types of SG. The biology and physiology of acinar cells (serous and mucous) is well documented (see [7–9] for comprehensive review); however, the

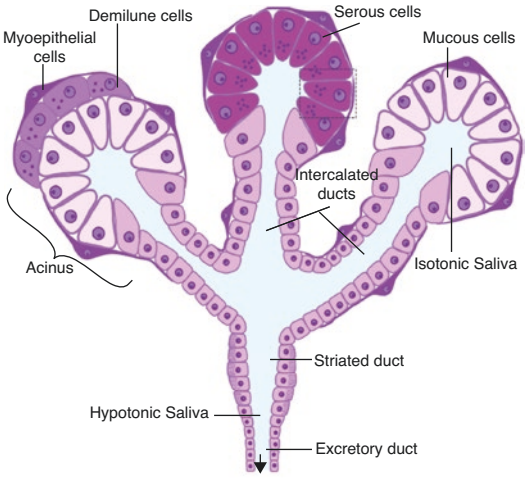

Fig. 4.1 Salivary gland (SG) secretory units. Representation of a mixed SG. SG contain three major cell types: acinar cells, myoepithelial cells, and ductal cells. The acinar cells (i.e., mucous, serous, and seromucous cells) compose the SG glandular secretory end piece, acini, that makes saliva. The acini and intercalated ducts are surrounded by the myoepithelial cells, which display between 4 and 8 processes that contract to squeeze saliva from the acini upon nerve stimulation. The saliva is then pushed through the duct system and into the oral cavity. Serous acinar cells produce a watery secretion containing proteins. Mucous acinar cells produce a viscous secretion rich in glycoproteins. Seromucous acinar cells produce secretions of both types. The duct secretes bicarbonate to keep the secreted proteins in a soluble state. Each of the major SG contains different proportions of each acinar cells. The salivary secretion found in the acini is isotonic and it becomes hypotonic as it moves through the intercalated and striated ducts. The gray box represents the serous acinar cell presented in Fig. 4.2

J. O. Maldonado · P. Pérez Riveros · J. A. Chiorini (✉)
AAV Biology Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA
e-mail: jchiorini@dir.nidcr.nih.gov

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exact physiology and pathophysiology of MEC is relatively unknown. In this article, we review the classical mechanisms of saliva production and secretion involving acinar and ductal cells and summarize the current knowledge regarding the MEC including their histology, structure, function, and role in the pathophysiology of SG.

4.2 General Morphology of Human Salivary Glands

The paired PG are the largest of the major SG, with a size of approximately 4 by 6 cm on the widest sections and weighing 15–30 g each. The PG are on each side of the face in the parotideomasseteric fascia, anteroinferior to external acoustic meatus, inferior to the zygomatic arch, superior to the inferior border of the mandible, and posterior to the anterior border of the masseter. The PG are loosely encapsulated by a fibroconnective tissue capsule exhibiting an inverted pyramidal shape. Uniquely, the PG is composed entirely of serous acini which secrete serous fluid, a protein-rich watery solution [9, 10].

At about half the size of the PG, the SMG measure approximately 5 by 1.5 cm and weigh 7–15 g each. SMG are firm organs with a dense fibroconnective tissue capsule and are shaped roughly like a triangular prism oriented just lateral to the anteroposterior axis in the submandibular space. The acini of this gland secrete both serous and mucus fluids owing to the roughly equal distribution of serous and mucous acini.

The third, and least voluminous, of the major SG are the SLG. These glands measure approximately 3 × 1.5 cm, are ellipsoid in shape, and produce a secretion that is a mixed, although mainly mucous fluid. MSG are numerous (600–1000), measure between 1 and 5 mm and are spheroid in shape. MSG are widely distributed in clusters in the submucosa throughout the upper aerodigestive tract (i.e., palate, uvula, oral mucosa, floor of the mouth, retromolar area, posterior tongue, peritonsillar area, paranasal sinuses, pharynx, and larynx), and primarily produce a mixed, although mainly mucin-rich secretion thought to provide lubrication and protection of the local mucosal structures [1, 2, 6, 8].

4.3 General Histology of the Salivary Glands

SG are compound exocrine tubuloacinar glands that are comprised of secretory units and ducts. The primary fluid secretion is synthesized in the acini and transported to the oral cavity via a network of specialized ducts (i.e., acini terminate in the intercalated ducts [ID]; the ID terminate in the striated ducts [SD]; the SD terminate in the excretory ducts [ED] which terminate in the oral cavity). During the salivary transport process, the ducts fine-tune the composition of electrolytes and regulate the salivary pH. The acinus structure is composed of mucous, serous, and/or seromucous cells (Fig. 4.1). Mucous acinar cells are columnar cells with a basally situated nucleus and apically oriented secretory granules. These cells synthesize, store, and release viscous glycoproteins that form mucus when hydrated. Serous acinar cells exhibit a similar structure, but synthesize, store, and release a protein secretion from secretory granules that may be glycosylated or non-glycosylated (Fig. 4.2). Seromucous acinar cells commonly exhibit a demilune shape and contain components of both mucinous and serous acinar cells [8, 11]. The MEC are located between the secretory cells and basal lamina of the acinus and the intercalated ducts. These cells lie on top of the basal portion of the acini and intercalated ducts, exhibit a flat body and multiple pseudopodia-like extensions that form a meshwork that when contracted, impart force onto the lumen of the acini to eject secretions of the primary saliva from the acinus via the ducts into the oral cavity (Figs. 4.1 and 4.3) [5, 6].

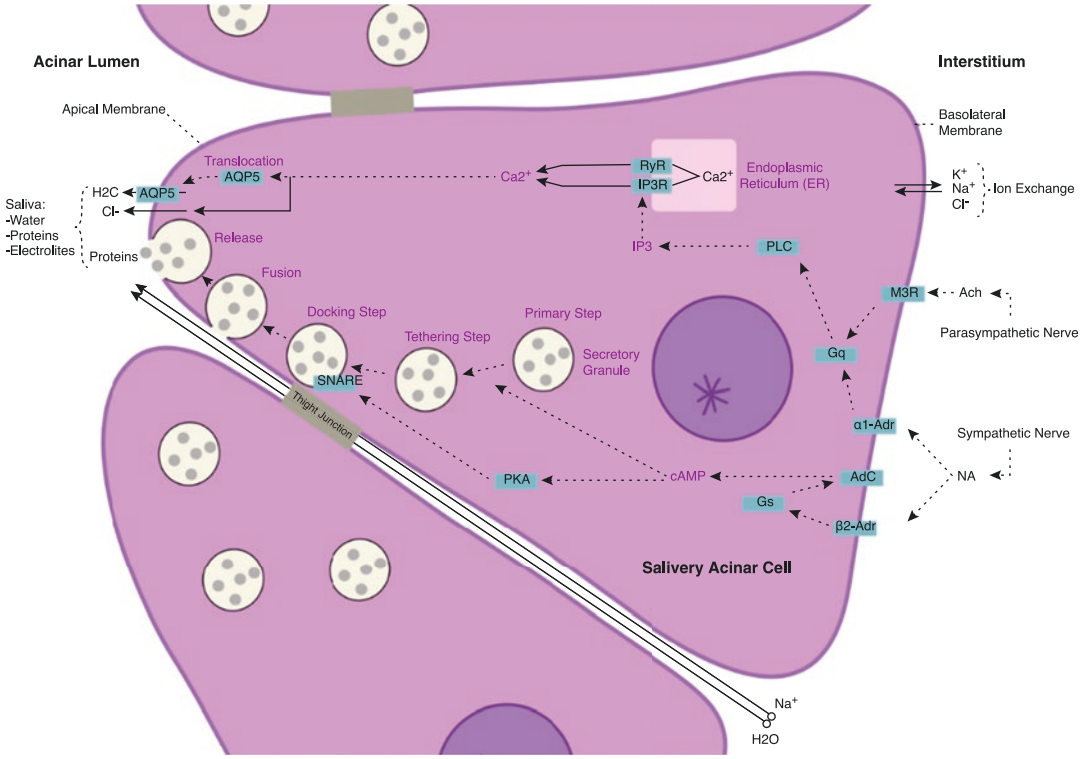

Fig. 4.2 Salivary secretion is controlled by sympathetic and parasympathetic nerves. Representation of a serous acinar cell. Salivary secretion is primarily dependent on acetylcholine (Ach) activation of the muscarinic M3 receptors (M3R) and alpha 1 adrenergic receptor (α1-Adr). Intracellular signaling is through the G proteins (Gq), phospholipase C (PLC), and the receptors for inositol triphosphate (IP_3R) and ryanodine (RyR). Activation of the IP_3R via inositol triphosphate (IP_3) results in an increase in intracellular calcium (Ca^{2+}) due to release from the endoplasmic reticulum (ER) and activation of the RyR by the released Ca^{2+}. This pathway may be activated, in part, by the sympathetic nerves by activating α1-Adr. Increased intracellular Ca^{2+} levels result in chloride release (Cl^-) from the apical side of the acinar cell, K^+ release from the basolateral side, and water flow through activation of the apical water channel aquaporin 5 (AQP5). Cell volume then recovers by activation of the ion channels and coupled transporters in the basolateral membrane of the acinar cell (i.e., K^+, Na^+, and Cl^-). The electrochemical gradient between the basal and apical acinar cell surfaces produces a transepithelial potential difference that induces Na^+ diffusion across the epithelial tight junction. Protein secretion is primarily driven by the release of noradrenaline (NA) from sympathetic nerves which activates beta 2 adrenoceptors (β_2-Adr), which in turn activates G proteins (Gs) and adenylate cyclase (Adc). This intracellular signaling results in an increase in cyclic AMP (cAMP), which then activates protein kinase A (PKA) and subsequently, activation of the SNARE pathway, leading to exocytosis of protein storage in granules and release into saliva. The exocytosis secretion process consists of vesicle trafficking, tethering, docking, fusion, and protein release

4.4 Salivary Secretion

In the basal physiologic state, saliva is a clear, mucoserous exocrine fluid that plays a vital role in daily activities such as talking and swallowing by providing mucosal lubrication. The latter reduces friction between hard materials, such as the teeth and food, and the oral soft tissues by providing smooth, low adherence surfaces. Lubrication permits the food bolus smooth transit through the esophagus and initiates the digestion process. Saliva serves an important role in taste by activating the taste buds and permitting tastant chemicals to be liberated and solubilized from

the food bolus. This saliva-tastant solution is ultimately diffused throughout the oral cavity and delivered to the taste pores for binding to their cognate receptor. Saliva functions to dilute and clear substances from the mouth, promotes tooth remineralization, buffers the pH, and influences the oral microbiome [7, 12].

4.4.1 Salivary Secretion Flow Rate

Whole saliva is a complex physiological fluid secreted by the major and MSG that consist of approximately 99% water and 1% of other components, such as electrolytes (e.g., potassium (K^+), bicarbonate (HCO_3^-), chloride (Cl^-), calcium (Ca^{2+}), sodium (Na^+), fluoride (F^-), magnesium (Mg^{2+}), and proteins (e.g., enzymes, mucosal glycoproteins, immunoglobulins A, G, and M, albumin, histatins, defensins), in addition to glucose, amino acids, and urea [1, 7]. A healthy individual produces an average daily flow of 0.5 to 1.5 L of saliva [13]. Each SG contributes a different percentage of unstimulated saliva throughout the day: SMG contribute the most (65%), followed by PG (20%), and MSG and SLG (~10%) and (~5%), respectively. The normal, healthy state, whole unstimulated saliva flow (WUSF) rate is 0.3–0.5 mL/min, thus WUSF above 0.3 mL/min is considered normal. WUSF <0.1 mL/min is considered clinical SG hypofunction and may result in xerostomia, the subjective feeling of dry mouth [14, 15]. After stimulation (e.g., food, citric acid, etc.), the PG can increase their production up to tenfold and contribute at least 50% of the total volume to whole stimulated saliva (WSS) [3]. The normal WSS flow is in the range of 2–5 mL/min.

4.4.2 Autonomic Control of Salivary Fluid and Protein Secretion

Saliva secretion is largely under the control of the autonomic nervous system with parasympathetic and sympathetic inputs. This dual innervation corresponds with two major secretory pathways: fluid and ion secretion, and protein exocytosis. The acinar cells present a different set of ion-transport proteins in the apical membrane compared to the basal membrane. Neighboring acinar cells, through tight junctions, aid in the maintenance of the cell polarity; these junctions allow the movement of water, small molecules, and selected ions, such as Na^+, that together with the net isotonic secretion by acinar cells leads to water movement and the formation of isotonic salivary secretion (Fig. 4.2).

The protein exocytosis is stimulated by sympathetic nerve fibers through synergistic action of alpha 1 (α1) and beta 2 (β2) adrenergic receptors. The β2 receptors activate adenylate cyclase to increase intracellular cyclic AMP (cAMP) and the α1 receptors increase Ca^{2+} to modulate the cAMP-activated secretory process. The increase of cAMP causes the docking, fusion, and exocytosis secretory granules that contain proteins and mucins. Parasympathetic stimulation works mainly through M3 receptors (although animal models and histological studies in human suggest the participation of M1 and M5 receptors as well [16]), which activates phospholipase C [17, 18]. This interaction increases the intracellular levels of Ca^{2+}, leading to aquaporin 5 (AQP5) channels translocation towards the apical pole of acinar cells and activation of ion transporters and channels, which results in the production and secretion of water and electrolytes (Fig. 4.2) [19].

Intracellular Ca^{2+} increase also synergizes with cAMP to augment protein secretion, while Ca^{2+} increase alone causes minimal protein secretion [20]. cAMP which increases through activation of the G protein-coupled adenylate cyclase activity in response to noradrenaline released from the sympathetic nerve fibers is the major mediator of protein secretion. Simultaneous activation of parasympathetic and sympathetic nerve fibers at physiological low stimulus intensity results in maximal elevation of proteins secretion through synergy between cAMP and Ca^{2+} signaling pathways [21–23]. Activation of the autonomic nerves results in fusion of the storage granules with the apical membrane of acinar cells and release of the protein content into the acini lumen (Fig. 4.2) [24, 25]. The neuronal control herein described makes minor contributions to

the MSG secretions under physiological conditions [7, 26].

4.4.3 Salivary Secretion Modification

Acinar cell polarity is necessary for the proper movement of salivary fluid and protein into the acinar lumen, then through the SG ductal network and into the oral cavity. In contrast to SG acinar cells, the SG ductal epithelial cells have water impermeable tight junctions that play an important role in the modification of the salivary secretion as it progresses through the ducts becoming hypotonic [27]. Lysozyme and lactoferrin are added to saliva in the intercalated ducts, while the striated ducts reabsorb Na^+ and Cl^-, and add K^- and bicarbonate to create the proper hypotonic salivary secretion [28]. Salivary secretion hypotonicity is affected by flow rate, thus stimulated salivary secretion presents higher Na^+ and Cl^- concentration. Conversely, during hyposalivation, levels of Na^+ and Cl^- are decreased.

Most of the knowledge accumulated to date on the secretion of salivary fluids was obtained by studying isolated acinar and ductal cells. A knowledge gap remains as to how these two cell types interact and function in concert with MEC to maintain SG healthy homeostasis and normal flow of saliva.

4.5 Myoepithelial Cells

4.5.1 Development and Structure

MEC are stellate-shaped cells located at the base of epithelial cells between the epithelial cells and the extracellular basal membrane of the terminal portion of most exocrine glands. Although MEC are of ectodermal origin, they present both epithelial and mesenchymal structure and function. An immunohistochemical study using several markers for MEC showed they appear early in the development of the SG (weeks 10–18). By weeks 19 to 24, the MEC start to express actin and begin to form actin microfilaments before acinar and ductal cells appear. Once acinar cells mature, the MEC elongate their cytoplasm. From weeks 25 to 32, the MEC further elongate, flatten, and develop contractile characteristics between weeks 33 and 40 [29]. These findings are in line with more recent studies using single cell RNA sequencing in an in vitro mouse model for SG development (Fig. 4.3).

The distribution of MEC in the major SG are similar; they surround the acini, intercalated ducts, and some striated ducts, but are absent in the excretory ducts (Fig. 4.1) [30, 31]. In the MSG, MEC surround the acini and its projections are present in the intercalated ducts, but not in the striated and excretory ducts. MEC surrounding acini present a star-shaped form, with numerous branching processes, up to 30 terminal processes (four to eight primary processes with two or more secondary branches for each primary process), a flattened nucleus, and a small peri-nuclear cytoplasm. In contrast, the MEC associated with intercalated ducts are fusiform and elongated with fewer processes (Fig. 4.3) [11, 31]. Due to the numerous MEC processes, all the acinar cells are in contact with MEC, thus the ratio of MEC to acinar cells is lower. Furthermore, acinar cells have a portion of their basolateral plasma membrane in direct contact with the basal lamina of the extracellular matrix (Figs. 4.1 and 4.3). A study of the major SG by Hardy et al. (1998), found that the distribution of MEC are more abundant in pure mucous acini than the pure serous acini of the SMG and SLG, with PG showing a lower distribution per surface area [30].

Most of the initial characterization of MEC has been done with electron microscopy and immunohistochemistry because they are difficult to identify in regular histology owing to their stellate shape. These cells are characterized by the expression of smooth muscle actin (SMA) (Fig. 4.3b), calponin, S100 calcium-binding protein, integrin β1 (CD29), and p63. The nucleus of MEC is fusiform and parallel to the long axis of the cell surrounded by a minima of organelles. The remaining cytoplasm is occupied by parallel arrays of microfilaments that gather in dense bodies

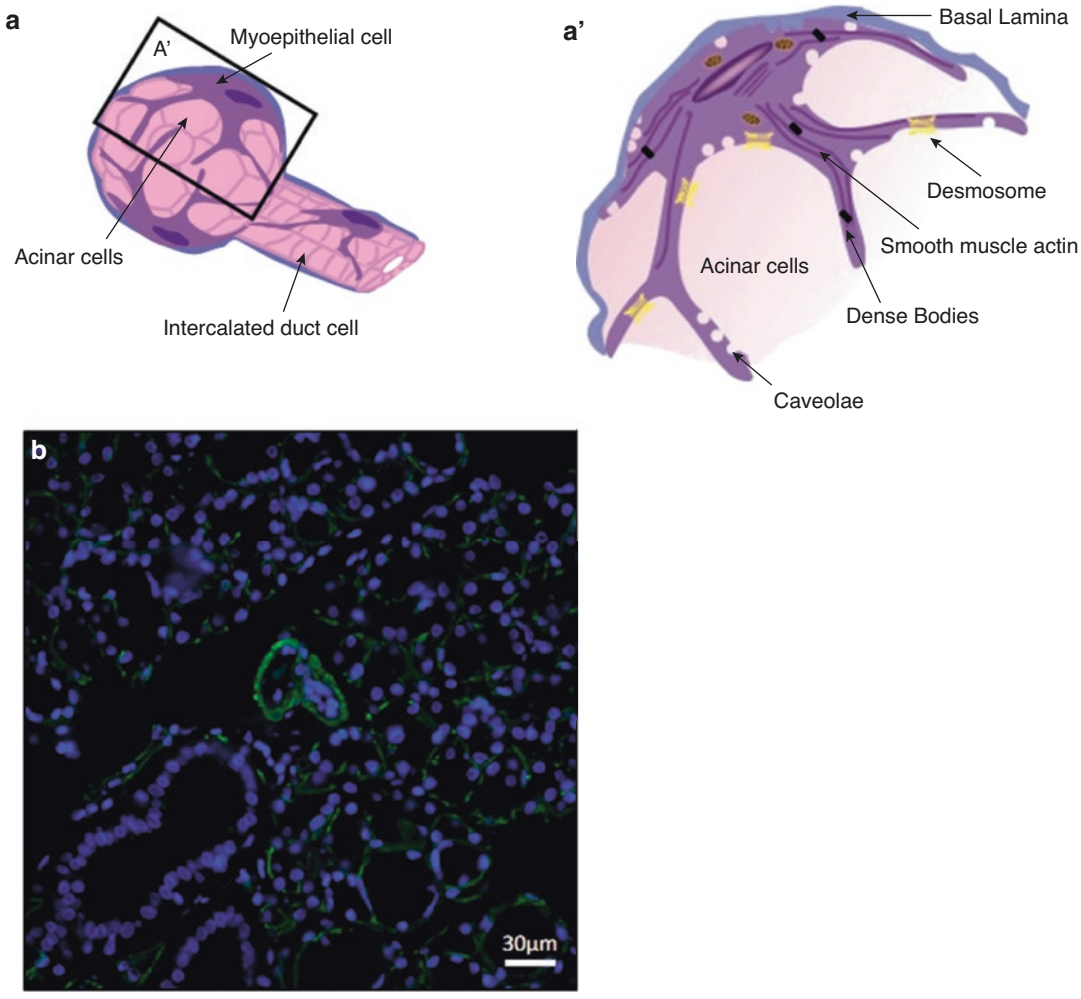

Fig. 4.3 Myoepithelial cells (MEC), relationship with acinar cells and principal structural characteristics. (**a**) Representation of an acini and the relationship with MEC. Per each acinus, there are about two to four MEC. The MEC of acini have several extensions that surround the acini like a basket. The intercalated duct has fewer MEC and they are more elongated with just two extensions. (**a′**) Representation of a single MEC showing the principal subcellular structural characteristics. (**b**) Immunofluorescence of smooth muscle actin in a human parotid gland, showing the typical staining of MEC (green). The extensions of the MEC appear as a discrete segment of staining

terminating into the plasma membrane. These structural characteristics correlate with the ability of MEC to contract. Numerous physiological studies in several animal models indicate that MEC contract under both sympathetic and parasympathetic nerve stimulation [32–34]. This makes physiologic sense since fluid and protein vesicle secretion are regulated chiefly by parasympathetic and sympathetic stimuli, respectively.

Ultrastructurally, MEC attach to the extracellular matrix through hemidesmosomes forming an interconnected meshwork across the basolateral surface of the acini or ducts. The area of the MEC that approximate the termination of nerve fibers are the caveolae [35]; however, the exact function of the caveolae in the physiology of MEC has not been studied. One can speculate that their distribution suggests a role similar to that of the caveolae in smooth muscle where they

play a role in coordinating the contractibility of the muscle. Caveolae, which are associated with stress fibers, could provide an important strategy for cells to deal with mechanical stresses (Fig. 4.3) [35]. MEC attach to the epithelial cells through desmosomes [36]; the latter are characterized by resisting mechanical stress because they adopt a hyper-adhesive state [37]. Given the adhesive properties of desmosomes, this interaction likely provides more than structural support, possibly promulgating a dynamic cell-to-cell communication network. In SG, despite being clearly identified in electron microscopy, the molecular structure of the desmosomes that connect epithelial cells to MEC has not been adequately described.

4.5.2 Functions of Myoepithelial Cells

Since the MEC were first described in the SG of cats, their principal role was thought to be a mechanical aid in the secretion of saliva, mostly through coordinated contraction and relaxation of the acini and in the ducts, respectively. Experimental work in the SG of dogs established that MEC played a minor role in the expulsion of saliva during basal flow, but plays an outsized role when secretion was required at increased rates such as during stimulation. This function is more relevant in the mucous glands and in response to adrenergic stimulation [38]. Stimulation of sympathetic nerves causes vasoconstriction and contraction of the MEC [38, 39]. It has been proposed that this contraction forces the saliva out of the acini and into the duct network, facilitating the secretion of a smaller volume of thick saliva that is rich in mucins.

Given the location of MEC (at the base of the epithelial cells), it is thought that MEC also act as support for the secretory cells; however, it is not clear what subcellular structures would be involved in this function and how important it is for the maintenance of the acinar and ductal cell function and survival. MEC secrete proteins such as fibronectin, laminin, and elastin, which makes them important contributors to the organization of the basal membrane and the proper attachment of the epithelial cells. Additionally, it is thought that the MEC processes aid in the rigidity and patency of the SG, preventing them from becoming obstructed, specifically during mastication when the SG undergo physical changes (e.g., the PG can be deformed easily during mastication and masseteric contraction due to the lower number of MEC) [40]. Given the differences in the number of MEC in different SG, we can speculate that this function would be more relevant in mucous and seromucous SG (SMG, SLG, and MSG) than in serous SG (PG) that deform more easily during mastication, thus releasing more secretions.

Microscopy studies of MEC have shown the presence of basal infoldings, pinocytotic vesicles, increased alkaline phosphatase and Mg^{2+}-dependent adenosine triphosphatase (ATPase) activity, and positive staining for iron-binding protein ferritin. These characteristics propose a role of MEC in the transport of metabolites in the secretory process. However, this is not well characterized [41–43].

4.5.3 Myoepithelial Cells in Salivary Gland Pathology

More than 70% of the MEC literature is related to SG neoplasia. This is due to the expression of myoepithelial markers as a common finding in SG tumors. Distinct tumor types show differences with regard to various MEC markers and can be useful for differential diagnosis of SG tumors. In other glandular tissues, like mammary glands, MEC appear to resist neoplastic transformation by producing a combination of proteins that have tumor-suppressor activity that inhibit tissue angiogenesis, invasion, metastasis, and can restrain and recapture invasive cancer cells [44]. However, this tumor suppressive program in SG MEC is not clear. MEC have the capacity to differentiate into epithelial as well as mesenchymal components in pleomorphic adenomas, the most common benign salivary tumor; these tumor cells have been termed the neoplastic myoepithelial

cells (NMEC). It is thought that MEC can modify the biological behavior of SG tumors. SG carcinomas with histopathological evidence of active participation of MEC are generally those taking origin from the intercalated ducts and secretory end pieces. These carcinomas tend to be classified as low grade with a low ability to metastasize. This correlation is an argument on the antitumoral activity of MEC; however, SG neoplasms are not extensively studied and the real role of MEC is not clear.

MEC have been implicated in other nonneoplastic disorders such as benign lymphoepithelial lesions and Sjögren's syndrome (SS). The latter is a systemic autoimmune disease that primarily affects salivary and lacrimal glands. Similar in function to AQP5 in the acinar cells, aquaporin 4 (AQP4) normally expressed in MEC is downregulated in SS. The presence of AQP4 in MEC suggests a role of these cells in the transport of water during saliva production and secretion. This phenomenon suggests that the capacity for water to flow across the membrane of MEC may be altered in SS [45]. Mouse models of SS present atrophy of MEC in lacrimal glands as a consequence of chronic inflammation [46, 47]. In MSG of SS patients, p63 nuclear labeling in SS MEC is preserved, whereas α-SMA cytoplasmic staining is strongly and significantly reduced when compared with healthy SG [48]. Although the physiological consequences of these changes have not been established, it may implicate reduced MEC contractile properties as contributing to reduced saliva flow in these patients [48].

Parotitis is clinically evident as a bilateral swelling of the PG and occur in diseases with severe and long-standing hormonal metabolic disturbances, or systemic immune dysfunction [49]. Glands from patients with sialadenosis (non-inflammatory, nonneoplastic swelling of SG such as parotitis) exhibit significant loss and thinning of the myofilament elements within the MEC, and consequently, a loss of mechanical support for the acini has been reported [50]. The authors proposed that a functional deficit of the MEC allows acinar cells to expand as secretory granules accumulate intracellularly. This acinar cell enlargement is thought to be a consequence of an autonomic neuropathy, secondary to severe metabolic or hormonal disorders; however, no further studies have been reported.

Although great advances have been made in understanding the mechanisms for head and neck radiation therapy (RT)-induced salivary hypofunction, no effective therapy exists to date to prevent SG damage or to regenerate SG tissues [51–53]. Moderate to high RT dosages are devastating to the SG tissues, thus resulting in lower salivary flow. Acinar epithelial cells present the highest degree of atrophy followed by ductal epithelia after a single dose of radiation therapy [52, 54, 55]. High radiation doses to the SG may result in the elimination of serous acini and a significant decrease in the number and size of mucous acini [51]. On the contrary, MEC have been shown to be less susceptible to radiation damage (i.e., structurally and enzymatically) in both humans and rats [51, 56, 57] and to some extent, resist atrophy. Similar results have been obtained in experimental models of SG atrophy and regeneration [58–60]. The implications for MEC resistance to RT and the development of therapy to recover or improve SG function are still not clear.

4.5.4 Recent Advances

It is clear that MEC represent an important cell type within the SG. Recent technological advances should provide unprecedented opportunities to better dissect the role of MEC in the physiology and functional properties will aid in the development of new therapies to restore the function of damaged SG. One example is the development of a purification method for MEC from human salivary tissue explants [61]. This work demonstrated that cells with characteristics similar to MEC in the tissue proved their contractile function in vitro.

Transgenic animal models, such as the *SMA*$^{CreErt2/+}$*:Rosa26-TdTomato*$^{fl/fl}$ mouse strain that allows genetic labeling of SMA+ MEC and pericytes, have also been successfully utilized to isolate MEC from lacrimal glands and SMG [62]. This could be used for RNA sequencing or cell

line development and paired with 3D culture techniques, which could open the door to better analyze their physiology and interaction with acinar cells and extracellular matrix. Also, the animal model could be instrumental in understanding the physiology of the MEC when paired with intravital two-photon microscopy tools that allow for real-time imaging of physiological processes [63].

4.6 Summary and Conclusion

It is clear that MEC play an important role in the normal function of SG. In this article, we briefly reviewed the biology and physiology of SG and the production and secretion of salivary fluids. We highlighted salient aspects of MEC physiology and function in normal and pathologic conditions involving the SG. This review emphasizes that although much is known about the MEC, further studies are needed to better elucidate their involvement in the production and secretion of salivary secretions. A clearer understanding of the integral nature of MEC in SG function is vital to develop new, effective therapies for SG diseases including salivary hypofunction (e.g., Sjögren's Syndrome, radiation-induced xerostomia) and SG neoplasms of purported myoepithelial origin (e.g., pleomorphic adenoma, myoepithelial cell carcinoma).

References

1. Edgar WM. Saliva: its secretion, composition and functions. Br Dent J. 1992;172(8):305–12.
2. Hand AR, Pathmanathan D, Field RB. Morphological features of the minor salivary glands. Arch Oral Biol. 1999;44(Suppl 1):S3–10.
3. Edgar WM. Saliva and dental health. Clinical implications of saliva: report of a consensus meeting. Br Dent J. 1990;169(3–4):96–8.
4. Amano O, et al. Anatomy and histology of rodent and human major salivary glands: overview of the Japan salivary gland society-sponsored workshop. Acta Histochem Cytochem. 2012;45(5):241–50.
5. Nagato T, et al. A scanning electron microscope study of myoepithelial cells in exocrine glands. Cell Tissue Res. 1980;209(1):1–10.
6. Patel SA, Barros JA. Introduction. In: Streckfus CF, editor. Advances in salivary diagnostics. Berlin, Heidelberg: Springer; 2015. p. 1–16.
7. Proctor GB. The physiology of salivary secretion. Periodontol 2000. 2016;70(1):11–25.
8. Holsinger FC, Bui DT. Anatomy, function, and evaluation of the salivary glands. In: Salivary gland disorders. Berlin: Springer; 2007. p. 1–16.
9. de Paula F, et al. Overview of human salivary glands: highlights of morphology and developing processes. Anat Rec (Hoboken). 2017;300(7):1180–8.
10. Berkovitz BK, Holland GR, Moxham BJ. Color atlas & textbook of oral anatomy, histology, and embryology. St. Louis, MO: Mosby; 1992.
11. Martinez-Madrigal F, Micheau C. Histology of the major salivary glands. Am J Surg Pathol. 1989;13(10):879–99.
12. Carpenter GH. The secretion, components, and properties of saliva. Annu Rev Food Sci Technol. 2013;4:267–76.
13. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. 2001;85(2):162–9.
14. Sreebny LM, Valdini A. Xerostomia. A neglected symptom. Arch Intern Med. 1987;147(7):1333–7.
15. Speight PM, Kaul A, Melsom RD. Measurement of whole unstimulated salivary flow in the diagnosis of Sjogren's syndrome. Ann Rheum Dis. 1992;51(4):499–502.
16. Ryberg AT, et al. Expression of muscarinic receptor subtypes in salivary glands of rats, sheep and man. Arch Oral Biol. 2008;53(1):66–74.
17. Nakamura T, et al. M(3) muscarinic acetylcholine receptor plays a critical role in parasympathetic control of salivation in mice. J Physiol. 2004;558(Pt 2):561–75.
18. Gautam D, et al. Cholinergic stimulation of salivary secretion studied with M1 and M3 muscarinic receptor single- and double-knockout mice. Mol Pharmacol. 2004;66(2):260–7.
19. Gallacher D, Smith P. Autonomic transmitters and Ca2+−activated cellular responses in salivary glands in vitro. In: Neural mechanisms of salivary gland secretion, vol. 11. Basel: Karger; 1999. p. 80–93.
20. MÖLLER K, et al. The role of protein kinase C in carbachol-induced and of cAMP-dependent protein kinase in isoproterenol-induced secretion in primary cultured Guinea pig parotid acinar cells. Biochem J. 1996;314(1):181–7.
21. Tanimura A, et al. Isoproterenol potentiates α-adrenergic and muscarinic receptor-mediated Ca2+ response in rat parotid cells. Am J Physiol Cell Physiol. 1999;276(6):C1282–7.
22. Bobyock E, Chernick W. Vasoactive intestinal peptide interacts with alpha-adrenergic-, cholinergic-, and substance-P-mediated responses in rat parotid and submandibular glands. J Dent Res. 1989;68(11):1489–94.
23. ASKING B. Sympathetic stimulation of amylase secretion during a parasympathetic background activity in the rat parotid gland. Acta Physiol Scand. 1985;124(4):535–42.

24. Segawa A, et al. Cell biology of human salivary secretion. Eur J Morphol. 2000;38(4):237–41.
25. Suzuki A, Iwata J. Molecular regulatory mechanism of exocytosis in the salivary glands. Int J Mol Sci. 2018;19(10):3208.
26. Proctor GB, Carpenter GH. Salivary secretion: mechanism and neural regulation. Monogr Oral Sci. 2014;24:14–29.
27. Baker OJ. Tight junctions in salivary epithelium. Biomed Res Int. 2010;2010:278948.
28. Pedersen AML, et al. Salivary secretion in health and disease. J Oral Rehabil. 2018;45(9):730–46.
29. Ianez RF, et al. Human salivary gland morphogenesis: myoepithelial cell maturation assessed by immunohistochemical markers. Histopathology. 2010;57(3):410–7.
30. Hardy G, Kramer B. The myoepithelium of human major salivary glands revisited. SADJ. 1998;53(7):371–5.
31. Redman RS. Myoepithelium of salivary glands. Microsc Res Tech. 1994;27(1):25–45.
32. Emmelin N, Garrett JR, Ohlin P. Neural control of salivary myoepithelial cells. J Physiol. 1968;196(2):381–96.
33. Emmelin N, Garrett JR, Ohlin P. Motor nerves of salivary myoepithelial cells in dogs. J Physiol. 1969;200(2):539–46.
34. Lung MA. Autonomic nervous control of myoepithelial cells and secretion in submandibular gland of anaesthetized dogs. J Physiol. 2003;546(Pt 3):837–50.
35. Echarri A, Del Pozo MA. Caveolae—mechanosensitive membrane invaginations linked to actin filaments. J Cell Sci. 2015;128(15):2747–58.
36. Chaudhry A, et al. Ultrastructure of normal human parotid gland with special emphasis on myoepithelial distribution. J Anat. 1987;152:1.
37. Garrod D, Kimura TE. Hyper-adhesion: a new concept in cell–cell adhesion. London: Portland Press; 2008.
38. Emmelin N, Gjorstrup P. On the function of myoepithelial cells in salivary glands. J Physiol. 1973;230(1):185–98.
39. Emmelin N, Gjörstrup P. Contraction of myoepithelial cells in secreting submandibular glands of dogs. Experientia. 1976;32(2):197–8.
40. Garrett J, Parsons P. Alkaline phosphatase and myoepithelial cells in the parotid gland of the rat. Histochem J. 1973;5(5):463–71.
41. Garrett JR, Emmelin N. Activities of salivary myoepithelial cells: a review. Med Biol. 1979;57(1):1–28.
42. Bogart BI. The fine structural localization of alkaline and acid phosphatase activity in the rat submandibular gland. J Histochem Cytochem. 1968;16(9):572–81.
43. Yoshihara T, Kanda T, Kaneko T. A cytochemical study on the salivary gland pleomorphic adenoma (mixed tumor) and the fetal and adult salivary gland. Arch Otorhinolaryngol. 1984;240(3):231–8.
44. Sirka OK, Shamir ER, Ewald AJ. Myoepithelial cells are a dynamic barrier to epithelial dissemination. J Cell Biol. 2018;217(10):3368–81.
45. Sisto M, et al. Abnormal distribution of AQP4 in minor salivary glands of primary Sjögren's syndrome patients. Autoimmunity. 2017;50(4):202–10.
46. Hawley D, et al. Myoepithelial cell-driven acini contraction in response to oxytocin receptor stimulation is impaired in lacrimal glands of Sjogren's syndrome animal models. Sci Rep. 2018;8(1):9919.
47. Nashida T, et al. Atrophy of myoepithelial cells in parotid glands of diabetic mice; detection using skeletal muscle actin, a novel marker. FEBS Open Biol. 2013;3:130–4.
48. Sisto M, et al. Reduced myofilament component in primary Sjogren's syndrome salivary gland myoepithelial cells. J Mol Histol. 2018;49(2):111–21.
49. Donath K, Seifert G. Ultrastructural studies of the parotid glands in sialadenosis. Virchows Arch A Pathol Anat Histol. 1975;365(2):119–35.
50. Ihrler S, et al. Pathogenesis of sialadenosis: possible role of functionally deficient myoepithelial cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;110(2):218–23.
51. Redman RS. On approaches to the functional restoration of salivary glands damaged by radiation therapy for head and neck cancer, with a review of related aspects of salivary gland morphology and development. Biotech Histochem. 2008;83(3–4):103–30.
52. Acauan MD, et al. Radiotherapy-induced salivary dysfunction: structural changes, pathogenetic mechanisms and therapies. Arch Oral Biol. 2015;60(12):1802–10.
53. Ship JA, Hu K. Radiotherapy-induced salivary dysfunction. Semin Oncol. 2004;31(6 Suppl 18):29–36.
54. Valdez IH, et al. Major salivary gland function in patients with radiation-induced xerostomia: flow rates and sialochemistry. Int J Radiat Oncol Biol Phys. 1993;25(1):41–7.
55. Konings AW, Coppes RP, Vissink A. On the mechanism of salivary gland radiosensitivity. Int J Radiat Oncol Biol Phys. 2005;62(4):1187–94.
56. Redman RS. Histologic changes in the salivary glands following radiation therapy. In: Salivary gland development and regeneration. Berlin: Springer; 2017. p. 75–91.
57. Chomette G, et al. Effects of irradiation on the submandibular gland of the rat. An enzyme histochemical and ultrastructural study. Virchows Arch A Pathol Anat Histol. 1981;391(3):291–9.
58. Maria O, et al. Effects of double ligation of Stensen's duct on the rabbit parotid gland. Biotech Histochem. 2014;89(3):181–98.
59. Takahashi S, et al. Changing myoepithelial cell distribution during regeneration of rat parotid glands. Int J Exp Pathol. 1999;80(5):283–90.

60. Burgess LK, Dardick I. Cell population changes during atrophy and regeneration of rat parotid gland. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(6):699–706.
61. Ozdemir T, et al. Bottom-up assembly of salivary gland microtissues for assessing myoepithelial cell function. Biomaterials. 2017;142:124–35.
62. Zyrianova T, Basova LV, Makarenkova H. Isolation of myoepithelial cells from adult murine lacrimal and submandibular glands. J Vis Exp. 2019;(148):e59602.
63. Schießl IM, Castrop H. Deep insights: intravital imaging with two-photon microscopy. Pflügers Arch Eur J Physiol. 2016;468(9):1505–16.

Innate Immune Dysregulation in Sjögren's Syndrome

5

Jeremy Kiripolsky and Jill M. Kramer

5.1 Introduction

Sjögren's syndrome (SS) is an autoimmune disease characterized by exocrine gland dysfunction in addition to many systemic manifestations including autoantibody production, pulmonary fibrosis, and B cell lymphoma [1]. SS primarily effects middle-aged women and occurs in two forms: primary (pSS) and secondary (sSS) [1]. Patients with pSS have SS in isolation, while those with sSS display another autoimmune connective tissue disease, such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) [2]. The disease etiopathology is poorly understood and there are currently no known treatments that target underlying disease mechanisms.

The adaptive immune response has long been recognized as an essential component of pSS disease development [3, 4]. Although adaptive immunity is a central player in disease, more recent work reveals an important role for the innate immune response in the development and progression of pSS. Since reliable markers for early pSS disease in humans have yet to be established, studies focusing on disease etiology in SS mouse models are critical to inform our knowledge of early disease events [5–8]. Murine studies examining disease kinetics show that activation of signaling networks involved in the innate immune response occurs early in the disease process and persists throughout the disease course [9–12]. Significantly, early activation of innate pathways in chronic inflammation results in subsequent recruitment and activation of adaptive immune cells in pSS mouse models [10–13].

Studies show heightened innate immune activation in both the exocrine glands and systemically in pSS patients. In particular, dysregulation of interferons (IFNs) and IFN-inducible genes are a hallmark of disease [14, 15]. IFNs are produced as a result of activation of many diverse innate immune sensors, including Toll-like receptors (TLRs) [16–18]. In addition, cell populations that are required for innate defense, including dendritic cells (DCs), macrophages, natural killer (NK) cells, innate lymphoid cells (ILCs), and salivary and lacrimal epithelium, are altered both locally and systemically in pSS patients and in various pSS mouse models of disease. Within this chapter we will examine

J. Kiripolsky
Department of Oral Biology, School of Dental Medicine, The University at Buffalo, State University of New York, Buffalo, NY, USA

J. M. Kramer (✉)
Department of Oral Biology, School of Dental Medicine, The University at Buffalo, State University of New York, Buffalo, NY, USA

Department of Oral Diagnostics Sciences, School of Dental Medicine, The University at Buffalo, State University of New York, Buffalo, NY, USA
e-mail: jkramer@buffalo.edu

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both the innate cell types and pathways that are dysregulated in pSS as well as putative causes of disease initiation and progression. We will discuss pattern recognition receptors (PRRs) and cognate ligands in the context of disease. We will provide an overview of the factors that govern pSS susceptibility, including genetics, infection, and alterations in the microbiome. Lastly, we will review emerging therapies that target innate immune dysregulation in pSS.

5.2 Innate Immune Pathways Mediate Disease

5.2.1 Toll-like Receptor Signaling: Murine Studies

Toll-like receptors are ancient pattern recognition receptors (PRRs) expressed on all cell types, including immune and epithelial cells [19]. TLRs recognize both pathogen-associated molecular patterns (PAMPs) derived from pathogens and danger-associated molecular patterns (DAMPs) from endogenous sources [20]. TLRs are found both on the cell surface and endosomal compartments and upon ligation they recruit adaptor molecules essential for signal transduction [20]. There are 10 distinct TLRs identified in humans and 12 in mice [21]. They signal either as homodimeric or heterodimeric complexes and many require additional co-receptors to mediate inflammation [21]. TLR activation triggers cytosolic signaling cascades that rely on myeloid differentiation primary response 88 (MyD88) or TIR-domain-containing adapter-inducing interferon-β (TRIF) adaptor molecules [22, 23]. Activation of TLR signaling pathways results in a myriad of inflammatory outcomes including the transcription of pro-inflammatory cytokines and chemokines [24].

TLR dysregulation is an important mechanism that contributes to chronic inflammation in numerous autoimmune diseases [24]. Accordingly, aberrant activation of TLR signaling pathways plays a critical role in the development of autoimmune diseases such as SLE, RA, and multiple sclerosis (MS) [24, 25]. While the role of TLRs is well understood in many autoimmune diseases, the activation and signaling of these receptors is poorly understood in the context of pSS. Studies in pSS patients are crucial to understand key events in disease pathogenesis, and mouse models remain invaluable in elucidating the mechanistic underpinnings of this disease. While a detailed review of these studies is provided elsewhere [26], we will summarize findings related to TLR signaling in SS in the following section.

One of the most well-studied SS murine models is the NOD/ShiLt strain. NOD/ShiLt animals exhibit secondary SS (sSS), as this model develops SS and type I diabetes concomitantly [27]. Studies in this strain suggest that TLR activation may contribute to salivary-specific disease manifestations, as NOD/ShiLt animals display increasing expression of *TLR1*, *TLR2*, *TLR3* *TLR4*, and *TLR9* in submandibular salivary gland (SMG) tissue with disease progression [9]. Interestingly, treatment of NOD/ShiLt animals with chloroquine, an inhibitor of endosomal acidification, resulted in diminished *TLR* levels and decreased lymphocytic infiltrates in SMG tissue. While the inhibitory effects of chloroquine are not limited to endosomal TLRs, this work supports a role for heightened TLR activation in salivary tissue in SS [9, 28].

Further studies in a related model, NOD.B10Sn-*H2*^{b}/J (NOD.B10) provide corroborative evidence for the importance of TLR activation in disease. The NOD.B10 model was derived from the NOD/ShiLtJ by replacing the diabetes-susceptible MHC locus with that from a healthy C57BL/10 animal [29]. Subsequent work revealed that NOD.B10 mice develop SS, but are protected from diabetes and thus NOD.B10 animals are a good model for the study of pSS [7, 29, 30]. Work in the NOD.B10 strain demonstrated that MyD88, an adaptor used by most TLRs and IL-1R family members, is required for both local and systemic pSS manifestations [22, 31]. In the NOD.B10 mouse model, systemic knockout of *MyD88* resulted in protection from loss of salivary flow and reduced prevalence of lymphocytic infiltrates in the lacrimal tissue and SMG derived from female mice [31]. *MyD88*-deficient NOD.B10 mice were also protected from extraglandular

manifestations including decreased lymphocytic infiltration of both the lung and kidney and diminished total and autoreactive antibodies when compared to the parental strain [31]. While it is important to point out that several IL-1R family members also rely on MyD88 for signal transduction, this work provides evidence that activation of TLR ligation likely contributes to chronic inflammation in pSS.

Elegant studies performed in NOD/ShiLt animals lead to the identification of the genetic loci required for the exocrine-related SS disease manifestations in this model, and when these NOD-derived loci are expressed in healthy C57BL/6 mice the animals develop pSS [32]. Microarray studies in this strain, termed C57BL/6.NOD-*Aec1Aec2*, revealed that several genes associated with innate immunity were dysregulated in SMG tissue early in the course of disease, including those related to *TLR3* activation [12]. Therefore, these data suggest multiple TLR pathways are integral to pSS, and activation of these signaling networks may represent an initial event in pSS disease pathogenesis.

Additional corroborative work demonstrates that ligation of TLR3, TLR4, or TLR5 in healthy mice results in development of a pSS-like disease. Specifically, when C57BL/6 mice were treated with the TLR3 agonist polyinosinic:polycytidylic acid (poly(I:C)) or the TLR4 agonist lipopolysaccharide (LPS), the mice exhibited reduced salivary and lacrimal flow and displayed increased expression of pro-inflammatory cytokines in lacrimal and SMG tissue [33–35]. Moreover, C57BL/6 mice treated with the TLR5 agonist FliC developed salivary inflammation and exhibited elevated serum levels of IgG and anti-Ro (SSA) autoantibodies [36]. In addition, agonism of TLR3 accelerated disease in the SS-susceptible New Zealand Black (NZB)/WF1 mouse model [33, 37], as TLR3 ligation resulted in upregulation of type I IFN and IFN-responsive genes and cytokines in SMG tissue and also reduced saliva production. Since lymphocytic infiltrates were not observed in the salivary tissue following this treatment, TLR3 activation of innate immune cells and salivary epithelial cells is likely to be an important source of exocrine inflammation in this model [37]. Thus, several lines of evidence from both pSS and sSS mouse models demonstrate the importance of TLR signaling in disease.

5.2.2 Human Studies Corroborate Findings in Mouse Models

Of significant clinical relevance, tissue derived from patients with pSS demonstrates dysregulation of numerous TLRs in both peripheral blood and salivary tissue [26]. Accordingly, salivary tissue from pSS patients shows increased expression of TLR1, TLR2, TLR4, and TLR6 and peripheral blood cells from pSS patients are hyper-responsive to TLR2, TLR4, and TLR6 agonism as compared to healthy controls [38, 39].

Moreover, salivary gland epithelial cells (SGECs) from pSS patients express functional TLR3 [39]. Ligation of TLR3 increases expression of TLR3 as well as co-stimulatory molecules [39–41]. Furthermore, minor salivary gland (MSG) biopsies from pSS patients show increased levels of a signaling intermediate employed by TLR3, RIPK3 kinase [41]. TLR3 ligation also promotes apoptosis in salivary tissue derived from both healthy controls and pSS patients [40–42]. Finally, stimulation of salivary cells with a TLR3 agonist induces secretion of BAFF [43], a potent mediator of B cell survival that plays a pivotal role in pSS disease [44, 45].

Finally, altered expression and activation of TLR7, TLR8, and TLR9 is described both locally and systemically in pSS patients [43, 46–48]. Expression of TLR7 and TLR9 is described in the epithelial cells and lymphocytes in pSS parotid glands [46]. Furthermore, studies in pSS salivary tissue reveal higher levels of *TLR7* and *TLR9* in the parotid and elevated *TLR8* and *TLR9* in MSG tissue as compared to controls [46, 49]. Moreover, TLRs are dysregulated in peripheral blood from pSS patients. Of particular relevance to the innate immune response, CD14+ monocytes derived from pSS patients with a positive IFN gene signature show elevated *TLR7* expression and decreased *TLR9* expression [47]. In addition, stimulation of DCs derived from pSS patients with the TLR7/8 agonist CL097 drives

enhanced maturation of this population [50]. Of note, TLR7 promotes inflammation in the related autoimmune disease, SLE, while TLR9 activation ameliorates this inflammatory phenotype [51], although it is unclear whether this paradigm extends to pSS as well. Taken together, these studies demonstrate a compelling role for innate immune activation via TLRs in the context of pSS, although the disease-relevant ligands and the pathologic consequences of activation of these receptors in specific tissues remain poorly understood.

5.2.3 Inflammasome Activation Is Implicated in pSS

The inflammasome is a large heteromeric protein complex that mediates innate immune defense [52]. Although there are several types of inflammasomes, the NLRP3 and AIM2 inflammasomes are the best studied in the context of autoimmunity to date [53]. Inflammasomes are activated by PAMPs and damage-associated molecular patterns (DAMPs) through both cell-surface and cytosolic receptors [53, 54]. Secretion of the pro-inflammatory cytokines IL-1β and IL-18 is a hallmark of inflammasome activation and these cytokines are elevated both locally and systemically in pSS mouse models and patients [53, 55–59]. One of the ways in which inflammasome activation occurs is through $P2X_7$ receptor ($P2X_7R$) signaling. The $P2X_7R$ is a ligand (ATP)-gated ionotropic P2XR that is expressed ubiquitously and is activated by ATP that is released extracellularly as a consequence of cellular damage [60]. This receptor is considered to be one of the most potent activators of the NLRP3 inflammasome and activation of this complex results in secretion of IL-1β and IL-18 [60].

Agonism of the $P2X_7R$ in salivary epithelium contributes to inflammation in the context of pSS. Significantly, the $P2X_7R$ plays a central role in salivary gland inflammation, as studies in mice reveal that the highly specific $P2X_7R$ agonist, Bz-ATP, induces robust sialadenitis [61]. Moreover, recent work in another pSS model (CD28−/− IFN γ−/− NOD.H-2^{h4}) showed $P2X_7R$ antagonism decreased salivary inflammation and protected against loss of salivary flow [62]. In addition, when the *$P2X_7R$* was deleted in *IL-14α* transgenic mice, another well-established model of pSS, animals showed reduced B and T cell infiltration in the salivary tissue [63].

This work is highly relevant to the human disease, as $P2X_7R$ expression is increased in pSS salivary tissue and polymorphisms in *$P2X_7R$* are identified in pSS patients [56, 64]. In addition, the inflammasome components *NLRP3*, *ASC*, and *Caspase-1* are elevated in MSG biopsies from pSS patients as compared to those of healthy controls [56]. Furthermore, IL-18 is increased in pSS saliva samples, providing corroborative evidence for activation of $P2X_7R$-mediated signaling in pSS salivary tissue [56]. In separate studies, both IL-1β and IL-18 were elevated in the sera and saliva of pSS patients [57, 65]. One study that stratified pSS patients by lymphoma risk found patients at high risk of developing lymphoma had higher IL-1β and IL-18 levels as compared to healthy controls [66]. Moreover, pSS patients with lymphoma had higher IL-18 levels than healthy controls and those that were at risk for lymphoma development [66].

Significantly, data indicate that inflammasome activation has important implications for development of B cell lymphoma in SS, as *$P2X_7R$*, *NLRP3*, *Caspase-1*, and *IL-18* expression increased with the number of lymphocytic foci and were higher in patients who developed non-Hodgkin's lymphoma during the course of the study [67]. Furthermore, SS patients who developed lymphoma had a threefold higher glandular expression of IL-18 than patients with SS alone [67]. While further studies are needed to determine whether inflammasome activation is an early event in lymphoma development, these results suggest that IL-18 may have utility as a biomarker for lymphoma in SS patients.

In addition to $P2X_7R$-mediated inflammasome activation, elegant work demonstrates pSS patients who are at high risk of lymphoma and those that have lymphoma manifest high levels of cell-free DNA (cf-DNA) associated with impaired DNase I activity [66]. Cell-free DNA is a known inflammasome agonist and these subsets

of pSS patients accumulate cf-DNA in the sera, salivary glands, and peripheral blood. In addition, pSS patients show increased expression of inflammasome-related proteins in sera [66]. Furthermore, pSS patients with lymphoma display more robust inflammasome dysregulation and higher cf-DNA levels than pSS patients who do not have lymphoma [66]. Taken together, this work suggests that inflammasome activation is an important disease mechanism that contributes to chronic inflammation and lymphoma development in pSS patients.

5.2.4 Emerging Work Describes a Role for Additional Innate Immune Sensors in pSS Pathogenesis

Activation of the STING pathway is emerging as an important disease mechanism in autoimmunity [68]. Cyclic GMP-AMP synthase (cGAS) is a PRR that binds to cytosolic DNA [68]. Upon ligation, cGAS mediates upregulation of inflammatory mediators, including type I IFNs, through engagement of STING [68]. Studies in a murine model provide compelling evidence that STING activation contributes to pSS pathogenesis. Indeed, treatment of C57BL/6 mice with the highly specific STING agonist dimethylxanthenone-4-acetic acid (DMXAA) resulted in focal lymphocytic sialadenitis along with decreased salivary production [69]. Moreover, DMXAA agonism led to enhanced expression of *IFNβ1*, *IL-6*, and *TNFa* in salivary gland tissue and these cytokines were also found in increased quantities in the sera [69]. Importantly, *IFNγ* was elevated in salivary tissue following DMXAA treatment and elegant work revealed that salivary Type I ILCs were expanded and identified this population as the primary source of IFNγ in SMG tissue in disease [69]. Of note, numerous PRRs, including STING, activate TANK-binding kinase-1 (TBK1) to mediate downstream signaling outcomes, ultimately resulting in induction of IFN-stimulated genes [70]. Increased phosphorylation of TBK1 was observed in plasmacytoid DCs (pDCs) derived from IFN-positive pSS patients as compared to healthy controls [70], providing support for a role for STING activation in the human disease.

IFN-inducible protein 16 (IFI16) is innate immune sensor that forms filamentous oligomers when activated by double-stranded DNA (dsDNA) [71]. Emerging work identified IFI16 filaments in salivary ductal epithelial cells and found that autoantibodies from pSS patients bind to IFI16 more efficiently in its filamentous state [71]. Finally, the RNA-sensing PRRs retinoic acid inducible gene 1 protein I (RIG-I) and melanoma-differentiation-associated gene 5 (MDA5) are dysregulated in the context of pSS [47]. Both proteins recognize viral nucleic acids [72–75]. A study using monocytes derived from pSS patients who were classified according to their IFN signature found that both RIG-I and MDA5 were upregulated in the pDCs and monocytes of IFN-positive patients [47]. Immunohistochemical studies of pSS salivary tissue revealed strong expression of both RIG-I and MDA5 within the lymphocytic foci [47]. Altogether, these data provide corroborative evidence that ligation of innate immune sensors by DNA derived from viral infection or damaged host cells could be an underlying disease mechanism driving pSS development.

Finally, the receptor tyrosine kinases Tyro-3, Axl, and Mer (collectively referred to as TAM receptors) are integral to many diverse cellular processes, including phagocytosis, inflammation, and development [76]. TAM receptors are expressed on many different cell types, including monocytes and macrophages [76]. These receptors play an essential role in mitigating excessive inflammatory responses through inhibition of type I IFN and TLR-mediated signals and by facilitating phagocytosis of apoptotic cells [77]. TAM receptors are implicated in numerous autoimmune diseases but remain poorly understood in pSS [76, 78]. However, since pSS is characterized by excessive IFN and TLR-mediated signaling and enhanced apoptosis is observed in pSS salivary tissue, TAM receptor activation likely contributes to disease [26, 78, 79]. Work done in a pSS mouse model at the clinical disease stage identifies dysregulation in TAM receptor genes and associated ligands within salivary tissue [78]. Thus, accumulating evidence points to a role for

numerous types of innate immune sensors in pSS and further studies are required to determine whether targeting of these may represent novel therapeutic approaches in disease.

There are many different innate immune populations that mediate pSS disease pathogenesis in both mice and humans, including DCs, macrophages, NK cells, ILCs, and SGECs. Since the role of these cells in disease have been covered extensively in recent reviews [1, 80], a concise summary of the role of each cell type in both murine and human disease is provided in Tables 5.1 and 5.2, respectively.

Table 5.1 Innate immune cells mediate pSS pathogenesis in murine models

Cell type	Tissue	Pathogenic finding	Mouse model
DCs	• SMG • Lacrimal gland	• Increased DCs in early inflammatory infiltrates following treatment with a TLR3 agonist [81] • Salivary DCs exhibit diminished co-stimulatory molecule expression [82] • CD11c + cells increase in SMG tissue with disease progression [83] • Salivary gland hypofunction associated with pDC accumulation in SMG [84] • pDCs modulate autoreactivity to the RNA-binding nuclear antigen La [85] • Ocular surface DCs are required for autoreactive T cell-mediated lacrimal keratoconjunctivitis [86]	• NZB/WF1 [81] • C57BL/6.NOD-*Aec1/Aec2* [82] • NOD/ShiLtJ [83] • Immunized (NZB x NZW)F1 [84] • Adoptive transfer of human La-specific CD4+ T cells [85] • Adoptive transfer of T cells using desiccating stress model [86]
Macrophages	• SMG • WAT • Lacrimal glands	• CCL22-resident macrophages increased in SMG, CCL22 induced T cell migration and IFNγ production [87] • Proportion and absolute numbers of macrophages higher in WAT; macrophages increased in cervical WAT around SMG. Elevated MCP-1 seen in SMG and WAT [88] • CD68+ cells increase in SMG tissue with disease progression [83] • Systemic depletion of macrophages resulted in improved tear secretion and ocular surface integrity [89]	• Thymectomized NFS/*sld* [87] • ArKO [88] • NOD/ShiLtJ [83] • *Aire*-deficient [89]
NK cells	• SMG	• NK cell-mediated apoptosis of CD4+ T cells [90] • Increased NK cells in early inflammatory infiltrates following treatment with a TLR3 agonist [81]	• mCMV model [90] • NZB/WF1 [81]
Glandular epithelial cells	• SMG • Lacrimal tissue • SGECs	• *TLR1*, *TLR2*, *TLR3*, *TLR4*, *TLR7*, *TLR9,* and *MyD88* expression increases with disease progression [9] • Salivary epithelial cells express TLR3 [37] • SGECs overexpress miR-146a and have diminished CD80 expression [82] • TLR3 agonism induces innate immune response in lacrimal gland tissue [35] • SGECs express elevated levels of co-stimulatory molecules and inflammatory cytokines [37, 91, 92] • Salivary epithelial cells express functional $P2X_7R$ [61, 62] • Lacrimal epithelial cells that lack IκB-ζ demonstrate SS-like inflammation [93]	• NOD/ShiLt [9, 92] • NZB/WF1 [37] • C57BL/6.NOD-*Aec1/Aec2* [82] • C57BL/6 [35] • RbAp48 Tg [91] • $P2X_7R$-deficient mice [61] • Tissue-specific ablation of *Nfkbiz* [93]
ILCs	• SMG	• Significant source of IFNγ in salivary tissue [69]	• DMXAA-treated C57BL/6 [69]

ArKO Aromatase knockout, *WAT* white adipose tissue, *RbAp48 Tg* retinoblastoma-associated protein 48 transgenic

Table 5.2 Dysregulation of the innate immune response is implicated in pSS in humans

Cell type	Tissue	Pathogenic finding in pSS
DCs	• Peripheral blood • MSG	• Circulating pDCs and moDCs secrete pro-inflammatory cytokines including IFNα in response to TLR agonists [50, 94–97] • Circulating pDC miRNA is dysregulated [98] • IFN-positive circulating pDCs have altered expression of innate immune sensors in [47] • pDCs and myeloid DCs are decreased in peripheral blood and increased in salivary tissue [99–101] • DCs accumulate with increased disease severity in salivary tissue [102] • Follicular DC networks observed in salivary tissue [103, 104]

Monocytes	• Peripheral blood
	• MSG
		• Peripheral CD14+ monocytes:
		  • Increased *TLR7* expression in IFN-positive patients; decreased *TLR9* expression in IFN-positive and negative patients [47]
		  • Show NLRP3 activation and heightened responsiveness to NLRP3 stimuli [66]
		  • Increased responsiveness to viral antigens following vaccination [105]
		• Increased salivary CD16+ monocytes [106]

Macrophages	• MSG	• Pro-inflammatory cytokine expression [58, 87] • Macrophage accumulation and expression of macrophage-associated genes correlate with disease severity [102, 107] • Exhibit evidence for NLRP3 activation and pyroptosis [66]
NK cells	• Peripheral blood • MSG	• Circulating levels of the NK-specific NCR3/NKp30 receptor increased in pSS patients [108] • NK cell accumulation in salivary tissue correlates with severity of loss of flow [108] • Significant source of IL-22 [109] • Decreased circulating NK cells [99]
Exocrine tissue	• pSS and control SGECs • MSG • A253 salivary gland cell line	• Increased TLR expression [38, 39, 41, 43, 46, 47] • Express functional TLRs [39, 41–43, 48, 80, 110, 111] • Co-stimulatory function [39, 112] • Pro-inflammatory cytokine secretion [58, 97, 112, 113] • Promote T cell differentiation [114] • Express functional ERs [115]
ILCs	• Peripheral blood	• ILC1 frequency in peripheral blood correlates with disease activity in pSS [116] • IFN-high pSS and SLE patients have higher levels of circulating ILC2s as compared to IFN-low patients [116]

MoDC monocyte-derived dendritic cell, *ER* estrogen receptor

5.3 Underlying Causes of Innate Immune Activation

5.3.1 PAMPs Contribute to the Development and Progression of pSS

Innate immunity is triggered by environmental stimuli such as viruses and bacteria and many microbial infections are implicated in autoimmune disease [117, 118]. Significantly, a subset of pSS patients display an IFN gene signature in both salivary tissue and peripheral blood, indicating dysregulation of genes that are modulated by IFNs [119–123]. Corroborative work in both mice and patients reveals high levels of both IFNα and IFNβ in salivary tissue and in peripheral blood [14, 49, 122, 124, 125]. Of note, pSS patients exposed to viral antigens through vaccination demonstrated heightened innate immune activation as compared to healthy controls [105]. It is well established that viral infections induce high levels of type 1 IFN, yet there are few studies that link viruses conclusively to pSS onset. However, several lines of evidence point to a role for cytomegalovirus (CMV), hepatitis delta virus (HDV), and Epstein–Barr virus (EBV) in the pathogenesis of pSS.

The role of CMV in pSS is somewhat controversial, as one study found innate immune activation by CMV may contribute to disease [126], although more recent work found either no differences in antibody titers to CMV between pSS patients and healthy controls [127] or that anti-CMV antibodies actually protect against SS development [128]. However, work in mice suggests CMV may be an etiologic agent in disease, as an autoimmune-prone model develops pSS-like disease more rapidly than controls when infected with murine CMV [129]. Thus, further work is needed to clarify whether CMV infection may be an etiologic agent in pSS disease development.

In addition, HDV may promote a pSS-like phenotype, as *HDV* transcripts were identified in 50% of pSS salivary tissue samples examined and expression of HDV antigens in murine salivary tissue resulted in reduced salivary flow, increased sialadenitis, and development of autoantibodies [130]. Interestingly, pSS patients with *HDV* transcript levels similar to healthy controls had more robust pSS manifestations than those with high levels, leading the authors of the study to hypothesize that detection of HDV may identify patients at early stages of disease [130].

Investigators have explored the link between EBV and pSS for many years [131]. Recent work found pSS patients had higher titers of antibodies to EBV [127] and antibodies against EBV were positively correlated with SS [128]. Furthermore, active EBV infection was identified in the tertiary lymphoid structures within salivary tissue of SS patients and autoreactive salivary B cells showed evidence of EBV infection [132]. Moreover, EBV reactivation was seen more commonly and was more strongly correlated with autoantibody positivity (anti-Ro and anti-La) and markers of B cell activation in pSS patients as compared to controls [133]. Finally, three separate studies suggest that EBV infection may promote lymphoma development in SS patients, although the total number of patients analyzed was small [134–136]. Thus, while viruses clearly mediate innate immune regulation in pSS, more work is needed to understand the role of viral infection in pSS development and progression.

Interestingly, a recent study found that history of microbial infection was associated with an increased risk of pSS [137]. This elegant case-control study examined cases of documented infection in 945 patients with pSS and 9048 controls using the Swedish National Patient Register [137]. Significantly, they found SS was preceded by an infection in 21% of pSS patients, while only 12% of matched controls had evidence of infection [137]. When patients were stratified according to the tissue-specific localization of infection, respiratory infections increased the risk of pSS regardless of autoantibody status, while urogenital and skin infections were positively associated with pSS development in patients who displayed anti-Ro and anti-La autoantibodies [137]. Taken together, these data suggest microbial insults and subsequent activation of the innate immune response may contribute to SS development in susceptible individuals.

5.3.2 Evidence for DAMPs in Innate Immune Activation in pSS

While many studies have focused on innate immune activation by PAMPs in the context of pSS (vide supra), there are a paucity of studies related to the significance of DAMP-mediated inflammation in disease. DAMPs are endogenous molecules that are generated as a consequence of tissue damage, thereby inducing "sterile" inflammation in the absence of microbial infection [138]. Many diverse ligands can act as DAMPs, including extra-cellular matrix (ECM) molecules, glycans, lipoproteins, and nucleic acids [139–141]. Chronic innate immune activation by DAMPs is a well-established disease mechanism in autoimmunity, including SLE, scleroderma, and RA [142–147]. While several studies implicate host-derived molecules in pSS, the specific DAMPs that drive disease and the relevant receptors and tissues that are activated by DAMPs remain to be elucidated [13, 26, 148]. As discussed previously (vide supra), diverse innate immune receptors are dysregulated in disease and most of these broadly recognize both DAMPs and PAMPs [139, 141, 149, 150].

In pSS, soluble ECM molecules that may serve as DAMPs are described within saliva and salivary cells, specifically biglycan, decorin, and versican [151, 152]. Moreover, fibronectin, calprotectin, and ectopically expressed salivary mucins may represent endogenous ligands that drive innate inflammation in the context of SS [153–159]. Finally, long interspersed nuclear element 1 (LINE-1 or L1) is a family of endogenous virus-like genomic retroelements that are increased in salivary tissue from pSS patients [95, 160]. Inappropriate expression of this retroelement activates innate immunity [161]. Significantly, L1 transcripts correlate with type I IFN in salivary tissue of pSS patients [95]. Moreover, L1 expression in pDCs or monocytes results in upregulation of *IFNα* and inhibition of TLR-7/TLR-8 reduced this expression in pDCs [95]. Thus, these data demonstrate the presence of DAMPs within salivary gland tissue and in the periphery may serve as chronic sources of inflammation in pSS. A summary of the innate immune receptors and putative ligands that are of relevance to pSS is provided in Fig. 5.1.

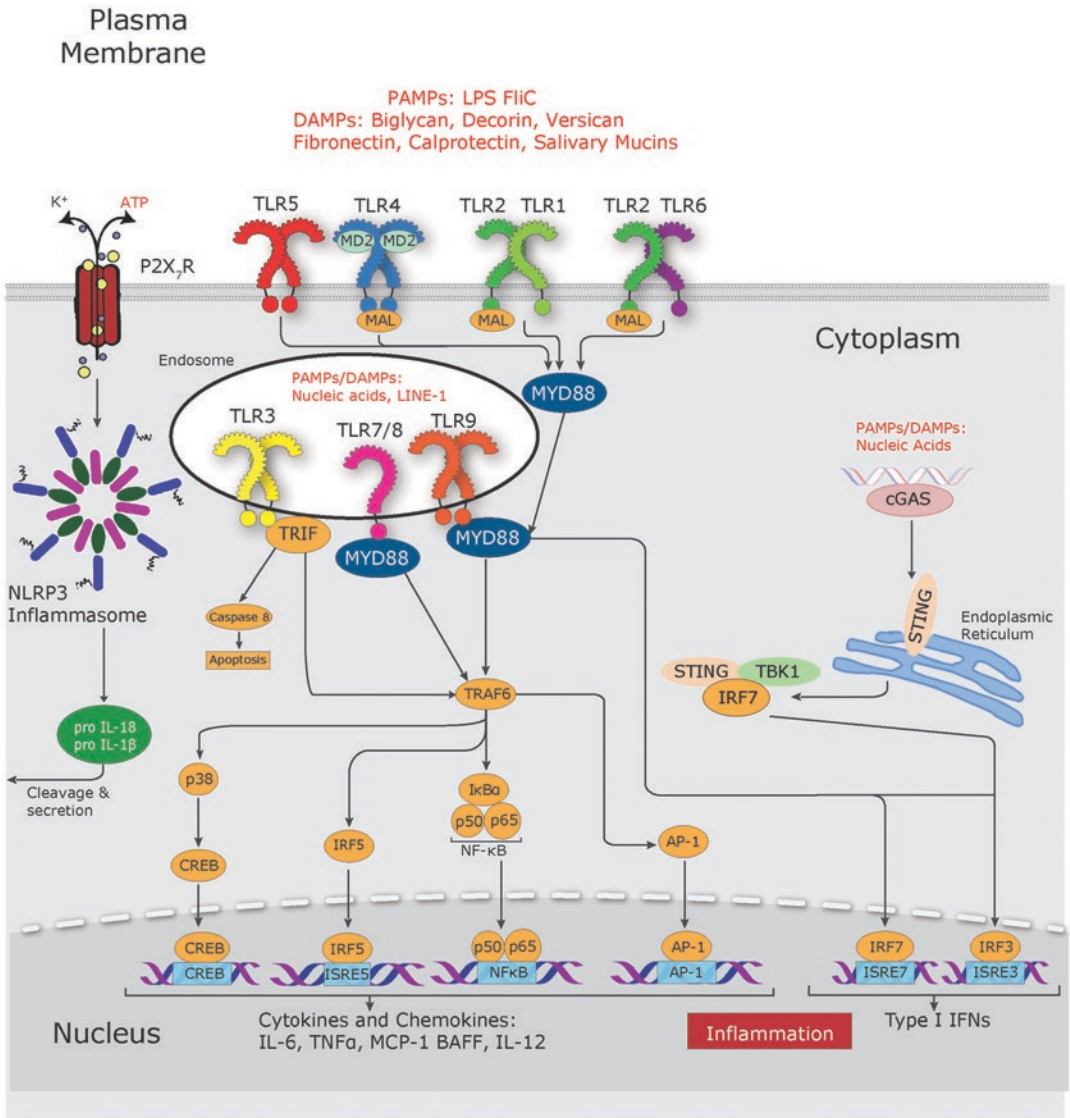

Fig. 5.1 Activation of innate immune sensors in pSS

5.3.3 Innate Immune Dysfunction Is Influenced by Genetics in pSS

While there are relatively few genetic studies conducted in pSS patients relative to other autoimmune conditions such as SLE or RA [162], several polymorphisms in genes related to innate immunity, and in IFN signaling in particular, are identified in pSS patients [163–166]. Indeed, a large-scale association study reported a strong association between polymorphisms in *IRF5-TNPO3*, *STAT4*, *IL-12A* and *TNIP1* and pSS disease [167]. IRF5 is a transcription factor that is expressed in B cells, monocyte, macrophages, and pDCs [168]. IRF5 mediates production of type I IFNs upon activation of PRRs by viral RNA and DNA [168]. IL-12 and STAT4 are also important in IFN-mediated responses, as many types of innate cells secrete IL-12 following PRR activation [169]. IL-12 activates a signaling cascade that relies on STAT4, leading to production of numerous inflammatory mediators, most notably IFNγ [169].

In contrast, TNIP1 is an important negative regulator of TLR-mediated inflammation [170]. Thus, decreased TNIP1 expression leads to heightened NFκB activity and hyperresponsiveness to TLR agonists [170]. Finally, a more recent study identified *OAS1* as a risk locus for pSS [165]. OAS1 is expressed by many different cell types (including monocytes) and is upregulated in response to type I IFNs during viral infection [165, 171]. The *OAS1* polymorphism observed in pSS patients is correlated with reduced enzymatic activity, potentially leading to impaired viral clearance [172]. Thus, while further work is needed to establish the way in which these polymorphisms shape the innate immune response in pSS, these studies provide evidence that underlying genetic susceptibility may contribute to the innate immune dysregulation observed in pSS.

In addition to genetic polymorphisms, alterations in epigenetic modifications of innate immune genes are described in pSS patients. Interestingly, a genome-wide methylation analysis performed in pSS MSG tissue found hypomethylation of *OAS2*, a gene that encodes an enzyme that functions in a similar manner as OAS1 [173]. Further work in salivary tissue from pSS patients and controls identified 57 genes that were enriched for differentially methylated positions. Many of these were associated with innate immunity, including the inflammasome-related gene *AIM2* [174]. Finally, a recently published study using peripheral blood of patients with SLE and pSS found a shared common DNA methylation pattern, although each disease also carried a distinct methylation gene signature [175]. Importantly, pathway analysis performed on the top 1000 unique genes annotated to the shared differentially methylated CpG sites between SLE and pSS patients revealed enrichment in genes associated with the innate immune system [175]. Thus, epigenetic changes likely play a key role in innate immune activation and function in pSS and further studies are warranted to understand how differential gene methylation in pSS governs innate immune activation.

Moreover, work examining regulation of innate immune function by microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) suggests an important role for these genetic regulatory mechanisms in pSS [176–178]. MiRNAs are small non-coding RNAs that bind complementary gene targets, resulting in post-translational silencing [179]. In addition, miRNAs target RNA for degradation and also mediate translational repression [180]. Thus, miRNAs have a profound effect on gene expression and miRNA dysregulation is linked to diverse pathoses, including autoimmunity [181]. Many studies demonstrate altered microRNA expression in innate immune cells in the context of pSS [82, 98, 182–185]. Of particular relevance, a recent study examined the miRNome of pDCs derived from pSS patients as compared to controls. The authors found levels of several microRNAs were altered in pSS, including those associated with apoptosis and type I IFN production [98]. In a separate study using type-2 conventional DCs, a miRNA that targets MSK-1 was increased in pSS patients as compared to healthy controls following TLR4 ligation [182]. MSK-1 likely contributes to inflammation in pSS, as this signaling intermediate controls production of IL-12, TNFα, and IL-6 [182]. Additional work

in mouse models and patients reveals an important role for miR-146a in the regulation of inflammation both locally and systemically in pSS [82, 184, 185]. Finally, miR-1248 expression in salivary epithelial cells downregulates ITPR3, a critical component of calcium signaling in salivary tissue [113]. Moreover, miR-1248 binds the RNA-sensing receptor RIG-1 and thereby induces IFNβ production in primary human salivary cells [113].

LncRNAs are also implicated in autoimmunity and play pivotal roles in the regulation of inflammation mediated by adaptive immune cells as well as DCs, macrophages, and monocytes [186]. LncRNAs control gene transcription by interactions with transcription factors and epigenetic modifiers, and also direct post-transcriptional events including mRNA stability and degradation [186]. A study using peripheral blood cells from pSS patients and healthy controls identified differential gene expression of numerous lncRNAs associated with innate immune function, including apoptosis, IFN signaling, and the inflammatory response [187]. Thus, accumulating evidence indicates an important role for both miRNAs and lncRNAs in innate immune activation in disease, and further studies will be instrumental in elucidating the role of these potent regulators of gene expression in pSS.

5.3.4 Sex Hormones May Contribute to the Striking Female Disease Prevalence Observed in pSS

Since pSS is seen predominately in women, many studies have focused on the role of estrogen and estrogen precursors in disease and we will review those reports that discuss the role of sex hormones as they relate to innate immune dysfunction in the context of pSS. Early work revealed that transgenic (Tg) expression of the retinoblastoma-associated protein 48 (RbAp48) induced estrogen deficiency-dependent apoptosis within exocrine tissue [188]. Specifically, RbAp48 overexpression in estrogen-deficient mice drives p53-mediated apoptosis in both salivary and lacrimal tissue [188]. In a corroborative study, the authors found mice developed an SS-like disease when RbAp48 was expressed under the control of the exocrine-specific parotid secretory protein promoter [91, 189]. Of relevance to innate immunity, lacrimal and salivary gland epithelial cells derived from RbAp48 Tg mice produced heightened IFNγ and IL-18. Moreover, RbAp48 Tg SGECs expressed elevated levels of MHC class II and the co-stimulatory molecules CD86, CD80, and ICAM-1. This expression carried functional significance, as RbAp48 Tg SGECs induced proliferation of CD4+ T cells in co-culture experiments [91]. Together, these data demonstrate a molecular mechanism by which estrogen deficiency drives tissue-specific overexpression of RbAp84, leading to an SS-like phenotype in mice [91].

More recent work used female Aromatase knockout (ArKO) mice to model estrogen deficiency, as aromatase is an enzyme that is required to convert androgens to estrogen [88, 190]. Interestingly, female ArKO mice developed an SS-like disease characterized by sialadenitis and autoantibody production [190]. When either ArKO splenocytes or bone marrow were transferred to *Rag2*-deficient mice that lacked adaptive immune cells, no inflammatory infiltrates were observed within the salivary or lacrimal glands of the recipient animals, suggesting an innate immune origin for these lesions [88]. Moreover, monocyte-chemotactic protein-1 (MCP-1) production by M1 macrophages within salivary glands and white adipose tissue (WAT) was increased in ArKO females as compared to controls [88]. Finally, treatment of pSS-prone Tx-NFS/*sld* mice with an aromatase inhibitor increased the severity of lymphocytic infiltration in both salivary and lacrimal tissue and resulted in an increase in CD11c+ DCs and F4/80+ macrophages in splenic tissue [88]. Thus, work in several murine models supports a role for estrogen deficiency in innate immune activation in pSS.

Significantly, studies using human salivary cells also demonstrate a role for estrogen in modulating the innate immune response [115]. Both estrogen receptor α (ERα) and ERβ are expressed in SGECs derived from MSG tissue [115]. These

receptors are functional, as estrogen treatment reduced IFNγ-inducible expression of the immuno-stimulatory molecule ICAM-1 [115]. This anti-inflammatory role of estrogen is corroborated by a recent study that examined over a thousand pSS patients and individuals with sicca symptoms in the absence of pSS [191]. The authors documented an association between pSS and lifetime sex hormone exposure. Indeed, women with pSS had reduced estrogen exposure and cumulative menstrual cycling as compared with sicca controls [191]. Therefore, work in both mouse models and pSS patients supports a role for estrogen deficiency in the pathogenesis of pSS, at least in part through attenuation of innate immune activation.

Recent findings in pSS and other autoimmune diseases point to an underlying genetic cause for the striking female predilection observed. Females express two X chromosomes and X-chromosome inactivation (XCI) is an important regulatory mechanism that allows for expression of only one allele [192]. Many genes that are essential for immunity are encoded on the X chromosome and abnormal XCI may lead to their aberrant expression [192]. Of relevance to pSS, both TLR7 and the endogenous retroelement L1 are located on the X chromosome and are known to escape XCI [193, 194]. The overexpression of these genes may contribute to the female disease predilection observed (vide supra) [195]. Thus, both genetic and hormonal factors that mediate innate immune function likely contribute to the striking female disease predilection observed in pSS.

5.3.5 The Microbiome Modulates the Innate Immune Response and Is Dysregulated in Autoimmunity

While the role of the microbiome in pSS pathogenesis remains incompletely understood, recent work demonstrates alterations in the microbiome in pSS patients in the oral cavity and in the gut [196–201], although a separate study found no difference in the salivary microbiome between pSS patients and those with salivary hypofunction unrelated to pSS [202]. While the role of the microbiome in innate immune activation in the context of pSS remains unclear [203], innate immune sensors are activated by PAMPs and microbial metabolites. Thus the microbiome likely plays a pivotal role in innate immune activation in both health and disease [204, 205]. Of particular relevance to pSS, the microbiota is altered by sex hormones and these have profound effects on the immune response in pSS (*vide supra*) [206–208].

Furthermore, emerging work demonstrates the importance of diet in shaping the microbiome and mediating innate immune activation in the context of autoimmunity. Indeed, *Lactobacillus reuteri* worsens lupus manifestations and disease severity is attenuated in animals that receive a starch-deficient diet [209]. Colonization with this bacteria resulted in increased pDCs and IFN production in lupus-prone TLR7.1 Tg mice, a lupus model in which TLR7 is overexpressed at levels between 8- and 16-fold higher than controls [209, 210]. Finally, disease severity is attenuated following blockade of TLR7, indicating an essential role for microbiome-induced activation of innate immune sensors in disease [209]. While future studies are needed to establish the molecular mechanisms that underlie microbial dysbiosis and innate immune activation in autoimmunity, alterations in the microbiome likely have profound effects on the development and chronicity of pSS.

5.4 Current and Emerging Therapeutics That Target Innate Immune Activation in pSS

5.4.1 Hydroxychloroquine (HCQ) Blocks Endosomal-Mediated TLR Signaling

HCQ is commonly used to manage pSS symptoms, particularly arthralgia, myalgia, and fatigue [211]. HCQ inhibits innate immune activation,

although the exact molecular targets of HCQ remain incompletely understood [212]. HCQ is an inhibitor of endosomal and lysosomal acidification and therefore blocks many different cellular processes including endosomal TLR signaling and autophagy [213, 214]. It is postulated that HCQ may also inhibit TLR activation by directly interacting with nucleic acids, thereby altering their structure in such a way that they are no longer recognized by cognate TLRs [28]. HCQ is thought to exert its effects primarily on macrophages and DCs [212]. Importantly, antigen presentation is also decreased by HCQ treatment, as this process requires a functional endocytic compartment [212].

Several recent studies have focused on HCQ for the management of pSS patients. In a randomized placebo-controlled trial of 77 pSS patients, HCQ reduced IFN scores and IFN-stimulated gene expression [215]. However, there was no change in the EULAR disease activity score or IgG and IgM antibody titers [215]. A separate study of 221 pSS patients reported HCQ reduced the incidence of extraglandular manifestations, although the authors noted that larger patient cohorts were required to confirm these findings [216]. Finally, a systematic review and meta-analysis of 4 trials that included 215 pSS patients in total found that HCQ-treated patients did not differ from placebo controls in terms of either objective or subjective reports of dry eyes and dry mouth [211]. Similar to the aforementioned study, the authors concluded that well-designed randomized control trials were needed to establish whether HCQ is efficacious in mitigating exocrine-specific and systemic pSS disease manifestations [211].

5.4.2 Blockade of TLRs Pathways Shows Promise in Clinical Trials

Several drugs are in clinical trials that block TLRs or TLR-activated signaling cascades in autoimmunity [217–219] and these are reviewed elsewhere [26, 220, 221]. One of the more recent drugs that inhibits innate immune activation is RSLV-132, a novel biologic that consists of a human RNase fused to the Fc portion of IgG1 [222]. This fusion protein is able to digest circulating RNA enzymatically, thereby preventing it from activating innate immune sensors (vide supra) [222]. A phase 1b randomized, placebo-controlled study with 32 SLE patients that evaluated the safety and tolerability of this treatment found no increase in serious adverse events as compared to controls, and results from this study suggest RSLV-132 may reduce the IFN signature in SLE [222]. Phase II clinical trials are ongoing in SLE patients [223, 224]. Given the early promising results in SLE, this biologic may also have efficacy in pSS patients, particularly those that are IFN-positive.

5.4.3 Cytokine Inhibitors Mitigate Innate Immune Activation

Numerous biologics that target pro-inflammatory cytokines or their cognate receptors integral to the innate immune response are either in clinical trials or approved for the treatment of autoimmunity [80, 225, 226], although none of these is approved for the treatment of pSS specifically. Ongoing clinical trials in pSS patients demonstrate that cytokine blockade is efficacious in pSS, at least in part through the attenuation of innate immune response [227]. In particular, therapies that block signals mediated by BAFF or IL-1β currently show promise for improving local and systemic pSS disease manifestations [228–232]. Moreover, many cytokine-targeted therapies that modulate innate immune activation are being explored in other autoimmune conditions and are likely to have utility in the management of pSS. These include biologics that target IL-1 family members, IL-6, IL-12, IL-17A, IL-22, IL-23 and type I IFNs [226, 227, 233]. Therapies directed against specific cytokines and receptors will likely mitigate exocrine-specific and systemic disease manifestations in pSS that are associated with aberrant innate immune activation.

5.4.4 Depletion of pDCs May Have Therapeutic Efficacy in pSS

Since pDCs are implicated in autoimmunity in both mice and humans [85, 94, 96], a novel therapeutic (termed VIB7734) is currently in phase I clinical trials that depletes this population specifically. An ongoing multi-center clinical trial is being conducted in patients with many different autoimmune conditions, including SS, to determine the safety and tolerability of the drug [234]. VIB7734 binds immunoglobulin-like transcript 7 (ILT7), a cell-surface molecule that is restricted to pDCs [234–236]. Depletion of pDCs in pSS may lead to the reduction of pro-inflammatory cytokine production both locally and systemically, particularly in patients who display an IFN gene signature (Table 5.2).

5.4.5 Targeting of the Microbiome May Represent a Novel Therapeutic Approach to Diminish Innate Immune Activation in pSS

Since the microbiome plays a central role in shaping the immune response, therapeutics that seek to modify the composition of the microbiota will likely be efficacious in the treatment of autoimmunity. Specifically, administration of pre- and probiotics and fecal transplantation may attenuate innate immune activation in disease [237]. Clinical trials to examine the efficacy of fecal transplant in several autoimmune conditions are being conducted, including MS, type I diabetes and SS. A phase I trial is ongoing in SS patients to evaluate the efficacy of FMP30, a drug that contains frozen human fecal microbiota [238]. Participants will be screened to determine whether their gut microbiota is skewed to resemble that of the donor at a 3-month time point post-treatment [238]. Subjects will also be screened for improvements in exocrine-specific and systemic disease manifestations [238]. Additional work focuses on the use of prebiotics and probiotics in MS and type I diabetes [239, 240], although this has not been tested in the context of pSS to date. Thus, understanding the ways in which the microbiome shapes the immune response will lead to novel approaches that use microbial-derived products to alter the patient's microbiome in order to mitigate self-directed immune responses.

5.5 Conclusion

In summary, activation of the innate immune system is complex and multifactorial. In the context of pSS, innate immune sensors that bind a wide array of host and microbial-derived ligands are implicated in disease. Many factors contribute to innate immune activation in pSS, such as genetics, sex, microbial infection, and the microbiome. Development of diagnostics and therapeutics should consider the diverse factors that contribute to disease susceptibility in pSS. Finally, a more sophisticated understanding of how the innate and adaptive arms of the immune system coalesce to orchestrate chronic inflammation in this disease will allow us to target specific pathways that drive disease, thereby mitigating both exocrine-specific and systemic disease manifestations.

References

1. Mavragani CP, Moutsopoulos HM. Sjogren's syndrome. Annu Rev Pathol. 2014;9:273–85.
2. Patel R, Shahane A. The epidemiology of Sjogren's syndrome. Clin Epidemiol. 2014;6:247–55.
3. Verstappen GM, Kroese FGM, Bootsma H. T cells in primary Sjogren's syndrome: targets for early intervention. Rheumatology (Oxford). 2019.
4. Nocturne G, Mariette X. B cells in the pathogenesis of primary Sjogren syndrome. Nat Rev Rheumatol. 2018;14(3):133–45.
5. Delaleu N, Nguyen CQ, Peck AB, Jonsson R. Sjögren's syndrome: studying the disease in mice. Arthritis Res Ther. 2011;13(3):217.
6. Cha S, Peck AB, Humphreys-Beher MG. Progress in understanding autoimmune exocrinopathy using the non-obese diabetic mouse: an update. Crit Rev Oral Biol Med. 2002;13(1):5–16.
7. Kiripolsky J, Shen L, Liang Y, Li A, Suresh L, Lian Y, et al. Systemic manifestations of primary Sjogren's syndrome in the NOD.B10Sn-H2b/J mouse model. Clin Immunol. 2017;183:225–32.

8. Park YS, Gauna AE, Cha S. Mouse models of primary Sjogren's syndrome. Curr Pharm Des. 2015;21(18):2350–64.
9. Shimizu S, Kurashige Y, Nishimura M, Yamazaki M, Sato J, Saitoh M, et al. Involvement of toll-like receptors in autoimmune sialoadenitis of the non-obese diabetic mouse. J Oral Pathol Med. 2012;41(7):517–23.
10. Nguyen CQ, Sharma A, Lee BH, She JX, McIndoe RA, Peck AB. Differential gene expression in the salivary gland during development and onset of xerostomia in Sjögren's syndrome-like disease of the C57BL/6.NOD-Aec1Aec2 mouse. Arthritis Res Ther. 2009;11(2):R56.
11. Nguyen CQ, Sharma A, She JX, McIndoe RA, Peck AB. Differential gene expressions in the lacrimal gland during development and onset of keratoconjunctivitis sicca in Sjogren's syndrome (SJS)-like disease of the C57BL/6.NOD-Aec1Aec2 mouse. Exp Eye Res. 2009;88(3):398–409.
12. Killedar SJ, Eckenrode SE, McIndoe RA, She JX, Nguyen CQ, Peck AB, et al. Early pathogenic events associated with Sjogren's syndrome (SjS)-like disease of the NOD mouse using microarray analysis. Lab Invest. 2006;86(12):1243–60.
13. Kramer JM. Early events in Sjogren's syndrome pathogenesis: the importance of innate immunity in disease initiation. Cytokine. 2014;67(2):92–101.
14. Yao Y, Liu Z, Jallal B, Shen N, Ronnblom L. Type I interferons in Sjogren's syndrome. Autoimmun Rev. 2013;12(5):558–66.
15. Li H, Ice JA, Lessard CJ, Sivils KL. Interferons in Sjogren's syndrome: genes, mechanisms, and effects. Front Immunol. 2013;4:290.
16. Uematsu S, Akira S. Toll-like receptors and type I interferons. J Biol Chem. 2007;282(21):15319–23.
17. Bai J, Liu F. The cGAS-cGAMP-STING pathway: a molecular link between immunity and metabolism. Diabetes. 2019;68(6):1099–108.
18. Nuka E, Ohnishi K, Terao J, Kawai Y. ATP/P2X7 receptor signaling as a potential anti-inflammatory target of natural polyphenols. PLoS One. 2018;13(9):e0204229.
19. Medzhitov R, Janeway C Jr. The toll receptor family and microbial recognition. Trends Microbiol. 2000;8(10):452–6.
20. O'Neill LA, Golenbock D, Bowie AG. The history of toll-like receptors—redefining innate immunity. Nat Rev Immunol. 2013;13(6):453–60.
21. Balka KR, De Nardo D. Understanding early TLR signaling through the Myddosome. J Leukoc Biol. 2019;105(2):339–51.
22. Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell. 1998;2(2):253–8.
23. Kawai T, Akira S. Toll-like receptor downstream signaling. Arthritis Res Ther. 2005;7(1):12–9.
24. Marshak-Rothstein A. Toll-like receptors in systemic autoimmune disease. Nat Rev Immunol. 2006;6:823.
25. Mohammad Hosseini A, Majidi J, Baradaran B, Yousefi M. Toll-like receptors in the pathogenesis of autoimmune diseases. Adv Pharm Bull. 2015;5(Suppl 1):605–14.
26. Kiripolsky J, Kramer JM. Current and emerging evidence for toll-like receptor activation in Sjogren's syndrome. J Immunol Res. 2018;2018:1246818.
27. Humphreys-Beher MG, Hu Y, Nakagawa Y, Wang PL, Purushotham KR. Utilization of the non-obese diabetic (NOD) mouse as an animal model for the study of secondary Sjogren's syndrome. Adv Exp Med Biol. 1994;350:631–6.
28. Kuznik A, Bencina M, Svajger U, Jeras M, Rozman B, Jerala R. Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. J Immunol. 2011;186(8):4794–804.
29. Wicker LS, Appel MC, Dotta F, Pressey A, Miller BJ, DeLarato NH, et al. Autoimmune syndromes in major histocompatibility complex (MHC) congenic strains of nonobese diabetic (NOD) mice. The NOD MHC is dominant for insulitis and cyclophosphamide-induced diabetes. J Exp Med. 1992;176(1):67–77.
30. Robinson CP, Yamachika S, Bounous DI, Brayer J, Jonsson R, Holmdahl R, et al. A novel NOD-derived murine model of primary Sjogren's syndrome. Arthritis Rheum. 1998;41(1):150–6.
31. Kiripolsky J, McCabe LG, Gaile DP, Kramer JM. Myd88 is required for disease development in a primary Sjogren's syndrome mouse model. J Leukoc Biol. 2017;102(6):1411–20.
32. Cha S, Nagashima H, Brown VB, Peck AB, Humphreys-Beher MG. Two NOD Idd-associated intervals contribute synergistically to the development of autoimmune exocrinopathy (Sjogren's syndrome) on a healthy murine background. Arthritis Rheum. 2002;46(5):1390–8.
33. Nandula SR, Dey P, Corbin KL, Nunemaker CS, Bagavant H, Deshmukh US. Salivary gland hypofunction induced by activation of innate immunity is dependent on type I interferon signaling. J Oral Pathol Med. 2013;42(1):66–72.
34. Wang CS, Wee Y, Yang CH, Melvin JE, Baker OJ. ALX/FPR2 modulates anti-inflammatory responses in mouse submandibular gland. Sci Rep. 2016;6:24244.
35. Zhou J, Jin JO, Du J, Yu Q. Innate immune signaling induces IL-7 production, early inflammatory responses, and Sjogren's-like Dacryoadenitis in C57BL/6 mice. Invest Ophthalmol Vis Sci. 2015;56(13):7831–8.
36. Higuchi T, Haruta I, Shibata N, Yanagisawa N, Yagi J. Flagellar filament structural protein induces Sjogren's syndrome-like sialadenitis in mice. Oral Dis. 2017;23(5):636–43.
37. Deshmukh US, Nandula SR, Thimmalapura PR, Scindia YM, Bagavant H. Activation of innate immune responses through toll-like receptor 3 causes a rapid loss of salivary gland function. J Oral Pathol Med. 2009;38(1):42–7.

38. Kwok SK, Cho ML, Her YM, Oh HJ, Park MK, Lee SY, et al. TLR2 ligation induces the production of IL-23/IL-17 via IL-6, STAT3 and NF-kB pathway in patients with primary Sjogren's syndrome. Arthritis Res Ther. 2012;14(2):R64.
39. Spachidou MP, Bourazopoulou E, Maratheftis CI, Kapsogeorgou EK, Moutsopoulos HM, Tzioufas AG, et al. Expression of functional toll-like receptors by salivary gland epithelial cells: increased mRNA expression in cells derived from patients with primary Sjögren's syndrome. Clin Exp Immunol. 2007;147(3):497–503.
40. Nakamura H, Horai Y, Suzuki T, Okada A, Ichinose K, Yamasaki S, et al. TLR3-mediated apoptosis and activation of phosphorylated Akt in the salivary gland epithelial cells of primary Sjögren's syndrome patients. Rheumatol Int. 2013;33(2):441–50.
41. Horai Y, Nakamura H, Nakashima Y, Hayashi T, Kawakami A. Analysis of the downstream mediators of toll-like receptor 3-induced apoptosis in labial salivary glands in patients with Sjogren's syndrome. Mod Rheumatol. 2016;26(1):99–104.
42. Manoussakis MN, Spachidou MP, Maratheftis CI. Salivary epithelial cells from Sjogren's syndrome patients are highly sensitive to anoikis induced by TLR-3 ligation. J Autoimmun. 2010;35(3):212–8.
43. Ittah M, Miceli-Richard C, Gottenberg JE, Sellam J, Eid P, Lebon P, et al. Viruses induce high expression of BAFF by salivary gland epithelial cells through TLR- and type-I IFN-dependent and -independent pathways. Eur J Immunol. 2008;38(4):1058–64.
44. Groom J, Kalled SL, Cutler AH, Olson C, Woodcock SA, Schneider P, et al. Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren's syndrome. J Clin Invest. 2002;109(1):59–68.
45. Youinou P, Pers JO. Disturbance of cytokine networks in Sjogren's syndrome. Arthritis Res Ther. 2011;13(4):227.
46. Zheng L, Zhang Z, Yu C, Yang C. Expression of toll-like receptors 7, 8, and 9 in primary Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(6):844–50.
47. Maria NI, Steenwijk EC, As IJ, van Helden-Meeuwsen CG, Vogelsang P, Beumer W, et al. Contrasting expression pattern of RNA-sensing receptors TLR7, RIG-I and MDA5 in interferon-positive and interferon-negative patients with primary Sjogren's syndrome. Ann Rheum Dis. 2016;76(4):721–30.
48. Shimizu T, Nakamura H, Takatani A, Umeda M, Horai Y, Kurushima S, et al. Activation of toll-like receptor 7 signaling in labial salivary glands of primary Sjogren's syndrome patients. Clin Exp Immunol. 2019;196(1):39–51.
49. Gottenberg JE, Cagnard N, Lucchesi C, Letourneur F, Mistou S, Lazure T, et al. Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjögren's syndrome. Proc Natl Acad Sci U S A. 2006;103(8):2770–5.
50. Vogelsang P, Jonsson MV, Dalvin ST, Appel S. Role of dendritic cells in Sjogren's syndrome. Scand J Immunol. 2006;64(3):219–26.
51. Nickerson KM, Christensen SR, Shupe J, Kashgarian M, Kim D, Elkon K, et al. TLR9 regulates TLR7- and MyD88-dependent autoantibody production and disease in a murine model of lupus. J Immunol. 2010;184(4):1840–8.
52. Lamkanfi M, Dixit VM. Inflammasomes and their roles in health and disease. Annu Rev Cell Dev Biol. 2012;28:137–61.
53. Shin JI, Lee KH, Joo YH, Lee JM, Jeon J, Jung HJ, et al. Inflammasomes and autoimmune and rheumatic diseases: a comprehensive review. J Autoimmun. 2019;103:102299.
54. Platnich JM, Muruve DA. NOD-like receptors and inflammasomes: a review of their canonical and non-canonical signaling pathways. Arch Biochem Biophys. 2019;670:4–14.
55. Bulosan M, Pauley KM, Yo K, Chan EK, Katz J, Peck AB, et al. Inflammatory caspases are critical for enhanced cell death in the target tissue of Sjögren's syndrome before disease onset. Immunol Cell Biol. 2009;87(1):81–90.
56. Baldini C, Rossi C, Ferro F, Santini E, Seccia V, Donati V, et al. The P2X7 receptor-inflammasome complex has a role in modulating the inflammatory response in primary Sjögren's syndrome. J Intern Med. 2013.
57. Bombardieri M, Barone F, Pittoni V, Alessandri C, Conigliaro P, Blades MC, et al. Increased circulating levels and salivary gland expression of interleukin-18 in patients with Sjogren's syndrome: relationship with autoantibody production and lymphoid organization of the periductal inflammatory infiltrate. Arthritis Res Ther. 2004;6(5):R447–56.
58. Sakai A, Sugawara Y, Kuroishi T, Sasano T, Sugawara S. Identification of IL-18 and Th17 cells in salivary glands of patients with Sjögren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. J Immunol. 2008;181(4):2898–906.
59. Delaleu N, Immervoll H, Cornelius J, Jonsson R. Biomarker profiles in serum and saliva of experimental Sjogren's syndrome: associations with specific autoimmune manifestations. Arthritis Res Ther. 2008;10(1):R22.
60. Di Virgilio F, Dal Ben D, Sarti AC, Giuliani AL, Falzoni S. The P2X7 receptor in infection and inflammation. Immunity. 2017;47(1):15–31.
61. Woods LT, Camden JM, Batek JM, Petris MJ, Erb L, Weisman GA. P2X7 receptor activation induces inflammatory responses in salivary gland epithelium. Am J Physiol Cell Physiol. 2012;303(7):C790–801.
62. Khalafalla MG, Woods LT, Camden JM, Khan AA, Limesand KH, Petris MJ, et al. P2X7 receptor antagonism prevents IL-1beta release from salivary epithelial cells and reduces inflammation in a mouse model of autoimmune exocrinopathy. J Biol Chem. 2017.

63. Woods LT, Camden JM, Khalafalla MG, Petris MJ, Erb L, Ambrus JL Jr, et al. P2Y2 R deletion ameliorates sialadenitis in IL-14alpha-transgenic mice. Oral Dis. 2018;24(5):761–71.
64. Lester S, Stokes L, Skarratt KK, Gu BJ, Sivils KL, Lessard CJ, et al. Epistasis with HLA DR3 implicates the P2X7 receptor in the pathogenesis of primary Sjögren's syndrome. Arthritis Res Ther. 2013;15(4):R71.
65. Blokland SLM, Flessa CM, van Roon JAG, Mavragani CP. Emerging roles for chemokines and cytokines as orchestrators of immunopathology in Sjogren's syndrome. Rheumatology (Oxford). 2019.
66. Vakrakou AG, Boiu S, Ziakas PD, Xingi E, Boleti H, Manoussakis MN. Systemic activation of NLRP3 inflammasome in patients with severe primary Sjogren's syndrome fueled by inflammagenic DNA accumulations. J Autoimmun. 2018;91:23–33.
67. Baldini C, Santini E, Rossi C, Donati V, Solini A. The P2X7 receptor-NLRP3 inflammasome complex predicts the development of non-Hodgkin's lymphoma in Sjogren's syndrome: a prospective, observational, single-centre study. J Intern Med. 2017;282(2):175–86.
68. Kumar V. A STING to inflammation and autoimmunity. J Leukoc Biol. 2019;106(1):171–85.
69. Papinska J, Bagavant H, Gmyrek GB, Sroka M, Tummala S, Fitzgerald KA, et al. Activation of stimulator of interferon genes (STING) and Sjogren syndrome. J Dent Res. 2018;97(8):893–900.
70. Bodewes ILA, Huijser E, van Helden-Meeuwsen CG, Tas L, Huizinga R, Dalm V, et al. TBK1: a key regulator and potential treatment target for interferon positive Sjogren's syndrome, systemic lupus erythematosus and systemic sclerosis. J Autoimmun. 2018;91:97–102.
71. Antiochos B, Matyszewski M, Sohn J, Casciola-Rosen L, Rosen A. IFI16 filament formation in salivary epithelial cells shapes the anti-IFI16 immune response in Sjogren's syndrome. JCI Insight. 2018;3(18):e120179.
72. Zhao Y, Karijolich J. Know thyself: RIG-I-like receptor sensing of DNA virus infection. J Virol. 2019;93(23):e01085.
73. Chiu YH, Macmillan JB, Chen ZJ. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. Cell. 2009;138(3):576–91.
74. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol. 2004;5(7):730–7.
75. Kang DC, Gopalkrishnan RV, Wu Q, Jankowsky E, Pyle AM, Fisher PB. Mda-5: an interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. Proc Natl Acad Sci U S A. 2002;99(2):637–42.
76. Wium M, Paccez JD, Zerbini LF. The dual role of TAM receptors in autoimmune diseases and cancer: an overview. Cell. 2018;7(10):166.
77. Rothlin CV, Carrera-Silva EA, Bosurgi L, Ghosh S. TAM receptor signaling in immune homeostasis. Annu Rev Immunol. 2015;33:355–91.
78. Witas R, Peck AB, Ambrus JL, Nguyen CQ. Sjogren's syndrome and TAM receptors: a possible contribution to disease onset. J Immunol Res. 2019;2019:4813795.
79. Nguyen CQ, Peck AB. The interferon-signature of Sjogren's syndrome: how unique biomarkers can identify underlying inflammatory and Immunopathological mechanisms of specific diseases. Front Immunol. 2013;4:142.
80. Kiripolsky J, McCabe LG, Kramer JM. Innate immunity in Sjogren's syndrome. Clin Immunol. 2017;182:4–13.
81. Nandula SR, Scindia YM, Dey P, Bagavant H, Deshmukh US. Activation of innate immunity accelerates sialoadenitis in a mouse model for Sjögren's syndrome-like disease. Oral Dis. 2011;17(8):801–7.
82. Gauna AE, Park YJ, Nayar G, Onate M, Jin JO, Stewart CM, et al. Dysregulated co-stimulatory molecule expression in a Sjogren's syndrome mouse model with potential implications by microRNA-146a. Mol Immunol. 2015;68(2 Pt C):606–16.
83. Roescher N, Lodde BM, Vosters JL, Tak PP, Catalan MA, Illei GG, et al. Temporal changes in salivary glands of non-obese diabetic mice as a model for Sjogren's syndrome. Oral Dis. 2012;18(1):96–106.
84. Deshmukh US, Ohyama Y, Bagavant H, Guo X, Gaskin F, Fu SM. Inflammatory stimuli accelerate Sjogren's syndrome-like disease in (NZB x NZW) F1 mice. Arthritis Rheum. 2008;58(5):1318–23.
85. Pan ZJ, Horton CG, Lawrence C, Farris AD. Plasmacytoid dendritic cells and type 1 interferon promote peripheral expansion of forkhead box protein 3(+) regulatory T cells specific for the ubiquitous RNA-binding nuclear antigen La/Sjogren's syndrome (SS)-B. Clin Exp Immunol. 2016;186(1):18–29.
86. Schaumburg CS, Siemasko KF, De Paiva CS, Wheeler LA, Niederkorn JY, Pflugfelder SC, et al. Ocular surface APCs are necessary for autoreactive T cell-mediated experimental autoimmune lacrimal keratoconjunctivitis. J Immunol. 2011;187(7):3653–62.
87. Ushio A, Arakaki R, Otsuka K, Yamada A, Tsunematsu T, Kudo Y, et al. CCL22-producing resident macrophages enhance T cell response in Sjogren's syndrome. Front Immunol. 2018;9:2594.
88. Iwasa A, Arakaki R, Honma N, Ushio A, Yamada A, Kondo T, et al. Aromatase controls Sjogren syndrome-like lesions through monocyte chemotactic protein-1 in target organ and adipose tissue-associated macrophages. Am J Pathol. 2015;185(1):151–61.
89. Zhou D, Chen YT, Chen F, Gallup M, Vijmasi T, Bahrami AF, et al. Critical involvement of macrophage infiltration in the development of Sjögren's

syndrome-associated dry eye. Am J Pathol. 2012;181(3):753–60.
90. Schuster IS, Wikstrom ME, Brizard G, Coudert JD, Estcourt MJ, Manzur M, et al. TRAIL+ NK cells control CD4+ T cell responses during chronic viral infection to limit autoimmunity. Immunity. 2014;41(4):646–56.
91. Ishimaru N, Arakaki R, Yoshida S, Yamada A, Noji S, Hayashi Y. Expression of the retinoblastoma protein RbAp48 in exocrine glands leads to Sjogren's syndrome-like autoimmune exocrinopathy. J Exp Med. 2008;205(12):2915–27.
92. Kramer JM, Klimatcheva E, Rothstein TL. CXCL13 is elevated in Sjogren's syndrome in mice and humans and is implicated in disease pathogenesis. J Leukoc Biol. 2013;94(5):1079–89.
93. Okuma A, Hoshino K, Ohba T, Fukushi S, Aiba S, Akira S, et al. Enhanced apoptosis by disruption of the STAT3-IκB-ζ signaling pathway in epithelial cells induces Sjögren's syndrome-like autoimmune disease. Immunity. 2013.
94. Hillen MR, Pandit A, Blokland SLM, Hartgring SAY, Bekker CPJ, van der Heijden EHM, et al. Plasmacytoid DCs from patients with Sjogren's syndrome are transcriptionally primed for enhanced pro-inflammatory cytokine production. Front Immunol. 2019;10:2096.
95. Mavragani CP, Sagalovskiy I, Guo Q, Nezos A, Kapsogeorgou EK, Lu P, et al. Expression of long interspersed nuclear element 1 Retroelements and induction of type I interferon in patients with systemic autoimmune disease. Arthritis Rheumatol. 2016;68(11):2686–96.
96. Zhao J, Kubo S, Nakayamada S, Shimajiri S, Zhang X, Yamaoka K, et al. Association of plasmacytoid dendritic cells with B cell infiltration in minor salivary glands in patients with Sjogren's syndrome. Mod Rheumatol. 2016;26(5):716–24.
97. Ittah M, Miceli-Richard C, Lebon P, Pallier C, Lepajolec C, Mariette X. Induction of B cell-activating factor by viral infection is a general phenomenon, but the types of viruses and mechanisms depend on cell type. J Innate Immun. 2011;3(2):200–7.
98. Hillen MR, Chouri E, Wang M, Blokland SLM, Hartgring SAY, Concepcion AN, et al. Dysregulated miRNome of plasmacytoid dendritic cells from patients with Sjogren's syndrome is associated with processes at the centre of their function. Rheumatology (Oxford). 2019;58:2305–14.
99. van der Kroef M, van den Hoogen LL, Mertens JS, Blokland SLM, Haskett S, Devaprasad A, et al. Cytometry by time of flight identifies distinct signatures in patients with systemic sclerosis, systemic lupus erythematosus and Sjogrens syndrome. Eur J Immunol. 2019;50(1):119–29.
100. Ozaki Y, Ito T, Son Y, Amuro H, Shimamoto K, Sugimoto H, et al. Decrease of blood dendritic cells and increase of tissue-infiltrating dendritic cells are involved in the induction of Sjogren's syndrome but not in the maintenance. Clin Exp Immunol. 2010;159(3):315–26.
101. Vogelsang P, Brun JG, Oijordsbakken G, Skarstein K, Jonsson R, Appel S. Levels of plasmacytoid dendritic cells and type-2 myeloid dendritic cells are reduced in peripheral blood of patients with primary Sjogren's syndrome. Ann Rheum Dis. 2010;69(6):1235–8.
102. Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjögren's syndrome. J Autoimmun. 2010;34(4):400–7.
103. Jonsson MV, Skarstein K. Follicular dendritic cells confirm lymphoid organization in the minor salivary glands of primary Sjogren's syndrome. J Oral Pathol Med. 2008;37(9):515–21.
104. Bombardieri M, Barone F, Humby F, Kelly S, McGurk M, Morgan P, et al. Activation-induced cytidine deaminase expression in follicular dendritic cell networks and interfollicular large B cells supports functionality of ectopic lymphoid neogenesis in autoimmune sialoadenitis and MALT lymphoma in Sjogren's syndrome. J Immunol. 2007;179(7):4929–38.
105. Bjork A, Thorlacius GE, Mofors J, Richardsdotter Andersson E, Ivanchenko M, Tingstrom J, et al. Viral antigens elicit augmented immune responses in primary Sjogren's syndrome. Rheumatology (Oxford). 2019;59(7):1651–61.
106. Wildenberg ME, Welzen-Coppens JM, van Helden-Meeuwsen CG, Bootsma H, Vissink A, van Rooijen N, et al. Increased frequency of CD16+ monocytes and the presence of activated dendritic cells in salivary glands in primary Sjogren syndrome. Ann Rheum Dis. 2009;68(3):420–6.
107. Greenwell-Wild T, Moutsopoulos NM, Gliozzi M, Kapsogeorgou E, Rangel Z, Munson PJ, et al. Chitinases in the salivary glands and circulation of patients with Sjogren's syndrome: macrophage harbingers of disease severity. Arthritis Rheum. 2011;63(10):3103–15.
108. Rusakiewicz S, Nocturne G, Lazure T, Semeraro M, Flament C, Caillat-Zucman S, et al. NCR3/NKp30 contributes to pathogenesis in primary Sjogren's syndrome. Sci Transl Med. 2013;5(195):195ra96.
109. Ciccia F, Guggino G, Giardina A, Ferrante A, Carrubbi F, Giacomelli R, et al. The role of innate and lymphoid IL-22-producing cells in the immunopathology of primary Sjogren's syndrome. Expert Rev Clin Immunol. 2014;10(4):533–41.
110. Sisto M, Lorusso L, Lisi S. TLR2 signals via NF-kappaB to drive IL-15 production in salivary gland epithelial cells derived from patients with primary Sjogren's syndrome. Clin Exp Med. 2017;17(3):341–50.
111. Kyriakidis NC, Kapsogeorgou EK, Gourzi VC, Konsta OD, Baltatzis GE, Tzioufas AG. Toll-like receptor 3 stimulation promotes Ro52/TRIM21 synthesis and nuclear redistribution in salivary gland epithelial

cells, partially via type I interferon pathway. Clin Exp Immunol. 2014;178(3):548–60.
112. Kapsogeorgou EK, Manoussakis MN. Salivary gland epithelial cells (SGEC): carriers of exquisite B7-2 (CD86) costimulatory molecules. J Autoimmun. 2010;35(3):188–91.
113. Jang SI, Tandon M, Teos L, Zheng C, Warner BM, Alevizos I. Dual function of miR-1248 links interferon induction and calcium signaling defects in Sjogren's syndrome. EBioMedicine. 2019;48:526–38.
114. Gong YZ, Nititham J, Taylor K, Miceli-Richard C, Sordet C, Wachsmann D, et al. Differentiation of follicular helper T cells by salivary gland epithelial cells in primary Sjogren's syndrome. J Autoimmun. 2014;51:57–66.
115. Tsinti M, Kassi E, Korkolopoulou P, Kapsogeorgou E, Moutsatsou P, Patsouris E, et al. Functional estrogen receptors alpha and beta are expressed in normal human salivary gland epithelium and apparently mediate immunomodulatory effects. Eur J Oral Sci. 2009;117(5):498–505.
116. Blokland SLM, van den Hoogen LL, Leijten EFA, Hartgring SAY, Fritsch R, Kruize AA, et al. Increased expression of Fas on group 2 and 3 innate lymphoid cells is associated with an interferon signature in systemic lupus erythematosus and Sjogren's syndrome. Rheumatology (Oxford). 2019;58(10):1740–5.
117. Getts DR, Chastain EM, Terry RL, Miller SD. Virus infection, antiviral immunity, and autoimmunity. Immunol Rev. 2013;255(1):197–209.
118. Alam J, Kim YC, Choi Y. Potential role of bacterial infection in autoimmune diseases: a new aspect of molecular mimicry. Immune Netw. 2014;14(1):7–13.
119. Nguyen CQ, Peck AB. The interferon-signature of Sjögren's syndrome: how unique biomarkers can identify underlying inflammatory and immunopathological mechanisms of specific diseases. Front Immunol. 2013;4:142.
120. Maria NI, Brkic Z, Waris M, van Helden-Meeuwsen CG, Heezen K, van de Merwe JP, et al. MxA as a clinically applicable biomarker for identifying systemic interferon type I in primary Sjogren's syndrome. Ann Rheum Dis. 2014;73(6):1052–9.
121. Wildenberg ME, van Helden-Meeuwsen CG, van de Merwe JP, Drexhage HA, Versnel MA. Systemic increase in type I interferon activity in Sjögren's syndrome: a putative role for plasmacytoid dendritic cells. Eur J Immunol. 2008;38(7):2024–33.
122. Emamian ES, Leon JM, Lessard CJ, Grandits M, Baechler EC, Gaffney PM, et al. Peripheral blood gene expression profiling in Sjögren's syndrome. Genes Immun. 2009;10(4):285–96.
123. Brkic Z, Maria NI, van Helden-Meeuwsen CG, van de Merwe JP, van Daele PL, Dalm VA, et al. Prevalence of interferon type I signature in CD14 monocytes of patients with Sjogren's syndrome and association with disease activity and BAFF gene expression. Ann Rheum Dis. 2012.
124. Kimoto O, Sawada J, Shimoyama K, Suzuki D, Nakamura S, Hayashi H, et al. Activation of the interferon pathway in peripheral blood of patients with Sjogren's syndrome. J Rheumatol. 2011;38(2):310–6.
125. Li H, Ice JA, Lessard CJ, Sivils KL. Interferons in Sjögren's syndrome: genes, mechanisms, and effects. Front Immunol. 2013;4:290.
126. Thorn JJ, Oxholm P, Andersen HK. High levels of complement fixing antibodies against cytomegalovirus in patients with primary Sjögren's syndrome. Clin Exp Rheumatol. 1988;6(1):71–4.
127. Burbelo PD, Ching KH, Morse CG, Alevizos I, Bayat A, Cohen JI, et al. Altered antibody profiles against common infectious agents in chronic disease. PLoS One. 2013;8(12):e81635.
128. Kivity S, Arango MT, Ehrenfeld M, Tehori O, Shoenfeld Y, Anaya JM, et al. Infection and autoimmunity in Sjogren's syndrome: a clinical study and comprehensive review. J Autoimmun. 2014;51:17–22.
129. Ohyama Y, Carroll VA, Deshmukh U, Gaskin F, Brown MG, Fu SM. Severe focal sialadenitis and dacryoadenitis in NZM2328 mice induced by MCMV: a novel model for human Sjogren's syndrome. J Immunol. 2006;177(10):7391–7.
130. Weller ML, Gardener MR, Bogus ZC, Smith MA, Astorri E, Michael DG, et al. Hepatitis Delta virus detected in salivary glands of Sjogren's syndrome patients and recapitulates a Sjogren's syndrome-like phenotype in vivo. Pathog Immun. 2016;1(1):12–40.
131. Maslinska M. The role of Epstein-Barr virus infection in primary Sjogren's syndrome. Curr Opin Rheumatol. 2019;31(5):475–83.
132. Croia C, Astorri E, Murray-Brown W, Willis A, Brokstad KA, Sutcliffe N, et al. Implication of Epstein-Barr virus infection in disease-specific autoreactive B cell activation in ectopic lymphoid structures of Sjogren's syndrome. Arthritis Rheumatol. 2014;66(9):2545–57.
133. Sanosyan A, Daien C, Nutz A, Bollore K, Bedin AS, Morel J, et al. Discrepancy of serological and molecular patterns of circulating Epstein-Barr virus reactivation in primary Sjogren's syndrome. Front Immunol. 2019;10:1153.
134. Mackrides N, Campuzano-Zuluaga G, Maque-Acosta Y, Moul A, Hijazi N, Ikpatt FO, et al. Epstein-Barr virus-positive follicular lymphoma. Mod Pathol. 2017;30(4):519–29.
135. Strunk JE, Schuttler C, Ziebuhr J, Stowasser M, Nohte M, Mayer K, et al. Epstein-Barr virus-induced secondary high-grade transformation of Sjogren's syndrome-related mucosa-associated lymphoid tissue lymphoma. J Clin Oncol. 2013;31(17):e265–8.
136. Kim CS, Choi YD, Choi JS, Bae EH, Ma SK, Kim SW. EBV-positive diffuse large B-cell lymphoma in a patient with primary Sjogren's syndrome and membranous glomerulonephritis. BMC Nephrol. 2012;13:149.

137. Mofors J, Arkema EV, Bjork A, Westermark L, Kvarnstrom M, Forsblad-d'Elia H, et al. Infections increase the risk of developing Sjogren's syndrome. J Intern Med. 2019;285(6):670–80.
138. Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol. 2010;10(12):826–37.
139. Bryant CE, Gay NJ, Heymans S, Sacre S, Schaefer L, Midwood KS. Advances in toll-like receptor biology: modes of activation by diverse stimuli. Crit Rev Biochem Mol Biol. 2015;50(5):359–79.
140. Piccinini AM, Midwood KS. DAMPening inflammation by modulating TLR signalling. Mediators Inflamm. 2010;2010.
141. Fermaintt CS, Sano K, Liu Z, Ishii N, Seino J, Dobbs N, et al. A bioactive mammalian disaccharide associated with autoimmunity activates STING-TBK1-dependent immune response. Nat Commun. 2019;10(1):2377.
142. Moreth K, Brodbeck R, Babelova A, Gretz N, Spieker T, Zeng-Brouwers J, et al. The proteoglycan biglycan regulates expression of the B cell chemoattractant CXCL13 and aggravates murine lupus nephritis. J Clin Invest. 2010;120(12):4251–72.
143. Schaefer L, Babelova A, Kiss E, Hausser HJ, Baliova M, Krzyzankova M, et al. The matrix component biglycan is proinflammatory and signals through toll-like receptors 4 and 2 in macrophages. J Clin Invest. 2005;115(8):2223–33.
144. Zeng-Brouwers J, Beckmann J, Nastase MV, Iozzo RV, Schaefer L. De novo expression of circulating biglycan evokes an innate inflammatory tissue response via MyD88/TRIF pathways. Matrix Biol. 2014;35:132–42.
145. Midwood K, Sacre S, Piccinini AM, Inglis J, Trebaul A, Chan E, et al. Tenascin-C is an endogenous activator of toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. Nat Med. 2009;15(7):774–80.
146. Piccinini AM, Zuliani-Alvarez L, Lim JM, Midwood KS. Distinct microenvironmental cues stimulate divergent TLR4-mediated signaling pathways in macrophages. Sci Signal. 2016;9(443):ra86.
147. Bhattacharyya S, Tamaki Z, Wang W, Hinchcliff M, Hoover P, Getsios S, et al. FibronectinEDA promotes chronic cutaneous fibrosis through Toll-like receptor signaling. Sci Transl Med. 2014;6(232):232ra50.
148. Kay JG, Kramer JM, Visser MB. Danger signals in oral cavity-related diseases. J Leukoc Biol. 2019;106(1):193–200.
149. Schaefer L. Complexity of danger: the diverse nature of damage-associated molecular patterns. J Biol Chem. 2014;289(51):35237–45.
150. Linder A, Hornung V. Mitochondrial dsRNA: a new DAMP for MDA5. Dev Cell. 2018;46(5):530–2.
151. Yamachika S, Brayer J, Oxford GE, Peck AB, Humphreys-Beher MG. Aberrant proteolytic digestion of biglycan and decorin by saliva and exocrine gland lysates from the NOD mouse model for autoimmune exocrinopathy. Clin Exp Rheumatol. 2000;18(2):233–40.
152. Vakrakou AG, Polyzos A, Kapsogeorgou EK, Thanos D, Manoussakis MN. Perturbation of transcriptome in non-neoplastic salivary gland epithelial cell lines derived from patients with primary Sjogren's syndrome. Data Brief. 2018;17:194–9.
153. Enger TB, Samad-Zadeh A, Bouchie MP, Skarstein K, Galtung HK, Mera T, et al. The Hippo signaling pathway is required for salivary gland development and its dysregulation is associated with Sjogren's syndrome. Lab Invest. 2013;93(11):1203–18.
154. Silvestre FJ, Puente A, Bagan JV, Castell JV. Presence of fibronectin peptides in saliva of patients with Sjogren's syndrome: a potential indicator of salivary gland destruction. Med Oral Patol Oral Cir Bucal. 2009;14(8):e365–70.
155. Nordal HH, Brun JG, Halse AK, Madland TM, Fagerhol MK, Jonsson R. Calprotectin (S100A8/A9), S100A12, and EDTA-resistant S100A12 complexes (ERAC) in primary Sjogren's syndrome. Scand J Rheumatol. 2014;43(1):76–8.
156. Cuida M, Halse AK, Johannessen AC, Tynning T, Jonsson R. Indicators of salivary gland inflammation in primary Sjogren's syndrome. Eur J Oral Sci. 1997;105(3):228–33.
157. Nicaise C, Weichselbaum L, Schandene L, Gangji V, Dehavay F, Bouchat J, et al. Phagocyte-specific S100A8/A9 is upregulated in primary Sjogren's syndrome and triggers the secretion of pro-inflammatory cytokines in vitro. Clin Exp Rheumatol. 2017;35(1):129–36.
158. Jazzar A, Shirlaw PJ, Carpenter GH, Challacombe SJ, Proctor GB. Salivary S100A8/A9 in Sjogren's syndrome accompanied by lymphoma. J Oral Pathol Med. 2018;47:900–6.
159. Barrera MJ, Aguilera S, Veerman E, Quest AF, Diaz-Jimenez D, Urzua U, et al. Salivary mucins induce a toll-like receptor 4-mediated pro-inflammatory response in human submandibular salivary cells: are mucins involved in Sjogren's syndrome? Rheumatology (Oxford). 2015;54(8):1518–27.
160. Mavragani CP, Nezos A, Sagalovskiy I, Seshan S, Kirou KA, Crow MK. Defective regulation of L1 endogenous retroelements in primary Sjogren's syndrome and systemic lupus erythematosus: role of methylating enzymes. J Autoimmun. 2018;88:75–82.
161. Crow MK. Long interspersed nuclear elements (LINE-1): potential triggers of systemic autoimmune disease. Autoimmunity. 2010;43(1):7–16.
162. Harris VM, Scofield RH, Sivils KL. Genetics in Sjogren's syndrome: where we are and where we go. Clin Exp Rheumatol. 2019;37 Suppl 118(3):234–9.
163. Nezos A, Makri P, Gandolfo S, De Vita S, Voulgarelis M, Crow MK, et al. TREX1 variants in Sjogren's syndrome related lymphomagenesis. Cytokine. 2019;132:154781.

164. Colafrancesco S, Ciccacci C, Priori R, Latini A, Picarelli G, Arienzo F, et al. STAT4, TRAF3IP2, IL10, and HCP5 polymorphisms in Sjogren's syndrome: association with disease susceptibility and clinical aspects. J Immunol Res. 2019;2019:7682827.
165. Li H, Reksten TR, Ice JA, Kelly JA, Adrianto I, Rasmussen A, et al. Identification of a Sjogren's syndrome susceptibility locus at OAS1 that influences isoform switching, protein expression, and responsiveness to type I interferons. PLoS Genet. 2017;13(6):e1006820.
166. Ice JA, Li H, Adrianto I, Lin PC, Kelly JA, Montgomery CG, et al. Genetics of Sjögren's syndrome in the genome-wide association era. J Autoimmun. 2012;39:57–63.
167. Lessard CJ, Li H, Adrianto I, Ice JA, Rasmussen A, Grundahl KM, et al. Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. Nat Genet. 2013;45(11):1284–92.
168. Jefferies CA. Regulating IRFs in IFN driven disease. Front Immunol. 2019;10:325.
169. Zundler S, Neurath MF. Interleukin-12: functional activities and implications for disease. Cytokine Growth Factor Rev. 2015;26(5):559–68.
170. Shamilov R, Aneskievich BJ. TNIP1 in autoimmune diseases: regulation of toll-like receptor signaling. J Immunol Res. 2018;2018:3491269.
171. Lee WB, Choi WY, Lee DH, Shim H, Kim-Ha J, Kim YJ. OAS1 and OAS3 negatively regulate the expression of chemokines and interferon-responsive genes in human macrophages. BMB Rep. 2019;52(2):133–8.
172. Bonnevie-Nielsen V, Field LL, Lu S, Zheng DJ, Li M, Martensen PM, et al. Variation in antiviral 2′,5′-oligoadenylate synthetase (2'5'AS) enzyme activity is controlled by a single-nucleotide polymorphism at a splice-acceptor site in the OAS1 gene. Am J Hum Genet. 2005;76(4):623–33.
173. Imgenberg-Kreuz J, Sandling JK, Almlof JC, Nordlund J, Signer L, Norheim KB, et al. Genome-wide DNA methylation analysis in multiple tissues in primary Sjogren's syndrome reveals regulatory effects at interferon-induced genes. Ann Rheum Dis. 2016;75(11):2029–36.
174. Cole MB, Quach H, Quach D, Baker A, Taylor KE, Barcellos LF, et al. Epigenetic signatures of salivary gland inflammation in Sjogren's syndrome. Arthritis Rheumatol. 2016;68(12):2936–44.
175. Imgenberg-Kreuz J, Almlof JC, Leonard D, Sjowall C, Syvanen AC, Ronnblom L, et al. Shared and unique patterns of DNA methylation in systemic lupus erythematosus and primary Sjogren's syndrome. Front Immunol. 2019;10:1686.
176. Cha S, Mona M, Lee KE, Kim DH, Han K. MicroRNAs in autoimmune Sjogren's syndrome. Genomics Inform. 2018;16(4):e19.
177. Shi H, Cao N, Pu Y, Xie L, Zheng L, Yu C. Long non-coding RNA expression profile in minor salivary gland of primary Sjogren's syndrome. Arthritis Res Ther. 2016;18(1):109.
178. Sandhya P, Joshi K, Scaria V. Long noncoding RNAs could be potential key players in the pathophysiology of Sjogren's syndrome. Int J Rheum Dis. 2015;18(8):898–905.
179. Jonas S, Izaurralde E. Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet. 2015;16(7):421–33.
180. Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: an overview of nuclear functions. Int J Mol Sci. 2016;17(10):1712.
181. Lam IKY, Chow JX, Lau CS, Chan VSF. MicroRNA-mediated immune regulation in rheumatic diseases. Cancer Lett. 2018;431:201–12.
182. Lopes AP, van Roon JAG, Blokland SLM, Wang M, Chouri E, Hartgring SAY, et al. MicroRNA-130a contributes to Type-2 classical DC-activation in Sjogren's syndrome by targeting mitogen- and stress-activated protein Kinase-1. Front Immunol. 2019;10:1335.
183. Williams AE, Choi K, Chan AL, Lee YJ, Reeves WH, Bubb MR, et al. Sjogren's syndrome-associated microRNAs in CD14(+) monocytes unveils targeted TGFbeta signaling. Arthritis Res Ther. 2016;18(1):95.
184. Pauley KM, Stewart CM, Gauna AE, Dupre LC, Kuklani R, Chan AL, et al. Altered miR-146a expression in Sjogren's syndrome and its functional role in innate immunity. Eur J Immunol. 2011;41(7):2029–39.
185. Zilahi E, Tarr T, Papp G, Griger Z, Sipka S, Zeher M. Increased microRNA-146a/b, TRAF6 gene and decreased IRAK1 gene expressions in the peripheral mononuclear cells of patients with Sjogren's syndrome. Immunol Lett. 2012;141(2):165–8.
186. Hur K, Kim SH, Kim JM. Potential implications of long noncoding RNAs in autoimmune diseases. Immune Netw. 2019;19(1):e4.
187. Dolcino M, Tinazzi E, Vitali C, Papa ND, Puccetti A, Lunardi C. Long non-coding RNAs modulate Sjogren's syndrome associated gene expression and are involved in the pathogenesis of the disease. J Clin Med. 2019;8(9):1349.
188. Ishimaru N, Arakaki R, Omotehara F, Yamada K, Mishima K, Saito I, et al. Novel role for RbAp48 in tissue-specific, estrogen deficiency-dependent apoptosis in the exocrine glands. Mol Cell Biol. 2006;26(8):2924–35.
189. Mikkelsen TR, Brandt J, Larsen HJ, Larsen BB, Poulsen K, Ingerslev J, et al. Tissue-specific expression in the salivary glands of transgenic mice. Nucleic Acids Res. 1992;20(9):2249–55.
190. Shim GJ, Warner M, Kim HJ, Andersson S, Liu L, Ekman J, et al. Aromatase-deficient mice spontaneously develop a lymphoproliferative autoimmune disease resembling Sjogren's syndrome. Proc Natl Acad Sci U S A. 2004;101(34):12628–33.
191. McCoy SS, Sampene E, Baer AN. Sjogren's syndrome is associated with reduced lifetime sex

hormone exposure: a case-control study. Arthritis Care Res (Hoboken). 2019;72(9):1315–22.
192. Syrett CM, Anguera MC. When the balance is broken: X-linked gene dosage from two X chromosomes and female-biased autoimmunity. J Leukoc Biol. 2019;106(4):919–32.
193. Chow JC, Ciaudo C, Fazzari MJ, Mise N, Servant N, Glass JL, et al. LINE-1 activity in facultative heterochromatin formation during X chromosome inactivation. Cell. 2010;141(6):956–69.
194. Souyris M, Cenac C, Azar P, Daviaud D, Canivet A, Grunenwald S, et al. TLR7 escapes X chromosome inactivation in immune cells. Sci Immunol. 2018;3(19):eaap8855.
195. Mougeot JL, Noll BD, Bahrani Mougeot FK. Sjogren's syndrome X-chromosome dose effect: an epigenetic perspective. Oral Dis. 2018;25(2):372–84.
196. Sharma D, Sandhya P, Vellarikkal SK, Surin AK, Jayarajan R, Verma A, et al. Saliva microbiome in primary Sjogren's syndrome reveals distinct set of disease associated microbes. Oral Dis. 2019;26(2):295–301.
197. Rusthen S, Kristoffersen AK, Young A, Galtung HK, Petrovski BE, Palm O, et al. Dysbiotic salivary microbiota in dry mouth and primary Sjogren's syndrome patients. PLoS One. 2019;14(6):e0218319.
198. van der Meulen TA, Harmsen HJM, Bootsma H, Liefers SC, Vich Vila A, Zhernakova A, et al. Dysbiosis of the buccal mucosa microbiome in primary Sjogren's syndrome patients. Rheumatology (Oxford). 2018;57(12):2225–34.
199. Mandl T, Marsal J, Olsson P, Ohlsson B, Andreasson K. Severe intestinal dysbiosis is prevalent in primary Sjogren's syndrome and is associated with systemic disease activity. Arthritis Res Ther. 2017;19(1):237.
200. Siddiqui H, Chen T, Aliko A, Mydel PM, Jonsson R, Olsen I. Microbiological and bioinformatics analysis of primary Sjogren's syndrome patients with normal salivation. J Oral Microbiol. 2016;8:31119.
201. de Paiva CS, Jones DB, Stern ME, Bian F, Moore QL, Corbiere S, et al. Altered mucosal microbiome diversity and disease severity in Sjogren syndrome. Sci Rep. 2016;6:23561.
202. Sembler-Moller ML, Belstrom D, Locht H, Enevold C, Pedersen AML. Next-generation sequencing of whole saliva from patients with primary Sjogren's syndrome and non-Sjogren's sicca reveals comparable salivary microbiota. J Oral Microbiol. 2019;11(1):1660566.
203. Tsigalou C, Stavropoulou E, Bezirtzoglou E. Current insights in microbiome shifts in Sjogren's syndrome and possible therapeutic interventions. Front Immunol. 2018;9:1106.
204. Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. Nature. 2016;535(7610):65–74.
205. Yurkovetskiy LA, Pickard JM, Chervonsky AV. Microbiota and autoimmunity: exploring new avenues. Cell Host Microbe. 2015;17(5):548–52.
206. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science. 2013;339(6123):1084–8.
207. Yurkovetskiy L, Burrows M, Khan AA, Graham L, Volchkov P, Becker L, et al. Gender bias in autoimmunity is influenced by microbiota. Immunity. 2013;39(2):400–12.
208. Markle JG, Frank DN, Adeli K, von Bergen M, Danska JS. Microbiome manipulation modifies sex-specific risk for autoimmunity. Gut Microbes. 2014;5(4):485–93.
209. Zegarra-Ruiz DF, El Beidaq A, Iniguez AJ, Lubrano Di Ricco M, Manfredo Vieira S, Ruff WE, et al. A diet-sensitive commensal Lactobacillus strain mediates TLR7-dependent systemic autoimmunity. Cell Host Microbe. 2019;25(1):113–27 e6.
210. Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town T, Ward JM, et al. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. Immunity. 2007;27(5):801–10.
211. Wang SQ, Zhang LW, Wei P, Hua H. Is hydroxychloroquine effective in treating primary Sjogren's syndrome: a systematic review and meta-analysis. BMC Musculoskelet Disord. 2017;18(1):186.
212. van der Heijden EHM, Kruize AA, Radstake T, van Roon JAG. Optimizing conventional DMARD therapy for Sjogren's syndrome. Autoimmun Rev. 2018;17(5):480–92.
213. Pasquier B. Autophagy inhibitors. CMLS. 2016;73(5):985–1001.
214. Wallace DJ, Gudsoorkar VS, Weisman MH, Venuturupalli SR. New insights into mechanisms of therapeutic effects of antimalarial agents in SLE. Nat Rev Rheumatol. 2012;8(9):522–33.
215. Bodewes ILA, Gottenberg JE, van Helden-Meeuwsen CG, Mariette X, Versnel MA. Hydroxychloroquine treatment downregulates systemic interferon activation in primary Sjogren's syndrome in the JOQUER randomized trial. Rheumatology (Oxford). 2019;59:107–11.
216. Demarchi J, Papasidero S, Medina MA, Klajn D, Chaparro Del Moral R, Rillo O, et al. Primary Sjogren's syndrome: Extraglandular manifestations and hydroxychloroquine therapy. Clin Rheumatol. 2017;36(11):2455–60.
217. Balak DM, van Doorn MB, Arbeit RD, Rijneveld R, Klaassen E, Sullivan T, et al. IMO-8400, a toll-like receptor 7, 8, and 9 antagonist, demonstrates clinical activity in a phase 2a, randomized, placebo-controlled trial in patients with moderate-to-severe plaque psoriasis. Clin Immunol. 2017;174:63–72.
218. Kelly PN, Romero DL, Yang Y, Shaffer AL 3rd, Chaudhary D, Robinson S, et al. Selective interleukin-1 receptor-associated kinase 4 inhibitors for the treatment of autoimmune disorders and lymphoid malignancy. J Exp Med. 2015;212(13):2189–201.

219. Brenke JK, Popowicz GM, Schorpp K, Rothenaigner I, Roesner M, Meininger I, et al. Targeting TRAF6 E3 ligase activity with a small-molecule inhibitor combats autoimmunity. J Biol Chem. 2018;293(34):13191–203.
220. Midwood KS, Piccinini AM, Sacre S. Targeting toll-like receptors in autoimmunity. Curr Drug Targets. 2009;10(11):1139–55.
221. Clanchy FI, Sacre SM. Modulation of toll-like receptor function has therapeutic potential in autoimmune disease. Expert Opin Biol Ther. 2010;10(12):1703–16.
222. Burge DJ, Eisenman J, Byrnes-Blake K, Smolak P, Lau K, Cohen SB, et al. Safety, pharmacokinetics, and pharmacodynamics of RSLV-132, an RNase-fc fusion protein in systemic lupus erythematosus: a randomized, double-blind, placebo-controlled study. Lupus. 2017;26(8):825–34.
223. A study of RSLV-132 in subjects with primary Sjogren's syndrome. 2019. https://clinicaltrials.gov/ct2/show/NCT03247686?term=RSLV-132&draw=2&rank=1. Accessed 12 Nov 2019.
224. A phase 2a of RSLV-132 in subjects with systemic lupus erythematosus (SLE). 2019. https://clinicaltrials.gov/ct2/show/NCT02660944?term=RSLV--132&draw=2&rank=2. Accessed 12 Nov 2019.
225. Benschop RJ, Chow CK, Tian Y, Nelson J, Barmettler B, Atwell S, et al. Development of tibulizumab, a tetravalent bispecific antibody targeting BAFF and IL-17A for the treatment of autoimmune disease. MAbs. 2019;11(6):1175–90.
226. Bodewes ILA, Bjork A, Versnel MA, Wahren-Herlenius M. Innate immunity and interferons in the pathogenesis of Sjogren's syndrome. Rheumatology (Oxford). 2019.
227. Retamozo S, Flores-Chavez A, Consuegra-Fernandez M, Lozano F, Ramos-Casals M, Brito-Zeron P. Cytokines as therapeutic targets in primary Sjogren syndrome. Pharmacol Ther. 2018;184:81–97.
228. Norheim KB, Harboe E, Goransson LG, Omdal R. Interleukin-1 inhibition and fatigue in primary Sjogren's syndrome—a double blind, randomised clinical trial. PLoS One. 2012;7(1):e30123.
229. De Vita S, Quartuccio L, Seror R, Salvin S, Ravaud P, Fabris M, et al. Efficacy and safety of belimumab given for 12 months in primary Sjogren's syndrome: the BELISS open-label phase II study. Rheumatology (Oxford). 2015.
230. Mariette X, Seror R, Quartuccio L, Baron G, Salvin S, Fabris M, et al. Efficacy and safety of belimumab in primary Sjogren's syndrome: results of the BELISS open-label phase II study. Ann Rheum Dis. 2015;74(3):526–31.
231. Seror R, Nocturne G, Lazure T, Hendel-Chavez H, Desmoulins F, Belkhir R, et al. Low numbers of blood and salivary natural killer cells are associated with a better response to belimumab in primary Sjogren's syndrome: results of the BELISS study. Arthritis Res Ther. 2015;17(1):241.
232. Quartuccio L, Salvin S, Corazza L, Gandolfo S, Fabris M, De Vita S. Efficacy of belimumab and targeting of rheumatoid factor-positive B-cell expansion in Sjogren's syndrome: follow-up after the end of the phase II open-label BELISS study. Clin Exp Rheumatol. 2016;34(2):311–4.
233. Nocturne G, Cornec D, Seror R, Mariette X. Use of biologics in Sjogren's syndrome. Rheum Dis Clin North Am. 2016;42(3):407–17.
234. Viela Bio. Product candidates VIB7734. 2019. https://vielabio.com/product-candidates/vib7734/. Accessed 19 Nov 2019.
235. Rissoan MC, Duhen T, Bridon JM, Bendriss-Vermare N, Peronne C, de Saint VB, et al. Subtractive hybridization reveals the expression of immunoglobulin-like transcript 7, Eph-B1, granzyme B, and 3 novel transcripts in human plasmacytoid dendritic cells. Blood. 2002;100(9):3295–303.
236. Ju XS, Hacker C, Scherer B, Redecke V, Berger T, Schuler G, et al. Immunoglobulin-like transcripts ILT2, ILT3 and ILT7 are expressed by human dendritic cells and down-regulated following activation. Gene. 2004;331:159–64.
237. De Luca F, Shoenfeld Y. The microbiome in autoimmune diseases. Clin Exp Immunol. 2019;195(1):74–85.
238. Galor A. Fecal microbial transplant (FMT) for Sjogrens syndrome. 2019. https://clinicaltrials.gov/ct2/show/NCT03926286?term=microbiome&cond=Autoimmune+Diseases&draw=2&rank=3. Accessed 19 Nov 2019.
239. Columbia University. Prebiotic vs. probiotic in multiple sclerosis (MS). 2019. https://clinicaltrials.gov/ct2/show/NCT04038541?term=NCT04038541&draw=2&rank=1. Accessed 19 Nov 2019.
240. Ismail HM. Effect of prebiotics on the gut microbiome profile and beta cell function. 2019. https://clinicaltrials.gov/ct2/show/NCT04114357?term=NCT04114357&draw=2&rank=1. Accessed 19 Nov 2019.

6 Dysregulation of Adaptive Immunity in Sjögren's Syndrome

Qing Yu

6.1 Introduction

SjÐgren's syndrome (SjS) is a chronic autoimmune disease that affects approximately four million Americans [1, 2]. SjS is characterized by progressive infiltration of salivary and lacrimal glands by T and B lymphocytes, increased levels of proinflammatory cytokines and autoantibodies, and the destruction and dysfunction of these exocrine glands and xerostomia (dry mouth) and xerophthalmia (dry eyes) as main clinical manifestations. SjS patients also frequently suffer from systemic inflammation and pathologies that can be painful and debilitating, and significantly compromise the life quality [3, 4]. Moreover, SjS patients also have a much higer risk of developing life-threatening B cell lymphoma compared to healthy people and those with other autoimmune diseases [5, 6]. SjS occurs either as primary SjS (pSjS) or as secondary SjS in association with other connective tissue diseases [3, 4]. The current treatments for SjS are mostly palliative, with no cure or effective biological therapies available [2, 7, 8]. There is therefore an urgent need to develop effective and targeted new therapeutic approaches for this autoimmune disease with high oral health impact. The etiology, pathological events, and immunopathogenic mechanisms of SjS still await more comprehensive elucidation, but studies in recent years have clearly demonstrated that T and B lymphocytes, the major cellular players of adaptive immunity, and the various cytokines and autoantibodies produced by these cells collectively contribute to the destruction and dysfunction of the salivary and lacrimal glands as well as pathologies in other affected organs. This chapter reviews the key recent findings in the functional involvement of T and B lymphocytes, and the cytokines mediating or affecting their function, in the pathogenesis and persistence of SjS.

6.2 Initiation of Adaptive Immune Responses

Many lines of evidence generated in the SjS research field have demonstrated that both genetic and epigenetic variations, along with environmental triggers, including viral infections, sex hormone changes, tissue injuries and microbiome alterations, all contribute to the initiation, amplification, and progression of autoimmune responses in SjS [6, 9–16]. Some of the early changes in exocrine gland epithelial cells, including apoptosis, upregulation of surface expression of costimulatory molecules, and production of a number of proinflammatory cytokines and chemokines, are

Q. Yu (✉)
The Forsyth Institute, Cambridge, MA, USA

Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, MA, USA
e-mail: qyu@forsyth.org

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believed to critically contribute to the activation of self-reactive T cells [17–20]. In addition, the augmented type 1 IFN-dependent gene expression and increased number and/or activity of innate immune cells, including NK cells, macrophages, and plasmacytoid dendritic cells (pDCs), which are the major cellular sources of type 1 IFNs, in the target exocrine glands likely promote antigen presentation and the activation of self-reactive T cells and B cells [17–21].

The activated CD4 T cells in the target exocrine glands or their draining lymph nodes, instructed or influenced by the signals from exocrine gland epithelial cells, antigen-presenting cells, other innate immune cells, and adaptive immune cells, subsequently differentiate into diverse effector T cell subsets, defined based on the cytokines they produce, signature transcriptional master regulators they express, and specialized effector functions they execute. The major effector T helper (Th) subsets include Th1, Th2, Th17, and T follicular helper (TFH) cells. As illustrated in Fig. 6.1, IL-12, IL-7, and IL-18 promotes activated CD4 T cells to differentiate into Th1 effector cells, which are characterized by the expression of T-bet and production of large amount of IFNγ as well as TNFα [22, 23]. Th1 cells and their cytokines play vital roles in all stages of SjS development through enhancing the recruitment and pathogenic activity of other immune cells, and by directly causing damages and dysfunction of target exocrine tissues [24–26]. Signals including IL-4 facilitate the generation of Th2 cells, which express GATA-3 and produce IL-4, IL-5, and IL-13, thereby providing help to B cell responses and autoantibody production [22, 23, 27–29]. Cytokines including IL-23, IL-6, TGFβ, IL-1, and IL-21 all have the ability to dictate or enhance the differentiation of activated CD4 T cells into Th17 cells, which express RORγt and produce IL-17 family cytokines and IL-21 to promote tissue inflammation and damage and assist B cell responses [30–34]. Cytokines including IL-21 and IL-6, along with costimulatory signals such as those mediated through ICOS, promote the generation of TFH cells, which express BCL6 and produce IL-21 to facilitate the formation and maintenance of germinal center and the germinal center B cell responses [35–37]. In addition to effector Th cell subsets, activated T cells can also be induced to differentiate into regulatory T cell (Treg) subsets with specialized immunosuppressive activity. Cytokines including TGFβ and IL-2, which are present in elevated amounts in SjS target tissues, induce the differentiation of some activated CD4 T cells into Foxp3-expressing Tregs that produce immune-regulatory cytokines such as TGFβ, IL-10, and IL-35 and dampen autoimmune responses [38–40].

The genetic/epigenetic factors, environmental triggers such as viral infections, and the activation of exocrine epithelial cells and innate immune cells also lead to the activation of B cells through a number of direct or indirect mechanisms, including activation of toll-like receptors, virus mimicking B cell receptor-like signaling, induction of BAFF production, and activation and generation of effector Th subsets that can facilitate B cell activation and function [5, 6, 8, 41]. Multiple B cell populations/subsets have been identified to be associated with SjS, including marginal zone (MZ) B cells, plasmablasts, plasma cells, and $CD27^+$ memory B cells, among others. The autoantibodies produced, such as the anti-muscarinic type 3 receptor (M3R), as the result of autoreactive B cell responses critically contribute to the development of exocrine gland dysfunction and damage [5, 6, 8, 41–43].

6.3 Th1 Cells and Cytokines

6.3.1 IFNγ

IFNγ is the hallmark cytokine of Th1 effector subset and the cytotoxic CD8 T (Tc1) cells, and a critical mediator of Th1/Tc1-dependent cellular immunity against intracellular microbes and tumor cells [23]. IFNγ is produced in large amount by Th1 cells in a variety of autoimmune inflammatory disorders, such as systemic lupus erythematosus (SLE) [44, 45] and rheumatoid arthritis (RA) [46], and promotes the pathogenesis of these diseases.

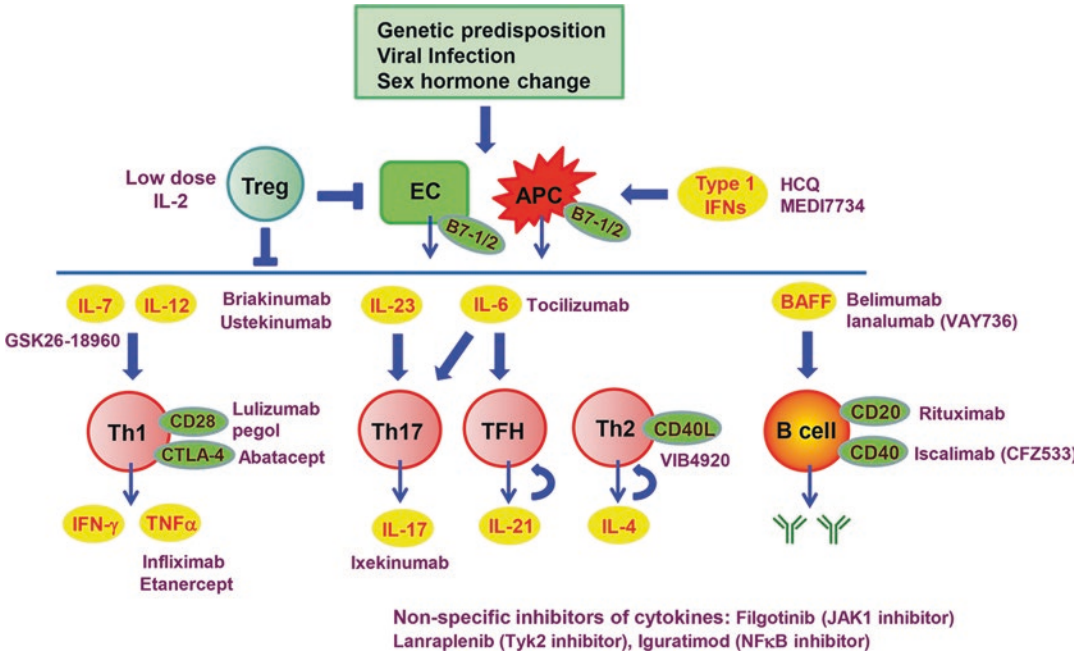

Fig. 6.1 Adaptive immunity in SS and the therapeutic development. This is an overview of the major adaptive immune players in SjS that are discussed in this chapter, and the corresponding therapeutics targeting these players that have been subjected to clinical trials. (**a**) Therapeutics targeting initial T cell activation: Lulizumab pegol targets the costimulatory molecule CD28, which transduces the critical second signal for T cell activation and proliferation. Hydroxychloroquine (HCQ) and MEDI7734, an inhibitor of ILT7 molecule on plasmacytoid dendritic cells, both have the ability to reduce the production of type 1 IFNs, which are critical promoters of antigen-presenting activity of professional APCs and exocrine gland epithelial cells, and enhancers of IL-7 and BAFF production (ClinicalTrials.gov Identifier:NCT02780674) [17, 18, 20, 221, 222]. (**b**) Therapeutics targeting Th1 cells and associated cytokines: GSK26-18960 is a humanized monoclonal antibody that blocks IL-7 receptor signaling (ClinicalTrials.gov Identifier: NCT03239600). Briakinumab and Ustekinumab are both humanized antibodies that block IL-12p40, a shared subunit of IL-12 and IL-23 [8] (ClinicalTrials.gov Identifier: NCT04093531). Infliximab, a monoclonal neutralizing antibody against TNFα, and Etanercept, a soluble TNF receptor 2-IgG1 Fc fusion protein, both inhibit TNFα signaling and function [65, 223]. (**c**) Therapeutics targeting Th2 cells and associated cytokines: VIB4920 is a fusion protein that inhibits CD40 ligand binding to CD40, and thereby disrupting Th2 help to B cells (ClinicalTrials gov Identifier: NCT04129164). (**d**) Therapeutics targeting Th17 cells and associated cytokines: Ixekizumab is a humanized neutralizing antibody against IL-17A and a promising candidate for clinical trials for SjS disease. Tocilizumab is a humanized monoclonal antibody that blocks IL-6 receptor to abrogate the function of the Th17-promoting cytokine IL-6 (ClinicalTrials gov Identifier: NCT04129164). (**e**) Therapeutics targeting TFH cells and associated cytokines: By inhibiting IL-6 receptor signaling and function, Tocilizumab may also diminish the generation of TFH cells as IL-6 has been shown to promote TFH differentiation from activated CD4 T cells. (**f**) Therapeutics enhancing Treg expansion and function: Low dose recombinant IL-2 has been tested since IL-2 plays an essential role in the generation, expansion, and suppressor function of Tregs [38, 149] (ClinicalTrials gov Identifier: NCT02464319). (**g**) Therapeutics targeting B cells: Rituximab, a monoclonal anti-CD20 antibody that depletes B cells, has been the most extensively investigated therapeutic for SjS, but the overall outcome has not been consistent and satisfactory, especially in randomized clinical trials [2, 8, 134, 224]. Belimumab, a humanized antibody neutralizing BAFF activity, and Ianalumab (VAY736), a humanized antibody blocking BAFF receptor, both inhibit BAFF-dependent B cell expansion and function [224–227]. Iscalimab (CFZ533) is a monoclonal anti-CD40 antibody that blocks its interaction with CD40 ligand to suppresses B cell activation (ClinicalTrials gov Identifier: NCT02291029). (**h**) Non-selective cytokine inhibitors: Filgotinib, an inhibitor of JAK1, and Lanraplenib, an inhibitor of Tyk2, interfere with the function of cytokines that depend on these two kinases for signal transduction (ClinicalTrials gov Identifier: NCT03100942). Iguratimod is a small molecule anti-inflammatory drug that suppresses NF-κB activity to inhibit the production of proinflammatory cytokines, including TNFα, IL-1, and IL-6, and also reduces the expression of a number of costimulatory molecules (ClinicalTrials gov Identifier: NCT03023592) [228]

IFNγ production is similarly elevated in salivary glands and saliva from patients with primary SjS (pSjS) compared to the control subjects [47, 48]. The T cell profile is skewed towards a Th1 in salivary glands, saliva and blood of pSjS patients compared to the healthy controls and non-SjS sicca patients [49, 50]. The magnitude of Th1 response is also directly correlated with the degree of sialadenitis [51].

In vivo functional studies in mouse disease models have supported a critical disease-promoting function of IFNγ in SjS. In female nonobese diabetic (NOD) mice that spontaneously develop both type 1 diabetes and SjS, ablation of IFNγ gene prevents the emergence of early, pre-immunological salivary tissue abnormalities, as well as the development and onset of salivary gland inflammation, damage, and secretory dysfunction [24, 25]. In accordance, neutralization of IFNγ with a monoclonal antibody in NOD mice with newly onset SjS disease markedly attenuates the severity of sialadenitis and improves salivary secretion, indicating an essential role of this cytokine in sustaining the established disease and suggesting a therapeutic promise of IFNγ neutralization [26].

The mechanisms underlying the actions of IFNγ in SjS include induction of apoptosis and dysfunction of salivary gland tissues [52], enhancement of MHC expression and antigen-presenting ability of epithelial cells, macrophages and dendritic cells in salivary glands [53, 54], activation of proinflammatory macrophages and NK cells, and augmentation of production of various chemokines, such as CXCR3 ligands and CXCL13, by epithelial cells and immune cells in the salivary glands [26, 55]. The importance of CXCR3 ligands, including CXCL9, -10, and -11, has been supported by the marked upregulation of their levels in salivary glands and tears of humans and mice afflicted with SjS [47, 55, 56], and by *in vivo* functional studies showing that blockade of CXCL10 in the MRL/lpr mouse model of SjS significantly impedes the development of sialadenitis [57]. Hence, upregulation of CXCL3 ligands and other chemokines in target tissues is a critical mechanism by which IFNγ promotes local autoimmune responses and SjS pathogenesis. Other effects of IFNγ, such as those on Tregs, M1 macrophages, and NK cells, in the context of SjS disease will need to be better defined and elucidated.

6.3.2 TNFα

Tumor necrosis factor-α (TNFα) is a proinflammatory cytokine that can be produced by Th1 cells in large amounts, and by other immune or non-immune tissue cells, during various immune and autoimmune responses. TNFα induces or exacerbates tissue inflammation and damage in numerous autoimmune or inflammatory diseases such as RA, psoriasis, and Crohn's disease [58–62]. TNFα levels are elevated in salivary glands and sera of SjS patients compared to non-SS sicca patients [48, 49]. It is detected in salivary gland-infiltrating Th1 and Tc1 cells and salivary gland epithelium [48, 63].

Studies in mouse models have provided strong evidence for a functional importance of TNFα in the pathogenesis of SjS. Inhibition of TNFα activity *in vivo* with pegylated soluble TNF receptor 1 impedes the development of SjS-like inflammation and secretory dysfunction of lacrimal and salivary glands in NOD mice [64, 65]. Accordingly, a neutralizing anti-TNFα antibody hinders the development of SjS-like sialadenitis and hyposalivation when given prior to disease onset, and reduces the disease severity when given after the disease onset, in the NOD model of SjS [26, 66]. These studies collectively demonstrate a pathogenic role of TNFα in the development and chronic persistence of SjS and suggest a therapeutic potential of TNFα antagonism for this disease.

Some of the reported key mechanisms underlying the pathogenic role of TNFα in SjS include induction of salivary gland cell apoptosis, alone or in cooperation with other proinflammatory cytokines such as IFNγ [67], disruption of the integrity of tight junction by reducing expression of claudin-1 [26, 53, 66], downregulation of water channel protein, aquaporin 5 (AQP5) [68, 69], and upregulation of the expression of various chemokines including CXCR3 ligands and

CXCL13 in salivary gland tissues [26, 70, 71]. One poorly understood, undesired outcome of TNFα antagonism is the development of anti-TNFα-induced lupus erythematosus as shown in humans and mice [66, 72, 73], characterized by the reversible production of autoantibodies during the course of anti-TNFα treatments generally without lupus-like clinical manifestations [73–75]. The functional implication and underlying mechanisms of this event await additional investigations.

6.3.3 IL-7

IL-7 is a cytokine mainly produced by stromal cells, epithelial cells, and certain other non-hematopoietic cells, and plays a critical role in the development and homeostasis of T cells at the physiological level [76, 77]. However, when produced at a greater amount in the target organs of a variety of autoimmune inflammatory conditions, IL-7 preferentially enhances the pathogenic autoimmune T cell responses, particularly Th1 (and Tc1) responses, to accelerate the development and sustain the persistence of these diseases as in the cases of SLE, RA, type-1 diabetes, EAE, and inflammatory bowel disease (IBD) [78–83]. Multiple reports have shown that the expression levels of IL-7 and its receptors are significantly upregulated in the salivary gland tissues of pSjS patients and positively correlated with the severity of the disease [84, 85].

A number of functional studies have provided convincing evidence for a critical pathogenic role of IL-7 in the development and persistence of SjS, predominantly through enhancing Th1 and Tc1 responses [78–83, 86]. IL-7 treatment of peripheral blood T cells from pSjS patients increases the production of Th1 and Th17 cytokines [84, 85]. *In vivo* blockade of IL-7 receptor function with a monoclonal antibody prevents, whereas administration of exogenous IL-7 markedly accelerates, the development and onset of SjS pathologies and hyposalivation in the C57BL/6.NOD-*Aec1*.*Aec2* mouse strain, a model of pSjS [63, 87]. The effect of IL-7 at the pre-diseased stage is closely associated with an enhanced Th1 and Tc1 responses and increased IFNγ and TNFα amounts in the salivary glands [63, 66]. Moreover, blockade of IL-7 receptor in female NOD mice that have newly established SjS disease significantly attenuates the SjS pathologies and hyposalivation, with the outcomes underlain by decreased Th1 and Tc1 responses in the salivary gland tissues [26]. Hence, IL-7 potentiates Th1/Tc1 responses to accelerate the development and sustain the chronic persistence of SjS. There are still many knowledge gaps in the understanding of IL-7 function in SjS, such as its impact on other Th subsets, Tregs, and innate immune cells. These are important subjects of future investigations.

6.3.4 IL-12 and IL-18

IL-12 is a potent Th1-promoting cytokine mainly produced by dendritic cells and macrophages [88, 89]. It drives the activated T cells to differentiate into Th1 effectors and also enhances their proliferation and IFNγ production [88–90]. Excessive production of IL-12 contributes to the pathogenesis of autoimmune inflammatory disorders including Crohn's disease, RA, psoriasis, and type 1 diabetes [91–93]. The amount of IL-12 is elevated in salivary gland tissues of SjS patients [94, 95]. IL-12 levels are similarly increased in the salivary glands of mouse models of SjS, at the early stage of disease development compared to the control mice [29, 96]. An IL-12-transgenic mouse strain on the SJL genetic background develops SjS-like exocrinopathy and hyposalivation [97]. However, properly designed loss-of-function studies in mouse models of SjS, which are still lacking, will be needed to elucidate the role of endogenous IL-12 in the pathogenesis and persistence of this disease.

IL-18 is an IL-1 family proinflammatory cytokine that affects the function of many immune and tissue cell types, thereby contributing to diverse inflammatory and autoimmune responses [98–100]. A prominent effect of IL-18 in adaptive immunity is to facilitate IL-12-induced Th1 differentiation and function [99, 101], thereby

promoting the development of multiple autoimmune inflammatory diseases such as RA, type 1 diabetes, and IBD [100–103]. IL-18 amounts are increased in the salivary glands, saliva, and sera of SjS patients with a positive correlation to the disease severity [85, 104, 105]. In addition, the level of IL-18 expression by macrophages and DCs in the salivary gland tissues of pSjS patients is positively associated with the degree of sialadenitis and incidence of lymphoma [94]. Nonetheless, the functional importance and specific actions of IL-18 in SjS still remain largely uncharacterized and will need a better delineation through both *in vivo* and *in vitro* functional studies.

6.4 Th2 Cells and Cytokines

6.4.1 IL-4

IL-4 is the signature cytokine produced by Th2 cells, which also reinforces Th2 cell differentiation while inhibiting that of Th1 cells [22, 106]. IL-4 plays a vital role in the development of asthma and allergy and provides critical help to the expansion and function of activated B cells [22, 106]. IL-4 has a protective effect against type 1 diabetes and RA [107, 108], but promotes SLE, an autoimmune disease in which autoreactive B cell responses are essentially required [44, 109]. IL-4 is detected in the salivary glands of some SjS patients, particularly those exhibiting a greater degree of B cell accumulation in the salivary glands [51, 110].

Deficiency of IL-4 gene or its downstream effector, STAT6, in the NOD mice or NOD.B10-H2b mice prevents the production of IgG1-type anti-M3R autoantibody and protects the normal salivary gland function [27–29]. IL-4 preferentially facilitates the production of IgG1-type anti-M3R, possibly by assisting the isotype switching process, without compromising the generation of other isotypes of this antibody or affecting salivary gland inflammation [27–29]. Therefore, IL-4 contributes to the development of SjS-like exocrine dysfunction, at least in part, through promoting the generation of the pathogenic, IgG1-type anti-M3R autoantibody. Additional functionalities of IL-4 in the SjS disease setting, such as its effect on M2 macrophages and interaction with other Th effector subsets, will be important topics to investigate.

6.4.2 IL-13

IL-13, another Th2-associated cytokine, bears partially overlapping functions with IL-4 [111, 112]. IL-13 targets B cells, mast cells, macrophages, and fibroblasts; promotes the development of allergy/asthma and tissue fibrosis; and plays a critical role in anti-helminth immunity [111, 112]. IL-13 amounts are elevated in patients with RA and SLE, and positively associated with the disease severity [113, 114]. IL-13 mRNA is detected in the exocrine glands of SjS patients [51, 115]. In mice, IL-13-producing Th2 cells are detected in the salivary gland-draining lymph nodes of the Id3-knockout mouse strain, a model of pSjS, but not in the control C57BL/6 mouse strain [116, 117]. More importantly, neutralization of IL-13 in Id3-knockout mice improves salivary secretion, and the effect is associated with a reduction in the number of mast cells in the target tissues, which is otherwise higher than that in the control C57BL/6 mice [116, 117]. Therefore, these findings strongly suggest that IL-13 derived from the Th2 cells contributes to the development of SjS-like hyposalivation in part by positively regulating mast cell number and activity.

6.5 Th17 Cells and Cytokines

6.5.1 IL-17 (IL-17A) and IL-23

IL-17 (IL-17A), the hallmark cytokine of Th17 effector cell subset, has a prominent proinflammatory function and drives the development of numerous autoimmune inflammatory diseases including multiple sclerosis, RA, and psoriasis

[31, 89, 118, 119]. IL-23, an IL-12 family cytokine and Th17-skewing cytokine, plays a pivotal role in the induction, stabilization, and expansion of Th17 cells [31, 89, 118]. IL-17 and IL-23 are both increased in the salivary glands and sera of pSjS patients [120–122], and along with their receptors, are detected in the lymphocytic infiltrates and ductal areas in the salivary glands of SjS patients, with IL-17 predominantly colocalizing with the CD4 T cells [121, 122]. In addition, a population of IL-17-producing $CD3^+CD4^-CD8^-$ T cells are also expanded in the blood and salivary glands of SjS patients [123].

Multiple *in vivo* investigations have convincingly demonstrated a functional importance of IL-17 in the pathogenesis of SjS. Blockade of IL-17 signaling through adenovirus-mediated expression of soluble IL-17 receptor impedes the development and onset of SjS and attenuates the established SjS disease in the C57BL/6.NOD-*Aec1Aec2* model [34]. Similarly, IL-17 gene deletion in the same mouse model markedly attenuates salivary gland inflammation and damage, and improves salivary secretion [124]. The complementary gain of function study shows that adenovirus vector-mediated IL-17 overexpression in wildtype C57BL/6 mice induces the development of SjS-like exocrinopathy [33]. In a salivary gland autoantigen-immunization-induced model of SjS, IL-17 deficiency prevents the development and onset of SjS-like salivary gland inflammation and dysfunction, which can be re-established by the adoptive transfer of Th17 cells [125].

Th17 cells and IL-17 exert SjS-promoting actions through multiple layers of mechanisms. They enhance the production of proinflammatory mediators, such as TNFα and matrix metalloproteinases, by exocrine gland epithelial cells and other exocrine tissue cells [32]. Adoptive transfer of Th17 cells leads to an increase in Th1 cells in a salivary gland autoantigen-immunization-induced SjS model [125]. In addition, adoptive transfer of Th17 cells and viral vector-mediated overexpression of IL-17 both lead to enhanced B cell responses and autoantibody production, whereas deletion of IL-17 gene results in a substantial reduction of germinal center B cells and plasma cells in salivary glands and a decrease in serum autoantibodies [33, 34], accompanied by a decline in the amount of multiple Th2 cytokines. Therefore, positive regulation of autoreactive B cell responses, in part through promoting Th2 cytokine production, may serve as one important mechanism by which IL-17 promotes SjS pathogenesis and persistence. Collectively, these findings depict an essential role of IL-17 in the pathogenesis and sustainment of SjS by engaging multiple cellular and molecular mechanisms, and identify IL-17 as a promising therapeutic target for this disease.

6.5.2 IL-22

IL-22 is an IL-10 family cytokine that can be produced by Th17, Th22 cells, among other immune cells [126–128]. It predominantly acts on non-immune tissue cells such as epithelial cells and keratinocytes to exert tissue-protective or proinflammatory, detrimental impacts in an intricate, tissue- and disease type-dependent manner [126–129]. Emerging evidence has supported the notion that IL-22 may play a disease-promoting pathogenic role in SjS. IL-22 and its receptor are both expressed at higher levels in the salivary glands of SjS patients compared to the control subjects, in which Th17 and NK cells are the principal producers of IL-22, while mononuclear cells, epithelial cells, and endothelial cells express IL-22 receptors [130–132]. IL-22 levels are also increased in the sera of SjS patients with a positive correlation with the disease severity [130–132].

An *in vivo* functional study using an adenovirus infection-induced SjS mouse model shows that deletion of IL-22 gene significantly curtails the accumulation of B cells and formation of ectopic germinal center-like structures in the salivary glands, and reduces autoantibody production [133]. This outcome is accompanied by failure to efficiently upregulate CXCL12 and -13, two B cell chemoattractants, by stromal cells residing in the ectopic germinal centers in the salivary glands [133]. Hence,

it is conceivable that IL-22 derived from Th17 cells contributes to the B cell response-enhancing effect of these Th17 cells in SjS, a hypothesis to be tested. The specific role and actions of IL-22 in SjS development, persistence, and SjS-associated B cell lymphomagenesis still remain largely undetermined and should be an important subject of investigation in the SjS research field.

6.6 TFH Cells and IL-21

TFH cells, characterized by the expression of transcription factor BCL6 and production of IL-21, critically contribute to the formation and maintenance of germinal centers and germinal center B cell responses [134]. The proportion of $CD4^+CD45RA^-CXCR5^+PD\text{-}1^+$ TFH cells is increased in the blood of pSjS patients compared to the healthy controls, with a positive association with the disease activity [32]. Identification of TFH cells based on the expression of IL-21 and surface markers PD-1, CXCL13, and ICOS-1 similarly shows that the frequency of these cells is elevated in the blood of pSjS patients compared to the healthy controls [32, 135, 136]. In salivary glands, the amount of TFH cells, defined as $PD\text{-}1^+ICOS^+$ T cells, is also increased in pSjS patients, constituting approximately 9% of the total CD4 T cells [137]. In addition, B-cell lymphoma 6 protein (BCL6), the key molecular marker of TFH cells, is detected in pSjS patient salivary gland tissues that contain germinal center-like structures [138]. Some of the TFH cells in the salivary glands may be recruited from the peripheral blood, while others may be generated locally in the glands in response to the TFH-promoting cytokines produced in greater amounts in salivary glands of pSjS patients compared to the control subjects, such as IL-6 and the TFH cytokine IL-21 itself [110, 138, 139]. For instance, it has been shown that salivary gland epithelial cells from pSjS patients can promote the generation of IL-21-producing TFH cells from the naïve CD4 T cells through IL-6 and ICOS ligand expression [140].

The hallmark effector cytokine produced by TFH cells is IL-21, a member of the common cytokine receptor γ chain (γc)-dependent cytokine family with Th17 and NKT cells as chief producers [141, 142]. IL-21 can exert diverse functionalities on various T cell subsets, other immune cells, and non-immune tissue cells [37, 141, 142]. It can potentiate plasma cell differentiation, augment germinal center B cell response, and reinforce TFH and Th17 cell differentiation [37, 118, 143, 144], to foster the pathogenesis of a variety of autoimmune and inflammatory disorders, including SLE, IBD, type 1 diabetes, and psoriasis [145, 146]. Consistent with an increase in TFH cells, IL-21 transcripts and protein, along with IL-21-producing T cells, are also detected at a significant amount in salivary glands of SjS patients [59, 147, 148]. In addition, IL-21 levels in the salivary glands and sera of pSjS patients, which are higher than those in control subjects, exhibit a positive correlation with the serum autoantibody levels [147].

Several *in vivo* functional studies have delineated an important contribution of TFH cells and IL-21 to the pathogenesis of SjS. Lentiviral vector-mediated local expression of IL-21 shRNA in submandibular glands of the NOD mice effectively hinders the development of sialadenitis and hyposalivation, which is accompanied by a diminished TFH response and B cell autoantibody production [36]. In a salivary gland autoantigen-immunization-induced SjS model, ablation of TFH cells through deletion of BCL6 gene, the master regulator of TFH differentiation, substantially attenuates SjS-like sialadenitis and autoantibody production and reduces the number of TFH cells, Th17 cells, and germinal center B cells in the salivary gland-draining lymph nodes [35]. Nevertheless, ablation of TFH cells does not have an impact on salivary secretion in this model [35]. The role of TFH cells in SjS pathogenesis, persistence, and B cell lymphomagenesis, as well as the underlying mechanisms of their actions, are still poorly defined. More studies that are comprehensive and in-depth will be needed using additional mouse models and experimental approaches.

6.7 Immune-Regulatory Lymphocytes and Cytokines

6.7.1 Tregs

The functional property and importance of Tregs in the pathogenesis and modulation of SjS has remained as a key knowledge gap in the SjS research field due to insufficient amount of studies and conflicting results [149–152]. While some reports show a reduced number of Tregs in salivary glands or blood of pSjS patients [149, 151, 152] and impaired suppressor activity of these cells [153], other studies reported an increased number of Tregs in the salivary glands of pSjS patients that are positively correlated with the severity of sialadenitis [150, 153, 154]. Several recent investigations have revealed that the proportion of activated memory Tregs in the blood is increased in pSjS patients compared to the healthy control counterparts [155, 156] and positively correlates with the disease activity [155]. In addition, the amount of total Foxp3-expressing Tregs is also shown to be positively associated with the severity of sialadenitis in SjS patients [157, 158].

Investigations of the functional activity of SjS-afflicted Tregs have also yielded conflicting results. One report shows a normal functionality of blood Tregs from pSjS patients [150] while another depicts an impaired immunosuppressive activity of these cells [153]. A recent study using the NOD mouse model of SjS shows that Treg cell-depletion in female NOD mice does not affect the disease development or severity, providing more evidence for an defective Treg suppressor function in the SjS disease environment [159]. The defects of Tregs may be attributed, at least in part, to the exposure to the proinflammatory signals in the target exocrine tissues, since Th1 cytokines IFNγ and IL-12, and Th17 cytokine IL-23 all have a documented ability to impair the function, identity, and stability of Tregs while conferring or enhancing the proinflammatory property of these cells [160–166]. A better understanding of the functional characteristics, *in vivo* role and regulation of Tregs in the SjS disease will significantly contribute to the development of novel, effective biological therapies for SjS and many other autoimmune inflammatory diseases.

6.7.2 IL-10

IL-10 is an extensively characterized immunosuppressive cytokine, and a potent regulator of both innate and adaptive immune responses under numerous circumstances [126, 167]. Originally classified as a Th2 cytokine, IL-10 can be produced by Th2 cells and Tregs, as well as macrophages, type 1 regulatory T cells (Tr1), and various other Th subsets [126, 167–169]. IL-10 attenuates the activation and function of antigen-presenting cells, Th1 cells and Th17 cells, among others [168–171], and prevents or attenuates a wide array of autoimmune/inflammatory diseases, including IBD, RA, EAE, and microbial infection-induced organ inflammations [171–173]. IL-10 also positively regulates B cell responses, enhancing the expansion, isotype switching, and antibody production of these cells [170, 171, 174, 175].

IL-10 concentrations are elevated in the saliva of SjS patients and correlate positively with the severity of xerophthalmia and xerostomia [176]. IL-10 levels are also increased in the sera of pSjS patients with a direct correlation with the magnitude of sialadenitis and the amount of serum IgG1 autoantibodies [177]. Transgenic IL-10 expression in exocrine gland tissues induces SjS-like exocrinopathy [178], whereas IL-10 gene deletion markedly curtails sialadenitis in the NOD model of this disease [179]. These findings demonstrate a pathogenic, SjS disease-promoting effect of IL-10 unlike its extensively reported immune-regulatory and anti-inflammatory activity in many other disease settings. The SjS-promoting activity of IL-10 is underpinned by the upregulation of Fas ligand in CD4 T cells [178], enhancement of ICAM-1 expression in exocrine tissues, acquisition of proinflammatory function by IFNα-primed innate immune cells [105, 180], and a likely augmentation of autoreactive B cell responses. Therefore, the current evidence has indicated that IL-10 may predomi-

nantly act as a disease-promoting pathogenic player in SjS, as in the cases of SLE and type 1 diabetes [105, 180]. Future investigations are needed to better delineate its action and role in SjS in a disease stage- and cellular producer-specific fashion.

6.7.3 Tfr Cells

T follicular regulatory cells (Tfr) are a group of Foxp3-expressing immune-regulatory T cells that bear similar phenotypic features to TFH cells but suppress TFH activities and curtail germinal center B cell responses in the germinal centers and other germinal center-like structures [181, 182]. Tfr cells are enriched in the blood of pSjS patients to a greater degree than TFH cells [155, 183], present in the salivary glands of the majority of the pSjS patients, and located mainly at the borders between the T and B cell zones outside of the ectopic germinal center-like structures [183].

In a salivary gland autoantigen-immunization-induced SjS model, ablation of Tfr cells through BCL6 gene deletion in Foxp3-expressing regulatory cells markedly accelerates the development of SjS-like pathologies, whereas ablation of TFH cells impedes the disease development [35]. However, depletion of Tfr cells does not affect salivary secretion in this study, raising the question whether Tfr and TFH cells are essentially required for this process. Additional investigations will be needed to comprehensively and rigorously elucidate the properties and actions of Tfr cells in the SjS disease using both human samples and mouse models.

6.8 B Cells

6.8.1 Major B Cell Subsets in the Target Exocrine Glands of SjS

The activation of autoreactive B cells in the context of SjS is initiated and potentiated by genetic and epigenetic predispositions, environmental triggers such as viral infections, and impact from various cell types in the microenvironment, including epithelial cells, innate immune cells, and multiple Th subsets that can provide help to B cells [6, 41].

Multiple B cell populations or subsets that may collectively contribute to SjS pathogenesis and persistence have been identified in the exocrine glands of SjS patients, including $CD27^+$ memory B cells, marginal zone B cells, plasmablasts, and plasma cells. (1) $CD27^+$ memory B cells: In pSjS patients, the number of circulating $CD27^+$ memory B cells is consistently decreased [184–189], which may result from the migration of these cells to the target exocrine glands or from the retention of these cells in the glands as supported by an increased amount of these cells detected in salivary glands of pSjS patients [190]. (2) Plasmablasts and plasma cells: A comprehensive single cell-based analysis demonstrates a decrease in the number of $CD27^+$ memory B cells, CD4 T cells, and plasmacytoid dendritic cells (pDCs) and an increase in the number of activated CD4- and CD8 T cells and plasmablasts in the blood of SjS patients, compared to the healthy control subjects [191]. The analysis also shows the presence of total B cells, plasma cells, and significant amount of effector CD8 T cells in the circulation of these pSjS patients. Some of these changes are positively associated with the severity of disease pathologies. In addition, the single cell-based profiling also reveals that plasma cells, including the long-lived plasma cells, constitute a significant proportion of the total B cells in the salivary glands of pSjS patients [191]. (3) Marginal zone (MZ) B cells: MZ B cells normally reside in the marginal zone of the spleen and are characterized by the expression of high levels of IgM, CD21, and CD1, combined with low levels of IgD, CD23, and CD5. They are generated through T cell-independent selection process and can rapidly elicit T cell-independent, first-line responses against certain blood-borne antigens with a lower activation threshold than the follicular B cells. A significant proportion of $CD27^+$ memory B cells in the salivary glands of pSjS patients express IgM and bear a MZ B cell-like phenotype, [192, 193], although more

definitive and thorough characterizations of these cells are still needed.

The functional importance of B cells as a whole has been revealed by multiple *in vivo* studies with mouse model of SjS [194, 195]. Depletion of B cells with an anti-CD20 antibody ameliorates the development of SjS-like sialadenitis and autoantibody production in the Id3-KO mouse strain, a model of pSjS [194]. The pathogenic T cells from the SjS mouse models cannot induce SjS-like disease when transferred into host mice lacking B cells [194–196]. In addition, deficiency of Th2 cytokine IL-4 prevents the development of salivary secretory dysfunction due to failure to produce IgG1-type autoantibodies, including anti-M3R, by B cells [27, 28].

A critical pathogenic role of MZ B cells in SjS has been demonstrated in several models of this disease. In the *Tnfsf13b*-transgenic mice in which the liver cells overexpress B cell growth factor B cell-activating factor (BAFF), which spontaneously develop SjS-like sialadenitis and hyposalivation, a significant proportion of the salivary gland-infiltrating B cells have a MZ B cell-like phenotype of $IgM^{hi}B220^{hi}CD5^{-}CD23^{lo/-}IgD^{lo/-}CD1^{hi}CD21^{hi}$ L-selectin^{lo}HSA^{++} [197, 198]. Importantly, genetic ablation of the MZ B cell population in these mice prevents the development of SjS-like sialadenitis without affecting that of nephritis [199]. In *Txlna*-transgenic mice that overexpress B cell growth factor IL-14 in activated T and B cells and in follicular dendritic cells, the splenic $CD19^{+}CD21^{+}IgM^{+}$ MZ B cell population is expanded, and genetic ablation of MZ B cells in these mice abolishes all the major characteristic pathologies of SjS including the autoantibody production [200, 201].

6.8.2 B Cells in Ectopic Germinal Centers

Ectopic germinal centers are detected in the salivary glands of approximately10–30% of pSjS patients [202, 203], and bear similar morphologies to the conventional germinal centers, defined as T and B cell aggregates containing proliferative cells and a network of follicular dendritic cells and activated tissue cells. The germinal center-like structures in the salivary glands contain apoptotic cells and anti-Ro/SSA and anti-La/SSB autoantibodies, with the amount of autoantibodies markedly higher in pSjS patients that have ectopic germinal centers than those without these structures [203]. Furthermore, the polymorphism of the CXCR5 gene, which encodes the signature chemokine receptor expressed by TFH cells, is associated with the pSjS incidence [204]. These findings suggest that the formation of the ectopic germinal center-like structures may contribute to the sustained autoreactive B cell responses in SjS.

As described in the earlier sections on T cells and cytokines of this chapter, the importance of multiple germinal center-promoting factors, including the TFH-IL-21 axis, IL-22, and CXCL13, in SjS pathogenesis has been demonstrated in mouse models, indirectly supporting a functional significance of germinal center-like structures in SjS. Nevertheless, more definitive and comprehensive investigations will be required to elucidate the role of ectopic germinal centers and germinal center B cells in the pathogenesis and sustainment of SjS and the associated B cell lymphomagenesis.

6.8.3 B Cell-Activating Factor (BAFF)

BAFF is a cytokine that plays a vital role in promoting the maturation, survival, expansion, and antibody production of B cells, including MZ B cells [205, 206]. BAFF, with both a membrane-bound form and a soluble form, can bind to the BAFF receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), or B-cell maturation antigen (BCMA), three receptors that are chiefly expressed by immature B cells, memory B cells, and plasma cells, respectively [205, 206]. A BAFF homologue protein named a proliferation-inducing ligand (APRIL), which is also a tumor necrosis factor ligand superfamily member, can also bind to TACI and BCMA to enhance B cell expansion, differentiation and antibody produc-

tion [207, 208], and has partially overlapping effects on B cells with BAFF.

The amount of BAFF is increased in the salivary glands of pSjS patients compared to the control subjects [198, 209], with a variety of cell types, including T and B cells, macrophages, dendritic cells, and epithelial cells, as cellular producers [209–212]. Apart from exocrine gland tissues, BAFF levels are also elevated in the blood and correlated with the levels of anti-SSA/Ro, anti-SSB/La, and the rheumatoid factor [186, 209]. Type I IFNs and IFNγ that are rich in SjS target tissues can induce BAFF production [87, 211, 213], and the apoptotic bodies released from salivary gland epithelial cells and autoantibodies can also stimulate BAFF production by epithelial cells through enhancing type 1 IFN production from the infiltrating immune cells [134, 210, 214]. In addition, virus-triggered signals have been shown to potentiate BAFF production by salivary gland epithelial cells through both type 1 IFN- and TLR-dependent and independent mechanisms [212].

Excessive amount of BAFF in the target tissues can positively affect multiple aspects of B cell responses, including the survival and proliferation of B cells, generation of plasma cells, production of autoantibodies, and lymphomagenesis of B cells [198, 215, 216]. The *Tnfsf13b*-transgenic mice, in which liver cells overexpress BAFF, develop pSjS-like hyposalivation and sialadenitis characterized by the presence of a large number of B cells in the salivary glands, with a significant proportion of them being MZ-like B cells [197, 198]. In addition, plasmid-mediated BAFF overexpression in NOD mice exacerbates leukocytic infiltration of salivary glands and enhances B cell differentiation, without significantly altering the formation of ectopic germinal centers [217]. The *in vivo* role of endogenously produced BAFF in SjS pathogenesis has also been revealed by several loss-of-function studies. ΔBAFF is a naturally arising physiological inhibitor of BAFF function, a minor splice variant of BAFF mRNA formed through deletion of a single exon deletion (exon 3 in human and exon 4 in mice) [218]. An exon-skipping approach that targets BAFF mRNA splicing to increase ΔBAFF amount and concomitantly reducing the full-length BAFF amount in salivary tissues of NOD mice causes a substantial decrease in B cells and plasma cells in the salivary glands and an attenuation of SjS-like sialadenitis and hyposalivation [219]. In addition, inhibition of BAFF activity in the NOD mice prior to SjS disease onset through administration of a soluble BAFF receptor dramatically reduces the number of B cells in the salivary glands and hinders the development of SjS-like sialadenitis and production of antinuclear antibodies [220]. Hence, current evidence points to an importance of BAFF in the autoreactive B cell responses and pathogenesis of SjS, although there are controversies about its *in vivo* significance for the ectopic germinal center formation in this disease.

In comparison to BAFF, the expression and function of APRIL in SjS in both humans and mice has remained largely uncharacterized. A better and more comprehensive understanding of the actions of both BAFF and APRIL and the underlying mechanisms of their effects will greatly advance the development of effective, B cell-targeted therapies for this disease.

6.9 Biological Therapies Targeting Adaptive Immunity in SjS

Recent SjS research has unequivocally demonstrated the importance of autoreactive T and B cells, and the cytokines that affect or mediate their functions, in development and persistence of this disease. As a result, significant efforts have been made to design and clinically evaluate a variety of therapeutics that can potentially abolish or dampen the activity of these adaptive immune players or their downstream signaling and effector pathways. Figure 6.1 depicts an overview of the major adaptive immune players in SjS discussed in this chapter, and the corresponding therapeutics targeting these players subjected to clinical trials. Despite partial positive outcomes in some of the clinical trials, the great majority of the therapeutics tested to date

have not demonstrated a consistent and satisfactory efficacy.

The underlying reasons for the lack of success in developing effective biological therapeutics for SjS, in contrast to a number of other major autoimmune inflammatory diseases, may include: (a) There is an insufficient and inadequate understanding of the precise function, role, and mechanisms of action for many of the principal immunological players in SjS. Many of the cellular and molecular players identified as associated with SjS in human patients need to be assessed for their *in vivo* functional importance using animal models. Conversely, many findings generated from mouse models are yet to be evaluated in human samples. (b) The effects and roles of an immunological player are often complex, fluid and context-dependent, in that the same player can be pathogenic in other diseases or organs but protective in SjS, and vice versa. Hence, application of therapeutic strategies effective for other disease conditions to SjS without a thoroughly delineated scientific basis may yield unsuccessful or even detrimental outcomes. Supporting this notion, multiple biologics, including those targeting TNFα, IL-6, CD20, and BAFF described above, have been successful in treating certain other inflammatory diseases, such as RA, SLE, and Crohn's disease, but fail to achieve desired outcome for SjS. (c) SjS is highly multi-factorial in its pathogenic mechanisms and heterogenous in its pathological manifestations, which significantly increases the complexity and difficulty in developing effective therapeutic strategies.

6.10 Conclusions

Remarkable advancement has been made in understanding various key aspects of the adaptive immunity governing the SjS disease. However, many critical knowledge gaps still remain to be filled, including the precise effects and the disease stage-dependent functions of T and B cell populations and cytokines, the mechanisms underlying the actions of these adaptive immune players, and the intricate interactions and cross-regulation among them during the development and persistence of local and systemic SjS pathologies. There is an urgent and crucial need to address these knowledge gaps, identify new therapeutic targets, and develop novel therapeutic approaches including those that simultaenously target multiple immune players and those that effectively target subgroups or even individual patients. The overall goal will be to establish effective and targeted treatments for this challenging and complex autoimmune disease and improve the oral and overall health of millions of people.

References

1. Fox PC. Autoimmune diseases and Sjogren's syndrome: an autoimmune exocrinopathy. Ann N Y Acad Sci. 2007;1098:15–21. Epub 2007/03/03. eng.
2. Cafaro G, Croia C, Argyropoulou OD, Leone MC, Orlandi M, Finamore F, et al. One year in review 2019: Sjogren's syndrome. Clin Exp Rheumatol. 2019;37 Suppl 118(3):3–15.
3. Katsifis GE, Moutsopoulos NM, Wahl SM. T lymphocytes in Sjogren's syndrome: contributors to and regulators of pathophysiology. Clin Rev Allergy Immunol. 2007;32(3):252–64. Epub 2007/11/10. eng.
4. Lee BH, Tudares MA, Nguyen CQ. Sjogren's syndrome: an old tale with a new twist. Arch Immunol Ther Exp (Warsz). 2009;57(1):57–66. Epub 2009/02/17. eng.
5. Cornec D, Devauchelle-Pensec V, Tobon GJ, Pers JO, Jousse-Joulin S, Saraux A. B cells in Sjogren's syndrome: from pathophysiology to diagnosis and treatment. J Autoimmun. 2012;39(3):161–7.
6. Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjogren's syndrome. Nat Rev Rheumatol. 2010;6(9):529–37.
7. Del Papa N, Vitali C. Management of primary Sjogren's syndrome: recent developments and new classification criteria. Therapeut Adv Musculoskelet Dis. 2018;10(2):39–54. Pubmed Central PMCID: 5784475.
8. Sada PR, Isenberg D, Ciurtin C. Biologic treatment in Sjogren's syndrome. Rheumatology (Oxford). 2015;54(2):219–30.
9. Bartoloni E, Alunno A, Gerli R. The dark side of Sjogren's syndrome: the possible pathogenic role of infections. Curr Opin Rheumatol. 2019;31(5):505–11.
10. Harris VM, Scofield RH, Sivils KL. Genetics in Sjogren's syndrome: where we are and where we go. Clin Exp Rheumatol. 2019;37 Suppl 118(3):234–9.

11. Konttinen YT, Fuellen G, Bing Y, Porola P, Stegaev V, Trokovic N, et al. Sex steroids in Sjogren's syndrome. J Autoimmun. 2012;39(1-2):49–56.
12. Maslinska M. The role of Epstein-Barr virus infection in primary Sjogren's syndrome. Curr Opin Rheumatol. 2019;31(5):475–83.
13. Porola P, Laine M, Virkki L, Poduval P, Konttinen YT. The influence of sex steroids on Sjogren's syndrome. Ann N Y Acad Sci. 2007;1108:426–32.
14. Argyropoulou OD, Valentini E, Ferro F, Leone MC, Cafaro G, Bartoloni E, et al. One year in review 2018: Sjogren's syndrome. Clin Exp Rheumatol. 2018;36 Suppl 112(3):14–26.
15. Tsigalou C, Stavropoulou E, Bezirtzoglou E. Current insights in microbiome shifts in Sjogren's syndrome and possible therapeutic interventions. Front Immunol. 2018;9:1106. Pubmed Central PMCID: 5976780.
16. Zhong D, Wu C, Zeng X, Wang Q. The role of gut microbiota in the pathogenesis of rheumatic diseases. Clin Rheumatol. 2018;37(1):25–34.
17. Kiripolsky J, McCabe LG, Kramer JM. Innate immunity in Sjogren's syndrome. Clin Immunol. 2017;182:4–13. Pubmed Central PMCID: 6025757.
18. Kramer JM. Early events in Sjogren's syndrome pathogenesis: the importance of innate immunity in disease initiation. Cytokine. 2014;67(2):92–101.
19. Nikolov NP, Illei GG. Pathogenesis of Sjogren's syndrome. Curr Opin Rheumatol. 2009;21(5):465–70. Pubmed Central PMCID: 2766246. Epub 2009/07/02. eng.
20. Pontarini E, Lucchesi D, Bombardieri M. Current views on the pathogenesis of Sjogren's syndrome. Curr Opin Rheumatol. 2018;30(2):215–21.
21. Nandula SR, Scindia YM, Dey P, Bagavant H, Deshmukh US. Activation of innate immunity accelerates sialoadenitis in a mouse model for Sjogren's syndrome-like disease. Oral Dis. 2011;17(8):801–7. Pubmed Central PMCID: 3192939.
22. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol. 2002;2(12):933–44.
23. Glimcher LH, Murphy KM. Lineage commitment in the immune system: the T helper lymphocyte grows up. Genes Dev. 2000;14(14):1693–711.
24. Cha S, Brayer J, Gao J, Brown V, Killedar S, Yasunari U, et al. A dual role for interferon-gamma in the pathogenesis of Sjogren's syndrome-like autoimmune exocrinopathy in the nonobese diabetic mouse. Scand J Immunol. 2004;60(6):552–65. Epub 2004/12/09. eng.
25. Roescher N, Tak PP, Illei GG. Cytokines in Sjogren's syndrome: potential therapeutic targets. Ann Rheum Dis. 2010;69(6):945–8. Pubmed Central PMCID: 3044243.
26. Zhou J, Yu Q. Anti-IL-7 receptor-alpha treatment ameliorates newly established Sjogren's-like exocrinopathy in non-obese diabetic mice. Biochim Biophys Acta Mol Basis Dis. 2018;1864(7):2438–47. Pubmed Central PMCID: 5963292.
27. Brayer JB, Cha S, Nagashima H, Yasunari U, Lindberg A, Diggs S, et al. IL-4-dependent effector phase in autoimmune exocrinopathy as defined by the NOD.IL-4-gene knockout mouse model of Sjogren's syndrome. Scand J Immunol. 2001;54(1-2):133–40. Epub 2001/07/06. eng.
28. Nguyen CQ, Gao JH, Kim H, Saban DR, Cornelius JG, Peck AB. IL-4-STAT6 signal transduction-dependent induction of the clinical phase of Sjogren's syndrome-like disease of the nonobese diabetic mouse. J Immunol. 2007;179(1):382–90. Pubmed Central PMCID: 2856075.
29. Gao J, Killedar S, Cornelius JG, Nguyen C, Cha S, Peck AB. Sjogren's syndrome in the NOD mouse model is an interleukin-4 time-dependent, antibody isotype-specific autoimmune disease. J Autoimmun. 2006;26(2):90–103.
30. Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol. 2008;8(5):337–48.
31. Martinez GJ, Nurieva RI, Yang XO, Dong C. Regulation and function of proinflammatory TH17 cells. Ann N Y Acad Sci. 2008;1143:188–211.
32. Verstappen GM, Corneth OBJ, Bootsma H, Kroese FGM. Th17 cells in primary Sjogren's syndrome: pathogenicity and plasticity. J Autoimmun. 2018;87:16–25.
33. Nguyen CQ, Yin H, Lee BH, Carcamo WC, Chiorini JA, Peck AB. Pathogenic effect of interleukin-17A in induction of Sjogren's syndrome-like disease using adenovirus-mediated gene transfer. Arthritis Res Ther. 2010;12(6):R220. Pubmed Central PMCID: 3046533.
34. Nguyen CQ, Yin H, Lee BH, Chiorini JA, Peck AB. IL17: potential therapeutic target in Sjogren's syndrome using adenovirus-mediated gene transfer. Lab Invest. 2011;91(1):54–62. Pubmed Central PMCID: 3012147.
35. Fu W, Liu X, Lin X, Feng H, Sun L, Li S, et al. Deficiency in T follicular regulatory cells promotes autoimmunity. J Exp Med. 2018;215(3):815–25. Pubmed Central PMCID: 5839755.
36. Liu H, Liu G, Gong L, Zhang Y, Jiang G. Local suppression of IL-21 in submandibular glands retards the development of Sjogren's syndrome in non-obese diabetic mice. J Oral Pathol Med. 2012;41(10):728–35.
37. Ma CS, Deenick EK, Batten M, Tangye SG. The origins, function, and regulation of T follicular helper cells. J Exp Med. 2012;209(7):1241–53. Pubmed Central PMCID: 3405510.
38. Chinen T, Kannan AK, Levine AG, Fan X, Klein U, Zheng Y, et al. An essential role for the IL-2 receptor in Treg cell function. Nat Immunol. 2016;17(11):1322–33. Pubmed Central PMCID: 5071159.
39. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012;30:531–64. Pubmed Central PMCID: 6066374.

40. Goschl L, Scheinecker C, Bonelli M. Treg cells in autoimmunity: from identification to Treg-based therapies. Semin Immunopathol. 2019;41(3):301–14.
41. Nguyen CQ, Cha SR, Peck AB. Sjogren's syndrome (SjS)-like disease of mice: the importance of B lymphocytes and autoantibodies. Front Biosci. 2007;12:1767–89.
42. Zuo J, Williams AE, Park YJ, Choi K, Chan AL, Reeves WH, et al. Muscarinic type 3 receptor autoantibodies are associated with anti-SSA/Ro autoantibodies in Sjogren's syndrome. J Immunol Methods. 2016;437:28–36. Pubmed Central PMCID: 5036946.
43. Park YS, Gauna AE, Cha S. Mouse models of primary Sjogren's syndrome. Curr Pharm Des. 2015;21(18):2350–64. Pubmed Central PMCID: 4425610.
44. Peng SL, Moslehi J, Craft J. Roles of interferon-gamma and interleukin-4 in murine lupus. J Clin Invest. 1997;99(8):1936–46. Pubmed Central PMCID: 508018.
45. Theofilopoulos AN, Koundouris S, Kono DH, Lawson BR. The role of IFN-gamma in systemic lupus erythematosus: a challenge to the Th1/Th2 paradigm in autoimmunity. Arthritis Res. 2001;3(3):136–41. Pubmed Central PMCID: 128889.
46. Boissier MC, Chiocchia G, Bessis N, Hajnal J, Garotta G, Nicoletti F, et al. Biphasic effect of interferon-gamma in murine collagen-induced arthritis. Eur J Immunol. 1995;25(5):1184–90.
47. Boumba D, Skopouli FN, Moutsopoulos HM. Cytokine mRNA expression in the labial salivary gland tissues from patients with primary Sjogren's syndrome. Br J Rheumatol. 1995;34(4):326–33.
48. Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjogren's syndrome. J Immunol. 1994;152(11):5532–9.
49. Kang EH, Lee YJ, Hyon JY, Yun PY, Song YW. Salivary cytokine profiles in primary Sjogren's syndrome differ from those in non-Sjogren sicca in terms of TNF-alpha levels and Th-1/Th-2 ratios. Clin Exp Rheumatol. 2011;29(6):970–6.
50. van Woerkom JM, Kruize AA, Wenting-van Wijk MJ, Knol E, Bihari IC, Jacobs JW, et al. Salivary gland and peripheral blood T helper 1 and 2 cell activity in Sjogren's syndrome compared with non-Sjogren's sicca syndrome. Ann Rheum Dis. 2005;64(10):1474–9. Pubmed Central PMCID: 1755251.
51. Mitsias DI, Tzioufas AG, Veiopoulou C, Zintzaras E, Tassios IK, Kogopoulou O, et al. The Th1/Th2 cytokine balance changes with the progress of the immunopathological lesion of Sjogren's syndrome. Clin Exp Immunol. 2002;128(3):562–8. Pubmed Central PMCID: 1906267.
52. Hu X, Ivashkiv LB. Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases. Immunity. 2009;31(4):539–50. Pubmed Central PMCID: 2774226.
53. Baker OJ, Camden JM, Redman RS, Jones JE, Seye CI, Erb L, et al. Proinflammatory cytokines tumor necrosis factor-alpha and interferon-gamma alter tight junction structure and function in the rat parotid gland Par-C10 cell line. Am J Physiol Cell Physiol. 2008;295(5):C1191–201. Pubmed Central PMCID: 2584989.
54. Odusanwo O, Chinthamani S, McCall A, Duffey ME, Baker OJ. Resolvin D1 prevents TNF-alpha-mediated disruption of salivary epithelial formation. Am J Physiol Cell Physiol. 2012;302(9):C1331–45. Pubmed Central PMCID: 3361948.
55. Ogawa N, Ping L, Zhenjun L, Takada Y, Sugai S. Involvement of the interferon-gamma-induced T cell-attracting chemokines, interferon-gamma-inducible 10-kd protein (CXCL10) and monokine induced by interferon-gamma (CXCL9), in the salivary gland lesions of patients with Sjogren's syndrome. Arthritis Rheum. 2002;46(10):2730–41. Epub 2002/10/18. eng.
56. Yoon KC, Park CS, You IC, Choi HJ, Lee KH, Im SK, et al. Expression of CXCL9, -10, -11, and CXCR3 in the tear film and ocular surface of patients with dry eye syndrome. Invest Ophthalmol Vis Sci. 2010;51(2):643–50. Pubmed Central PMCID: 3258976.
57. Hasegawa H, Inoue A, Kohno M, Muraoka M, Miyazaki T, Terada M, et al. Antagonist of interferon-inducible protein 10/CXCL10 ameliorates the progression of autoimmune sialadenitis in MRL/lpr mice. Arthritis Rheum. 2006;54(4):1174–83.
58. Matsuno H, Yudoh K, Katayama R, Nakazawa F, Uzuki M, Sawai T, et al. The role of TNF-alpha in the pathogenesis of inflammation and joint destruction in rheumatoid arthritis (RA): a study using a human RA/SCID mouse chimera. Rheumatology (Oxford). 2002;41(3):329–37.
59. McGuire HM, Vogelzang A, Ma CS, Hughes WE, Silveira PA, Tangye SG, et al. A subset of interleukin-21+ chemokine receptor CCR9+ T helper cells target accessory organs of the digestive system in autoimmunity. Immunity. 2011;34(4):602–15.
60. Mease PJ. Tumour necrosis factor (TNF) in psoriatic arthritis: pathophysiology and treatment with TNF inhibitors. Ann Rheum Dis. 2002;61(4):298–304. Pubmed Central PMCID: 1754073.
61. Alimohammadi N, Koosha F, Rafeian-Kopaei M. Current, new, and future therapeutic targets in inflammatory bowel disease: a systematic review. Curr Pharmaceut Des. 2020.
62. Digby-Bell JL, Atreya R, Monteleone G, Powell N. Interrogating host immunity to predict treatment response in inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2020;17(1):9–20.
63. Jin JO, Kawai T, Cha S, Yu Q. Interleukin-7 enhances the Th1 response to promote the development of Sjogren's syndrome-like autoimmune exocrinopathy

in mice. Arthritis Rheum. 2013;65(8):2132–42. Pubmed Central PMCID: 3729733.
64. Steinfeld SD, Demols P, Salmon I, Kiss R, Appelboom T. Infliximab in patients with primary Sjogren's syndrome: a pilot study. Arthritis Rheum. 2001;44(10):2371–5.
65. Mariette X, Ravaud P, Steinfeld S, Baron G, Goetz J, Hachulla E, et al. Inefficacy of infliximab in primary Sjogren's syndrome: results of the randomized, controlled Trial of Remicade in Primary Sjogren's Syndrome (TRIPSS). Arthritis Rheum. 2004;50(4):1270–6.
66. Zhou J, Kawai T, Yu Q. Pathogenic role of endogenous TNF-alpha in the development of Sjogren's-like sialadenitis and secretory dysfunction in non-obese diabetic mice. Lab Invest. 2017;97(4):458–67. Pubmed Central PMCID: 5376226.
67. Kamachi M, Kawakami A, Yamasaki S, Hida A, Nakashima T, Nakamura H, et al. Regulation of apoptotic cell death by cytokines in a human salivary gland cell line: distinct and synergistic mechanisms in apoptosis induced by tumor necrosis factor alpha and interferon gamma. J Lab Clin Med. 2002;139(1):13–9.
68. Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. J Biol Chem. 1999;274(29):20071–4.
69. Lai Z, Yin H, Cabrera-Perez J, Guimaro MC, Afione S, Michael DG, et al. Aquaporin gene therapy corrects Sjogren's syndrome phenotype in mice. Proc Natl Acad Sci U S A. 2016;113(20):5694–9. Pubmed Central PMCID: 4878512.
70. Choi K, Ni L, Jonakait GM. Fas ligation and tumor necrosis factor alpha activation of murine astrocytes promote heat shock factor-1 activation and heat shock protein expression leading to chemokine induction and cell survival. J Neurochem. 2011;116(3):438–48.
71. Algood HM, Lin PL, Yankura D, Jones A, Chan J, Flynn JL. TNF influences chemokine expression of macrophages in vitro and that of CD11b+ cells in vivo during Mycobacterium tuberculosis infection. J Immunol. 2004;172(11):6846–57.
72. Aringer M, Smolen JS. The role of tumor necrosis factor-alpha in systemic lupus erythematosus. Arthritis Res Ther. 2008;10(1):202. Pubmed Central PMCID: 2374473.
73. Almoallim H, Al-Ghamdi Y, Almaghrabi H, Alyasi O. Anti-tumor necrosis factor-alpha induced systemic lupus erythematosus. Open Rheumatol J. 2012;6:315–9. Pubmed Central PMCID: 3504723.
74. Aringer M, Steiner G, Graninger WB, Hofler E, Steiner CW, Smolen JS. Effects of short-term infliximab therapy on autoantibodies in systemic lupus erythematosus. Arthritis Rheum. 2007;56(1):274–9.
75. Aringer M, Graninger WB, Steiner G, Smolen JS. Safety and efficacy of tumor necrosis factor alpha blockade in systemic lupus erythematosus: an open-label study. Arthritis Rheum. 2004;50(10):3161–9.
76. Fry TJ, Mackall CL. The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance. J Immunol. 2005;174(11):6571–6.
77. Surh CD, Sprent J. Homeostasis of naive and memory T cells. Immunity. 2008;29(6):848–62.
78. Gonzalez-Quintial R, Lawson BR, Scatizzi JC, Craft J, Kono DH, Baccala R, et al. Systemic autoimmunity and lymphoproliferation are associated with excess IL-7 and inhibited by IL-7Ralpha blockade. PLoS One. 2011;6(11):e27528. Pubmed Central PMCID: 3213145.
79. Lawson BR, Gonzalez-Quintial R, Eleftheriadis T, Farrar MA, Miller SD, Sauer K, et al. Interleukin-7 is required for CD4(+) T cell activation and autoimmune neuroinflammation. Clin Immunol. 2015;161(2):260–9. Pubmed Central PMCID: 4658267.
80. Arbelaez CA, Glatigny S, Duhen R, Eberl G, Oukka M, Bettelli E. IL-7/IL-7 receptor signaling differentially affects effector CD4+ T cell subsets involved in experimental autoimmune encephalomyelitis. J Immunol. 2015;195(5):1974–83. Pubmed Central PMCID: 4546887.
81. Lee LF, Logronio K, Tu GH, Zhai W, Ni I, Mei L, et al. Anti-IL-7 receptor-alpha reverses established type 1 diabetes in nonobese diabetic mice by modulating effector T-cell function. Proc Natl Acad Sci U S A. 2012;109(31):12674–9. Pubmed Central PMCID: 3412026.
82. Totsuka T, Kanai T, Nemoto Y, Makita S, Okamoto R, Tsuchiya K, et al. IL-7 Is essential for the development and the persistence of chronic colitis. J Immunol. 2007;178(8):4737–48.
83. Willis CR, Seamons A, Maxwell J, Treuting PM, Nelson L, Chen G, et al. Interleukin-7 receptor blockade suppresses adaptive and innate inflammatory responses in experimental colitis. J Inflamm. 2012;9(1):39. Pubmed Central PMCID: 3551718.
84. Bikker A, Kruize AA, Wenting M, Versnel MA, Bijlsma JW, Lafeber FP, et al. Increased interleukin (IL)-7Ralpha expression in salivary glands of patients with primary Sjogren's syndrome is restricted to T cells and correlates with IL-7 expression, lymphocyte numbers and activity. Ann Rheum Dis. 2012;71(6):1027–33.
85. Bikker A, van Woerkom JM, Kruize AA, Wenting-van Wijk M, de Jager W, Bijlsma JW, et al. Increased expression of interleukin-7 in labial salivary glands of patients with primary Sjogren's syndrome correlates with increased inflammation. Arthritis Rheum. 2010;62(4):969–77.
86. Hartgring SA, Willis CR, Alcorn D, Nelson LJ, Bijlsma JW, Lafeber FP, et al. Blockade of the interleukin-7 receptor inhibits collagen-induced arthritis and is associated with reduction of T cell activity and proinflammatory mediators. Arthritis Rheum. 2010;62(9):2716–25.
87. Jin JO, Shinohara Y, Yu Q. Innate immune signaling induces interleukin-7 production from salivary gland cells and accelerates the development of primary

Sjogren's syndrome in a mouse model. PLoS One. 2013;8(10):e77605. Pubmed Central PMCID: 3798297.
88. O'Garra A, Murphy KM. From IL-10 to IL-12: how pathogens and their products stimulate APCs to induce T(H)1 development. Nat Immunol. 2009;10(9):929–32.
89. Vignali DA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. Nat Immunol. 2012;13(8):722–8.
90. Lazarevic V, Glimcher LH. T-bet in disease. Nat Immunol. 2011;12(7):597–606.
91. Boniface K, Blom B, Liu YJ, de Waal Malefyt R. From interleukin-23 to T-helper 17 cells: human T-helper cell differentiation revisited. Immunol Rev. 2008;226:132–46.
92. Li R, Zheng X, Popov I, Zhang X, Wang H, Suzuki M, et al. Gene silencing of IL-12 in dendritic cells inhibits autoimmune arthritis. J Transl Med. 2012;10:19. Pubmed Central PMCID: 3293054.
93. Trembleau S, Penna G, Gregori S, Giarratana N, Adorini L. IL-12 administration accelerates autoimmune diabetes in both wild-type and IFN-gamma-deficient nonobese diabetic mice, revealing pathogenic and protective effects of IL-12-induced IFN-gamma. J Immunol. 2003;170(11):5491–501.
94. Manoussakis MN, Boiu S, Korkolopoulou P, Kapsogeorgou EK, Kavantzas N, Ziakas P, et al. Rates of infiltration by macrophages and dendritic cells and expression of interleukin-18 and interleukin-12 in the chronic inflammatory lesions of Sjogren's syndrome: correlation with certain features of immune hyperactivity and factors associated with high risk of lymphoma development. Arthritis Rheum. 2007;56(12):3977–88.
95. Szodoray P, Alex P, Brun JG, Centola M, Jonsson R. Circulating cytokines in primary Sjogren's syndrome determined by a multiplex cytokine array system. Scand J Immunol. 2004;59(6):592–9.
96. Yanagi K, Haneji N, Hamano H, Takahashi M, Higashiyama H, Hayashi Y. In vivo role of IL-10 and IL-12 during development of Sjogren's syndrome in MRL/lpr mice. Cell Immunol. 1996;168(2):243–50.
97. Vosters JL, Landek-Salgado MA, Yin H, Swaim WD, Kimura H, Tak PP, et al. Interleukin-12 induces salivary gland dysfunction in transgenic mice, providing a new model of Sjogren's syndrome. Arthritis Rheum. 2009;60(12):3633–41. Pubmed Central PMCID: 2849131.
98. Davis BK, Wen H, Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev Immunol. 2011;29:707–35.
99. Dinarello CA. IL-18: A TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol. 1999;103(1 Pt 1):11–24.
100. Kawayama T, Okamoto M, Imaoka H, Kato S, Young HA, Hoshino T. Interleukin-18 in pulmonary inflammatory diseases. J Interferon Cytokine Res. 2012;32(10):443–9.
101. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol. 2001;19:423–74.
102. Oikawa Y, Shimada A, Kasuga A, Morimoto J, Osaki T, Tahara H, et al. Systemic administration of IL-18 promotes diabetes development in young nonobese diabetic mice. J Immunol. 2003;171(11):5865–75.
103. Volin MV, Koch AE. Interleukin-18: a mediator of inflammation and angiogenesis in rheumatoid arthritis. J Interferon Cytokine Res. 2011;31(10):745–51.
104. Becker H, Pavenstaedt H, Willeke P. Emerging treatment strategies and potential therapeutic targets in primary Sjogren's syndrome. Inflamm Allergy Drug Targets. 2010;9(1):10–9.
105. Bulosan M, Pauley KM, Yo K, Chan EK, Katz J, Peck AB, et al. Inflammatory caspases are critical for enhanced cell death in the target tissue of Sjogren's syndrome before disease onset. Immunol Cell Biol. 2009;87(1):81–90.
106. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat Rev Immunol. 2010;10(4):225–35. Pubmed Central PMCID: 3496776.
107. Rapoport MJ, Jaramillo A, Zipris D, Lazarus AH, Serreze DV, Leiter EH, et al. Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. J Exp Med. 1993;178(1):87–99. Pubmed Central PMCID: 2191073.
108. van Roon JA, van Roy JL, Gmelig-Meyling FH, Lafeber FP, Bijlsma JW. Prevention and reversal of cartilage degradation in rheumatoid arthritis by interleukin-10 and interleukin-4. Arthritis Rheum. 1996;39(5):829–35.
109. Nakajima A, Hirose S, Yagita H, Okumura K. Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. J Immunol. 1997;158(3):1466–72.
110. Ohyama Y, Nakamura S, Matsuzaki G, Shinohara M, Hiroki A, Fujimura T, et al. Cytokine messenger RNA expression in the labial salivary glands of patients with Sjogren's syndrome. Arthritis Rheum. 1996;39(8):1376–84.
111. McKenzie AN, Culpepper JA, de Waal MR, Briere F, Punnonen J, Aversa G, et al. Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. Proc Natl Acad Sci U S A. 1993;90(8):3735–9. Pubmed Central PMCID: 46376.
112. Wynn TA. IL-13 effector functions. Annu Rev Immunol. 2003;21:425–56.
113. Spadaro A, Rinaldi T, Riccieri V, Taccari E, Valesini G. Interleukin-13 in autoimmune rheumatic diseases: relationship with the autoantibody profile. Clin Exp Rheumatol. 2002;20(2):213–6.
114. Xu Z, Chen Y. Determination of serum interleukin-13 and nerve growth factor in patients with systemic lupus erythematosus and clinical significance. J Huazhong Univ Sci Technolog Med Sci. 2005;25(3):360–1.

115. Villarreal GM, Alcocer-Varela J, Llorente L. Differential interleukin (IL)-10 and IL-13 gene expression in vivo in salivary glands and peripheral blood mononuclear cells from patients with primary Sjogren's syndrome. Immunol Lett. 1996;49(1-2):105–9.
116. Mahlios J, Zhuang Y. Contribution of IL-13 to early exocrinopathy in Id3-/- mice. Mol Immunol. 2011;49(1-2):227–33. Pubmed Central PMCID: 3205188.
117. Belle I, Mahlios J, McKenzie A, Zhuang Y. Aberrant production of IL-13 by T cells promotes exocrinopathy in Id3 knockout mice. Cytokine. 2014;69(2):226–33. Pubmed Central PMCID: 4127355.
118. Awasthi A, Kuchroo VK. Th17 cells: from precursors to players in inflammation and infection. Int Immunol. 2009;21(5):489–98. Pubmed Central PMCID: 2675030.
119. Nakajima K. Critical role of the interleukin-23/T--helper 17 cell axis in the pathogenesis of psoriasis. J Dermatol. 2012;39(3):219–24.
120. Nguyen CQ, Hu MH, Li Y, Stewart C, Peck AB. Salivary gland tissue expression of interleukin-23 and interleukin-17 in Sjogren's syndrome: findings in humans and mice. Arthritis Rheum. 2008;58(3):734–43. Pubmed Central PMCID: 2850059.
121. Sakai A, Sugawara Y, Kuroishi T, Sasano T, Sugawara S. Identification of IL-18 and Th17 cells in salivary glands of patients with Sjogren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. J Immunol. 2008;181(4):2898–906.
122. Mieliauskaite D, Dumalakiene I, Rugiene R, Mackiewicz Z. Expression of IL-17, IL-23 and their receptors in minor salivary glands of patients with primary Sjogren's syndrome. Clin Dev Immunol. 2012;2012:187258. Pubmed Central PMCID: 3259502.
123. Alunno A, Bistoni O, Bartoloni E, Caterbi S, Bigerna B, Tabarrini A, et al. IL-17-producing CD4-CD8- T cells are expanded in the peripheral blood, infiltrate salivary glands and are resistant to corticosteroids in patients with primary Sjogren's syndrome. Ann Rheum Dis. 2012;72(2):286–92.
124. Voigt A, Esfandiary L, Wanchoo A, Glenton P, Donate A, Craft WF, et al. Sexual dimorphic function of IL-17 in salivary gland dysfunction of the C57BL/6.NOD-Aec1Aec2 model of Sjogren's syndrome. Sci Rep. 2016;6:38717. Pubmed Central PMCID: 5153841.
125. Lin X, Rui K, Deng J, Tian J, Wang X, Wang S, et al. Th17 cells play a critical role in the development of experimental Sjogren's syndrome. Ann Rheum Dis. 2015;74(6):1302–10.
126. Ouyang W, O'Garra A. IL-10 family cytokines IL-10 and IL-22: from basic science to clinical translation. Immunity. 2019;50(4):871–91.
127. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. Immunity. 2008;28(4):454–67. Pubmed Central PMCID: 3424508.
128. Colonna M. Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity. Immunity. 2009;31(1):15–23.
129. Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat Immunol. 2011;12(5):383–90.
130. Ciccia F, Guggino G, Rizzo A, Ferrante A, Raimondo S, Giardina A, et al. Potential involvement of IL-22 and IL-22-producing cells in the inflamed salivary glands of patients with Sjogren's syndrome. Ann Rheum Dis. 2012;71(2):295–301.
131. Lavoie TN, Stewart CM, Berg KM, Li Y, Nguyen CQ. Expression of interleukin-22 in Sjogren's syndrome: significant correlation with disease parameters. Scand J Immunol. 2011;74(4):377–82. Pubmed Central PMCID: 3250060.
132. Matsui K, Sano H. T helper 17 cells in primary Sjogren's syndrome. J Clin Med. 2017;6(7):65. Pubmed Central PMCID: 5532573.
133. Barone F, Nayar S, Campos J, Cloake T, Withers DR, Toellner KM, et al. IL-22 regulates lymphoid chemokine production and assembly of tertiary lymphoid organs. Proc Natl Acad Sci U S A. 2015;112(35):11024–9. Pubmed Central PMCID: 4568258.
134. Nocturne G, Mariette X. B cells in the pathogenesis of primary Sjogren syndrome. Nat Rev Rheumatol. 2018;14(3):133–45.
135. Wei L, Laurence A, Elias KM, O'Shea JJ. IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. J Biol Chem. 2007;282(48):34605–10. Pubmed Central PMCID: 2323680.
136. Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature. 2017;542(7639):110–4. Pubmed Central PMCID: 5349321.
137. Fonseca VR, Romao VC, Agua-Doce A, Santos M, Lopez-Presa D, et al. The ratio of blood T follicular regulatory cells to T follicular helper cells marks ectopic lymphoid structure formation while activated follicular helper T cells indicate disease activity in primary Sjogren's syndrome. Arthritis Rheumatol. 2018;70(5):774–84.
138. Maehara T, Moriyama M, Hayashida JN, Tanaka A, Shinozaki S, Kubo Y, et al. Selective localization of T helper subsets in labial salivary glands from primary Sjogren's syndrome patients. Clin Exp Immunol. 2012;169(2):89–99. Pubmed Central PMCID: 3406368.
139. Pollard RP, Abdulahad WH, Bootsma H, Meiners PM, Spijkervet FK, Huitema MG, et al. Predominantly proinflammatory cytokines decrease after B cell depletion therapy in patients with primary Sjogren's syndrome. Ann Rheum Dis. 2013;72(12):2048–50.

140. Gong YZ, Nititham J, Taylor K, Miceli-Richard C, Sordet C, Wachsmann D, et al. Differentiation of follicular helper T cells by salivary gland epithelial cells in primary Sjogren's syndrome. J Autoimmun. 2014;51:57–66.
141. Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. Annu Rev Immunol. 2008;26:57–79.
142. Spolski R, Leonard WJ. The Yin and Yang of interleukin-21 in allergy, autoimmunity and cancer. Curr Opin Immunol. 2008;20(3):295–301. Pubmed Central PMCID: 2546604.
143. Chen Z, O'Shea JJ. Th17 cells: a new fate for differentiating helper T cells. Immunol Res. 2008;41(2):87–102.
144. Yi JS, Cox MA, Zajac AJ. Interleukin-21: a multifunctional regulator of immunity to infections. Microbes Infect. 2010;12(14–15):1111–9. Pubmed Central PMCID: 2998550.
145. Monteleone G, Sarra M, Pallone F. Interleukin-21 in T cell-mediated diseases. Discov Med. 2009;8(42):113–7.
146. Sarra M, Franze E, Pallone F, Monteleone G. Targeting interleukin-21 in inflammatory diseases. Expert Opin Ther Targets. 2011;15(6):695–702.
147. Kang KY, Kim HO, Kwok SK, Ju JH, Park KS, Sun DI, et al. Impact of interleukin-21 in the pathogenesis of primary Sjogren's syndrome: increased serum levels of interleukin-21 and its expression in the labial salivary glands. Arthritis Res Ther. 2011;13(5):R179. Pubmed Central PMCID: 3308114.
148. Kwok SK, Lee J, Yu D, Kang KY, Cho ML, Kim HR, et al. A pathogenetic role for IL-21 in primary Sjogren syndrome. Nat Rev Rheumatol. 2015;11(6):368–74.
149. Miao M, Hao Z, Guo Y, Zhang X, Zhang S, Luo J, et al. Short-term and low-dose IL-2 therapy restores the Th17/Treg balance in the peripheral blood of patients with primary Sjogren's syndrome. Ann Rheum Dis. 2018;77(12):1838–40.
150. Gottenberg JE, Lavie F, Abbed K, Gasnault J, Le Nevot E, Delfraissy JF, et al. CD4 CD25high regulatory T cells are not impaired in patients with primary Sjogren's syndrome. J Autoimmun. 2005;24(3):235–42.
151. Liu MF, Lin LH, Weng CT, Weng MY. Decreased CD4+CD25+bright T cells in peripheral blood of patients with primary Sjogren's syndrome. Lupus. 2008;17(1):34–9.
152. Li X, Li X, Qian L, Wang G, Zhang H, Wang X, et al. T regulatory cells are markedly diminished in diseased salivary glands of patients with primary Sjogren's syndrome. J Rheumatol. 2007;34(12):2438–45.
153. Szodoray P, Papp G, Horvath IF, Barath S, Sipka S, Nakken B, et al. Cells with regulatory function of the innate and adaptive immune system in primary Sjogren's syndrome. Clin Exp Immunol. 2009;157(3):343–9. Pubmed Central PMCID: 2745027.
154. Sarigul M, Yazisiz V, Bassorgun CI, Ulker M, Avci AB, Erbasan F, et al. The numbers of Foxp3 + Treg cells are positively correlated with higher grade of infiltration at the salivary glands in primary Sjogren's syndrome. Lupus. 2010;19(2):138–45.
155. Verstappen GM, Meiners PM, Corneth OBJ, Visser A, Arends S, Abdulahad WH, et al. Attenuation of follicular helper T cell-dependent B cell hyperactivity by Abatacept treatment in primary Sjogren's syndrome. Arthritis Rheumatol. 2017;69(9):1850–61.
156. Miyara M, Chader D, Sage E, Sugiyama D, Nishikawa H, Bouvry D, et al. Sialyl Lewis x (CD15s) identifies highly differentiated and most suppressive FOXP3high regulatory T cells in humans. Proc Natl Acad Sci U S A. 2015;112(23):7225–30. Pubmed Central PMCID: 4466753.
157. Christodoulou MI, Kapsogeorgou EK, Moutsopoulos NM, Moutsopoulos HM. Foxp3+ T-regulatory cells in Sjogren's syndrome: correlation with the grade of the autoimmune lesion and certain adverse prognostic factors. Am J Pathol. 2008;173(5):1389–96. Pubmed Central PMCID: 2570129.
158. Katsifis GE, Rekka S, Moutsopoulos NM, Pillemer S, Wahl SM. Systemic and local interleukin-17 and linked cytokines associated with Sjogren's syndrome immunopathogenesis. Am J Pathol. 2009;175(3):1167–77. Pubmed Central PMCID: 2731135.
159. Barr JY, Wang X, Kreiger PA, Lieberman SM. Salivary-gland-protective regulatory T-cell dysfunction underlies female-specific sialadenitis in the non-obese diabetic mouse model of Sjogren syndrome. Immunology. 2018;155(2):225–37. Pubmed Central PMCID: 6142283.
160. Overacre-Delgoffe AE, Chikina M, Dadey RE, Yano H, Brunazzi EA, Shayan G, et al. Interferon-gamma drives Treg fragility to promote anti-tumor immunity. Cell. 2017;169(6):1130–41 e11. Pubmed Central PMCID: 5509332.
161. Dominguez-Villar M, Baecher-Allan CM, Hafler DA. Identification of T helper type 1-like, Foxp3+ regulatory T cells in human autoimmune disease. Nat Med. 2011;17(6):673–5. Pubmed Central PMCID: 3675886.
162. Feng T, Cao AT, Weaver CT, Elson CO, Cong Y. Interleukin-12 converts Foxp3+ regulatory T cells to interferon-gamma-producing Foxp3+ T cells that inhibit colitis. Gastroenterology. 2011;140(7):2031–43. Pubmed Central PMCID: 3109200.
163. Tarique M, Saini C, Naqvi RA, Khanna N, Sharma A, Rao DN. IL-12 and IL-23 modulate plasticity of FoxP3(+) regulatory T cells in human leprosy. Mol Immunol. 2017;83:72–81.
164. Izcue A, Hue S, Buonocore S, Arancibia-Carcamo CV, Ahern PP, Iwakura Y, et al. Interleukin-23 restrains regulatory T cell activity to drive T cell-dependent colitis. Immunity. 2008;28(4):559–70. Pubmed Central PMCID: 2292821.
165. Kannan AK, Su Z, Gauvin DM, Paulsboe SE, Duggan R, Lasko LM, et al. IL-23 induces regulatory

T cell plasticity with implications for inflammatory skin diseases. Sci Rep. 2019;9(1):17675. Pubmed Central PMCID: 6881359.
166. Petermann F, Rothhammer V, Claussen MC, Haas JD, Blanco LR, Heink S, et al. gammadelta T cells enhance autoimmunity by restraining regulatory T cell responses via an interleukin-23-dependent mechanism. Immunity. 2010;33(3):351–63. Pubmed Central PMCID: 3008772.
167. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol. 2010;10(3):170–81.
168. Soukou S, Brockmann L, Bedke T, Gagliani N, Flavell RA, Huber S. Role of IL-10 receptor signaling in the function of CD4+ T-regulatory type 1 cells: T-cell therapy in patients with inflammatory bowel disease. Crit Rev Immunol. 2018;38(5):415–31.
169. Roncarolo MG, Gregori S, Bacchetta R, Battaglia M, Gagliani N. The biology of T regulatory type 1 cells and their therapeutic application in immune-mediated diseases. Immunity. 2018;49(6):1004–19.
170. Brooks DG, Walsh KB, Elsaesser H, Oldstone MB. IL-10 directly suppresses CD4 but not CD8 T cell effector and memory responses following acute viral infection. Proc Natl Acad Sci U S A. 2010;107(7):3018–23. Pubmed Central PMCID: 2840337.
171. Moore KW, de Waal MR, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001;19:683–765.
172. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993;75(2):263–74.
173. Roers A, Siewe L, Strittmatter E, Deckert M, Schluter D, Stenzel W, et al. T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. J Exp Med. 2004;200(10):1289–97. Pubmed Central PMCID: 2211912.
174. Beebe AM, Cua DJ, de Waal Malefyt R. The role of interleukin-10 in autoimmune disease: systemic lupus erythematosus (SLE) and multiple sclerosis (MS). Cytokine Growth Factor Rev. 2002;13(4-5):403–12.
175. Levy Y, Brouet JC. Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. J Clin Invest. 1994;93(1):424–8. Pubmed Central PMCID: 293803.
176. Bertorello R, Cordone MP, Contini P, Rossi P, Indiveri F, Puppo F, et al. Increased levels of interleukin-10 in saliva of Sjogren's syndrome patients. Correlation with disease activity. Clin Exp Med. 2004;4(3):148–51.
177. Perrier S, Serre AF, Dubost JJ, Beaujon G, Plazonnet MP, Albuisson E, et al. Increased serum levels of interleukin 10 in Sjogren's syndrome; correlation with increased IgG1. J Rheumatol. 2000;27(4):935–9.
178. Saito I, Haruta K, Shimuta M, Inoue H, Sakurai H, Yamada K, et al. Fas ligand-mediated exocrinopathy resembling Sjogren's syndrome in mice transgenic for IL-10. J Immunol. 1999;162(5):2488–94.
179. Rajagopalan G, Kudva YC, Sen MM, Marietta EV, Murali N, Nath K, et al. IL-10-deficiency unmasks unique immune system defects and reveals differential regulation of organ-specific autoimmunity in non-obese diabetic mice. Cytokine. 2006;34(1–2):85–95.
180. Sharif MN, Tassiulas I, Hu Y, Mecklenbrauker I, Tarakhovsky A, Ivashkiv LB. IFN-alpha priming results in a gain of proinflammatory function by IL-10: implications for systemic lupus erythematosus pathogenesis. J Immunol. 2004;172(10):6476–81.
181. Sage PT, Sharpe AH. T follicular regulatory cells. Immunol Rev. 2016;271(1):246–59.
182. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, et al. Foxp3+ follicular regulatory T cells control the germinal center response. Nat Med. 2011;17(8):975–82. Pubmed Central PMCID: 3182542.
183. Fonseca VR, Romao VC, Agua-Doce A, Santos M, Lopez-Presa D, Ferreira AC, et al. The ratio of blood T follicular regulatory cells to T follicular helper cells marks ectopic lymphoid structure formation while activated follicular helper T cells indicate disease activity in primary Sjogren's syndrome. Arthritis Rheumatol. 2018;70(5):774–84.
184. Bohnhorst JO, Bjorgan MB, Thoen JE, Natvig JB, Thompson KM. Bm1-Bm5 classification of peripheral blood B cells reveals circulating germinal center founder cells in healthy individuals and disturbance in the B cell subpopulations in patients with primary Sjogren's syndrome. J Immunol. 2001;167(7):3610–8.
185. Hansen A, Lipsky PE, Dorner T. B cells in Sjogren's syndrome: indications for disturbed selection and differentiation in ectopic lymphoid tissue. Arthritis Res Ther. 2007;9(4):218. Pubmed Central PMCID: 2206371.
186. Hansen A, Gosemann M, Pruss A, Reiter K, Ruzickova S, Lipsky PE, et al. Abnormalities in peripheral B cell memory of patients with primary Sjogren's syndrome. Arthritis Rheum. 2004;50(6):1897–908.
187. Roberts ME, Kaminski D, Jenks SA, Maguire C, Ching K, Burbelo PD, et al. Primary Sjogren's syndrome is characterized by distinct phenotypic and transcriptional profiles of IgD+ unswitched memory B cells. Arthritis Rheumatol. 2014;66(9):2558–69. Pubmed Central PMCID: 4160119.
188. Szabo K, Papp G, Szanto A, Tarr T, Zeher M. A comprehensive investigation on the distribution of circulating follicular T helper cells and B cell subsets in primary Sjogren's syndrome and systemic lupus erythematosus. Clin Exp Immunol. 2016;183(1):76–89. Pubmed Central PMCID: 4687513.
189. Hamza N, Bos NA, Kallenberg CG. B-cell populations and sub-populations in Sjogren's syndrome. Presse Med. 2012;41(9 Pt 2):e475–83.

190. Hansen A, Odendahl M, Reiter K, Jacobi AM, Feist E, Scholze J, et al. Diminished peripheral blood memory B cells and accumulation of memory B cells in the salivary glands of patients with Sjogren's syndrome. Arthritis Rheum. 2002;46(8):2160–71.
191. Mingueneau M, Boudaoud S, Haskett S, Reynolds TL, Nocturne G, Norton E, et al. Cytometry by time-of-flight immunophenotyping identifies a blood Sjogren's signature correlating with disease activity and glandular inflammation. J Allergy Clin Immunol. 2016;137(6):1809–21 e12.
192. Daridon C, Pers JO, Devauchelle V, Martins-Carvalho C, Hutin P, Pennec YL, et al. Identification of transitional type II B cells in the salivary glands of patients with Sjogren's syndrome. Arthritis Rheum. 2006;54(7):2280–8.
193. Vossenkamper A, Lutalo PM, Spencer J. Translational mini-review series on B cell subsets in disease. Transitional B cells in systemic lupus erythematosus and Sjogren's syndrome: clinical implications and effects of B cell-targeted therapies. Clin Exp Immunol. 2012;167(1):7–14. Pubmed Central PMCID: 3248081.
194. Guo Z, Li H, Han M, Xu T, Wu X, Zhuang Y. Modeling Sjogren's syndrome with Id3 conditional knockout mice. Immunol Lett. 2011;135(1-2):34–42. Pubmed Central PMCID: 3025308.
195. Hayakawa I, Tedder TF, Zhuang Y. B-lymphocyte depletion ameliorates Sjogren's syndrome in Id3 knockout mice. Immunology. 2007;122(1):73–9. Pubmed Central PMCID: 2265983.
196. Li H, Dai M, Zhuang Y. A T cell intrinsic role of Id3 in a mouse model for primary Sjogren's syndrome. Immunity. 2004;21(4):551–60.
197. Mackay F, Woodcock SA, Lawton P, Ambrose C, Baetscher M, Schneider P, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. J Exp Med. 1999;190(11):1697–710. Pubmed Central PMCID: 2195729.
198. Groom J, Kalled SL, Cutler AH, Olson C, Woodcock SA, Schneider P, et al. Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjogren's syndrome. J Clin Invest. 2002;109(1):59–68. Pubmed Central PMCID: 150825.
199. Fletcher CA, Sutherland AP, Groom JR, Batten ML, Ng LG, Gommerman J, et al. Development of nephritis but not sialadenitis in autoimmune-prone BAFF transgenic mice lacking marginal zone B cells. Eur J Immunol. 2006;36(9):2504–14.
200. Shen L, Zhang C, Wang T, Brooks S, Ford RJ, Lin-Lee YC, et al. Development of autoimmunity in IL-14alpha-transgenic mice. J Immunol. 2006;177(8):5676–86.
201. Shen L, Gao C, Suresh L, Xian Z, Song N, Chaves LD, et al. Central role for marginal zone B cells in an animal model of Sjogren's syndrome. Clin Immunol. 2016;168:30–6. Pubmed Central PMCID: 4940264.
202. Risselada AP, Looije MF, Kruize AA, Bijlsma JW, van Roon JA. The role of ectopic germinal centers in the immunopathology of primary Sjogren's syndrome: a systematic review. Semin Arthritis Rheum. 2013;42(4):368–76.
203. Salomonsson S, Jonsson MV, Skarstein K, Brokstad KA, Hjelmstrom P, Wahren-Herlenius M, et al. Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjogren's syndrome. Arthritis Rheum. 2003;48(11):3187–201.
204. Lessard CJ, Li H, Adrianto I, Ice JA, Rasmussen A, Grundahl KM, et al. Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. Nat Genet. 2013;45(11):1284–92. Pubmed Central PMCID: 3867192.
205. Bossen C, Schneider P. BAFF, APRIL and their receptors: structure, function and signaling. Semin Immunol. 2006;18(5):263–75.
206. Chan VS, Tsang HH, Tam RC, Lu L, Lau CS. B-cell-targeted therapies in systemic lupus erythematosus. Cell Mol Immunol. 2013;10(2):133–42. Pubmed Central PMCID: 4003049.
207. Kalled SL, Ambrose C, Hsu YM. The biochemistry and biology of BAFF, APRIL and their receptors. Curr Dir Autoimmun. 2005;8:206–42.
208. Mackay F, Ambrose C. The TNF family members BAFF and APRIL: the growing complexity. Cytokine Growth Factor Rev. 2003;14(3-4):311–24.
209. Lavie F, Miceli-Richard C, Quillard J, Roux S, Leclerc P, Mariette X. Expression of BAFF (BLyS) in T cells infiltrating labial salivary glands from patients with Sjogren's syndrome. J Pathol. 2004;202(4):496–502.
210. Daridon C, Devauchelle V, Hutin P, Le Berre R, Martins-Carvalho C, Bendaoud B, et al. Aberrant expression of BAFF by B lymphocytes infiltrating the salivary glands of patients with primary Sjogren's syndrome. Arthritis Rheum. 2007;56(4):1134–44.
211. Lavie F, Miceli-Richard C, Ittah M, Sellam J, Gottenberg JE, Mariette X. B-cell activating factor of the tumour necrosis factor family expression in blood monocytes and T cells from patients with primary Sjogren's syndrome. Scand J Immunol. 2008;67(2):185–92.
212. Ittah M, Miceli-Richard C, Gottenberg JE, Sellam J, Eid P, Lebon P, et al. Viruses induce high expression of BAFF by salivary gland epithelial cells through TLR- and type-I IFN-dependent and -independent pathways. Eur J Immunol. 2008;38(4):1058–64.
213. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol. 2002;3(9):822–9. Pubmed Central PMCID: 4621779.
214. Mauri C, Blair PA. Regulatory B cells in autoimmunity: developments and controversies. Nat Rev Rheumatol. 2010;6(11):636–43.
215. Ittah M, Miceli-Richard C, Lebon P, Pallier C, Lepajolec C, Mariette X. Induction of B cell-activating factor by viral infection is a general phenomenon, but the types of viruses and mech-

anisms depend on cell type. J Innate Immun. 2011;3(2):200–7.
216. Thabet Y, Le Dantec C, Ghedira I, Devauchelle V, Cornec D, Pers JO, et al. Epigenetic dysregulation in salivary glands from patients with primary Sjogren's syndrome may be ascribed to infiltrating B cells. J Autoimmun. 2013;41:175–81.
217. Ding J, Zhang W, Haskett S, Pellerin A, Xu S, Petersen B, et al. BAFF overexpression increases lymphocytic infiltration in Sjogren's target tissue, but only inefficiently promotes ectopic B-cell differentiation. Clin Immunol. 2016;169:69–79.
218. Gavin AL, Ait-Azzouzene D, Ware CF, Nemazee D. DeltaBAFF, an alternate splice isoform that regulates receptor binding and biopresentation of the B cell survival cytokine, BAFF. J Biol Chem. 2003;278(40):38220–8. Pubmed Central PMCID: 3792716.
219. Roescher N, Vosters JL, Alsaleh G, Dreyfus P, Jacques S, Chiocchia G, et al. Targeting the splicing of mRNA in autoimmune diseases: BAFF inhibition in Sjogren's syndrome as a proof of concept. Mol Ther. 2014;22(4):821–7. Pubmed Central PMCID: 3982500.
220. Sharma A, Kiripolsky J, Klimatcheva E, Howell A, Fereidouni F, Levenson R, et al. Early BAFF receptor blockade mitigates murine Sjogren's syndrome: concomitant targeting of CXCL13 and the BAFF receptor prevents salivary hypofunction. Clin Immunol. 2016;164:85–94. Pubmed Central PMCID: 4780410.
221. Gottenberg JE, Ravaud P, Puechal X, Le Guern V, Sibilia J, Goeb V, et al. Effects of hydroxychloroquine on symptomatic improvement in primary Sjogren syndrome: the JOQUER randomized clinical trial. JAMA. 2014;312(3):249–58.
222. Bodewes ILA, Gottenberg JE, van Helden-Meeuwsen CG, Mariette X, Versnel MA. Hydroxychloroquine treatment downregulates systemic interferon activation in primary Sjogren's syndrome in the JOQUER randomized trial. Rheumatology (Oxford). 2020;59(1):107–11. Pubmed Central PMCID: 6909893.
223. Sankar V, Brennan MT, Kok MR, Leakan RA, Smith JA, Manny J, et al. Etanercept in Sjogren's syndrome: a twelve-week randomized, double-blind, placebo-controlled pilot clinical trial. Arthritis Rheum. 2004;50(7):2240–5.
224. Mavragani CP, Moutsopoulos HM. Sjogren's syndrome: old and new therapeutic targets. J Autoimmun. 2020;110:102364.
225. De Vita S, Quartuccio L, Seror R, Salvin S, Ravaud P, Fabris M, et al. Efficacy and safety of belimumab given for 12 months in primary Sjogren's syndrome: the BELISS open-label phase II study. Rheumatology (Oxford). 2015;54(12):2249–56.
226. Mariette X, Seror R, Quartuccio L, Baron G, Salvin S, Fabris M, et al. Efficacy and safety of belimumab in primary Sjogren's syndrome: results of the BELISS open-label phase II study. Ann Rheum Dis. 2015;74(3):526–31.
227. Dorner T, Posch MG, Li Y, Petricoul O, Cabanski M, Milojevic JM, et al. Treatment of primary Sjogren's syndrome with ianalumab (VAY736) targeting B cells by BAFF receptor blockade coupled with enhanced, antibody-dependent cellular cytotoxicity. Ann Rheum Dis. 2019;78(5):641–7.
228. Xie S, Li S, Tian J, Li F. Iguratimod as a new drug for rheumatoid arthritis: current landscape. Front Pharmacol. 2020;11:73. Pubmed Central PMCID: 7054862.

7 B Cell Expansion and Neoplasia in Sjögren's Syndrome

Austin Anderson, Erin Hudson, Lijun Yang, Winnie W. Hui, Shuhong Han, Haoyang Zhuang, Robert Thoburn, and Westley H. Reeves

7.1 Introduction

Sjögren's syndrome (SS) is a progressive systemic autoimmune disease that causes chronic inflammation of the salivary, lacrimal, and other exocrine glands and B cell hyperactivity leading to the production of characteristic autoantibodies such as anti-Ro (SS-A) and La (SS-B). Dry eyes (xerophthalmia) and dry mouth (xerostomia) are prominent symptoms, though there are a host of other symptoms including fatigue, neurological problems (such as peripheral neuropathies and neuromyelitis optica), joint pain, vasculitis, Raynaud's phenomenon, tubulointerstitial nephritis, and lymphadenopathy. SS is associated with a 16–40-fold increase in the risk of B cell lymphoma [1, 2]. A retrospective study of 723 SS patients followed for a mean of 6 years showed a 10-year risk of developing lymphoproliferative disease (mostly B cell lymphoma) of 3.9% [3]. Typically, SS lymphomas develop extranodally at the sites of inflammation, most commonly within the inflamed salivary glands.

A. Anderson · E. Hudson · W. W. Hui · S. Han
H. Zhuang · R. Thoburn · W. H. Reeves (✉)
Division of Rheumatology, Allergy, and Clinical Immunology, Department of Medicine, College of Medicine, University of Florida, Gainesville, FL, USA
e-mail: Austin.anderson@medicine.ufl.edu; hudsonem15@ufl.edu; Winnie.hui@medicine.ufl.edu; shuhong.han@medicine.ufl.edu; haoyang.zhuang@medicine.ufl.edu; rthoburn@ufl.edu; westley.reeves@medicine.ufl.edu; whreeves@ufl.edu

L. Yang
Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA
e-mail: yanglj@pathology.ufl.edu

7.2 Salivary Gland Inflammation in SS

Chronic inflammation of the major salivary glands is characteristic of SS and may be manifested by painful or painless enlargement of the parotid or submandibular glands (Fig. 7.1a). The minor salivary glands of the lip (labial glands) are similarly affected and are frequently biopsied for diagnostic purposes (Fig. 7.1b, c). In contrast to chronic sialadenitis in which the glands are replaced by fibrotic tissue, the acini are preserved early in the course of SS (Fig. 7.1b, yellow arrows), but are destroyed later on in the disease. The presence of lymphoplasmacytic "foci" consisting of ≥50 lymphocytes or plasma cells is pathognomonic (Fig. 7.1b, black arrow). A focus score of ≥1 per 4 mm^2 is considered diagnostic. However, similar foci are found in hepatitis C virus (HCV) infection (Fig. 7.1c, black arrow) and sometimes HIV infection [4–7].

The salivary gland infiltrates in SS consist of $CD4^+$ T cells, B cells, and plasma cells [8, 9]. $CD27^+$ memory B cells are lower in the peripheral blood of SS patients compared with controls,

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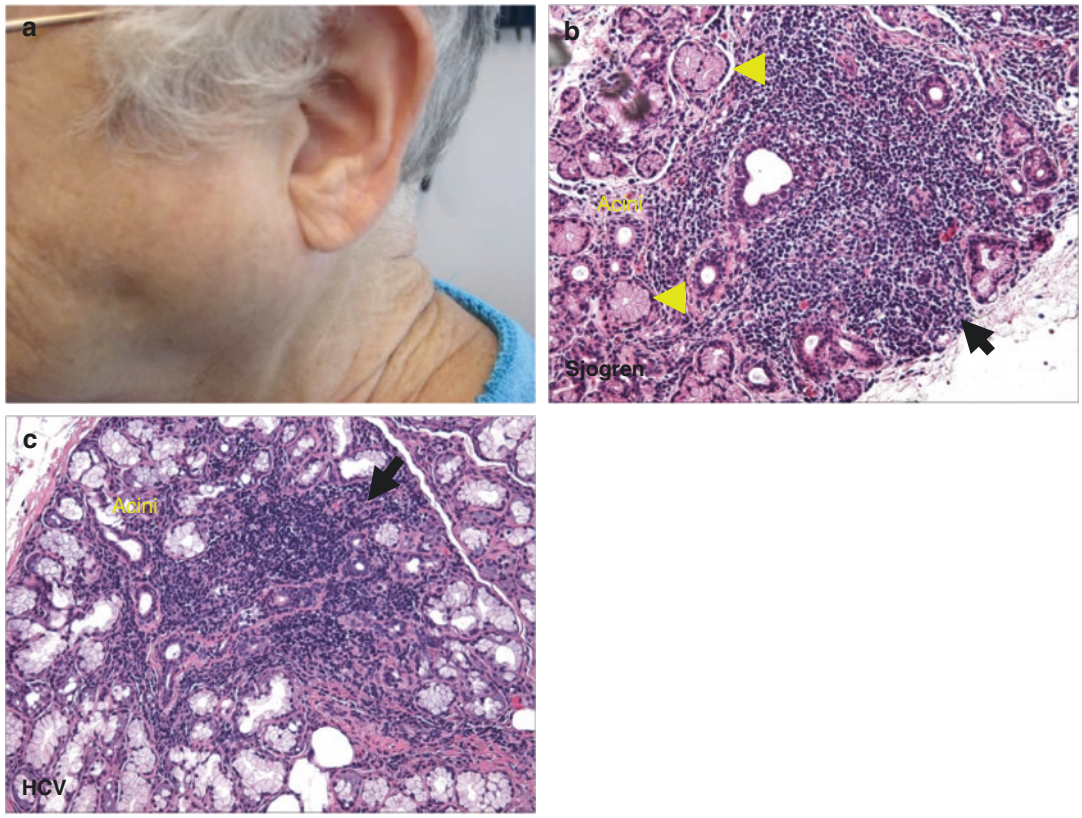

Fig. 7.1 Lymphoplasmacytic infiltrates in SS compared with HCV infection. (**a**) Left parotid enlargement in a patient with primary SS. (**b**) H&E stained labial salivary gland biopsy from a patient with primary SS showing a typical lymphoplasmacytic focus (collection of >50 lymphocytes and/or plasma cells, arrow) and preservation of the glandular architecture (acini). (**c**) H&E stained labial salivary gland from a patient with chronic HCV infection showing a focus (arrow) and preservation of the acini (400×)

and accumulate within the inflamed salivary glands. The B cell infiltrate in SS consists of transitional (T1) B cells (IgD^+, IgM^-, $CD21^+$, $CD23^+$) and marginal zone-like B cells (IgD^-, IgM^+, $CD21^\pm$, $CD23^\pm$) with small numbers of B1 cells ($CD20^+$, $CD5^+$) and numerous $CD27^+$ memory B cells [8, 9]. Although most of the cells infiltrating the salivary glands are T cells, the infiltrates may contain clonally expanded plasma cells, resulting in a high frequency of benign monoclonal or oligoclonal gammopathy, which may be associated with B cell neoplasia [1, 10–12].

In the course of the inflammatory response, the infiltrates can become organized into structures resembling B cell follicles, a process known as lymphoid neogenesis [13]. Secondary lymphoid follicles consist of three zones: an inner germinal center surrounded by a darkly stained mantle zone and an outer marginal zone (Fig. 7.2). Germinal center B cells undergo antigen- and T cell-dependent proliferation, class switching, and affinity maturation, generating antibody-producing plasma cells and memory B cells. Reactive germinal centers usually show clear polarity with both dark and light zones that can be clearly highlighted by anti-Ki-67 immunohistochemical staining. The dark zone contains highly proliferative centroblasts and the light zone comprises less proliferative centrocytes, which eventually differentiate into either memory B cells or immunoblasts, plasmablasts, and/or plasma cells. Mantle zone B cells are naïve (unstimulated) B cells that are $IgM^+/IgD^+/CD5^+/CD10^-/CD23^{-/+}$. Marginal zone (MZ) B cells

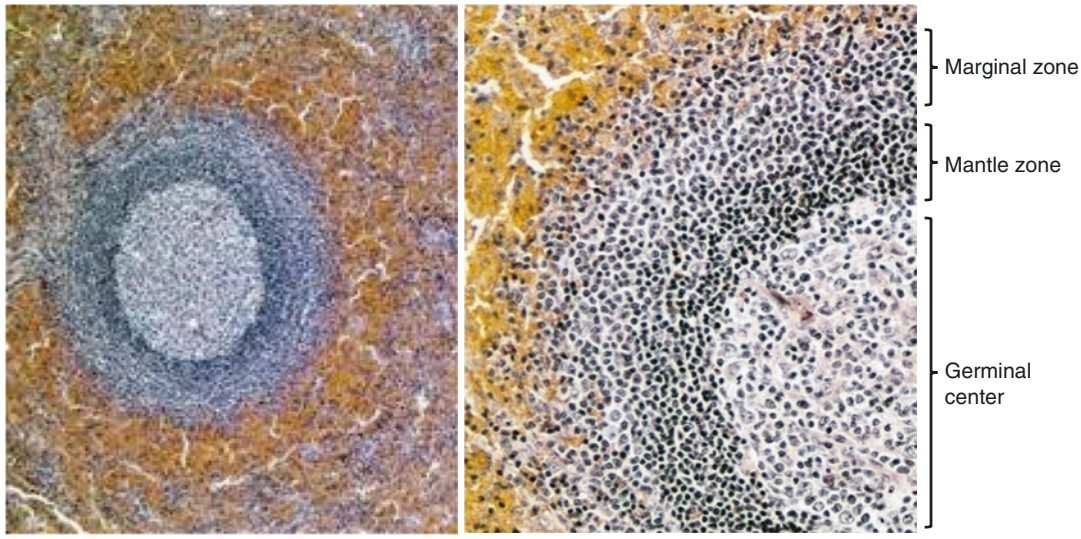

Fig. 7.2 Anatomy of secondary lymphoid follicles. Low power (left, 200×) and high power (right, 400×) micrographs of a secondary lymphoid follicle stained with H&E. Marginal zone, mantle zone, and a germinal center are indicated

rapidly generate antibody responses to both T cell-dependent and T cell-independent antigens [14]. They are positive for B cell markers, such as CD20 and CD19, but lack CD5, CD23, CD10, and IgD expression.

The presence of germinal center-like structures and memory B cells in the salivary gland infiltrates raises the possibility that antigenic stimulation is involved in the B cell proliferation of SS [15]. Further evidence comes from the similar histopathologic findings and pathogenic mechanisms seen chronic HCV infection, which may mimic primary SS [16] (Fig. 7.1c). Like SS, HCV infection is an autoimmune process marked by the production of rheumatoid factors (RFs), some of which are cryoglobulins (Fig. 7.3). Moreover, clonal B cell expansions also develop within the salivary glands of some patients with HCV infection [17] and extranodal lymphoma within the salivary glands occasionally has been reported [18]. Polyclonal B cell activation in both SS and HCV infection may evolve to monoclonal/oligoclonal gammopathy, and a subset of patients go on to develop B cell lymphoma [17, 19]. Interestingly, clonal diversity analysis (ratio of replacement to silent mutations) in the heavy-chain third complementarity determining regions (CDR3) compared with framework regions of these neoplasms in both SS and HCV infection is consistent with a chronic antigenic stimulus [15, 20, 21].

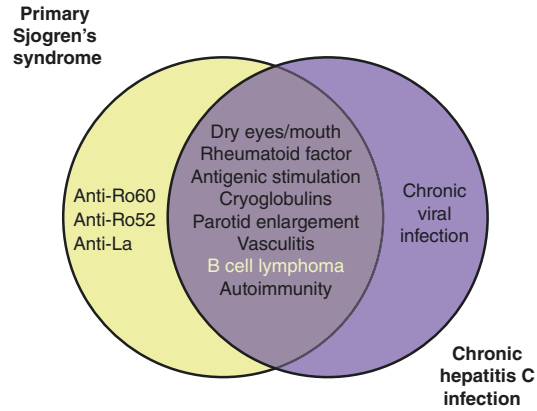

Fig. 7.3 Overlapping immunological and clinical features of SS and HCV infection. Immunological features (production of rheumatoid factor and cryoglobulins, evidence of chronic antigenic stimulation) and clinical features (autoimmune-mediated salivary gland enlargement and vasculitis, extranodal B cell lymphoma) overlap considerably in SS and chronic HCV infection. However, there are differences: chronic viral infection is likely to provide the antigenic stimulation in patients with HCV infection, but the antigenic stimulus in SS remains unknown. Also, anti-Ro (SS-A) and La (SS-B) antibodies are not seen in patients with HCV infection without coexisting SS

7.3 Extranodal Lymphomas in SS

In response to chronic inflammation, extranodal MZ lymphoma (MZL) can arise in a variety of sites with mucosa-associated lymphoid tissue (MALT), including the stomach (in association with *Helicobacter pylori* infection) [22], thyroid gland, salivary glands, and bronchus-associated lymphoid tissue (BALT) [23–26]. In *H. pylori*-associated MALT lymphomas, the B cell clone undergoing malignant transformation can be detected in the inflamed stomach (gastritis) preceding the onset of lymphoma [27] and the lymphoma can often be treated successfully with antibiotics to eradicate the organism [28]. In SS, these neoplasms most commonly arise within the inflamed parotid glands, and less commonly the submandibular or labial salivary glands [29, 30], lacrimal glands [14], or BALT [26]. The neoplastic B cells, which closely resemble MZ B cells from secondary lymphoid tissue of the gut or lymph nodes ("nodal MZL"), or the spleen ("splenic MZL"), are thought to arise in response to chronic immune or autoimmune stimulation and may undergo differentiation into plasma cells [31]. Therefore, distinguishing MZL from other types of small B cell lymphomas can be challenging [32]. Due to the propensity of some extranodal MZL to undergo plasmacytic differentiation [31, 33], distinguishing them from lymphoplasmacytic lymphoma or plasma cell neoplasms can sometimes be difficult. The faction of plasma cells ($CD20^-$) may increase following the elimination of $CD20^+$ B cells by rituximab therapy [34, 35].

The occasional transformation of gastric MALT lymphomas into $CD20^-$ plasma cell tumors after rituximab monotherapy suggests that "plasmacytoma of the GI tract" may actually originate as a MALT lymphoma [34]. It is important to uncover plasma cell differentiation because the $CD20^-$ plasma cells derived from a $CD20^+$ MZL are refractory to rituximab (anti-CD20 monoclonal antibody) [35]. One sign that clonal plasma cells have developed is the appearance of a monoclonal gammopathy, although many patients with plasma cell differentiation histologically do not present with paraproteins. Generally, the paraprotein has the same heavy and light chain as the original MZL and in one case, the plasma cell tumor exhibited the MALT lymphoma-specific t(11;18)(q21;q21) translocation [35]. In view of the possible outgrowth of $CD20^-$ plasma cell variants, combination chemotherapy with rituximab plus other drugs active against plasma cell disorders (such as R-CHOP) has been advocated [35].

The pathological diagnosis of extranodal MZL depends on several key features including the presence of reactive germinal centers, interfollicular MZ expansion, and lymphoepithelial lesions caused by infiltration of the tumor cells into the overlying epithelium [32]. Nearly all MZLs arising in the parotid gland exhibit monocytoid morphological features. Plasmacytic differentiation is seen in about a third of cases. Flow cytometry is helpful for identifying a clonal κ or λ light chain-restricted B cell population that lacks CD5, CD23, and CD10 expression. Most of these tumors express μ heavy chain, and when there is significant plasmacytic differentiation, this may lead to the production of an IgM paraprotein. Cytogenetic studies may be helpful to confirm the diagnosis. Pathognomonic abnormalities for extranodal MZL include apoptosis inhibitor-2 *(API2)/MALT1*, *IGH/MALT1*, and *IGH/BCL10* translocations [32]. The API2/MALT1 fusion protein activates NFκB, promoting B cell survival. However, these abnormalities are present in less than half of cases. The overall frequency of MALT1 translocation is about 20% [1]. Clinically, extranodal MZL is an indolent neoplasm that often remains localized to the salivary gland, but can disseminate to other locations, frequently to other sites with MALT [1].

7.3.1 Frequency of SS Among Patients with Salivary Gland-Associated MZL

Although common in SS, salivary gland MALT lymphoma is not unique to SS. In one study of 247 patients, 101 (47%) had an autoimmune

disorder and 84 (33%) had SS [36]. Other autoimmune diagnoses included rheumatoid arthritis (RA) (4%), Raynaud's phenomenon (3%), scleroderma (2%), and miscellaneous (8%). The remaining 146 patients (53%) did not have a diagnosed autoimmune condition.

7.3.2 Case 1: 58-Year-Old Woman with SS and Persistent Right Parotid Swelling

In 1980, the patient noted the onset of fatigue, dry eyes, and dry mouth and in 1982 she developed recurrent bilateral parotid swelling (worse on the right than on the left). She was seen by a rheumatologist in 1997 because of parotid gland swelling, oral, ocular, and vaginal dryness, and arthralgias. Her Schirmer test was abnormal. ANA test was strongly positive (1:1280 speckled pattern) with positive anti-Ro (SS-A) and La (SS-B) antibodies as well as positive RF. Globulin level was 4.4 g/dL. In 2001 she was referred to the University of Florida because of sicca symptoms and tender right parotid swelling. The parotid swelling initially fluctuated, but later became persistent. A CT scan revealed a right parotid mass (Fig. 7.4a), which was surgically excised. Pathology showed a well-encapsulated mass consisting of atypical lymphocytes with monocytoid appearance and lymphoepithelial lesions (infiltration of the salivary gland epithelium by neoplastic B cells), which are characteristic of extranodal MZL (Fig. 7.4b, c). Flow cytometry showed a monoclonal population of $CD19^+/CD20^+$ B cells that exhibited surface immunoglobulin κ light chain restriction with little or no expression of CD5, CD23 (a minor subpopulation of $CD23^+$ cells was not part of the monoclonal κ-restricted lymphoma population), and CD10 (Fig. 7.4d, e), supporting the diagnosis of MZL. Additional imaging did not show any evidence of lymphadenopathy and she did not undergo further therapy for lymphoma. She has now remained disease-free for 18 years.

7.4 Lymphocytic Interstitial Pneumonia and B Cell Neoplasia

Although the human lung does not normally contain prominent collections of organized lymphoid tissue [24], during infection or other inflammatory processes, rudimentary bronchus-associated lymphoid tissue can be induced to expand (iBALT) [24, 37]. This inducible lymphoid tissue is thought to represent tertiary (ectopic) lymphoid tissue, a process known as lymphoid neogenesis. BALT contains prominent B cell follicles with mature B cells, often in the absence of adjacent T cell areas. In the setting of infection or other antigenic stimulation, the B cell zones may contain germinal centers [24]. Germinal center B cells downregulate IgD expression and may exhibit class switching to IgG, IgA, or IgE. Reactive B cell follicles containing germinal centers also may contain $CD4^+$ T cells and the germinal centers contain oligoclonal B cells undergoing T cell-dependent clonal expansion [38]. Lung biopsies from patients with RA- or SS-associated lung disease consistently contain iBALT with extensive germinal center formation [39].

7.4.1 Case 2: 50-Year-Old Female Non-Smoker with SS and Multiple Pulmonary Nodules

In 1999, the patient developed dry eyes and dry mouth with an abnormal Schirmer test and positive anti-Ro (SS-A) and La (SS-B) antibodies. A chest X-ray in 2004 revealed four small, poorly visualized, non-calcified pulmonary nodules (one of these, located in the right upper lobe, is indicated by an * in Fig. 7.5a). Skin testing for tuberculosis was negative. The pulmonary lesions were followed by serial CT scans. On a CT scan performed in 2008, the nodule in her right upper lobe had enlarged (Fig. 7.5b) and a wedge resection was performed to exclude lung cancer. Pathology revealed a dense lymphocytic infiltrate

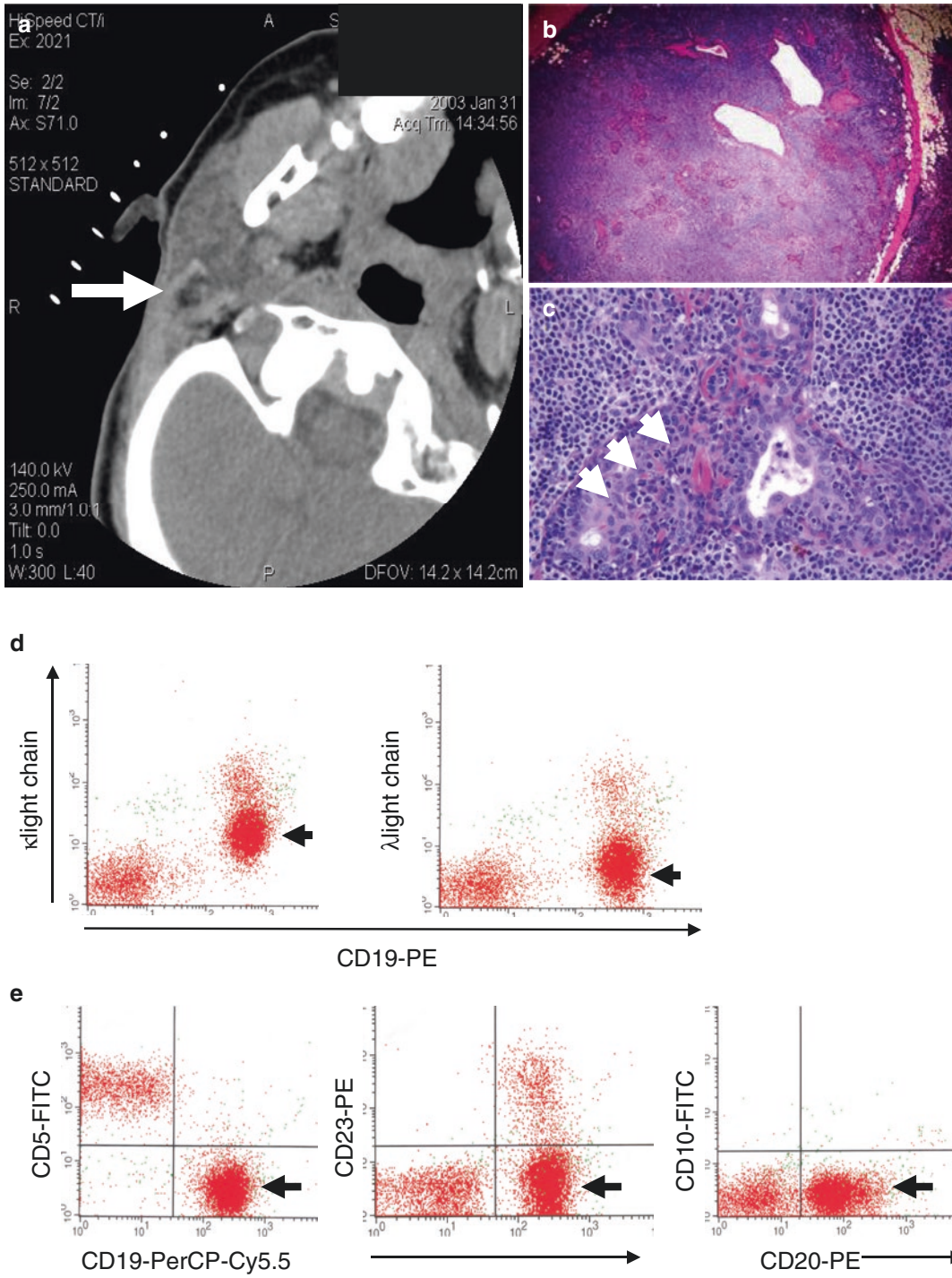

Fig. 7.4 Patient with SS and persistent unilateral parotid enlargement. (**a**) CT scan showing right parotid mass (long arrow). (**b**) H&E staining of the excised parotid mass (×40). (**c**) H&E staining showing typical lymphoepithelial lesions (short arrows) (×400). (**d**) Flow cytometry of salivary B cells showing a monoclonal, κ light chain-restricted B cell population (arrow). (**e**) Flow cytometry of the salivary gland cells showing the absence of CD5, CD23, and CD10 staining on the neoplastic κ-restricted $CD19^+/CD20^+$ B cells (arrows). A small population of $CD19^+CD23^+$ B cells was distinct from the κ-restricted neoplastic B cell population. PE, phycoerythrin; FITC, fluorescein isothiocyanate; PerCP-Cy5.5, peridinin-chlorophyll-Cy5.5

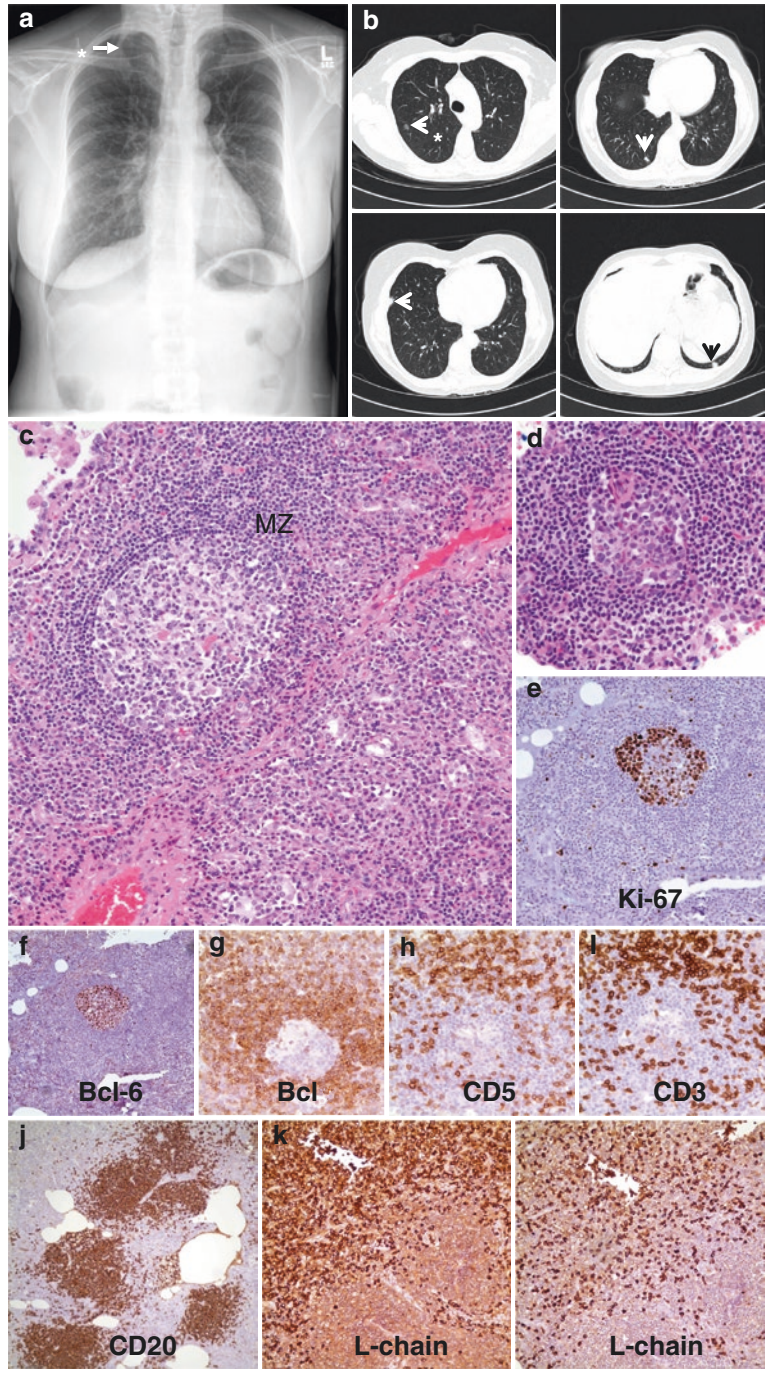

Fig. 7.5 Patient with SS and enlarging lung mass. (**a**) Chest X-ray from 2004 showing small, poorly visualized, non-calcified pulmonary nodules. A nodule in the right upper lobe is indicated by an arrow and an asterisk (*). (**b**) CT scan of the chest from 2008 showing the four nodules (arrows). The enlarging right upper lobe nodule, which was biopsied, is indicated by an asterisk (*). (**c**) H&E staining of a lymphoid follicle from the biopsied lung mass (×200). (**d**) H&E staining of a germinal center from the lung mass (×200). (**e**) Ki-67 staining (immunohistochemistry) of the germinal center from the mass (×200). (**f–i**) Bcl-6, Bcl-2, CD5, and CD3 staining (immunohistochemistry) of the germinal center from the mass (**f–i** ×400; (**j**), ×100; **k** ×200). J, CD20 staining (immunohistochemistry) of B cells in the lung nodule (×10). (**k**) Immunohistochemical staining of polyclonal plasma cells in the lung nodule (left, κ light chain, right, λ light chain) (×20)

with expanded MZ B cells along with reactive lymphoid follicles confirmed by the presence of Ki-67$^+$, BCL6$^+$, and BCL2$^-$ cells showing a phenotype consistent with reactive germinal center B cells (Fig. 7.5c–g). The germinal centers were surrounded by B cells with weaker Bcl-2 expression than the activated (CD3$^+$/CD5$^+$) T cells (Fig. 7.5g–i). The lung nodules also contained numerous CD20$^+$ B cells and polyclonal plasma cells with κ light chain predominance (Fig. 7.5j, k). The presence of germinal centers in the 2008 biopsy of her right upper lobe nodule along with the fact that the nodule was present in exactly the same location for 4 years before enlarging suggests the possibility of ongoing antigenic stimulation within the lung nodule [40]. Although germinal centers typically have a lifespan of less than a month, chronic germinal centers have been reported in chronic viral infection and have been associated with the appearance of self-reactive clones that may evade negative selection and promote genomic instability, leading to the development of autoantibodies and B cell lymphomas [40]. The nature of the antigen responsible for chronic germinal center formation in this case of SS with a benign B cell expansion was not determined. However, in contrast to this patient, neoplastic transformation is seen in some patients with SS, resulting in BALT lymphoma [25, 26].

7.4.2 Lymphoproliferation in SS Lung

The diagnosis of extranodal MZL of the lung in SS may be difficult since B cell proliferation can range from benign lymphoproliferation within BALT to lymphocytic interstitial pneumonia (LIP) to low-grade B cell lymphomas and MZL [33, 41]. Molecular analysis to detect monoclonal immunoglobulin heavy-chain (*IGH*) rearrangements plays an important role in distinguishing benign B cell proliferations from MZL.

LIP, the classic pulmonary lesion seen in 15% of SS patients [42], is defined by the presence of dense interstitial infiltrates consisting of T cells, plasma cells, and histiocytes, and often the formation of germinal centers containing CD20$^+$ B cells [41]. LIP also is seen in other autoimmune disorders and in HIV infection. Although it has been considered a form of interstitial pneumonia for many years, LIP is also a lymphoproliferative process. However, it remains unclear whether it represents a precursor of B cell lymphoma [33]. Viral infections, such as Epstein–Barr virus, HIV, and HHV-8, have been proposed as etiologic agents, but this possibility remains controversial. Clinical manifestations include dyspnea, cough, chest pain, and occasionally hemoptysis. Radiographic findings typically include reticular or reticulonodular infiltrates and ground glass infiltrates, with centrilobular nodules and ground glass infiltrates on high resolution CT (HRCT) scan (see Fig. 7.6 bottom panel). There is clinical and histological overlap with follicular bronchitis, a milder condition characterized by peribronchial lymphoid follicles [41]. The B cells in both LIP and follicular bronchitis are polyclonal, as determined by PCR amplification of *IGH* genes from the lesions. The differential diagnosis includes extrinsic allergic alveolitis, nodular lymphoid hyperplasia (lymphoid proliferations forming one or more isolated lung nodules) [33], and pseudolymphoma [41]. The first two are polyclonal, whereas pseudolymphomas typically are clonal and express B cell surface markers consistent with true lymphoma [41]. Primary pulmonary lymphomas are unusual (0.5–2% of all primary lung neoplasms) [41, 42]. Multiple nodules or alveolar opacities associated with air bronchograms may be visualized on HRCT scan. Histologically, lymphoepithelial lesions and germinal centers are seen. High grade tumors are often diffuse and necrotic [33, 41]. Nodal B cell lymphoma also can directly invade from adjacent mediastinal nodes or metastasize from the blood. Although detection of rearranged *IGH* genes by PCR is characteristic of B cell lymphomas, LIP also can exhibit small populations of clonal cells, consistent with the possibility that B cell lymphomas and LIP are part of a continuum. All these disorders, both

polyclonal and monoclonal, are thought to arise from BALT [43].

The standard treatment of LIP is corticosteroids plus steroid-sparing immunosuppressive drugs [42]. B cell depletion with rituximab has shown promising improvement of salivary and lacrimal flow and interstitial lung disease in SS [44, 45]. There also is a report of successful treatment of LIP with rituximab [46]. In view of the importance of T cells in the inflammatory infiltrates, abatacept (CTLA4-Ig) has been tested in small numbers of SS patients with encouraging results [47, 48] and successful treatment of LIP with abatacept plus tacrolimus has been reported [49]. Although there are no published data to suggest that aggressive treatment of LIP can prevent the development of B cell lymphoma, that possibility warrants further study.

7.5 Other Forms of Lymphoma in SS

Although MZL is most typical, other types of B cell lymphoma may develop in SS. In one study, the prevalence of MZL was 44%, diffuse large B cell lymphoma (DLBCL) 24%, and follicular lymphoma 24% [50]. T cell lymphomas and Hodgkin lymphomas are rare. There is not much information specific to SS patients with B cell lymphomas other than MZL.

7.5.1 DLBCL

DLBCL generally is much more aggressive than MZL. It is classified into germinal center-like (GCB) and activated B cell (ABC) subtypes on the basis of transcriptional profiling and expression of

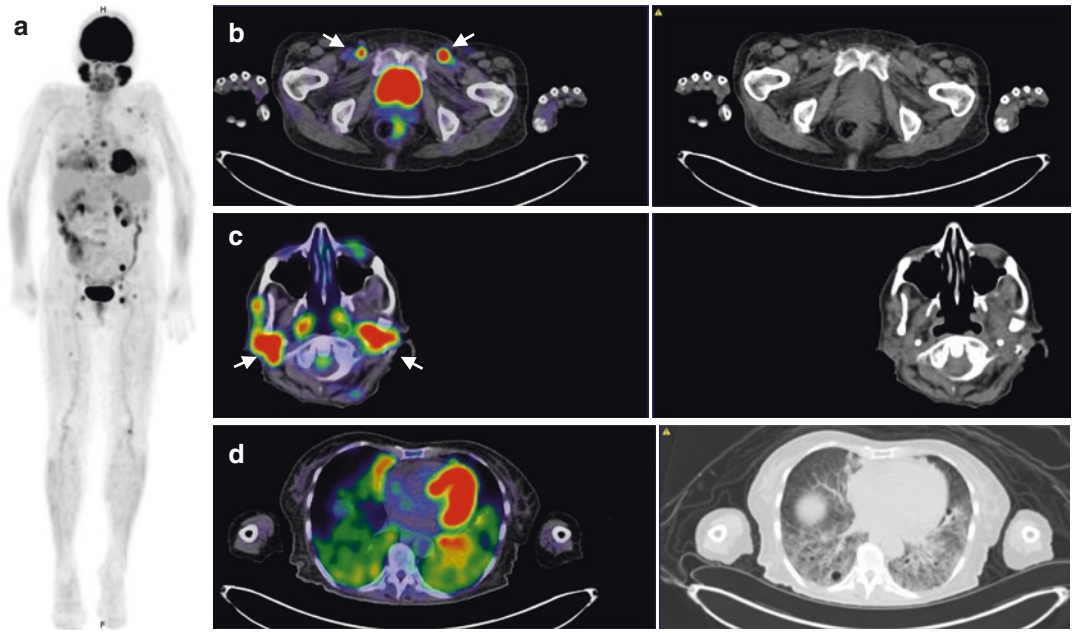

Fig. 7.6 Patient with SS and follicular lymphoma. (**a**) PET scan obtained in 12/2016 showing widespread fluorodeoxyglucose (FDG)-avid lymphadenopathy in a SS patient with probable follicular lymphoma. (**b**) *left,* PET scan showing FDG-avid groin lymph nodes; *right,* CT scan of the same region. (**c**) *left,* PET scan showing FDG uptake in the bilateral parotid glands; *right,* CT scan of the same region. (**d**) *left,* PET scan showing diffuse FDG uptake in the lungs; *right,* CT scan of the same region. (**e**) Histopathology of follicular lymphoma. Panels show the typical findings of Grade 3 follicular lymphoma in another patient (a different patient than shown in **a**–**d**). H&E section shows vaguely nodular and diffuse areas with predominantly medium to large cells with increased centroblasts (X100, inset X400). Immunohistochemical studies demonstrate diffuse $CD20^{+}$/$Pax5^{+}$ B cells that are also positive for BCL2 and BCL6 with approximately 30–50% Ki-67 labeling index. CD21 immunohistochemistry highlights some residual follicular dendritic cell meshwork

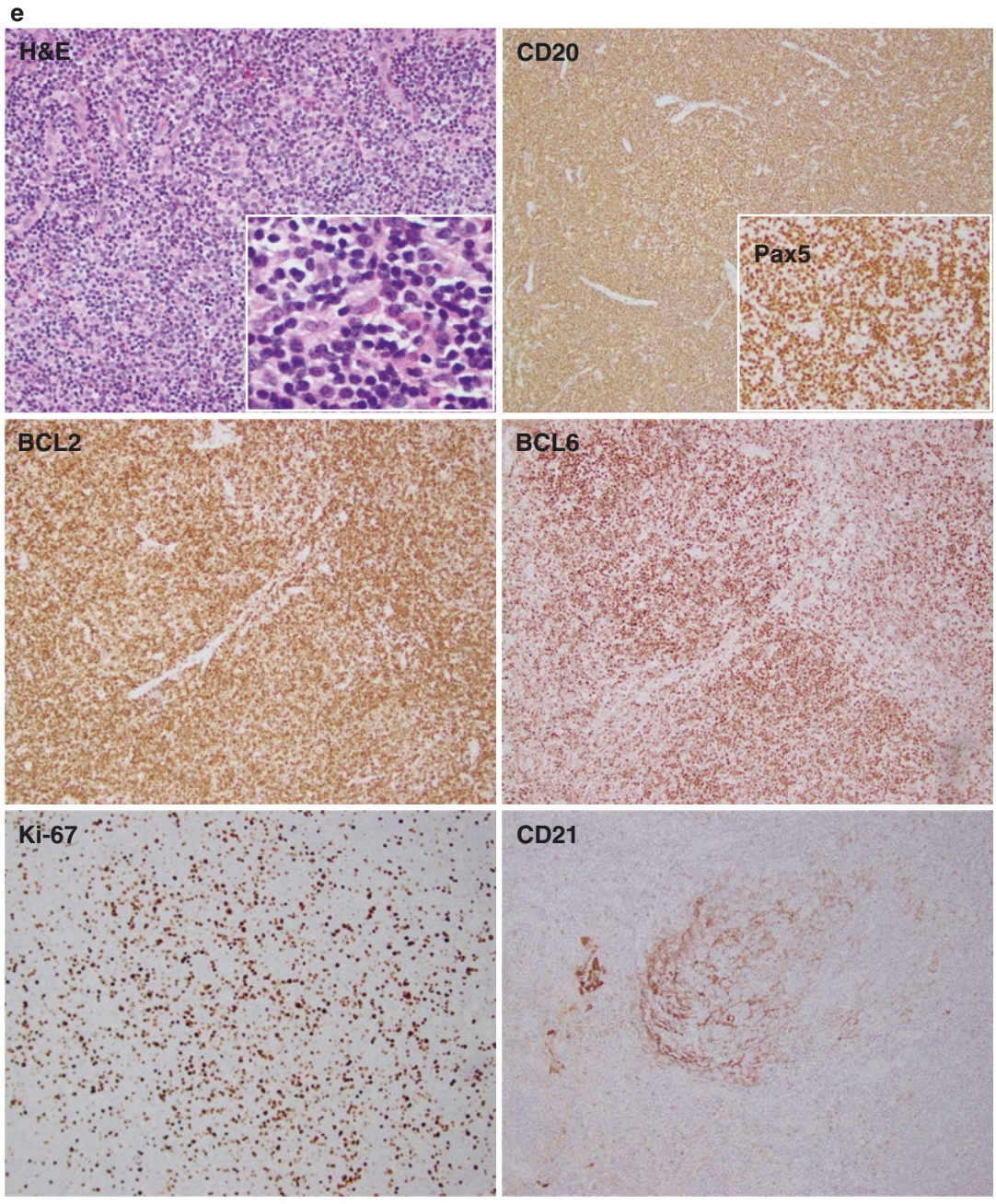

Fig. 7.6 (continued)

CD10, Bcl6, cyclin D2, and MUM1 [51, 52]. Five-year survival of patients with GCB is 76% compared with 34% for patients with non-GCB DLBCL [52]. Bcl6 or CD10 expression is associated with a better prognosis, whereas MUM1 or cyclin D2 expression is associated with a worse prognosis.

A recent study examined DLBCL in 18 SS patients [53]. Extranodal DLBCL was found in 14, most commonly arising in the lungs (6 patients) or stomach (2 patients) rather than the salivary gland. The onset was late in the course of SS with a median of 20.5 years from the initial SS

symptom to the diagnosis of DLBCL. In contrast to the favorable prognosis of MZL, the median survival after diagnosis of DLBCL is 3 months with an overall 5-year survival rate of 37.5%. Based on CD10, BCL6, and MUM1 staining, 9 of 12 typable lymphomas were of the non-GCB DLBCL subtype [53]. In another study of B cell lymphoma in SS patients, 169 had MZL and 25 had DLBCL [54]. Six had both MZL and DLBCL, and analysis of *IGH* rearrangements revealed that the MZL and DLBCL were clonally related in 5 of the cases, despite different localization of the MZL and DLBCL. Presumably DLBCL in the remaining 19 patients with no MZL arose de novo. DLBCL in these patients had a high Ki-67 proliferation index. Although the numbers were small, there was no evidence that DLBCL arising from transformation of MZL was more aggressive than de novo DLBCL [54]. A potential role for Epstein–Barr virus in the transformation of MZL to DLBCL has been reported [55].

7.5.2 Follicular Lymphoma

In patients with SS, the risk of MZL and DLBCL is increased 30–40-fold and ninefold, respectively, whereas the risk of follicular lymphoma is increased fourfold [56]. However, there are few detailed studies of SS patients with this B cell lymphoma [57]. Follicular lymphoma usually is indolent (median survival >15 years) with a follicular lymphoid structure and germinal center phenotype [58]. It has a long pre-malignant phase in which B cells with a t(14;18) chromosomal translocation (85% of cases) accumulate additional mutations that promote transformation or clonal expansion. The t(14;18) translocation places the *BCL2* oncogene under control of the *IGH* promoter.

The underlying mechanisms leading to follicular lymphoma are controversial [59–61]. It has been suggested that most follicular lymphomas develop via an antigen independent route, whereas about 20% are antigen-selected [62]. The tumor cells express typical GC markers, such as BCL6, activation induced deaminase, and CD10, but not CD5 [58]. Although extranodal involvement is uncommon [63], there are reports of follicular lymphoma arising in the parotid gland [64]. It is estimated that 30% of primary salivary gland lymphomas are follicular lymphomas [65]. These tumors bear some resemblance to MZL in having an indolent course along with co-existing myoepithelial sialadenitis, suggesting that chronic inflammation plays a role in pathogenesis.

7.5.3 Case 3: 79-Year-Old Woman with SS and Probable Follicular Lymphoma

The patient, who had a 50-year history of chronic leukopenia and 20 years of bilateral (left > right) parotid enlargement, was seen for evaluation of sicca symptoms and lymphadenopathy. She developed joint pain 6 years earlier, responsive to a short course of prednisone. Anti-Ro (SS-A) and La (SS-B) antibodies were positive and she was given a diagnosis of SS, although a Schirmer test was normal at the time. She subsequently developed progressive xerostomia, dental decay, and dry eyes. In September 2016, she developed left inguinal lymphadenopathy, 20-pound unintentional weight loss, and fever of 99–101.3 °F. A PET scan showed multiple areas of uptake in the liver, lungs, bone, and multiple lymph nodes, but not the parotid glands. Bone marrow biopsy showed marrow damage and multiple small lymphoid aggregates suggestive of an autoimmune process, but no lymphoma. A left inguinal lymph node biopsy revealed extensive necrosis and was non-diagnostic. She was referred to Rheumatology in November 2016 and was found to have dry eyes (Schirmer test 1 mm right, 0 mm left), xerostomia, poor dentition, inguinal and axillary lymphadenopathy, and basilar rales, greater on the left than the right. Her white blood count was 1300/mm^3, hemoglobin 10.4 g/dL, platelets 207,000/mm^3, and creatinine 0.58 mg/dL. ESR was 108 mm/h, ANA 1:160 (positive anti-Ro/SS-A and La SS-B), and RF 4130 IU/mL. Total IgG was 2699 mg/dL, IgA 137 mg/dL, IgM 872 mg/dL. Serum protein electrophoresis showed polyclonal hypergammaglobulinemia without a monoclonal gammopathy. Alkaline phosphatase initially was 288 IU/L but progres-

sively increased to 647 IU/L with normal transaminases. A second lymph node biopsy (axillary node) was again non-diagnostic. A CT-PET imaging study in December 2016 revealed widespread lymphadenopathy (Fig. 7.6a) with some fluorodeoxyglucose (FDG)-avid lymph nodes (Fig. 7.6b), as well as uptake in the parotid glands (Fig. 7.6c) and lungs (Fig. 7.6d). A PET-CT guided core biopsy of an FDG-avid inguinal lymph node (Fig. 7.6b) was consistent with a B cell lymphoma with κ-restricted clonal B cells that were $CD10^{+}CD5^{-}$ by flow cytometry, consistent with follicular lymphoma. Histology was inadequate due to extensive necrosis, but there was a focal increase in large centroblasts suggesting transformation into intermediate grade of follicular lymphoma. Figure 7.6e shows the typical histological features of follicular lymphoma in another patient who did not have SS. Our patient was treated with 6 cycles of cyclophosphamide, doxorubicin, rituximab, and vincristine. Her lymphadenopathy decreased markedly and the alkaline phosphatase normalized. CT scan in 2018 showed changes suggestive of LIP but no evidence of recurrent lymphoma. Diffusion capacity for carbon monoxide (DLCO) was 53% predicted. The patient refused lung biopsy. It was felt that her lymphoma therapy may have arrested progression of LIP and it was decided to monitor her lung disease by CT scan. A follow-up PET-CT scan in 2019 showed evidence of recurrent disease with multiple areas of lymphadenopathy, diffuse uptake in the lungs, hilar/mediastinal adenopathy, probable lymphomatous involvement of the parotid glands (more prominent on the right), and enlarging FDG-avid lymph nodes adjacent to the right parotid gland. RF was 63 IU/mL. She received an additional 4 courses of rituximab, but declined treatment with other agents. A repeat PET-CT scan 2 months later showed no suspicious FDG uptake.

7.6 RF B Cells, Cryoglobulins, and Lymphoma Risk

About two-thirds of SS patients produce IgM or IgA RFs [66] and many of the B cell lymphomas in SS produce RFs. RFs are autoantibodies that recognize the Fc portion of IgG. They are most commonly of the IgM class, but IgG, IgA, IgE, and IgD RFs also are seen. High levels of RF are associated with RA, but are not specific, as they also are present in many patients with SS or chronic HCV infection. Lower levels are seen in individuals with other infections (e.g., bacterial endocarditis) [67] and in some healthy individuals [66]. IgM RF produced during infections or by healthy individuals show little somatic hypermutation or class switching and may facilitate the clearance of immune complexes and antigen presentation [68]. In contrast, RFs produced in RA, SS, and chronic HCV infection tend to have higher titers and may exhibit higher affinity due to somatic hypermutation as indicated by a higher ratio of replacement to silent mutations in the heavy-chain CDR3 compared with framework regions [69].

7.6.1 Cryoglobulins and Preferential Immunoglobulin Heavy Chain (V_H) Gene Usage

Some of the RFs in HCV infection and SS are cryoglobulins, which precipitate out of solution at temperatures below 37 °C and dissolve again on re-warming. They are classified as Type I (self-associating monoclonal IgG or IgM), Type II (monoclonal IgM RF + polyclonal IgG), and Type III (polyclonal IgM + polyclonal IgG, usually with the IgM component having RF activity). Mixed cryoglobulins (Types II and III) consist of more than one immunoglobulin class, and are common in HCV infection (~30%) and SS (15–30%) [66, 70]. They sometimes cause cryoglobulinemic vasculitis, a small vessel vasculitis that in SS patients has a 36% mortality [71]. Mixed cryoglobulinemia is a predictive factor for the development of lymphoma in SS [72]. Cryoglobulins in HCV infection and in SS-associated MZL preferentially utilize the *V_H1–69* heavy chain, often in combination with *kv325* light-chain (Wa idiotype) [73–75]. About half of MZL arising in the salivary glands express *V_H1–69*. In contrast, only about 7% of gastric

MALT lymphomas express $V_H1–69$, and this gene segment is used only rarely in thyroid MALT lymphomas [76–79]. When recombinant heavy and light chains from salivary MALT lymphomas are transfected into a cell line, a high percentage of the recombinant lymphoma-derived antibodies exhibit RF activity [76]. Thus, there is strong evidence implicating $V_H1–69^+$ RFs in the pathogenesis of MZL in SS. Although it remains unclear why the $V_H1–69$ H-chain is preferentially utilized by cryoglobulins and MZL in SS and HCV infection, there is some information about the potential role of RF B cells.

7.6.2 Role of Infection in Pathogenesis of HCV-Induced MZL

In view of the similarities between SS and chronic HCV infection (Fig. 7.3), an understanding of MZL development in HCV infection may be relevant to SS. Chronic HCV infection can drive non-malignant proliferation of monoclonal B cells producing a polyreactive "natural" autoantibody with RF specificity [75]. These clonal expansions disappear when the infection is cleared by antiviral therapy, strongly suggesting that they are driven by the virus [80]. The expanded B cells have a memory phenotype, and it has been proposed that HCV-driven expansion of memory B cells expressing $V_H1–69$ generates a mutant B cell that escapes growth control mechanisms, resulting in lymphoma.

The $V_H1–69$ H-chain is expressed in approximately 1.7% of the peripheral blood B cells in healthy individuals, but is highly represented among neutralizing antibodies reactive with the HCV E2 glycoprotein and also in neutralizing antibodies against HIV-1 and influenza virus [81, 82]. Along with the biased usage of $V_H1–69$ by B cell lymphomas, these data suggest that chronic antigenic stimulation by the HCV E2 glycoprotein might lead to B cell proliferation and ultimately malignant transformation. More recently, it has been hypothesized that RF B cells are stimulated by IgG-containing immune complexes containing viral (HCV) or self RNA or DNA. Thus, IgG may be a key autoantigen in chronic inflammation that provides a growth advantage to RF B cells, promoting lymphomagenesis [83]. Consistent with that model, somatic hypermutation and affinity maturation in RF B cells can take place outside of germinal centers and are driven by TLR7/8/9-mediated innate immune responses to nucleic acids contained within immune complexes [84, 85]. Thus, there is evidence that the neoplastic B cells may develop extrafollicularly, although an antigen-stimulated, T cell-driven, germinal center-dependent mechanism cannot be completely excluded.

7.7 Risk Factors for Lymphomagenesis in SS

The risk of developing B cell lymphoma increases with the duration of disease: ~4% at 5 years, 10% at 10 years, and 18% after 20 years [86]. Additional risk factors include parotid enlargement, low serum C4 levels, and/or $CD4^+$ T cell lymphopenia [86–89]. The evidence that cryoglobulinemia is an independent risk factor is controversial [86]. The formation of germinal center-like structures (ectopic lymphoid tissue, see Fig. 7.5) within the salivary glands is strongly associated with increased risk of B cell lymphoma [90]. Overexpression of BAFF is associated with autoimmune disease (SLE and SS) and genetic variants within the *BAFF* promoter region are associated with an increased risk of B cell lymphoma in SS patients [91]. Similarly, a genetic variant (His159Tyr) of the BAFF receptor is associated with SS-associated MZL, possibly due to increased activation of the alternative NFκB signaling pathway [92].

7.8 Prevalence of B Cell Lymphoma in Primary Compared with Secondary SS

A pooled analysis of self-reported SS and risk of non-Hodgkin lymphoma in eight prior studies suggested that secondary SS is associated with a

higher risk of B cell lymphoma than primary SS [56]. However, this has not been our experience. We examined the prevalence of B cell lymphoma in patients with physician-diagnosed primary and secondary SS who were enrolled in the University of Florida SS Registry from 2000–2020. All registry patients undergo detailed formal evaluation for SS as well as other autoimmune diseases, including SLE, scleroderma, polymyositis/dermatomyositis, RA, and primary biliary cirrhosis. A search of our registry identified 430 patients with sicca symptoms (Table 7.1). Of these, a total of 184 patients had probable or definite SS (126 primary and 58 secondary) based on the 2002 ACR/EULAR Consensus Criteria [93]. One of the 58 patients with secondary SS had a history of B cell lymphoma (1.7%) in comparison with eight of 126 (6.3%) with primary SS (Table 7.1). Six of the primary SS patients had MZL, one DLBCL, and one probable follicular lymphoma. Four patients with other malignancies were included in the primary SS group: one patient each with renal cell carcinoma, malignant carcinoid syndrome, sarcoma, and breast cancer. Although the groups were mostly similar, the percentage of white patients was higher in the primary (83.3%) compared with secondary (63.8%) SS group and the prevalence of RF was higher in the primary (57.8%) compared with secondary (41.5%) SS groups (Table 7.1). Of the eight patients with B cell lymphoma (seven Primary, one Secondary) who were tested for RF, all were positive. Overall, the 10-year risk of developing B cell lymphoma in our cohort was 5.6% for patients with primary SS and 1.3% for patients with secondary SS (Table 7.1).

Table 7.1 Lymphoma in the UF Sjögren's syndrome registry

	Primary SS	Secondary SS
Number of patients	126	58
% female	98.4% (124)	96.6% (56)
% white	83.3% (105)	63.8% (37)
Age range (mean years)	31–100 (67.5)[a]	24–89 (62)[b]
Years followed, range (mean)	1–19 (12)	1–20 (13)
Lymphoma per 10 years followed[c]	5.6%	1.3%
Anti-Ro and/or La positive	80.3% (98/122)	83.9% (47/56)
Ro^+ and/or La^+ with lymphoma	85.7% (6/7)	100% (1/1)
Ro^+ and La^+ with lymphoma	71.4% (5/7)	100% (1/1)
Rheumatoid factor positive	57.8% (63/109)	41.5% (22/53)
RF^+ with lymphoma	100% (7/7)	100% (1/1)
Ro/La^- and RF^-	11.7% (14/120)	9.3% (5/54)
B cell lymphoma (%)	6.3% (8)	1.7% (1)
Marginal zone	4.8% (6)	1.7% (1)
DLBCL	0.8% (1)	0% (0)
Other B cell lymphoma	0.8% (1)	0% (0)

[a]4 deceased
[b]7 deceased
[c][(# of lymphomas / Σ years followed) × 10 years] × 100%

Although our cohort is smaller than the pooled analysis reported previously [56], it has the advantage that the diagnoses of SS and other autoimmune disorders were confirmed using established clinical criteria rather than being based on self-reported diagnoses. The frequency of B cell lymphoma we saw in patients with primary SS (6.3%) was consistent with the 2.7–9.8% prevalence reported in the literature. The explanation for the higher frequency of lymphoma in primary SS seen in our cohort compared with the pooled data reported previously is uncertain [56]. It might reflect differences in the application of diagnostic criteria for several disorders. Alternatively, it could reflect the fact that our secondary SS cohort was biased toward a primary diagnosis of SLE (reflecting our clinical practice) and included relatively few patients with a primary diagnosis of RA. Interestingly, the prevalence of RF was lower in our secondary SS patients than in those with primary SS and the only patient in the secondary SS group who developed lymphoma had a primary diagnosis of RA (Table 7.1). As there is a strong association of serum RF with B cell lymphoma in SS [94] and in view of the role of RF B cells in the pathogenesis of lymphoma in these patients (see above), we speculate that more extensive studies might reveal that patients with Secondary SS and RA may have a higher risk of B cell lymphoma than secondary SS patients with SLE. Larger studies

Table 7.2 Anti-Ro and La antibodies in SS patients (combined primary and secondary) with and without lymphoma

	B cell lymphoma[a]	No lymphoma[a]
Ro and La both positive	6	71
Ro only or Ro and La both negative	2	69
Totals	8	140

[a]*Limited to the subset of patients in whom both anti-Ro (SS-A) and La (SS-B) antibodies had been tested; P = 0.18 (Chi-square)*

of well-characterized patients with primary compared with secondary SS will be necessary to definitively address this possibility.

It also has been suggested that patients with both anti-Ro and anti-La autoantibodies are at increased risk for B cell lymphoma, but this remains controversial [reviewed in [95]]. Six of the eight SS patients with B cell lymphoma for whom data were available were positive for both anti-Ro and anti-La antibodies, whereas one was positive for anti-Ro alone and one was anti-Ro$^-$ and La$^-$ (Table 7.2). We did not detect a statistically significant association of B cell lymphoma with positivity for both anti-Ro and La, but our cohort is too small to address this question definitively.

7.9 Therapy of Extranodal B Cell Lymphoma in SS

The possible role of chronic antigenic stimulation in the pathogenesis of MZL suggests that the disease might be treated by removing the source of antigenic stimulation. This strategy is effective in some patients with gastric MZL (treatment of *H. pylori* infection) [22] or HCV-associated lymphoma (treated with antiviral therapy) [80]. Unfortunately, although there is evidence of antigenic stimulation in SS-associated lymphomas, the nature of the antigen or autoantigen remains unclear. Thus, other strategies are necessary.

The prognosis of SS patients with MZL is good. In one long-term retrospective study of 247 patients with salivary gland MZL (of which 33% has SS), 76% presented with limited disease, the median progression-free survival after primary therapy was 9.3 years, and the median overall survival was 18.3 years [36]. In that study, 57% of patients received only local therapy (surgery, radiation, or both), 37% received initial systemic therapy (47% were treated with rituximab), and 6% were observed. There was no difference in the outcome between patients receiving initial surgery, radiation, chemotherapy, or chemoimmunotherapy. In early disease, there was no benefit of local therapy (radiation or surgery) over systemic therapy. A better overall survival was associated with having a diagnosis of SS or receiving rituximab therapy. About a third of the 247 patients had progressive disease in the same salivary gland or regional nodes (29% of patients with progressive disease), the contralateral salivary gland (21%), or at distant sites (31%) and 39% had relapses (MALT lymphoma in all but two cases, one DLBCL and one mantle zone lymphoma).

The potential long-term risks compared with benefits of therapy must be weighed carefully when designing a plan of treatment. Xerostomia is a frequent side effect of radiation therapy or parotidectomy, which could compound preexisting xerostomia resulting from SS. In addition, parotid surgery can be disfiguring and risks facial nerve injury. On the other hand, rituximab therapy of GI MALT lymphoma can lead to the selection of CD20$^-$ cells exhibiting plasmacytic differentiation, suggesting that rituximab may not be the optimal therapy for MALT lymphomas with significant plasmacytic features [35].

7.9.1 Therapy of DLBCL

The standard treatment for DLBCL regardless of subtype is R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) [96]. Patients treated with this regimen have an overall survival of >60%. In patients with refractory or recurrent disease, standard therapy is autologous stem cell transplantation, although therapy with chimeric antigen receptor (CAR) T cells has been approved as well [97]. However, toxicity (cytokine storm and neurotoxicity) can

complicate CAR T-cell therapy. At present, the therapy of DLBCL in patients with SS is the same as in patients without SS, although there are no large studies comparing R-CHOP therapy with other forms of treatment in SS.

7.10 Conclusion

SS is an autoimmune disease characterized by chronic inflammation and lymphoid neogenesis in the salivary and other exocrine glands. The high frequency of B cell lymphomas, predominantly extranodal MZL, in patients with primary SS emphasizes the close relationship between chronic inflammation and B cell neoplasia. RF-producing B cells, especially those expressing the *V_H1–69* immunoglobulin heavy chain, are a hallmark of the B cell lymphomas developing in the inflamed parotid glands of SS patients. A better understanding of why these particular B cells are selected for neoplastic transformation may lead to improved approaches for preventing and treating this complication.

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References

1. Manganelli P, Fietta P, Quaini F. Hematologic manifestations of primary Sjogren's syndrome. Clin Exp Rheumatol. 2006;24(4):438–48.
2. De Re V, De Vita S, Marzotto A, Rupolo M, Gloghini A, Pivetta B, et al. Sequence analysis of the immunoglobulin antigen receptor of hepatitis C virus-associated non-Hodgkin lymphomas suggests that the malignant cells are derived from the rheumatoid factor-producing cells that occur mainly in type II cryoglobulinemia. Blood. 2000;96(10):3578–84.
3. Ioannidis JP, Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjogren's syndrome. Arthritis Rheum. 2002;46(3):741–7.
4. Jorgensen C, Legouffe MC, Perney P, Coste J, Tissot B, Segarra C, et al. Sicca syndrome associated with hepatitis C virus infection. Arthritis Rheum. 1996;39(7):1166–71.
5. Coll J, Gambus G, Corominas J, Tomas S, Esteban JI, Guardia J. Immunohistochemistry of minor salivary gland biopsy specimens from patients with Sjogren's syndrome with and without hepatitis C virus infection. Ann Rheum Dis. 1997;56(6):390–2.
6. Itescu S, Winchester R. Diffuse infiltrative lymphocytosis syndrome: a disorder occurring in human immunodeficiency virus-1 infection that may present as a sicca syndrome. Rheum Dis Clin North Am. 1992;18(3):683–97.
7. Kordossis T, Paikos S, Aroni K, Kitsanta P, Dimitrakopoulos A, Kavouklis E, et al. Prevalence of Sjogren's-like syndrome in a cohort of HIV-1-positive patients: descriptive pathology and immunopathology. Br J Rheumatol. 1998;37(6):691–5.
8. Hansen A, Odendahl M, Reiter K, Jacobi AM, Feist E, Scholze J, et al. Diminished peripheral blood memory B cells and accumulation of memory B cells in the salivary glands of patients with Sjogren's syndrome. Arthritis Rheum. 2002;46(8):2160–71.
9. Daridon C, Pers JO, Devauchelle V, Martins-Carvalho C, Hutin P, Pennec YL, et al. Identification of transitional type II B cells in the salivary glands of patients with Sjogren's syndrome. Arthritis Rheum. 2006;54(7):2280–8.
10. Freimark B, Fantozzi R, Bone R, Bordin G, Fox R. Detection of clonally expanded salivary gland lymphocytes in Sjogren's syndrome. Arthritis Rheum. 1989;32:859–69.
11. Youinou P, Le Corre R, Dueymes M. Autoimmune diseases and monoclonal gammopathies. Clin Exp Rheumatol. 1996;14(Suppl 14):S55–S8.
12. Burg N, Swendeman S, Worgall S, Hla T, Salmon JE. Sphingosine 1-phosphate receptor 1 signaling maintains endothelial cell barrier function and protects against immune complex-induced vascular injury. Arthritis Rheumatol. 2018;70(11):1879–89.
13. Kratz A, Campos-Neto A, Hanson MS, Ruddle NH. Chronic inflammation caused by lymphotoxin is lymphoid neogenesis. J Exp Med. 1996;183:1461–72.
14. Palamar M, Ozsan N, Sahin F. Bilateral lacrimal gland lymphoma in Sjogren syndrome. Case Rep Ophthalmol Med. 2016;2016:2798304.
15. Stott DI, Hiepe F, Hummel M, Steinhauser G, Berek C. Antigen-driven clonal proliferation of B cells within the target tissue of an autoimmune disease. The salivary glands of patients with Sjogren's syndrome. J Clin Invest. 1998;102(5):938–46.
16. Travaglino A, Varricchio S, Pace M, Iuliano A, Picardi M, Tranfa F, et al. Hepatitis C virus in MALT-lymphoma of the ocular adnexa. Pathol Res Pract. 2020;216:152864.
17. Gasparotto D, De Vita S, De Re V, Marzotto A, De Marchi G, Scott CA, et al. Extrasalivary lymphoma development in Sjogren's syndrome: clonal evolution from parotid gland lymphoproliferation and role of local triggering. Arthritis Rheum. 2003;48(11):3181–6.

18. Zucca E, Bertoni F, Roggero E, Bosshard G, Cazzaniga G, Pedrinis E, et al. Molecular analysis of the progression from helicobacter pylori-associated chronic gastritis to mucosa-associated lymphoid-tissue lymphoma of the stomach. N Engl J Med. 1998;338(12):804–10.
19. Flores-Chavez A, Kostov B, Solans R, Fraile G, Maure B, Feijoo-Masso C, et al. Severe, life-threatening phenotype of primary Sjogren's syndrome: clinical characterisation and outcomes in 1580 patients (GEAS-SS Registry). Clin Exp Rheumatol. 2018;36 Suppl 112(3):121–9.
20. Ramos-Casals M, Garcia-Carrasco M, Cervera R, Rosas J, Trejo O, de la Red G, et al. Hepatitis C virus infection mimicking primary Sjogren syndrome. A clinical and immunologic description of 35 cases. Medicine (Baltimore). 2001;80(1):1–8.
21. De Re V, De Vita S, Marzotto A, Gloghini A, Pivetta B, Gasparotto D, et al. Pre-malignant and malignant lymphoproliferations in an HCV-infected type II mixed cryoglobulinemic patient are sequential phases of an antigen-driven pathological process. Int J Cancer. 2000;87(2):211–6.
22. Stathis A, Chini C, Bertoni F, Proserpio I, Capella C, Mazzucchelli L, et al. Long-term outcome following helicobacter pylori eradication in a retrospective study of 105 patients with localized gastric marginal zone B-cell lymphoma of MALT type. Ann Oncol. 2009;20(6):1086–93.
23. Ferreri AJ, Govi S, Ponzoni M. Marginal zone lymphomas and infectious agents. Semin Cancer Biol. 2013;23(6):431–40.
24. Iwata Y, Matsushita T, Horikawa M, DiLillo DJ, Yanaba K, Venturi GM, et al. Characterization of a rare IL-10-competent B-cell subset in man that parallels mouse regulatory B10 cells. Blood. 2010;117(2):530–41.
25. Ingegnoli F, Sciascera A, Galbiati V, Corbelli V, D'Ingianna E, Fantini F. Bronchus-associated lymphoid tissue lymphoma in a patient with primary Sjogren's syndrome. Rheumatol Int. 2008;29(2):207–9.
26. Papiris SA, Kalomenidis I, Malagari K, Kapotsis GE, Harhalakis N, Manali ED, et al. Extranodal marginal zone B-cell lymphoma of the lung in Sjogren's syndrome patients: reappraisal of clinical, radiological, and pathology findings. Respir Med. 2007;101(1):84–92.
27. Hussell T, Isaacson PG, Crabtree JE, Spencer J. The response of cells from low-grade B-cell gastric lymphomas of mucosa-associated lymphoid tissue to helicobacter pylori. Lancet. 1993;342(8871):571–4.
28. Zucca E, Bertoni F. The spectrum of MALT lymphoma at different sites: biological and therapeutic relevance. Blood. 2016;127(17):2082–92.
29. Chen LY, Tsai MH, Tsai LT, Lu HM, Jan CI. Primary Sjogren's syndrome initially presenting as submandibular mucosa-associated lymphoid tissue lymphoma: a case report. Oncol Lett. 2016;11(2):921–4.
30. Keszler A, Adler LI, Gandolfo MS, Masquijo Bisio PA, Smith AC, Vollenweider CF, et al. MALT lymphoma in labial salivary gland biopsy from Sjogren syndrome: importance of follow-up in early detection. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115(3):e28–33.
31. Tonami H, Matoba M, Kuginuki Y, Yokota H, Higashi K, Yamamoto I, et al. Clinical and imaging findings of lymphoma in patients with Sjogren syndrome. J Comput Assist Tomogr. 2003;27(4):517–24.
32. Molina TJ, Lin P, Swerdlow SH, Cook JR. Marginal zone lymphomas with plasmacytic differentiation and related disorders. Am J Clin Pathol. 2011;136(2):211–25.
33. Sambataro G, Sambataro D. Letter to editor: "interstitial pneumonia with autoimmune features: clinical, radiologic and histological characteristics and outcome in a series of 57 patients". Respir Med. 2017;127:65–6.
34. Woehrer S, Streubel B, Chott A, Hoffmann M, Raderer M. Transformation of MALT lymphoma to pure plasma cell histology following treatment with the anti-CD20 antibody rituximab. Leuk Lymphoma. 2005;46(11):1645–9.
35. Troch M, Kiesewetter B, Dolak W, Jaeger U, Puspok A, Mullauer L, et al. Plasmacytic differentiation in MALT lymphomas following treatment with rituximab. Ann Hematol. 2012;91(5):723–8.
36. Jackson AE, Mian M, Kalpadakis C, Pangalis GA, Stathis A, Porro E, et al. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue of the salivary glands: a multicenter, international experience of 248 patients (IELSG 41). Oncologist. 2015;20(10):1149–53.
37. GeurtsvanKessel CH, Willart MA, Bergen IM, van Rijt LS, Muskens F, Elewaut D, et al. Dendritic cells are crucial for maintenance of tertiary lymphoid structures in the lung of influenza virus-infected mice. J Exp Med. 2009;206(11):2339–49.
38. Thorbecke GJ, Amin AR, Tsiagbe VK. Biology of germinal centers in lymphoid tissue. FASEB J. 1994;8(11):832–40.
39. Rangel-Moreno J, Hartson L, Navarro C, Gaxiola M, Selman M, Randall TD. Inducible bronchus-associated lymphoid tissue (iBALT) in patients with pulmonary complications of rheumatoid arthritis. J Clin Invest. 2006;116(12):3183–94.
40. Mesin L, Ersching J, Victora GD. Germinal center B cell dynamics. Immunity. 2016;45(3):471–82.
41. Barbareschi M, Ferrero S, Aldovini D, Leonardi E, Colombetti V, Carboni N, et al. Inflammatory pseudotumour of the lung. Immunohistochemical analysis on four new cases. Histol Histopathol. 1990;5(2):205–11.
42. Flament T, Bigot A, Chaigne B, Henique H, Diot E, Marchand-Adam S. Pulmonary manifestations of Sjogren's syndrome. Eur Respir Rev. 2016;25(140):110–23.
43. Koss MN. Pulmonary lymphoid disorders. Semin Diagn Pathol. 1995;12(2):158–71.

44. Souza FB, Porfirio GJ, Andriolo BN, Albuquerque JV, Trevisani VF. Rituximab effectiveness and safety for treating primary Sjogren's syndrome (pSS): systematic review and meta-analysis. PLoS One. 2016;11(3):e0150749.
45. Chen MH, Chen CK, Chou HP, Chen MH, Tsai CY, Chang DM. Rituximab therapy in primary Sjogren's syndrome with interstitial lung disease: a retrospective cohort study. Clin Exp Rheumatol. 2016;34(6):1077–84.
46. Swartz MA, Vivino FB. Dramatic reversal of lymphocytic interstitial pneumonitis in Sjogren's syndrome with rituximab. J Clin Rheumatol. 2011;17(8):454.
47. Meiners PM, Vissink A, Kroese FG, Spijkervet FK, Smitt-Kamminga NS, Abdulahad WH, et al. Abatacept treatment reduces disease activity in early primary Sjogren's syndrome (open-label proof of concept ASAP study). Ann Rheum Dis. 2014;73(7):1393–6.
48. Tsuboi H, Matsumoto I, Hagiwara S, Hirota T, Takahashi H, Ebe H, et al. Effectiveness of abatacept for patients with Sjogren's syndrome associated with rheumatoid arthritis. An open label, multicenter, one-year, prospective study: ROSE (rheumatoid arthritis with Orencia trial toward Sjogren's syndrome Endocrinopathy) trial. Mod Rheumatol. 2016;26(6):891–9.
49. Panchabhai TS, Farver C, Highland KB. Lymphocytic interstitial pneumonia. Clin Chest Med. 2016;37(3):463–74.
50. Ramos-Casals M, la Civita L, de Vita S, Solans R, Luppi M, Medina F, et al. Characterization of B cell lymphoma in patients with Sjogren's syndrome and hepatitis C virus infection. Arthritis Rheum. 2007;57(1):161–70.
51. Lossos IS. Diffuse large B cell lymphoma: from gene expression profiling to prediction of outcome. Biol Blood Marrow Transplant. 2008;14(1 Suppl 1):108–11.
52. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103(1):275–82.
53. Gorodetskiy VR, Probatova NA, Vasilyev VI. Characteristics of diffuse large B-cell lymphoma in patients with primary Sjogren's syndrome. Int J Rheum Dis. 2020;23(4):540–8.
54. Gorodetskiy VR, Probatova NA, Radenska-Lopovok SG, Ryzhikova NV, Sidorova YV, Sudarikov AB. Clonal relationship of marginal zone lymphoma and diffuse large B-cell lymphoma in Sjogren's syndrome patients: case series study and review of the literature. Rheumatol Int. 2020;40(3):499–506.
55. Camacho Castaneda FI, Dotor A, Manso R, Martin P, Prieto Pareja E, Palomo Esteban T, et al. EBV-associated large B-cell lymphoma transformation in marginal zone B-cell lymphoma. A series of four cases. Histopathology. 2020;77(1):112–22.
56. Ekstrom Smedby K, Vajdic CM, Falster M, Engels EA, Martinez-Maza O, Turner J, et al. Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph consortium. Blood. 2008;111(8):4029–38.
57. Infante De La Torre JR, Duran Barquero C, Garcia Bernardo L, Groiss J, Rayo Madrid JI, Serrano Vicente J, et al. Coexistence of two different types of lymphoma in a patient with Sjogren's syndrome. The usefulness of the PET-CT. Rev Esp Med Nucl Imagen Mol. 2012;31(1):28–30.
58. Carbone A, Roulland S, Gloghini A, Younes A, von Keudell G, Lopez-Guillermo A, et al. Follicular lymphoma. Nat Rev Dis Primers. 2019;5(1):83.
59. Zuckerman NS, McCann KJ, Ottensmeier CH, Barak M, Shahaf G, Edelman H, et al. Ig gene diversification and selection in follicular lymphoma, diffuse large B cell lymphoma and primary central nervous system lymphoma revealed by lineage tree and mutation analyses. Int Immunol. 2010;22(11):875–87.
60. Garcia-Alvarez M, Alonso-Alvarez S, Prieto-Conde I, Jimenez C, Sarasquete ME, Chillon MC, et al. Immunoglobulin gene rearrangement IGHV3-48 is a predictive marker of histological transformation into aggressive lymphoma in follicular lymphomas. Blood Cancer J. 2019;9(7):52.
61. Sachen KL, Strohman MJ, Singletary J, Alizadeh AA, Kattah NH, Lossos C, et al. Self-antigen recognition by follicular lymphoma B-cell receptors. Blood. 2012;120(20):4182–90.
62. Scherer F, van der Burgt M, Kielbasa SM, Bertinetti-Lapatki C, Duhren von Minden M, Mikesch K, et al. Selection patterns of B-cell receptors and the natural history of follicular lymphoma. Br J Haematol. 2016;175(5):972–5.
63. Freedman A, Jacobsen E. Follicular lymphoma: 2020 update on diagnosis and management. Am J Hematol. 2020;95(3):316–27.
64. Feinstein AJ, Ciarleglio MM, Cong X, Otremba MD, Judson BL. Parotid gland lymphoma: prognostic analysis of 2140 patients. Laryngoscope. 2013;123(5):1199–203.
65. Kojima M, Nakamura S, Ichimura K, Shimizu K, Itoh H, Masawa N. Follicular lymphoma of the salivary gland: a clinicopathological and molecular study of six cases. Int J Surg Pathol. 2001;9(4):287–93.
66. Newkirk MM. Rheumatoid factors: what do they tell us? J Rheumatol. 2002;29(10):2034–40.
67. Williams RC Jr, Kunkel HG. Rheumatoid factor, complement, and conglutinin aberrations in patients with subacute bacterial endocarditis. J Clin Invest. 1962;41:666–75.
68. Roosnek E, Lanzavecchia A. Efficient and selective presentation of antigen-antibody complexes by rheumatoid factor B cells. J Exp Med. 1991;173:487–9.
69. Mageed RA, Borretzen M, Moyes SP, Thompson KM, Natvig JB. Rheumatoid factor autoantibodies in health and disease. Ann N Y Acad Sci. 1997;815:296–311.
70. Tzioufas AG, Manoussakis MN, Costello R, Silis M, Papadopoulos NM, Moutsopoulos HM. Cryoglobulinemia in autoimmune rheumatic diseases. Evidence of circulating monoclonal cryoglobu-

lins in patients with primary Sjogren's syndrome. Arthritis Rheum. 1986;29(9):1098–104.
71. Ramos-Casals M, Anaya JM, Garcia-Carrasco M, Rosas J, Bove A, Claver G, et al. Cutaneous vasculitis in primary Sjogren syndrome: classification and clinical significance of 52 patients. Medicine (Baltimore). 2004;83(2):96–106.
72. Kapsogeorgou EK, Voulgarelis M, Tzioufas AG. Predictive markers of lymphomagenesis in Sjogren's syndrome: from clinical data to molecular stratification. J Autoimmun. 2019;104:102316.
73. Agnello V. The etiology and pathophysiology of mixed cryoglobulinemia secondary to hepatitis C virus infection. Semin Immunopathol. 1997;19:111–29.
74. Gorevic PD, Frangione B. Mixed cryoglobulinemia cross-reactive idiotypes: implications for the relationship of MC to rheumatic and lymphoproliferative diseases. Semin Hematol. 1991;28(2):79–94.
75. Carbonari M, Caprini E, Tedesco T, Mazzetta F, Tocco V, Casato M, et al. Hepatitis C virus drives the unconstrained monoclonal expansion of VH1-69-expressing memory B cells in type II cryoglobulinemia: a model of infection-driven lymphomagenesis. J Immunol. 2005;174(10):6532–9.
76. Bende RJ, Aarts WM, Riedl RG, de Jong D, Pals ST, van Noesel CJ. Among B cell non-Hodgkin's lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity. J Exp Med. 2005;201(8):1229–41.
77. Miklos JA, Swerdlow SH, Bahler DW. Salivary gland mucosa-associated lymphoid tissue lymphoma immunoglobulin V(H) genes show frequent use of V1-69 with distinctive CDR3 features. Blood. 2000;95(12):3878–84.
78. Sato Y, Nakamura N, Nakamura S, Sakugawa S, Ichimura K, Tanaka T, et al. Deviated VH4 immunoglobulin gene usage is found among thyroid mucosa-associated lymphoid tissue lymphomas, similar to the usage at other sites, but is not found in thyroid diffuse large B-cell lymphomas. Mod Pathol. 2006;19(12):1578–84.
79. Sakuma H, Nakamura T, Uemura N, Chiba T, Sugiyama T, Asaka M, et al. Immunoglobulin VH gene analysis in gastric MALT lymphomas. Mod Pathol. 2007;20(4):460–6.
80. Mazzaro C, Franzin F, Tulissi P, Pussini E, Crovatto M, Carniello GS, et al. Regression of monoclonal B-cell expansion in patients affected by mixed cryoglobulinemia responsive to alpha-interferon therapy. Cancer. 1996;77(12):2604–13.
81. Chan CH, Hadlock KG, Foung SK, Levy S. V(H)1-69 gene is preferentially used by hepatitis C virus-associated B cell lymphomas and by normal B cells responding to the E2 viral antigen. Blood. 2001;97(4):1023–6.
82. Tzarum N, Giang E, Kong L, He L, Prentoe J, Augestad E, et al. Genetic and structural insights into broad neutralization of hepatitis C virus by human VH1–69 antibodies. Sci Adv. 2019;5(1):eaav1882.
83. Bende RJ, Janssen J, Wormhoudt TA, Wagner K, Guikema JE, van Noesel CJ. Identification of a novel stereotypic IGHV4-59/IGHJ5-encoded B-cell receptor subset expressed by various B-cell lymphomas with high affinity rheumatoid factor activity. Haematologica. 2016;101(5):e200–3.
84. Shlomchik MJ, Euler CW, Christensen SC, William J. Activation of rheumatoid factor (RF) B cells and somatic hypermutation outside of germinal centers in autoimmune-prone MRL/lpr mice. Ann N Y Acad Sci. 2003;987:38–50.
85. Herlands RA, Christensen SR, Sweet RA, Hershberg U, Shlomchik MJ. T cell-independent and toll-like receptor-dependent antigen-driven activation of autoreactive B cells. Immunity. 2008;29(2):249–60.
86. Talotta R, Sarzi-Puttini P, Atzeni F. Microbial agents as putative inducers of B cell lymphoma in Sjogren's syndrome through an impaired epigenetic control: the state-of-the-art. J Immunol Res. 2019;2019:8567364.
87. Theander E, Henriksson G, Ljungberg O, Mandl T, Manthorpe R, Jacobsson LT. Lymphoma and other malignancies in primary Sjogren's syndrome: a cohort study on cancer incidence and lymphoma predictors. Ann Rheum Dis. 2006;65(6):796–803.
88. Ismail F, Mahmoud A, Abdelhaleem H, Mamdoh A, Geneidy M, Kamal E. Primary Sjogren's syndrome and B-non-Hodgkin lymphoma: role of CD4+ T lymphocytopenia. Rheumatol Int. 2013;33(4):1021–5.
89. Nishishinya MB, Pereda CA, Munoz-Fernandez S, Pego-Reigosa JM, Rua-Figueroa I, Andreu JL, et al. Identification of lymphoma predictors in patients with primary Sjogren's syndrome: a systematic literature review and meta-analysis. Rheumatol Int. 2015;35(1):17–26.
90. Theander E, Vasaitis L, Baecklund E, Nordmark G, Warfvinge G, Liedholm R, et al. Lymphoid organisation in labial salivary gland biopsies is a possible predictor for the development of malignant lymphoma in primary Sjogren's syndrome. Ann Rheum Dis. 2011;70(8):1363–8.
91. Nezos A, Papageorgiou A, Fragoulis G, Ioakeimidis D, Koutsilieris M, Tzioufas AG, et al. B-cell activating factor genetic variants in lymphomagenesis associated with primary Sjogren's syndrome. J Autoimmun. 2014;51:89–98.
92. Papageorgiou A, Mavragani CP, Nezos A, Zintzaras E, Quartuccio L, De Vita S, et al. A BAFF receptor His159Tyr mutation in Sjogren's syndrome-related lymphoproliferation. Arthritis Rheumatol. 2015;67(10):2732–41.
93. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European consensus group. Ann Rheum Dis. 2002;61(6):554–8.
94. Baldini C, Ferro F, Luciano N, Bombardieri S, Grossi E. Artificial neural networks help to identify disease subsets and to predict lymphoma in primary Sjogren's

syndrome. Clin Exp Rheumatol. 2018;36 Suppl 112(3):137–44.
95. Scofield RH, Fayyaz A, Kurien BT, Koelsch KA. Prognostic value of Sjogren's syndrome autoantibodies. J Lab Precis Med. 2018;3:92.
96. Coccaro N, Anelli L, Zagaria A, Perrone T, Specchia G, Albano F. Molecular complexity of diffuse large B-cell lymphoma: can it be a roadmap for precision medicine? Cancers (Basel). 2020;12(1):185.
97. Chow VA, Shadman M, Gopal AK. Translating anti-CD19 CAR T-cell therapy into clinical practice for relapsed/refractory diffuse large B-cell lymphoma. Blood. 2018;132(8):777–81.

Part III

Improved SS Diagnosis and Therapeutics

8 Recurrent Vs. Persistent Salivary Gland Swelling in Differential Diagnosis of Sjögren's Syndrome

Indraneel Bhattacharyya and Hardeep K. Chehal

8.1 Introduction

Sjögren's syndrome (SS) is a chronic, systemic, autoimmune condition characterized by diminished function of the salivary and lacrimal glands [1]. This disease is highly prevalent in middle-aged and older females. Children and males may also be affected. The exact etiopathogenesis of this condition is unknown. Though not a hereditary disease, genetic involvement is evident. There are two forms of the disease. In the *Primary form,* only the lacrimal and salivary glands are affected, whereas the *Secondary form* is associated with an additional autoimmune disease such as rheumatoid arthritis, systemic lupus, and scleroderma.[1]

There is no single diagnostic test for SS. Diagnosis of SS should be suspected in patients with a daily persistence of dry eyes and dry mouth 3 months or more [2]. These patients should undergo a thorough medical history and physical examination, including ocular examination, salivary function tests, selected blood tests, tests to determine autoimmunity and exclude other diseases mimicking SS [2].

Glandular involvement is the clinical hallmark of the disease and may vary from mild to severe. Clinically, the parotid gland is most commonly affected. The parotid gland may appear normal or they may be enlarged in one-third to one-half of the patients [3]. This enlargement is usually bilateral, asymptomatic or patients may experience slight tenderness which may be persistent or periodic. The enlargement is a result of focal lymphocytic infiltration of the salivary glands. This inflammatory destruction of the salivary glands causes xerostomia which in turn may lead to bacterial sialadenitis [3]. Lacrimal glands show similar involvement leading to xerophthalmia [3].

Labial salivary gland biopsy remains an important diagnostic test. Five or more minor salivary glands are harvested and examined histopathologically. Focal presence of 50 or more lymphocytes and plasma cells helps determine the focus score. A focus score of ≥ 1 (i.e., one or more focus of 50 or more cells per 4 mm^2 area of glandular tissue) is considered supportive for the diagnosis of SS. This test is however not 100% reliable [3]. The majority of the effects of this disease result from the destruction of the exocrine glands by the lymphocytic infiltrates. Serologic testing for diagnosis include anti-Ro/SSA and anti-La/SSB antibodies, rheumatoid factor, and antinuclear antibodies (ANA).

I. Bhattacharyya (✉)
Department of Oral and Maxillofacial Diagnostic Sciences, University of Florida, Gainesville, FL, USA
e-mail: ibhattacharyya@dental.ufl.edu

H. K. Chehal
Department of Diagnostic Sciences, School of Dentistry, Creighton University, Omaha, NE, USA
e-mail: HardeepChehal@creighton.edu

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Anti-Ro/SSA and anti-La/SSB may also be found in other autoimmune diseases [4].

In 2016, an international set of classification criteria for primary SS were developed and validated using approaches approved by both the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) [5]. The criteria included 5 objective measures. A total score of ≥4 confirmed a diagnosis of primary SS [5]. The 5 measures are:

1. Labial salivary gland with focal lymphocytic sialadenitis and focus score of ≥1 foci/4 mm^2 = 3 score.
2. Anti-SSA/Ro positive = 3 score.
3. Ocular Staining Score ≥ 5 (or van Bijsterveld score ≥ 4) in at least 1 eye = 1 score.
4. Schirmer's test ≤5 mm/5 min in at least 1 eye = 1 score.
5. Unstimulated whole saliva flow rate ≤ 0.1 mL/min = 1 score.

Differential diagnosis of SS includes diseases that cause dry mouth and dry eyes. Exclusion of these diseases must be done on basis of medical history, physical examination, biopsy, and serologic testing for other autoimmune conditions. SS may also present with persistent or recurrent swelling or enlargement of the parotid gland. However, since many other conditions may present with similar parotid enlargement, this chapter discusses some of the common conditions that may be considered in the differential diagnosis of parotid swelling.

8.2 Idiopathic

8.2.1 Chronic Recurrent Non-Specific Parotitis

Chronic recurrent non-specific parotitis results from unilateral or bilateral inflammation and swelling of the parotid gland alternating with periods of remission [6]. Since the parotitis is often recurrent, it is also called recurrent chronic parotitis. This condition may be subclassified as suppurative or non-suppurative [7]. Most cases are seen in middle-aged females [8]. Children are rarely affected [9]. The etiology is still uncertain though several factors have been implicated, namely inflammation from a bacterial (polymicrobial pathogens) or viral (HIV, EBV, Cytomegalovirus, coxsackievirus) infection, hereditary or genetic factors (HLA involvement), metabolic conditions (DM), or immune-mediated conditions (SS) [10]. The major consequence of all these associated factors is xerostomia and this condition is seen most often in patients with chronic xerostomia.

Clinical manifestations include pain and tenderness as well as hyperthermia. Cervical lymphadenopathy is uncommon [11]. Extraoral examination reveals that unilateral or bilateral enlargement of the parotid glands causes facial asymmetry or distortion (see Fig. 8.1). Intraorally, the parotid papillae may be elevated with the discharge of pus, resulting in a salty taste. Mucosa may be dry with ropy or bubbly saliva.

Radiographic images are very helpful in diagnosing this entity. Sialography, conventional and magnetic resonance imaging (MRI) based, shows changes in either the duct or gland or both (see Fig. 8.2). "'Sausaging"' of the Stenson duct, due to dilation and constrictions, may be seen. The gland parenchyma also shows due to marked inflammation and fibrosis [11]. Normally, in a computed tomography (CT) scan, the parotid

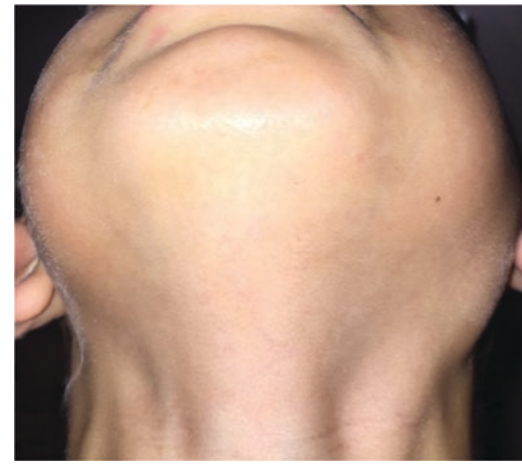

Fig. 8.1 A 43-year-old female with recurrent chronic non-specific parotitis. She reports multiple episodes of bilateral non-specific parotid enlargement with discomfort and mild hyperthermia

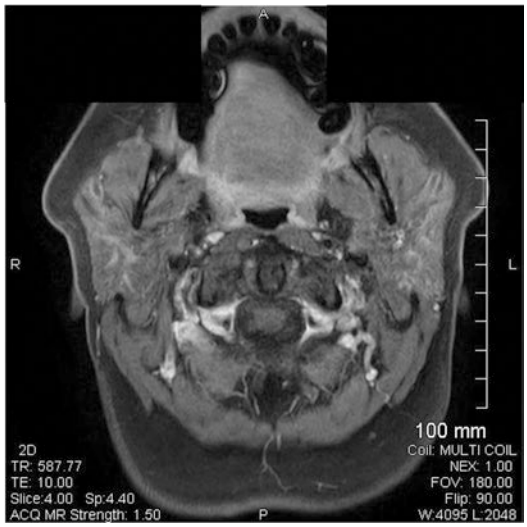

Fig. 8.2 MRI scan demonstrating bilateral prominence of the parotid glands with moderate prominence of the parotid ducts. No diffuse edema or enhancement in the parotid glands

gland parenchyma shows areas of radiolucency representing normal fat scattered among the normal areas of density. In chronic parotitis, these radiolucent areas are replaced by increased radiodense areas representing loss of fat and establishment of fibrosis and calcifications. Microscopically, acini atrophy as a result of infiltration of plasma cells and lymphocytes as well as fibrosis is evident. Ductal ectasias are also a common finding [3].

Treatment ranges from conservative to aggressive depending on the stage of the disease as well as number of recurrences. Early treatment modalities include antibiotics and analgesics supplemented by oral hygiene regimens and hydration reminder to patients since most common cause of chronic sialadenitis is retrograde bacterial infection as a result of xerostomia and glandular atrophy. Sialography acting as a diagnostic as well as a treatment has been professed, since it acts as a ductal lavage and helps in clearing the mucus plug or cellular debris causing obstruction [12]. Aggressive treatment includes ductal ligation, superficial parotid lobectomy, or parotidectomy. Complications include facial nerve palsy, Frey's syndrome, salivary fistula, or sialocele. Facial nerve palsy is usually transient.

In SS, similar unilateral or bilateral recurrent swelling associated with dry eyes is seen. Medical history, immunological profile, and labial salivary gland biopsy are crucial for its diagnosis.

8.2.2 Juvenile Recurrent Parotitis

Juvenile recurrent parotitis (JRP) is defined as a recurrent inflammatory parotitis in children and adolescents which is non-obstructive and non-suppurative in nature. The cause of this condition is not well understood [13]. It is a rare condition and characterized by multiple episodes of swelling of the parotid with associated pain and constitutional symptoms such as fever, malaise, and loss of appetite over a period of years. This condition is second only to mumps (viral parotitis) [14]. It is commonly seen in ages 3 to 6 without any gender predilection. However, a female gender bias is noted when symptoms appear later in life. JRP frequently goes undiagnosed and is often mistaken for mumps or pharyngitis. It typically presents with parotid swelling, pain, and fever lasting for a few days (see Figs. 8.3 and 8.4). Usually seen unilaterally, bilateral involvement is occasionally seen. Variable number of recurrences are seen annually. The disease process is usually self-limiting and symptoms are rarely seen after puberty [15]. Usually the disease does not leave permanent damage, but in severe cases destruction of glandular parenchyma and functionality of the gland is noted. It is important to note that recurrent parotitis may be the first presenting symptom for SS in pediatric patients [16].

Though the exact etiology is not known, several factors have been suggested including heredity, viral or bacterial infection, allergy, or SS. Partial obstruction and dilation of the duct is usually present [16].

Microscopically, cystic ductal dilatation with periductal lymphocytic infiltration also known as sialectasis is seen. Fibrosis, scarring, and decrease in parenchyma are also reported. Previously sialography was considered to be the most useful imaging modality. It is an invasive procedure, requiring patient cooperation and operator expertise. The use of salivary gland ultrasound imag-

ing has now surpassed this technique in terms of specificity and sensitivity. It is also a noninvasive, faster and cheaper technique making it a first-line diagnostic imaging tool [17].

Moreover, MRI also helps in accurately visualizing sialectasis and signal intensity changes in the parotid gland depending upon the phase of the disease (acute vs. chronic inflammation) [16]. Quenin and colleagues found sialendoscopy more sensitive than ultrasonography [17].

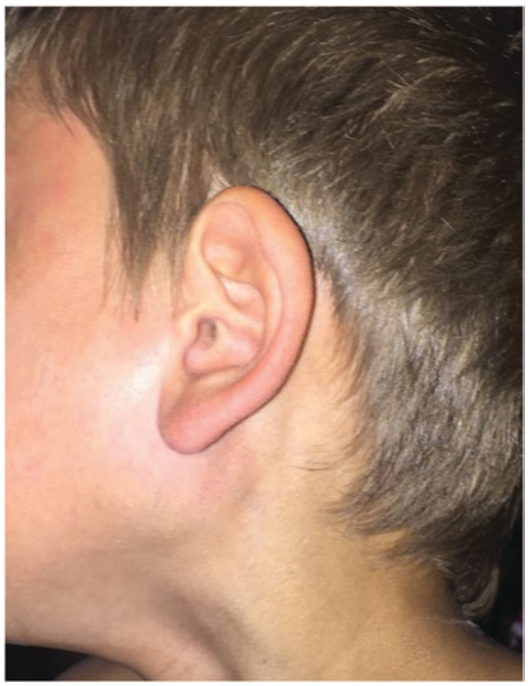

Fig. 8.3 A 7-year-old male with recurrent juvenile parotitis

In most cases, the symptoms resolve spontaneously after puberty, but all children should be screened to exclude SS, lymphoma, and HIV infection [18, 19]. Supportive therapy including hydration, analgesics, parotid massage, chewing gum use, sialogogue agents such as lemon candy are helpful in relieving symptoms [15]. In more severe cases, sialoendoscopic techniques with lavage and dilation of ducts have shown to be very helpful [19]. Since some of pediatric patients can present JRP as one of the initial signs of SS or develop SS later in life, a close clinical follow-up or a rheumatologist referral is suggested. In addition, dental health professionals as well pediatricians should be familiar with this condition [20].

8.2.3 Sarcoidosis

Sarcoidosis is a chronic, multisystem and granulomatous disease characterized by the formation of noncaseating granulomas. The etiology of sarcoidosis remains obscure. It most frequently (70%–90%) involves the lungs presenting with a variable clinical manifestation. Minor and major glands are infrequently involved. A persistent enlargement of the parotid gland is noted in 5%–30% [21] of the patients. This is a disease of the adult and most patients are in the third to fifth decades of life. It is rarely seen in children [22]. A distinct female predilection is noted [23]. In the USA, a higher prevalence is seen in women of African American origin. Lungs and related

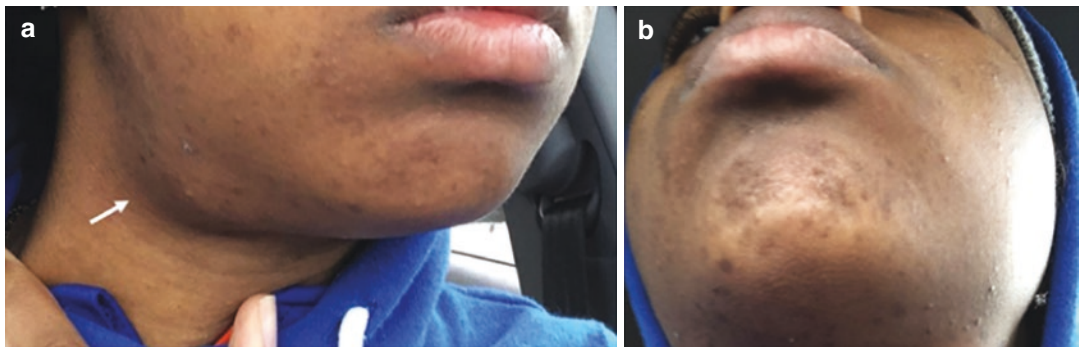

Fig. 8.4 Bilateral submandibular gland swelling of unknown cause in a 16-year-old female. Patient reports recurrent swelling with pain

lymph nodes are involved in 70%–90% of cases. In the head and neck region, any structure may be involved. Symptomatic ocular involvement is seen in up to 80% of patients and asymptomatic CNS involvement is seen in up to 25% of patients [24]. Genetics play an important role in disease manifestation. HLA DR3, 11, 12, 14, 15, and 17 are positively implicated [25] in most cases. Non-HLA locus-associated genes include annexin A11 [26].

Clinical manifestation includes low-grade fever, malaise, weight loss lymphadenopathy, splenomegaly, and hepatomegaly. The heart, central nervous system, and kidneys may also be affected. Parotid gland involvement results in an asymptomatic, persistent swelling which may be unilateral or bilateral. Painful swelling occurs in about 6% of cases [27]. In rare cases, parotid involvement is seen with facial nerve palsy, anterior uveitis, and fever. This classic triad is known as Heerfordt syndrome [28]. This syndrome is uncommonly seen in the USA. Uveitis manifests as eye pain and redness, photosensitivity, dark floating spots in visual field, and decreased vision. Heerfordt syndrome is self-limiting.

Radiographic imaging of the chest shows bilateral hilar lymphadenopathy. Bilateral enlargement of the parotid gland results in the "'Panda sign"—a symmetrical uptake of radio-gallium isotope occurs at sites of inflammation (lacrimal and parotid glands) during gallium scintigraphy. This sign may also be seen in SS and head and neck radiation cases. It is highly specific for sarcoidosis [29].

Histologic features consist of classic noncaseating granuloma formation. Schumann bodies (laminated basophil calcifications) are often seen within the granulomas. Langhans or foreign body type of giant cells are seen scattered among the histiocytes (see Fig. 8.5). Epithelioid cells of the granulomas produce angiotensin-converting enzyme (ACE). However, elevated serum ACE levels are detected in only 60% of sarcoidosis patients [30]. Hypercalciuria, hypercalcemia, eosinophilia, anemia, leukopenia, thrombocytopenia, elevated serum alkaline phosphate levels, and elevated erythrocyte sedimentation rate may also be seen.

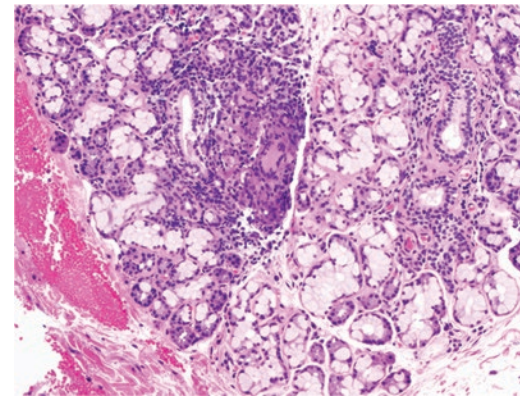

Fig. 8.5 Microscopic image from labial salivary gland biopsy demonstrating a focus of chronic non-necrotizing granulomatous inflammation. Langhans type of giant cell intermixed with epithelioid histiocytes and lymphocytes is seen within the lobule. Scattered chronic inflammation is seen elsewhere in the gland. The patient was diagnosed with sarcoidosis. Hematoxylin and eosin, 5× magnification

Treatment may not be necessary in mild cases as the disease may remit. Progressive cases are treated with prednisone as the first line of treatment (20–40 mg of prednisone daily for 1–3 months, thereafter tapering the dose to 5–10 mg/day for at least 1 year). Recurrence/relapse is seen in 20%–70% of cases. Cases not responding to corticosteroids may be treated with Infliximab (monoclonal antibody against tumor necrosis factor alpha) [31]. Other corticosteroid sparing drugs include azathioprine, chloroquine, hydroxychloroquine, methotrexate, tetracycline derivatives, and chlorambucil. Mortality rates increase in sarcoidosis and most deaths related to the disease are attributed to cardiac, central nervous system or pulmonary involvement [31]. An increasing mortality over the past few decades has been suggested especially among Blacks [31].

SS may show similar clinical presentations. Medical history, immunological profile, and labial salivary gland biopsy are crucial for the diagnosis of SS and are usually distinctly different from sarcoidosis.

8.3 Reactive

8.3.1 Sialadenosis

Sialadenosis or sialosis is an uncommon condition of the salivary glands characterized by asymptomatic, bilateral, diffuse swelling of the major salivary glands, particularly the parotid gland [32, 33]. Usually sialadenosis does not affect the function of the salivary glands. Several systemic conditions are associated with sialadenosis including diabetes mellitus and malnutrition chiefly, alcoholism related and bulimia. It is usually seen in adults and is rarely reported in children.

8.3.1.1 Alcohol Sialadenosis (Alcohol Sialosis)

Sialadenosis is a noninflammatory and nonneoplastic condition of the salivary glands that results in chronic, diffuse bilateral swelling of the salivary glands. It results in disturbances of metabolism and secretion of the affected gland. Chronic alcoholism may result in sialadenosis with a notable clinical enlargement of the parotid gland [32]. The exact pathogenesis is still elusive but it has been associated with diabetes, alcoholism, and malnutrition [33], with alcoholism being the most common predisposing factor. The most accepted hypothesis for this enlargement is related to dysregulation of the autonomic innervation, which in turn is thought to increase acinar protein synthesis. This increased secretion is accommodated in the acini by a size increase from the normal 40 μm to 100 μm manifesting clinically as a bilateral parotid gland enlargement [34]. In addition, dysregulation of the autonomic innervation also results in decrease in the contractile myoepithelial cells surrounding the acinus and the intercalated ducts resulting in inability of the acinar cells in clearing accumulated secretory granules and subsequent enlargement of the glands [35].

Clinical manifestation of sialadenosis typically results in asymptomatic, slow-growing, progressive, and bilateral enlargement of the parotid gland. Xerostomia is rarely noted [36]. It shows equal predilection between the genders and is usually seen after the fourth decade of life.

Radiographic imaging may be helpful. An anteroposterior radiograph shows an increased distance between the mandibular ramus and major duct and its branches depicting glandular hypertrophy. Sialography usually exhibits a "leafless tree." This pattern corresponds with compression of the ducts due to acinar hypertrophy. In computed tomography, the hypertrophic gland looks dense with minimal fat tissue. In some cases of chronic alcohol induced sialadenosis, there is fat infiltration showing as reduced gland density [37].

Histologic features consist of acinar hyperplasia due to increased accumulation of secretory granules, normal or decrease in size of excretory ductal lumen, reduction in the fatty as well as fibrous tissues and no inflammatory cell infiltrate [38].

Treatment is related to treating alcoholism. Discontinuation of consumption of alcohol may result in a decrease of parotid swelling. However, prognosis is variable.

SS syndrome may show similar clinical presentations. Medical history, immunological profile, and labial salivary gland biopsy are crucial for the diagnosis of SS and can be distinctly different from SS. Sialography is a useful diagnostic tool for distinguishing between SS and parotid enlargement related to alcoholism, particularly in later stages.

8.3.1.2 Bulimia/Malnutrition-Related Sialadenosis

Bulimia-related sialadenosis results in a noninflammatory enlargement of the salivary glands, especially the parotid gland. Minor salivary glands may also show sialadenosis [39]. The etiology of this process is obscure, but it has been hypothesized that peripheral autonomic dysregulation results in increased secretion and deranged metabolism of the acini, resulting in hypertrophy and enlargement [40]. Some investigators state that a decreased serum electrolyte content (hypokalemia, hypochloremia) and a metabolic alkalosis aid in the diagnosis of bulimia [41]. However, other investigators have found no evidence of electrolyte imbalance in bulimia [42]. Therefore,

electrolyte imbalance even though important may be an adjunct diagnostic tool requiring clinical correlation.

Clinically the parotid gland shows bilateral, slowly enlarging, and asymptomatic changes. Submandibular glands may also be affected. Xerostomia is present [43]. Minor salivary glands may also be enlarged in some cases. Greater prevalence is seen in young women compared to men and manifests as binge eating and emesis thereafter [37]. The frequency of emesis varies. Salivary secretion is usually clear and does not decrease in quantity.

Radiographic imaging is very similar to that seen in alcohol-related sialadenosis especially on an anteroposterior image of the affected side. In computed tomography, the hypertrophic gland shows an increase in density [37].

Histologic features are also similar to those seen in alcohol-related sialadenosis. These include acinar hyperplasia, normal or decreased excretory ductal lumen, decrease in parenchymal fat, and minimal inflammation.

Treatment for bulimia-related sialadenosis is not required. Once bulimia is addressed, a gradual reduction in the parotid gland swelling is seen [44]. However, in some cases, the swelling may remain unresolved in spite of treatment for bulimia. In such cases, surgery may be a management option [45].

SS shows similar bilateral parotid swellings. Sialography can be a useful tool in the diagnosis of sialadenosis especially in the later stages of the disease, when changes are caused by acinar swelling along with compression of the proximal ducts in sialadenosis.

8.3.2 Radiation Sialadenitis

Radioactive iodine 131 (^{131}I) is an effective adjunct treatment for thyrotoxicosis (goiter) as well as functional and well-differentiated thyroid carcinomas (follicular and papillary) [46]. This is due to the ability of normal thyroid gland to absorb and concentrate it. ^{131}I causes mutation and death of cells that absorb it. When therapeutic doses (high dose) of ^{131}I is administered, it only kills the cells absorbing it, namely normal and cancerous thyroid cells. Approximately, 24% of the therapeutic dose for thyroid pathologies is excreted into the saliva because epithelial lining of the salivary ducts extracts iodine from the periductal capillaries and secrete it into the ductal lumen [47]. ^{131}I from the saliva is then taken up by the salivary glands, with greater ability of serous cells to concentrate it as compared to mucous cells. This accounts for greater prevalence of parotid gland radiation sialadenitis as compared to other salivary glands [48]. Radiation salivary gland sialadenitis, although rare, is dose-dependent and may be acute, chronic, and/or recurrent [49]. Radiation damage to the salivary gland is permanent.

Clinically, bilateral parotid gland pain and swelling immediately after administration of a high therapeutic dose of ^{131}I are the prominent features of acute radiation sialadenitis [50]. The condition occurs as a result of glandular inflammation and resolves spontaneously within a few days. In case of chronic radiation sialadenitis, xerostomia is a prominent feature. This is a result of direct and irreversible glandular parenchymal damage (unilateral or bilateral) by ^{131}I and may have a significant effect on the quality of life of patient [51]. Transient radiation sialadenitis results from additional ^{131}I administration [46].

Scintigraphy is a widely accepted tool for evaluation of glandular dysfunction. It shows a ^{131}I dose-dependent reduction of salivary function. Sialography is contraindicated in case of acute radiation sialadenitis as it may worsen the inflammatory reaction. CT serves to assess the volume of the salivary gland, with a reduced volume indicating in salivary gland pathology and dysfunction [52]. MRI is not routinely used as a diagnostic tool in cases of radiation sialadenitis [47].

Treatment modalities are aimed towards prevention and include: (1) increased fluid intake, (2) candy sucking and chewing gum for at least 7 days post-radiotherapy, (3) external massage of affected glands, (4) anti-inflammatory, cholinergic, and/or steroidal medications, and (5) sialendoscopy, especially in cases with partial ductal obstruction [53]. Steroidal medications are added in case pain and swelling last for more than 48 h.

8.3.3 Obstructive

8.3.3.1 Sialolithiasis

Formation of calcified concrescence in the salivary glands is a well-known phenomenon that may typically cause an intermittent enlargement of the gland. The most commonly affected gland is the submandibular glands mainly due to their thick calcium rich mucoid secretion and the long tortuous pathway of the Wharton's duct. Sialoliths usually cause recurrent or intermittent enlargement with accompanying pain and discomfort especially during mealtimes. Sialoliths form due to deposition of calcium salts, primarily calcium oxalate and calcium phosphate, around an organic nidus composed of desquamated cells, bacteria, and/or mucin. These may be found in the gland parenchyma or the ductal system of the glands [54]. Approximately 10% to 20% of all sialoliths are found in the parotid gland. The etiology is still obscure. Factors such as alterations of salivary composition, gout, smoking, and diuretic medication have been implicated [55]. SS syndrome patients do not have increased prevalence to the formation of sialoliths [56].

Clinically, the patient may be asymptomatic or may experience pain, swelling, or both as a result of obstruction of salivary flow from the gland. Signs and symptoms are pronounced during mealtimes and may last for several hours. Dermal erythema in region of the effected gland as well as xerostomia is often present [57]. Location and size of the sialolith play a role in the manifestations of the signs and symptoms. Ductal stones and those greater than 1.5 cm cause greater pain and swelling [55]. Intraoral examination may reveal redness and edema of the ductal orifice. Greater prevalence is seen in males in their second to fifth decades of life and most cases are unilateral with multiple calculi [58]. Occasionally bilateral parotid sialoliths have been reported [59]. Sialolithiasis in children is a rare phenomenon [60].

Imaging modalities include conventional radiographs, sialography, sonography, computed tomography (CT)/MRI and scintigraphy (see Fig. 8.6). Conventional films have limited value in detecting parotid gland sialoliths as up to 40%

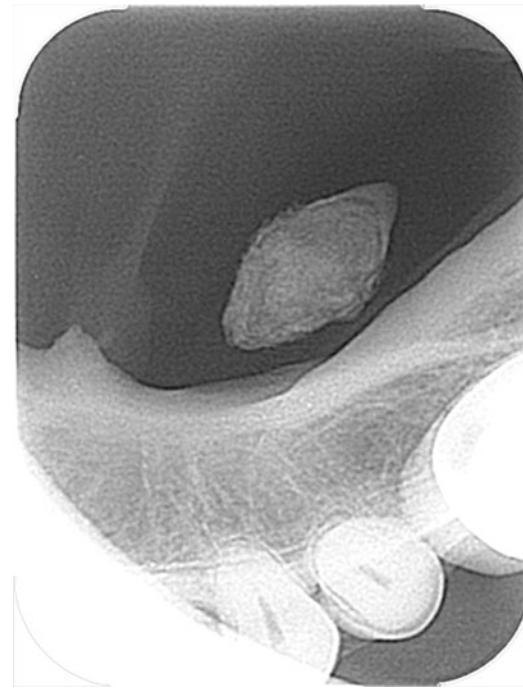

Fig. 8.6 Sialiolith measuring at least 2 cm inside the left submandibular (Wharton) duct

of these are radiolucent [60]. Sialography is useful in detecting calculi in the ductal system but with limited value as it cannot be used in cases of acute infection in the gland or in cases where a patient is allergic to the contrast dye [61]. CT and MRI do not require a contrast injection and ductal canalization [57]. Of the two, MRI has greater accuracy in detecting the sialolith. Scintigraphy is used in cases when sialography is not indicated.

Histologic features include spherical eosinophilic to basophilic variably mineralized and variably calcified concrescence exhibiting concentric laminations with or without bacterial colonization. Viable tissue is rarely seen.

Treatment modalities include invasive and noninvasive procedures. Noninvasive procedures include gland massage after each meal, daily hydration with water (up to 1.5 L), and use of sialagogues. Invasive treatment includes lithotripsy, sialendoscopy, and surgery [55].

SS typically presents with bilateral parotid swelling as opposed to unilateral swelling seen in sialolithiasis. In addition, SS-associated enlarge-

ment of the parotid is usually asymptomatic and persistent, whereas sialolith-associated swelling is intermittent and symptomatic. Bilateral and multiple sialolithiasis in the parotid gland in a patient has been reported in literature [62], but is extremely rare.

8.4 Developmental

The parotid glands are ectodermally derived structures. They develop around the sixth week of intrauterine life and are the first of the major salivary glands to develop. Their capsule, however, develops after those of the submandibular and sublingual glands. This is of critical importance, as the lymphatic system develops before the parotid gland is encapsulated, unlike the other major salivary glands, and may create entrapment of the lymphoid tissue within the developing gland resulting in developmental anomalies. Vascular anomalies within the parotid gland are extremely rare and are divided into two broad categories based on endothelial characteristics, namely, vascular malformations and hemangiomas.

8.4.1 Lymphangiomas

Lymphangiomas are benign congenital malformations of the lymphatic system and are most commonly seen in the head and neck area which is rich in lymphatics [63]. The lymphatic system arises from six primitive lymphatic sacs during the sixth week of intrauterine life. Lymphangiomas most likely arise as a result of failure of one of these sacs to connect to the rest of the lymphatic system and the development of a cystic developmental anomaly. The exact etiology, however, is obscure. Three forms of lymphangiomas have been described, namely, capillary, cavernous, and cystic. Cystic lymphangiomas tend to infiltrate the surrounding structures, leading to surgical complications. In the head and neck region, most cases are found in the posterior triangle of the neck. They are uncommon in the parotid gland and, when present, are congenital [64]. A few cases of adult parotid gland lymphangiomas have been reported.

Clinically, it presents as a soft, cystic, and painless swelling which enlarges over time. Rapid enlargement may result from infection or trauma. MRI is the most accurate tool and it displays a thin walled multicystic lesion [65]. Histologically, the lesion is composed of lymphatic channels of various caliber in loose connective tissue stroma. Peripheral lymphoid aggregates, mast cells, and plasma cells are present. The cystic spaces are filled with eosinophilic proteinaceous fluid [66].

Conservative surgery is the treatment of choice. Alternatively, sclerosing agents such as OK-432 and bleomycin have been used, but have shown inconsistent results. Recurrence rate of 10%–38% has been reported from incomplete surgical excision [67]. Radiation therapy should be avoided due to the risk of malignant transformation [68].

8.4.2 Arteriovenous Malformations

Vascular malformations are extremely common, relatively static and congenital in nature with a normal rate of endothelial cell turnover [69]. They are caused by disturbances in the late stages of angiogenesis and result in the arteriovenous anastomosis which occur during embryonic life. These lesions usually do not involute. Vascular malformations are classified according to the type of vessel involved (capillary, venous, lymphatic, and/or arteriovenous). They are further categorized as low-flow lesions (capillary, lymphatic, and/or venous) and high-flow lesions (arteriovenous). Shunt from a high-pressure region to a low-pressure region can produce a characteristic bruit. About 14%–65% of vascular malformations are found in the head and neck region [70]. Parotid gland vascular malformations are extremely rare. Majority of the cases are found in the superior lobe of the parotid gland.

Clinically, vascular malformations are slow-growing painless swellings. Venous malformations are typically blue in color, easily compressible, and grow proportionately with the

patient. Arteriovenous malformations are high-flow lesions exhibiting palpable bruit. The overlying skin is warm to touch and may be painful or ulcerated [71]. Enlargement of the parotid mass may occur during dependent head position, tilting the head forward or clenching of teeth. This phenomenon is pathognomonic and is called the "turkey wattle" sign, named after the wattle, seen in turkeys which is a red vascular structure noted in the neck of the turkey [72]. A wide age range of 3 months and 74 years has been reported. Some authors report a female predilection while others report equal gender bias. MRI is the diagnostic tool of choice and shows the differentiation between high-flow and low-flow lesions. High-flow lesions demonstrate signal flow voids with appearance of serpentine channels [73]. Histopathologically, numerous proliferative dilated blood vessels which may be congested, thrombosed or with calcifications may be seen. In most cases, treatment is sought mainly for cosmetic purposes. Surgical resection remains the gold standard [74].

8.4.3 Hemangiomas

Hemangiomas are benign vascular tumors exhibiting a rapid rate of endothelial proliferation. They are caused by a failure of differentiation in the early stages of embryogenesis [75] and are categorized as capillary and cavernous. Cavernous hemangiomas occur in adults and are rare. Capillary hemangiomas are more common in children and may spontaneously regress before adolescence [76]. About 65% of all hemangiomas arise in the head and neck region with the majority occurring in the parotid glands [77]. Hemangiomas are also classified as congenital and infantile types. Congenital hemangiomas manifest at birth, whereas infantile hemangiomas typically occur after the first few weeks of life. Congenital types are further subclassified into non-involuting congenital hemangioma (NICH) and rapidly involuting congenital hemangioma (RICH) [78]. Hemangiomas of the parotid gland are the most common tumors of salivary glands in children [79].

Clinically, parotid hemangiomas present as a poorly defined soft tissue swellings which may be smooth or bosselated ("strawberry skin"), non-tender, and non-fluctuant. The overlying skin may have a bluish-purple hue which is a helpful diagnostic clue. Approximately 90% arise in the first three decades of life [80]. Most show rapid growth during the first year of life and gradually regress by around 9 years of age. They are more common in females [81]. Rarely, dyspnea and dysphagia are reported and is chiefly related to large size of the tumor. Esthetics, intralesional bleeding, and acute thrombosis leading to pain and deformity is of main concern [82]. Clinical diagnosis of parotid hemangioma is aided by the "turkey wattle" sign (described above), which is not present in case of extraparotid lesions [83]. Reddi's sign is another helpful aid in which hemangioma becomes distended by blocking the venous flow [83]. MRI is the diagnostic tool of choice and typically demonstrates a lobulated lesion. The microscopic features are characterized by proliferative dilated vascular spaces engorged with blood. Calcifications are often seen [78]. Surgical resection is not favored for treatment, given the high probability of regression of parotid hemangiomas. Systemic corticosteroids and propranolol have been used as a treatment modality with some measure of success [84].

8.5 Inflammatory/Immune Mediated

8.5.1 Immunoglobulin G4-Related Sialadenitis

Immunoglobulin G4-related sialadenitis (IgG4) is one of the systemic manifestations of IgG4-related systemic disease (IgG4-RSD). IgG4-RSD is a chronic fibro-inflammatory condition that may affect nearly every organ system, leading to progressive organ failure [85]. The two main features of the disease include elevation of serum IgG4 concentrations and the set of unique histopathologic characteristics. The hallmarks of the disease include two main characteristics shared

by the affected organs: tissue damage related to inflammation and fibrosis [86]. The organ failure is metachronous with organs added one at a time over the disease course spanning from months to years [87]. It was first reported in the pancreas. Most frequently, the exocrine glands (pancreas, salivary, lacrimal, kidneys, thyroid) as well as lungs and aorta are affected [88]. The exact etiology is unclear. However, it has been proposed that upregulations of T helper 2 cells and T regulatory cells occur in response to an unknown antigen. The upregulation of these T-cells results in production of interleukins (IL-4, IL-5, IL-10, IL-13), interferons-ϒ (IFN-ϒ), and transforming growth factor-β. These cytokines are responsible for: 1. fibroblast activation, 2. class switching of B cell from IgM to IgE and IgG4, 3. increased number of eosinophils in blood and the tissue, and 4. elevated serum IgE [89].

Clinically, there is a predilection for middle-aged to elderly males. The onset of the disease is subacute and systemic features are seen in less than 10% of patients. Salivary glands involvement results in bilateral enlargement of the involved gland. The submandibular gland is involved at a higher frequency as compared to parotid or sublingual gland [90]. Xerostomia may occur and it can improve with immunosuppression such as steroids, unlike SS [91].

CT scans of the head and neck show symmetrical enlargements of the affected glands. Serum IgG4 is elevated in a majority of patients though this relationship with the elevated IgG4 levels and disease activity is still not fully understood. Some patients have normal IgG4 levels despite classic disease features while others achieve remission with elevated IgG4 levels in the serum [89]. Evidence suggests that the ratio of IgG4 to total IgG in serum may be a more useful diagnostic tool.

Histopathologic studies reveal four major hallmarks of the disease (see Fig. 8.7): (1) a dense lymphoplasmacytic infiltrate with a high percentage of IgG4 positive plasma cells, (2) storiform fibrosis, (3) mild to moderate tissue eosinophilia, and (4) obliterative phlebitis. An elevated ratio of IgG4 positive cells to IgG positive cells greater than 40% in tissues with substantial fibrosis often supports the diagnosis of the disease [92].

Successful therapy with corticosteroids administered for 2–4 weeks has been reported. A low-dose maintenance steroid therapy of 6–24 months has been proposed to maintain remission. The predictive risk factors for relapse include: (1) persistent high serum levels of IgG4 before and after treatment and (2) multiorgan involvement [93]. Steroid sparing drugs are used in cases of relapsing disease. However, there is no firm evidence that these drugs work [94].

8.6 Infectious

8.6.1 Bacterial Sialadenitis

Sialadenitis is the inflammation of salivary glands. It is classified according to the histologic and etiologic factors [95]. Bacterial sialadenitis is a result of bacterial infection which may occur due to: (1) retrograde infection of the ducts and parenchyma of the involved gland by bacteria in the oral cavity and/or (2) due to decreased salivary flow from the gland [96]. Bacterial sialadenitis may affect any salivary gland (major or minor), but most commonly affects the parotid gland followed by the submandibular gland. Based on duration and histologic features, the infection may be classified as acute or chronic. Based on causative factors, the infection may be classified as bacterial, viral, obstructive, granulomatous, post-treatment, necrotizing, or LE (HIV-related). Bacterial sialadenitis is usually diagnosed by history and a thorough physical, imaging and biopsy examination. Here we will discuss bacterial sialadenitis since it is the most common cause of sialadenitis.

8.6.1.1 Streptococcal/Staphylococcal Sialadenitis

Acute bacterial sialadenitis of the parotid gland is most commonly caused by *staphylococcus aureus* [97]. Streptococcal species causing acute bacterial parotitis include *S. pneumoniae, S. pyogenes,* and *Hemophilus influenzae.* Fibronectin found in saliva promotes adherence of these bacteria. Gram negative bacteria (*E. coli, Klebsiella pneumoniae, P. aeruginosa*)

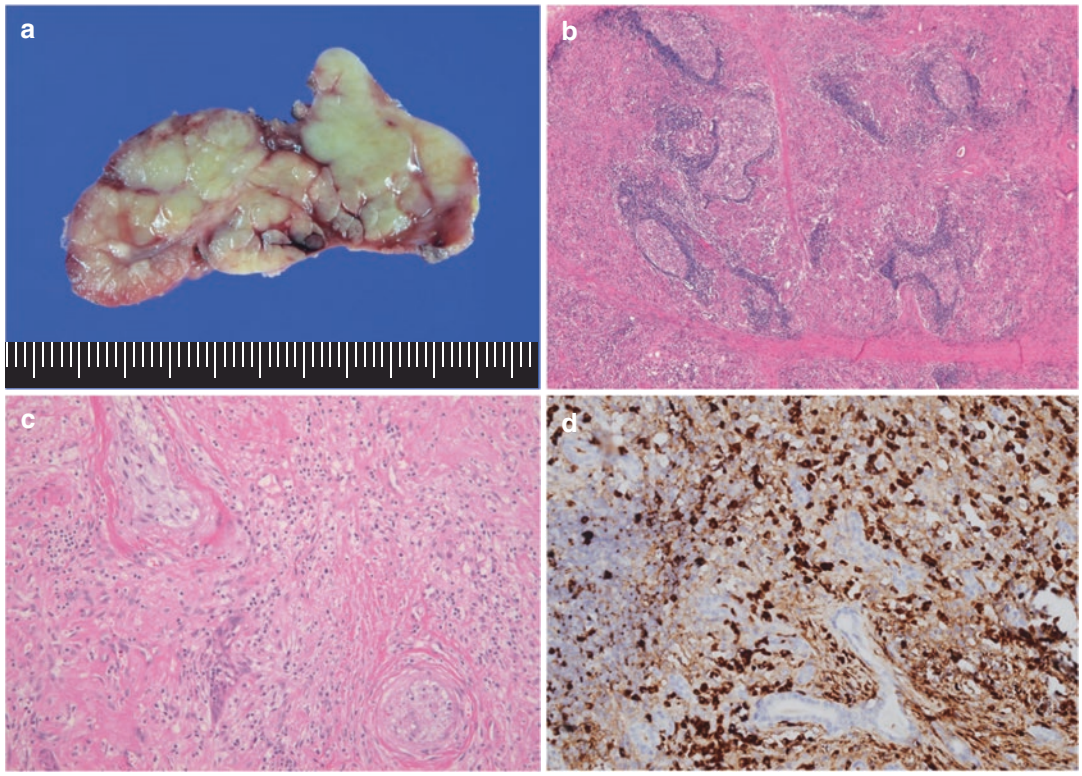

Fig. 8.7 The excised salivary gland preserves the lobular architecture with diffuse enlargement and multifocal fibrosclerotic areas (**a**). Histologic findings. The submandibular glands show lymphoplasmacytic infiltration with septal fibrosis (**b**) and obliterative phlebitis (**c**). Immunohistochemical results show numerous IgG4-positive cells (**d**) (IgG4 +cells, >60/high power fields). (Reprinted with permission from Ji Seon Bae, Joo Young Kim, Sang Hak Han, Seung Ho Choi, Kyung Ja Cho. IgG4-Related Sclerosing Sialadenitis: Report of Three Cases. J Pathol Transl Med. 2011;45 (0): S36–S40)

cause acute bacterial sialadenitis in pediatric and hospitalized patients. Infrequently, suppurative infections may result from *Arachnia spp., Haemophilus influenzae, Treponema pallidum,* cat-scratch *bacillus,* and *Eikenella corrodens* [98]. Clinically, acute bacterial sialadenitis is characterized by a sudden onset of swelling of the involved gland (unilateral or bilateral). The swelling is accompanied by pain and fever [99]. Pain may intensify during mealtimes and may be accompanied by trismus. Erythema and swelling are present in the preauricular area which may spread to the submandibular and submental areas. In case of suppurative bacterial sialadenitis, purulent discharge from the Stenson's duct is diagnostic. Various imaging modalities may be used in diagnosis of sialadenitis. CT images show gland enlargement and abscess formation may also be detected (a focal area of fluid attenuation). MRI demonstrates asymmetric diffuse enlargements of the parotid glands, that may be associated with a dilated duct. Imaging has an impact on management [100]. Systemic antibiotics therapy is the treatment of choice after blood culture is obtained and the causative bacteria identified [101]. Additionally, when abscess is present, drainage of the abscess may be required.

Chronic bacterial sialadenitis is caused by unresolved acute sialadenitis, resulting in glandular destruction. This results in alteration of the salivary chemistry including enzymes, leading to sialectasis, ductal dilation, atrophy of glandular tissues, and lymphocytic cell infiltration [102]. The chronic inflammatory state eventually leads to fibrosis (see Fig. 8.8). In

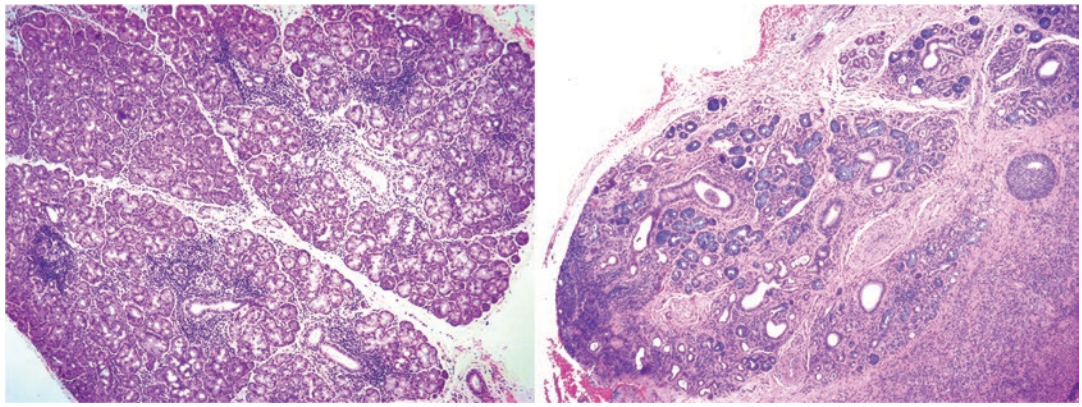

Fig. 8.8 Image on the left represents histologic features typical of Sjogren's syndrome from labial salivary gland biopsy demonstrating multiple foci of >50 lymphocytes scattered within relatively well-preserved salivary gland lobules. Ductal ectasia and periductal inflammation is noted. Image on the right is from chronic sclerosing sialadenitis demonstrating fibrosis and inflammatory destruction of acini with persistence of dilated salivary gland ducts. Some acini are still visible. Squamous metaplasia of ducts are also seen. Hematoxylin and eosin, 5× magnification

comparison, labial salivary biopsy from SS shows preservation of the acinar and lobular architecture with scattered foci of >50 lymphocytes. Clinically, recurrent and unilateral parotid swelling is noted which may or may not be painful [103]. Intraorally, suppurative drainage from the orifice of the Stenson duct has been reported. In time, fibrosis produces a firm tumor-like mass (Kuttner tumor) in the affected gland [101]. Kuttner tumor is seen most frequently in the submandibular gland as opposed to the parotid gland and may clinically mimic a malignant salivary gland tumor. Imaging features are varied depending on the duration and severity of the inflammatory reaction. Irregularly enlarged (sausage-shaped) main duct and central ductal dilation is the hallmark of recurrent chronic sialadenitis. These may or may not be accompanied with dystrophic calcifications. Ductal ectasia, fatty infiltration, and volume loss are seen in imaging during late stages of the disease [104]. Histologically, chronic bacterial sialadenitis is characterized by a chronic inflammatory cell infiltrate mainly lymphocytes, varying degrees of acini loss, and duct dilatation are noted. Extensive interstitial fibrosis develops over time and there may be squamous metaplasia of the duct epithelium. Calculus formation may be seen in the dilated ducts [102]. Treatment is challenging. The best approach in management is conservative which includes rehydration, analgesics, and long-term antibiotic therapy. Surgical intervention should be the last resort since the possibility of scarring, facial nerve damage, and cosmetic deformities may occur.

8.6.1.2 Parotid Tuberculosis (TB)

Parotid gland tuberculosis (TB) is a granulomatous infection caused by *Mycobacterium tuberculosis.* Primary TB of the parotid gland is extremely rare since saliva has an inhibitory effect on *Mycobacterium tuberculosis* and the continuous flow of the saliva from the salivary glands removes the bacteria [105]. Parotid involvement is an extrapulmonary manifestation of TB and spreads to the parotid glands via direct extension from a nearby site in the oral cavity or via the vascular or lymphatic channels [106]. Classification of this infection is based on the extent of involvement of the gland, namely, local or diffuse form [107]. In the localized form, intraglandular lymph nodes are affected, whereas the diffuse form results from the involvement of the parotid gland parenchyma [108]. Both forms may coexist as well.

Clinically, TB of the parotid presents as an asymptomatic, slowly growing, unilateral nodular or diffuse enlargement. Occasionally, bilateral involvement may be seen. Overlying skin is of normal color and temperature, but may exhibit formation of a fistula with drainage of pus in some cases. The parotid gland is the most common site amongst the salivary glands. Majority of cases occur between 20 and 60 years of age with a slight female predilection [109]. Pediatric cases as well as a congenital case has also been reported [110]. The growth occurs over a course of few months making it indistinguishable from a salivary gland malignancy clinically or sonographically. Histological examination is essential for a definitive diagnosis [111].

Radiographic imaging studies have limited value in diagnosing this condition. Ultrasonography imaging studies are done merely to evaluate the site (nodular/parenchymal) and density (solid/cystic) of the lesion. A definitive diagnosis is based on isolation and identification of the mycobacterium from a diagnostic specimen [112] as well as the biopsy specimen. FNA cytology and skin tests may show inconsistent results, especially in adults [111].

Histologic features, the gold standard for diagnosis, demonstrate classic caseous granulomatous inflammation, either intranodular, parenchymal, or both with discrete or confluent aggregates of epithelioid histiocytes intermixed with lymphocytes and multinucleated Langhans giant cells.

Treatment of salivary TB is similar to recommended treatment of TB and consists primarily of chemotherapy, provided the diagnosis is obtained via ancillary diagnostic methods. Lesions usually resolve following anti-TB treatment [113]. Surgery may be required when ancillary methods cannot deliver a diagnosis [111].

SS, unlike parotid TB, typically presents with bilateral parotid swelling and is usually also asymptomatic. However, rare bilateral cases of parotid TB have been reported in literature and may completely mimic clinical presentation of SS, especially if they are asymptomatic and slow growing.

8.6.2 Viral Sialadenitis

8.6.2.1 Mumps

Mumps, a self-limiting infection, is caused by mumps virus (MuV), an RNA virus belonging to the *Paramyxoviridae* family. It is typically associated with temporary bilateral, symptomatic enlargement of the parotid. It is transmitted via inhalation of the virus from respiratory droplets or via the direct contact with oral secretions of an infected individual [114]. Children between ages of 5–9 years are susceptible to the virus and it is the most common cause of a painful parotid gland swelling in children [115]. Recent outbreaks have involved adolescents and adults as well. The virus may also cause meningitis, encephalitis, orchitis, pancreatitis, myocarditis, and nephritis. Most cases are resolved within a few weeks. Paralysis, seizures, cranial nerve palsies, and deafness may occur in long-standing cases [116]. Salivary virus shedding has been reported from 1 week before to 1 week after the onset of parotid gland swelling [117].

Clinically, half to one-third of the cases are symptomatic and symptoms are preceded by an incubation period of 16–18 days which is followed by headache, malaise, anorexia, myalgia, and fever (prodromal stage). Characteristic features in symptomatic cases include the acute, bilateral, and painful enlargement of the parotid glands [118]. Severe cases may cause masticatory difficulties, speech difficulties, and trismus. Intraorally, the ductal orifice is erythematous and edematous.

Radiographic imaging shows enlargement of the parotid gland, loss of normal echotexture, multiple hypoechogenic lymph nodules, and increased vascularity on power Doppler. CT and MRI are helpful in detecting meningoencephalitis. Early stages demonstrate normal brain structure. Hydrocephalus may be detected in the last stages [119].

Histologic features show mononuclear infiltration in the perivascular and interstitial region of the parotid gland. This results in edema, hemorrhage and edema in the glandular and ductal epithelial cells [116]. However, diagnosis is done on the basis of reverse transcription-

polymerase chain reaction (RT-PCR), a sensitive and specific diagnostic technique [120]. Virus-specific IgM can also be measured by direct or indirect ELISA techniques 7–10 days after the onset of clinical symptoms and aids in diagnosis [121].

Treatment primarily consists of supportive care as mumps is a self-limiting condition. No targeted antiviral therapy is available [122].

SS also typically presents with bilateral parotid swelling which is usually asymptomatic. However, mumps is self-limiting and usually accompanied by severe pain, discomfort, fever, malaise, and loss of appetite.

8.6.2.2 Epstein–Barr Virus-Related Parotid Gland Lesions

Epstein–Barr virus (EBV) also known as human herpes virus-4 (HHV-4) is an enveloped double-stranded DNA herpesvirus that is transmitted via the saliva. Greater than 90% of the adult population is infected worldwide [123] and it is shed even in apparently healthy individuals [124]. EBV infects epithelial cells and B-lymphocytes, and has been implicated in several inflammatory, benign and malignant processes. Of the benign salivary gland lesions, EBV has been found in lymphoepithelial (LE) cysts and in Warthin tumor, the second most common benign salivary gland tumor. EBV has also been detected in undifferentiated carcinomas as well as malignant LE lesions of the parotid gland.

EBV-associated bilateral and multiple LE cysts are a common manifestation of HIV infections. Histologically, LE cysts show lymphoid hyperplasia in association with the cyst wall. Several studies were conducted to detect the role of EBV in the pathogenesis of HIV-associated LE cysts since EBV is a lymphotropic virus. However, the role of EBV in salivary gland disease remains controversial with no definitive evidence [124–127]. It is understood that EBV is found more frequently in benign LE cysts than in normal parotid gland.

EBV-associated Warthin tumor has also been reported. Warthin tumor is a slow-growing unilateral or bilateral benign salivary gland tumor. It is almost exclusively seen in parotid glands and has a male predilection. Histologically, it comprises of a double layered oncocytic epithelium associated with a lymphoid stroma. EBV has been detected in both the epithelial and lymphoid component of Warthin tumor. Presence of EBV in the tumor does not necessarily implicate it as an etiologic agent. Several authors employed in situ hybridization to evacuate the role of EBV and concluded that EBV is not involved in the pathogenesis of Warthin tumor [128, 129].

EBV-associated LE carcinoma (LEC) of the parotid gland is a rare malignancy occurring mainly in the East Asian population. Most cases occur in the fifth decade of life with a female predilection. Histologically, there is a malignant transformation of the ductal and glandular inclusions in the intraparotid lymph nodes. The association of EBV with LEC in American patients was reported to be 44.2%, and studies have reported that EBV infection in immunocompromised patients may play a strong role in LEC [130, 131].

8.6.2.3 HIV-Associated Sialadenitis

8.6.2.3.1 HIV-Related Bilateral Parotid Gland Lymphoepithelial Cysts

HIV-related parotid gland involvement typically results in bilateral parotid gland swelling [133]. Bilateral lymphoepithelial (LE) cysts of the parotid glands are one of the numerous pathologies that may arise in an HIV patient. In non-HIV-infected patients, most LE cysts arise in the oral cavity (oral LE cyst) or the lateral cervical region (branchial cleft cyst). LE cysts have been reported in the parotid gland in approximately 1% to 10% of HIV-infected patient and may be an indicator of HIV infection in these patients since the parotid gland is an uncommon site for benign LE cysts [103]. The incidence is even higher in children with HIV disease. In up to 80% of the HIV-related cases, the cysts are bilateral and multicentric, and are pathognomonic for HIV infections [134]. HIV-associated LE cysts usually occur before full blown AIDS has developed. The exact etiology of the development of these lesions is still unknown. Currently, two hypotheses prevail: (1) Due to HIV infection, reactive

lymphoproliferation occurs in the intraparotid lymph nodes, resulting in entrapment of glandular epithelium within the lymph nodes and the development of LE cysts and (2) Lymphoid proliferation of the intraparotid lymph nodes and development of glandular dysplasia occurs as a result of migration of the HIV-infected lymphoid cells in the parotid gland. Subsequently, ductal obstruction occurs, giving rise to LE cysts.

Clinically, parotid LE cyst is an important early head and neck manifestation of HIV disease. It is usually accompanied by cervical lymphadenopathy (90%), fatigue, night sweats, diarrhea, and weight loss [135]. Parotid glands develop slow-growing and painless swellings bilaterally. Unilateral swelling in a HIV positive patient is rare. Facial palsy and xerostomia are unusual [136].

Radiographically, multiple thin walled cysts may be detected via ultrasound examinations, MRI, and CT scans.

Fine needle aspiration cytology (FNAC) and biopsy specimens are useful diagnostic tools. FNAC reveals foamy macrophages, and squamous cells in a proteinaceous background [137]. These cells have a benign appearance. Biopsy is still the golden standard for a definitive diagnosis and reveals multiple epithelial cell-lined cysts associated with dense lymphoid tissue. Normal parotid parenchyma is replaced by the lymphoid tissue. The follicles of the lymph nodes in these cases are larger as compared to those of normal lymph nodes. Numerous macrophages are seen within the germinal centers of the lymph nodes [138]. HIV-1 p24 antigen immunostaining is another diagnostic tool. Intrafollicular macrophages and follicular dendritic cells are positive for these antigens in most lesions [136]. Blood analysis show a CD8 lymphocytosis due to HIV antigens [139].

Treatment is sought due to cosmetic concerns of the patient [134]. To date the treatment modality has not been streamlined. The proposed treatments include: (1) close watch, (2) repeated aspirations, (3) antiretroviral medication, (4) Low-dose radiation therapy, (5) injection sclerotherapy, and (6) parotidectomy [140]. Close monitoring and follow-up is highly advised because these patients are at a higher risk of developing lymphomas [141].

8.7 Neoplastic Lesions

Salivary gland neoplasms are rare and the majority of these are benign (80%). Majority of cases arise in the sixth decade with an equal prevalence in both sexes. About 80% of cases arise in the parotid gland. In minor glands, palate is the site of prevalence [3]. Etiology is still obscure. Possible risk factors include therapeutic radiation for other head and neck cancers, radiation exposure, occupational exposures (rubber manufacturing and woodworking), employment at hairdressers or beauty shops, history of previous cancer related to Epstein–Barr virus, HIV infection, Hodgkin lymphoma, and immunosuppression [142]. Benign tumors present as a slow-growing mass either within the major salivary gland or intraorally. Sudden growth, pain, fixation of tumor mass, ipsilateral facial nerve paralysis, and cervical lymphadenopathy are some of the characteristic features of a malignant tumor [143].

Pleomorphic adenoma is the most common benign salivary gland tumor and can be seen in the major as well as minor salivary glands. It carries a 10% risk of malignant transformation [143]. Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor and is the most common salivary carcinoma in children. It can also be seen in the major or minor salivary glands with the parotid gland being the most common extraoral site. Approximately 50–80% of MECs harbor MAML 2 gene fusion [144]. Adenoid cystic carcinoma (AdCC) is also a malignant salivary which can be seen in major or minor salivary glands. It has a propensity for perineural invasion and has a relatively indolent course with a survival rate of 15–20 years. These tumors metastasize early because of their propensity for perineural invasion and hematological spread, with the lungs being the most common site. Approximately 60% of AdCC show MYB-NFIB gene fusion [145]. Acinic cell carcinoma (ACC) is a low to intermediate malignancy arising primarily in the parotid gland. An upregulation of NR4A3 transcription factor is noted [146].

Due to high prevalence of salivary gland neoplasms observed in the parotid gland and clinical

similarities to SS-associated parotitis or lymphoma, this section will focus on common benign and malignant lesions in the parotid glands.

8.7.1 Pleomorphic Adenoma of the Parotid Gland

Pleomorphic adenoma is the most common benign salivary gland tumor [147]. Majority of cases are seen in the parotid gland. Intraorally, the tumor is most commonly seen on the palate. Long-standing cases may cause disFigurement, facial nerve damage and undergo malignant transformations. This malignant change may be in the form of: (1) carcinoma ex-pleomorphic adenoma (75% of epithelial cells show malignant transformation), (2) carcinosarcoma (true malignant mixed tumor) formed by both epithelial and mesenchymal cells, or (3) metastasizing pleomorphic adenoma (cervical lymph nodes or distant metastasis of benign pleomorphic adenoma) [148]. Of the three, carcinoma ex-pleomorphic adenoma is the most common form of malignant transformation and may spread via infiltration to adjacent tissues or spread to distant sites via lymphatic and vascular channels [149]. The exact etiology of this transformation is not known [150].

Clinically, pleomorphic adenoma is typically a solitary, slow-growing, and painless tumor. It is most commonly encountered between fourth and seventh decades of life with occasional cases reported in children and adolescents. There is a slightly increased prevalence in females as compared to males. Its size ranges from 1 to 5 cm, and facial palsy is uncommon [151]. Most of the tumors originate from the superficial lobe of the parotid gland and present as a swelling overlying the mandibular ramus in front of the ear or as a neck mass (see Fig. 8.9). Tumors arising from the deep lobe or involving the entire gland have also been reported [152]. Pleomorphic adenomas are of concern because long-standing cases may undergo malignant transformation. Rapid increase in size of long-standing tumors is a strong indication of malignant transformation [153].

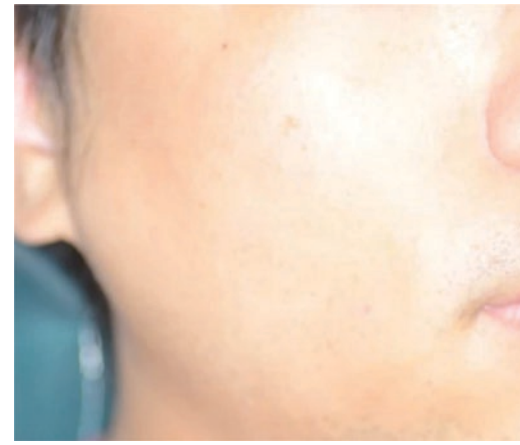

Fig. 8.9 A 37-year-old male with a well-defined firm movable slowly enlarging mass of the left parotid region. An incisional biopsy revealed pleomorphic adenoma

Radiographic imaging is useful in detecting the size and extent of the tumor. Ultrasound imaging reveals a well-defined, homogenous lesion with poor vascularization. Computed radiographic images are useful in lesions involving the palate, especially if bony involvement is suspected [147].

Histologic features reveal a well-demarcated lesion surrounded partially or completely by a capsule of variable thickness. The tumor is composed of ductal and myoepithelial cells and shows a marked diversity between different areas of the same tumor or different tumors (hence the name pleomorphic) (see Fig. 8.10). These epithelial and myoepithelial cells are seen in a mesenchymal-like background which may be hypo- or hyper-cellular. No sign of malignancy is seen in either of the components [154].

Treatment primarily consists of surgical resection. Superficial parotidectomy is sufficient in most cases as majority of the lesions involve the superficial lobe of the gland. In partially encapsulated cases, recurrence have been reported. Most of the complications (facial nerve paralysis or Frey syndrome) are related to the association of the lesion with the facial nerve [155].

SS, unlike pleomorphic adenoma of the parotid gland, typically presents with bilateral parotid swelling. The swellings in both cases are usually asymptomatic.

8.7.2 Warthin Tumor

Warthin tumor (WT) is also known as papillary cystadenoma lymphomatosum and is the second most common tumor of the parotid glands after pleomorphic adenoma. It is a benign tumor of salivary glands with an incidence rate of approximately 5–10% [156, 157]. These lesions produce persistent painless swelling of the parotid that slowly enlarge producing a delimited nodule with smooth outline, floor of the mouth, or under the chin.

WT are purported to arise from epithelial inclusions in intra- or peri-parotid lymph nodes [156, 158]. This concept is supported by the observation that the epithelial and mesenchymal components of the parotid gland are not encapsulated or demarcated at the early stages of development. This allows proliferation of the epithelial inclusions inside the lymphoid aggregates in response to unknown stimuli [158]. Moreover, this hypothesis could also explain the overwhelmingly frequent localization of WT in the parotid.

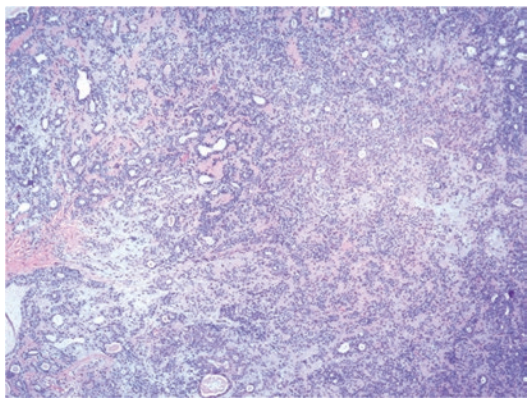

Fig. 8.10 Photomicrograph from pleomorphic adenoma demonstrating proliferation of myoepithelial cells forming interconnecting strands, islands, and clusters with pseudo-ductal spaces. The stromal tissues range from chondroid to myxoid to densely hyalinized. Both sparsely cellular and densely cellular areas are noted. Hematoxylin and eosin, 5× magnification

WT may present bilaterally in the parotid in a synchronous or metachronous manner in up to 15% of cases [156]. Multifocal tumors have also been reported [156]. It is more common in males between 60 and 70 years of age (see Fig. 8.11). This male predilection and bilateral occurrence is possibly linked to higher rates of smoking. Caucasians are affected significantly more when compared to other races [157]. WT has been strongly linked to tobacco smoking [156]. Obesity and EBV have also been implicated as etiologic factors [156, 159, 160]. Rare cases of malignant transformation have been reported with development of diffuse large B-cell lymphoma, mucoepidermoid carcinoma, and squamous cell carcinoma [156]. Interestingly, WT has also been reported to occur simultaneously with other non-salivary and extra-salivary tumors, such as breast, lung or thyroid carcinoma, or lymphomas [156].

Diagnosis of WT can be performed by FNAC with high degree of sensitivity and positive predictive value [161]. ^{99m}Tc-pertechnetate scintigraphy demonstrates pathognomonic accumulation

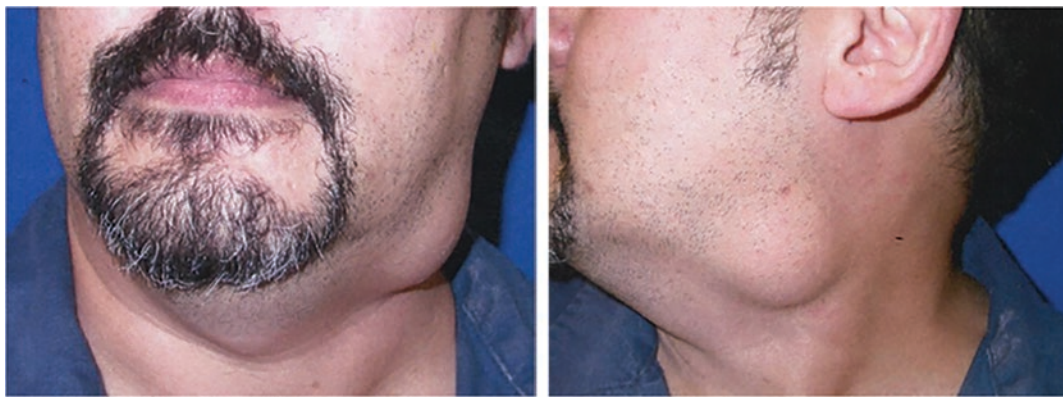

Fig. 8.11 A 47-year-old male with a 4-year history of slow-growing swelling of the tail of the parotid. Biopsy revealed Warthin tumor

of the radioisotope in WT [162]. Contrast-enhanced two-phase CT uniquely demonstrates early enhancement and washout of contrast agent, which is very helpful in distinguishing WT from other parotid tumors [163]. Color Doppler ultrasonography is also useful in separating WT from other benign tumors of the parotid [164].

Microscopically, the tumor is composed of a mixture of multiple cystic cavities lined by ductal epithelium demonstrating numerous papillary infoldings protruding into the cystic lumina. These structures are distributed in a variably dense lymphoid stroma. Frequently germinal centers are seen in the stroma [157] (see Fig. 8.12).

Superficial parotidectomy is the preferred mode of treatment due to the usually superficial localization of the tumor in the parotid [164]. An approximately 10% rate of recurrence has been reported, but many recurrences may actually be metachronous tumors rather than true recurrence [164].

8.7.3 Adenocarcinomas of the Parotid Gland

Primary salivary gland malignancies are rare and comprise of approximately 5% of all head and neck malignancies [165]. Of the salivary glands, only 25% of malignancies arise in the parotid gland [166]. The etiologies are still obscure, although radiation exposure has strongly been implicated and a linear-dose dependent relationship has also been established [167]. They occur more commonly in adult males [168]. Majority of patients present with an asymptomatic swelling. Rapid enlargement of lesion, pain, paresthesia, and facial nerve paralysis indicate malignancies [169]. Facial nerve palsy is seen in nearly 33% of cases with the incidence increasing to 50% in cases of advanced malignancies of the parotid gland [170]. Of all the various salivary adenocarcinomas, the ones which most frequently involve the parotid gland include: MEC, ACC, and carcinoma ex-pleomorphic adenoma.

MEC is the most common malignancy of the parotid gland and constitutes about 30% of parotid malignancies [171]. They occur over a wide age range (second to seventh decades of life) and are the most common malignant salivary gland malignancy occurring in children. Histologically mucin-secreting cells, intermediate cells, and epidermoid cells are seen. Lesions are graded as low-, intermediate-, or high-grade tumor (see Fig. 8.13). In general, MEC has low local invasiveness and low metastatic potential. However, high-grade tumors typically metasta-

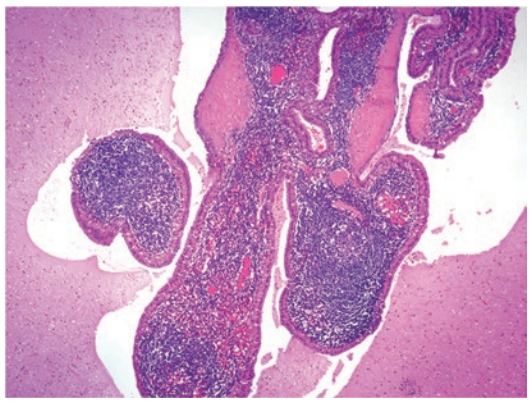

Fig. 8.12 Microscopic image from the patient above. Warthin tumor demonstrating papillary projections of pseudostratified columnar ciliated ductal epithelial cells surrounded by pale eosinophilic mucoid cystic content. The connective tissue cores within the papillary projections containing sheets of lymphocytes. Hence, the descriptive term papillary cystadenoma lymphomatosum. Hematoxylin and eosin, 5× magnification

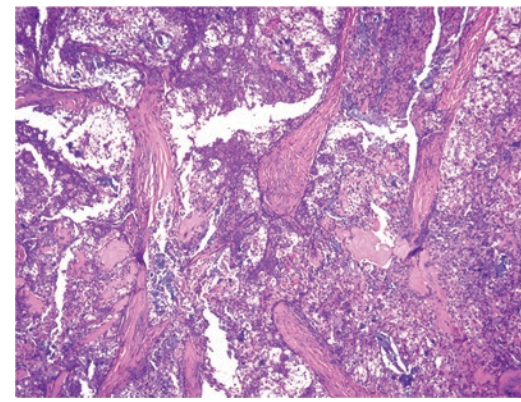

Fig. 8.13 Microscopic image from an intermediate grade mucoepidermoid carcinoma demonstrating a lobular proliferation of epithelial cells interspersed by cells exhibiting cleared cytoplasm and scattered basophilic cells containing mucous. Dense fibrous bands are noted between the lobules. Hematoxylin and eosin, 5× magnification

size to regional lymph nodes. Treatment modalities include a combination of radiation therapy and surgery. The overall 10-years survival rate is approximately 50% [172].

ACC accounts for 10% of malignant parotid tumors [173]. About 81% to 98% of ACC occur in the parotid glands. These tumors show a broad age range (second to seventh decades of life) and a female predilection. Histologically, these well-circumscribed tumors show an infiltrative growth pattern and a serous acinar cell differentiation (see Fig. 8.14). Other cell types present include intercalated ductal, vacuolated, and clear cells. The growth pattern may be variable [174]. ACC are asymptomatic and typically are considered as intermediate-grade malignancy which rarely metastasize. However, recent studies have suggested that a subset of ACCs may be associated with an aggressive behavior [174]. Since most lesions are seen in the superficial lobe, ACC is best treated by lobectomy. Parotidectomy is needed for deep seated tumors. The overall 5-year disease-specific survival is estimated to be around 91%. Recurrences and metastases after 3 to 10 years are common [175].

Carcinoma ex-pleomorphic adenoma is an aggressive neoplasm with poor prognosis [176]. They constitute approximately 25% of all parotid malignancies. Most patients are in the sixth to eighth decades of life. Typically, a long-standing asymptomatic mass in the parotid area exhibiting sudden rapid growth is reported. Rapid increase in size is often associated with facial nerve involvement giving rise to pain, paresthesia, and facial nerve palsy. Skin ulcerations, dysphagia, and lymphadenopathy have also been reported [177]. Histopathologically, the malignant component is infiltrative, ill-defined and has foci of necrosis and hemorrhage [177]. Surgical excision (lobectomy or parotidectomy) is the treatment of choice, based on the location of the tumor. A 5-year survival rate is as low as 37% [177].

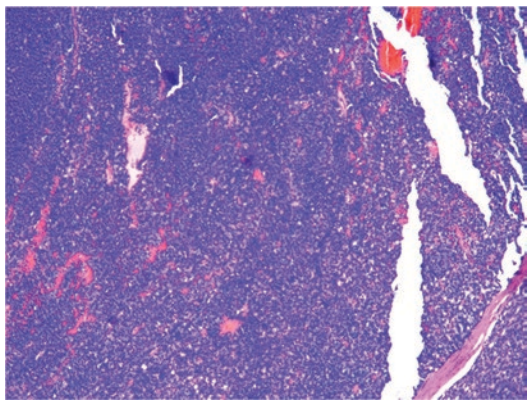

Fig. 8.14 Microscopic image from acinic cell carcinoma demonstrating sheets of deeply basophilic neoplastic acinar cells with little intervening stroma and dilated capillaries. Hematoxylin and eosin, 5× magnification

8.7.4 Primary Parotid Lymphoma

Primary parotid gland lymphoma is an extremely rare malignancy. The following criteria have been suggested for a lymphoma to be considered primary: (1) gland involvement should be the first clinical manifestation of the disease, (2) the lymphoid infiltrate should be malignant, and (3) the biopsy result should show involvement of the parenchyma of the gland instead of the adjacent lymph nodes or soft tissues alone [178]. The lymphoma may arise within the parotid gland lymph nodes (nodal) or within the parenchyma of the gland (extranodal). The true distinction between these two groups is difficult to establish because nodal lymphoma may involve the surrounding glandular parenchyma and may be confused with an extranodal lymphoma [179]. Salivary gland lymphomas may be Hodgkin lymphomas (HL) or non-Hodgkin. lymphomas (NHL). The most common primary parotid NHLs are the mucosa associated lymphoid tissue (MALT) lymphoma [180] (see Fig. 8.15). Unlike the aerodigestive tract, there is a lack of MALT in the salivary glands. The precursor lesion for MALT lymphoma is a benign lymphoid lesion (BLL), characterized by B cells interdigitating with the ductal epithelial cells, which arise as a result of stimulation of the adjacent lymphoid tissue. The primary parotid lymphomas are low-grade as compared to other extranodal NHLs [181].

Clinically, most cases are seen in middle-aged males and the prevalence increases with age.

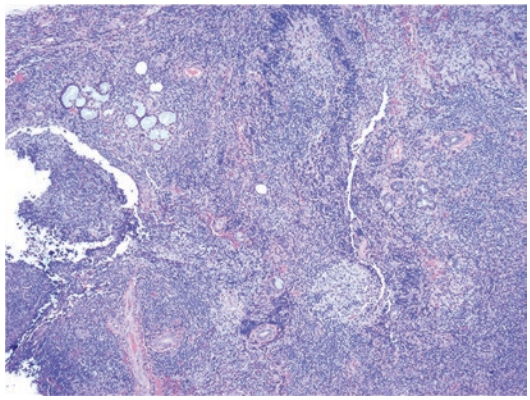

Fig. 8.15 Microscopic image from a follicular lymphoma involving minor salivary glands. Complete effacement of both acinar and lobular architecture with occasional acini and scattered dilated ducts visible. The lymphocytes are present in sheets and occasional clusters. Hematoxylin and eosin, 5× magnification

Some studies show no gender predilection [182]. Pediatric cases with primary parotid lymphomas have also reported in literature [182]. Following MALT lymphomas, the next two most common types of lymphoma involving the salivary glands include follicular lymphoma and diffuse large B-cell lymphoma [181]. The malignancy manifests as a unilateral, painless, slowly enlarging mass. Pain, facial nerve, paralysis, and involvement of the underlying tissues or overlying skin have also been reported [182]. Intraorally, xerostomia and periductal orifice swelling may be observed.

Radiographic imaging is useful in assessing the extent, shape, and size of the lesion [183]. MRI and CT scan are equally effective. Positron emission tomography-computed tomography (PET-CT) is used to detect metastasis [184]. Biopsy is the gold standard in establishing a definitive diagnosis. Immunohistochemical studies aid in further classifying the lymphoma types.

Treatment modalities majorly consist of radiation and chemotherapy. Radiotherapy is reserved for localized cases and chemotherapy for disseminated cases. Some authors advocate superficial lobectomy/parotidectomy, a low risk procedure, in any parotid salivary mass to establish a definitive diagnosis as many parotid gland tumors are epithelial in origin and are often associated with a lymphoid infiltrate. Most patients have Ann Arbor Stage I or stage II disease at time of diagnosis [183].

SS typically presents with bilateral parotid swelling which is usually asymptomatic. It must be kept in mind that the risk of NHL, primary parotid gland lymphoma, and primary parotid gland MALT lymphoma is increased to 6.5-, 250-, and 1000-folds, respectively in the setting of SS patient [182].

8.8 Conclusion

SS is a systemic autoimmune disease with a wide range of clinical symptoms including recurrent or persistent salivary gland enlargement especially of the parotid which can closely mimic several other diseases. These diseases encompass a myriad of etiologies including idiopathic, reactive, developmental, inflammatory/immune, infectious or neoplastic. It is of paramount importance for the clinician to be cognizant of SS, as well as the differential diagnosis, in order to manage the patient appropriately in a timely fashion. Though benign, SS can significantly alter the patient's quality of life as well as predispose to a malignancy. Diagnosis of SS can be challenging due to complexity of the disease pathogenesis and heterogeneous presentations, which often requires a multidisciplinary approach among health care workers to arrive at a definitive diagnosis. In this chapter, we have discussed some of the more common differential diagnoses of parotid enlargement.

References

1. Romas-Casals M, Tzioufas AG, Font J. Primary Sjogren's syndrome: new clinical and therapeutic concepts. Ann Rheum Dis. 2005;64:347.
2. Vitali C, Moutsopoulos HM, Bombardieri S. The European Community study group on diagnostic criterion for Sjogren's syndrome. Sensitivity and specificity of tests for ocular and oral involvement in Sjogren's syndrome. Ann Rheum Dis. 1994;53:637.
3. Neville BW, Damm DD, Allen CM, Chi AC. Oral & maxillofacial pathology. 4th ed. St. Louis, MO: WB Saunders, Elsevier; 2016. p. 429–30.

4. García-Carrasco M, Mendoza-Pinto C, Jiménez-Hernández C, Jiménez-Hernández M, Nava-Zavala A, Riebeling C. Serologic features of primary Sjögren's syndrome: clinical and prognostic correlation. Int J Clin Rheumatol. 2012;7(6):651–9.
5. Shiboski CH, Shiboski SC, Seror R, et al. 2016 American College of Rheumatology/European league against rheumatism classification criteria for primary Sjogren's syndrome. A consensus and data-driven methodology involving three international patient cohorts. Arthritis Rheumatol. 2017;69(1):35–45.
6. Nahlieli O, Bar T, Shacham R, Eliav E, Nakar LH. Management of chronic recurrent parotitis: current therapy. J Oral Maxillofac Surg. 2004;62:1150.
7. Patel P, Scott S, Cunningham S. J Am Osteopath Assoc. 2017;117(12):e137–40.
8. Mahalakshmi S, Kandula S, Shilpa P, Kokila G. Chronic recurrent non-specific parotitis: a case report and review. Ethiop J Health Sci. 2017;27(1):95–100.
9. Joshi M, Phansalkar D, Singh Z. Chronic juvenile recurrent parotitis: a retrospective study in a tertiary care hospital in South India. Int J Contemp Pediatr. 2016;3(2):389–93.
10. Al-Dajani N, Wootton SH. Cervical lymphadenitis, suppurative parotitis, thyroiditis, and infected cysts. Infect Dis Clin North Am. 2007;21920:523–41.
11. Mandel L, Whitek EL. Chronic parotitis diagnosis and treatment. J Am Dent Assoc. 2001;132(12):1707–11.
12. Reddy SS, Rakesh N, Raghav N, Devaraju D, Bijjal SG. Sialography: report of 3 cases. Indian J Dent Res. 2009;20:499–502.
13. Ericson S, Zetterlund B, Ohman J. Recurrent parotitis and sialectasis in childhood. Clinical, radiologic, immunologic, bacteriologic and histologic study. Ann Otol Rhinol Laryngol. 1991;100:527–35.
14. Chitre VV, Premchandra DJ. Recurrent parotitis. Arch Dis Child. 1997;77:359–63.
15. Katz P, Hartl DM, Guerre A. Treatment of juvenile recurrent parotitis. Otolaryngol Clin North Am. 2009;42:1087–91.
16. Papadopoulou-Alataki E, Dogantzis P, Chatziavramidis A, et al. Juvenile recurrent Parotitis: the role of Sialendoscopy. Int J Inflam. 2019;2019:7278907.
17. Gadodia A, Seith A, Sharma R, Thakar A. MRI and MR sialography of juvenile recurrent parotitis. Pediatr Radiol. 2010;40(8):1405–10.
18. Quenin S, Plouin-Gaudon I, Marchal F, Froehlich P, Disant F, Faure F. Juvenile recurrent parotitis. Arch Otolaryngol Head Neck Surg. 2008;134(7):715–9.
19. Nahlieli O, Shacham R, Shlesinger M, Eliav E. Juvenile recurrent parotitis: a new method of diagnosis and treatment. Pediatrics. 2004;114:9–12.
20. Mandel L, Surattanont F. Bilateral parotid swelling. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;93:221–37.
21. Ungprasert P, Crowson CS, Matteson EL. Clinical characteristics of parotid gland Sarcoidosis: a population-based study. JAMA Otolaryngol Head Neck Surg. 2016:142–503.
22. Yagin Y, Celik M, Elbistanli MS, Kayhan FT. An unusual entity of sarcoidosis: acute parotitis. Otolaryngol Open J. 2016;2(1):28–30.
23. Radochova V, Radocha J, Laco J, Slezak R. Oral manifestations of sarcoidosis: a case report and review of literature. J Indian Soc Periodontol. 2016;20:627–9.
24. Tuisku IS, Konttinen YT, Soinila S, Karma A, Tervo TM. Neurosarcoidosis mimicking Sjögren's syndrome. Acta Ophthalmol Scand. 2004;82:599–602.
25. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet. 2003;361(9363):1111–8.
26. Hofmann S, Franke A, Fischer A, Jacobs G, Nothnagel M, Gaede KI, et al. Genome-wide association study identifies ANXA11 as a new susceptibility locus for sarcoidosis. Nat Genet. 2008;40(9):1103–6. Erratum in: Nat Genet. 2009 Apr;41(4):504.
27. Chapman MN, Fujita A, Sung EK, et al. Sarcoidosis in the head and neck: an illustrative review of clinical presentations and imaging findings. AJR Am J Roentgenol. 2017;208(1):66–75.
28. Broggi G, Reggio E, Giuliano L, Palmucci S, Caltabiano R, Lanzafame S. Parotid gland involvement in Heerfordt syndrome. Pathologica. 2017;109:418–20.
29. Cakmak SK, Gonul M, Gul U, Gunduz H, Han O, Kulacoglu S. Sarcoidosis involving the lacrimal, submandibular, and parotid glands with panda sign. Dermatol Online J. 2009;15(3):8–12.
30. Patoulias D, Keyrttopoulos P. Diagnostic dilemma between sarcoidosis and primary Sjögren's syndrome: mimicry, concomitant or coincidental? An up-to-date clinican's perspective. Folia Med Cracov. 2018;58(1):5–23.
31. Gerke AK. Morbidity and mortality in sarcoidosis. Curr Opin Pulm Med. 2014;20(5):472–8.
32. Khairnar MR, Wadgave U, Khairnar SM. Effect of alcoholism on Oral health: a review. J Alcohol Drug Depend. 2017;5(3):1–4.
33. Mandel L, Vakkas J, Saqi A. Alcoholic (beer) Sioalosis. J Oral Maxillofac Surg. 2006;6:402–5.
34. Donath K, Seifert G. Ultrastructural studies of the parotid glands in sialadenosis. Virchows Arch A Pathol Anat Histol. 1975;365:119–35.
35. Ihrler S, Rath C, Zengel P, et al. Pathogenesis of sialadenosis: possible role of functionally deficient myoepithelial cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;110:218–23.
36. Krishnamurthy S, Vasudeva SB, Vijayasaraty S. Salivary gland disorders: a comprehensive review. World J Stomatol. 2015;4(2):56–71.
37. Mandel L, Hamele-Bena D. Alcoholic parotid sialadenosis. J Am Dent Assoc. 1997;128(10):1411–5.
38. Chen S, Paul BC, Myssiorek D. An algorithm approach to diagnosing bilateral parotid enlargements. Otolaryngol Head Neck Surg. 2013;148(5):732–9.
39. Aframian DJ. Anorexia/ bulimia-related sialadenosis of palatal minor salivary glands. J Oral Pathol Med. 2005;34(6):383.

40. Lo RL, Campisi G, Di Fede O, Panzarella V, Lo Muzio L. Oral manifestations of eating disorders: a critical review. Oral Dis. 2008;14(6):479–84.
41. Mandel L. Serum electrolytes in bulimic patients with parotid swellings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96:414–9.
42. Dynesen AW, Bardow A, Petersson B, Nielsen LR, Nauntofte B. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106(5):696–707.
43. Mehler PS, Rylander M. Bulimia Nervosa—medical complications. J Eat Disord. 2015;3:12.
44. Price C, Schmidt MA, Adam EJ, Lacey H. Parotid gland enlargement in eating disorders: an insensitive sign? Eat Weight Disord. 2008;13:e79–83.
45. Garcia BG, Ferrer AD, Jimenez ND, Granados FJA. Bilateral parotid Sialadenosis associated with long-standing bulimia: a case report and literature review. J Maxillofac Oral Surg. 2018;17(2):1171–211.
46. Mandel L, Liu F. Salivary gland injury resulting from exposure to radioactive iodine. J Am Dent Assoc. 2007;138(12):1582–7.
47. Gonzalez ME, Muttikkal THE, Rehm PK. Sialadenitis following low dose 1-131 diagnostic thyroid scan with Thyrogen (recombinant human thyroid stimulating hormone-thyrotropin alfa). J Radiol Case Rep. 2015;9(6):44–9.
48. Maier H, Bihl H. Effect of radioactive iodine therapy on parotid gland function. Acta Otolaryngol. 1987;103(3–4):318–24.
49. Lei L, Velasco O, Nobay F. Iodine mumps: a case repot of complicated radioactive iodine causing sialadenitis. Am J Emerg Med. 2012;30(3):512.e5–6.
50. Dreyer JO, Sakuma Y, Seifert G. Radiation-induced sialadenitis. Pathologe. 1989;10:165–70.
51. Hoffman HT, Chaudhry US, Menda Y, Robinson RA. Current management of radioiodine sialadenitis. Curr Otorhinolaryngol Rep. 2014;2:70–84.
52. Nabaa B, Takahashi K, Sasaki T, Okizaki A, Aburano T. Assessment of salivary gland dysfunction after radioiodine therapy for thyroid carcinoma using non-contrast-enhanced CT: the significance of changes in volume and attenuation of the glands. Am J Neuroradiol. 2012;33:1964–70.
53. Kim JW, Kim DH, Kim KT, Kim TY, Ko KJ, Choi JS, Kim YM. Sialoendoscopy: endoscopic diagnosis and treatment of the salivary gland disease. Kor J Otolayryngol Head Neck Surg. 2005;48(3):373–9.
54. Junior VM, Netto R, Janini MER, Azevedo AB, de Andrade VM. Dystrophic calcification vs sialolithiasis in a pediactric parotid gland: a case report. J Clin Exp Dent. 2019;11(5):e496–9.
55. Duong LT, Kakiche T, Ferre F, Nawrocki L, Bouattour A. Management of anterior submandibular sialolithiasis. J Oral Med Surg. 2019;25:16.
56. Kraaij S, Karagozoglu KH, Forouzanfar T, Veerman ECI, Brand HS. Salivary stones: symptoms aetiology, biochemical composition and treatment. Br Dent Jr. 2014;217:E23.
57. Mustafa AB, Syed KB, Bhat MYS, Basheer SA, Elnager M, Baig F. Sialolithiasis involving parotid glands: a case report and literature review. Indian J Stomatol. 2015;6(3):76–8.
58. Kirkendall J. Sialolithiasis: sonographic detection of salivary stones. J Diagn Med Sonogr. 2013;29(1):36–9.
59. Ottaviani F, Galli A, Lucia MB, Ventura G. Bilateral parotid sialolithiasis in a patient with acquired immunodeficiency syndrome and immunoglobulin G multiple myeloma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997;83:552–4.
60. Kim H, Song WS, Kim YJ, Kim WD. Parotid sialolithiasis in a two-year-old boy. Korean J Pediatr. 2013;56(10):451–5.
61. Moghe S, Pillai A, Thomas S, Nair P. Parotid sialolithiasis. BMJ Case Rep. 2012.
62. Konstantinidis I, Paschaloudi S, Triaridis S, Fyrmpas G, Sechlidis S, Constantinidis J. Bilateral multiple sialolithiasis of the parotid gland in a patient with Sjögren's's syndrome. Acta Otorhinolaryngol Ital. 2007;27:41–4.
63. Kandakure VT, Thakur GV, Thote AR, Kausar AJ. Cervical lymphangiomas in adult. Int J Otorhinolaryngol Clin. 2012;4:147–50.
64. Henke AC, Cooley ML, Hughes JH, Timmerman TG. Fine needle aspiration cytology of lymphangiomas of the parotid gland in an adult. Diagn Cytopathol. 2001;24:126–8.
65. Mandel L. Parotid area lymphangioma in an adult: case report. J Oral Maxillofac Surg. 2004;62:1320–3.
66. Sahoo N, Mohapatra D, Mohanty P, Das P, Das D. Intra parotid cystic lymphangioma masquerading as a neoplasm, a rare entity at unusual site. J Med Sci Res. 2019;10:39–41.
67. Shah GH, Deshpande MD. Lymphatic malformations in adult patients: a rare case. J Oral Maxillofac Surg. 2010;9:284–8.
68. Berri T, Azizi S. Large cystic lymphangioma of the parotid gland in the adult. Egypt J Ear Nose Throat Allied Sci. 2014;15(3):259–61.
69. Abramowicz S, Marshall CJ, Dolwick MF, Cohen D. Vascular malformations of the temporomandibular joint. Report of a case and review of literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103:203–6.
70. Kobayashi K, Nakao K, Kishishita S, Tamaruya N, Monobe H, Saito K, Kihara A. Vascular malformations of the head and neck. Auris Nasus Larynx. 2013;40:89–92.
71. Fevurly RD, Fishman SJ. Vascular anomalies in pediatrics. Surg Clin N Am. 2012;92:769–800.
72. Saeed WR, Koihe PS, Smith FW, Murray GI. The 'Turkey wattle' sign revisited: diagnostic parotid vascular malformations in the adult. Br J Plast Surg. 1997;50(1):43–6.
73. Bhatia C, Dalal S, Sattibabu V, Beniwal JP. Vascular malformation of the parotid gland: a rare case report. Int Surg J. 2017;4(6):2018–83.
74. Achache M, Fakhry N, Varoquaux A, Coulibaly B, Michel J, Lagier A, Antonini F, Turner F, Dessi P, Giovanni A. Management of vascular malformations

of the parotid area. Eur Ann Otorhinolaryngol Head Neck Dis. 2013;130(2):55–60.
75. Shailaja SR, Manika, Manjuka M, Kumar LV. Arteriovenous malformation of the mandible and parotid gland. Dentomaxillofac Radiol. 2012;41:609–14.
76. Ponniah I, Kumar PS, Karunakaran K, Shankar KA, Kumaran MG, Preeti LN. Hemangioma in minor salivary glands: real or illusion. Diagn Pathol. 2006;1:21.
77. Lara-Sanchez H, Peral-Cagigal B, Madrigal-Rubiales B, Verrier-Hernandez A. Cavernous hemangioma of the parotid gland in adults. J Clin Exp Dent. 2014;6(5):e592–4.
78. Suwal R, Guan J, Ang XY, Khatri KS, Nyimi BF. A rare case report of parotid cavernous hemangioma in an adult. BJMMR. 2017;19(5):1–5.
79. Rio G, Cosat NA, Fernandes JS, Torrao H, Silva PO. Parotid hemangioma in a child: an uncommon imaging diagnosis. Acta Radiologica Portuguesa. 2019;31(2):19–22.
80. Elsayed SA, Alahmady HH. Heamangioma in parotid gland encroaching on the temporomandibular joint: a case report. Tanta Dent J. 2014;11:109–13.
81. Bentz BG, Hughes CA, Ludemann JP, Maddalozzo J. Masses of the salivary gland region in children. Arch Otolaryngol Head Neck Surg. 2000;126(2):1435–9.
82. Popescu E, Trandafir V, Negru D, Dobrin N, Trandafir D. Hemangioma of the parotid gland: case report. Rev Med Chir Soc Med Nat Lasi. 2013;117(1):227–32.
83. Reddi BR. A clinical observation to diagnose parotid hemangioma. Indian J Surg. 2013;75:S401–3.
84. Mantadakis E, Tsouvala E, Deftereos S, Danielides V, Chatzimichael A. Involution of a large parotid hemangioma with oral propranolol: an illustrative report and review of the literature. Case Rep Pediatr. 2012;2012:353812.
85. Takeuchi M, Ohno K, Takata K, Gion Y, Tachibana T, Orita Y, Yoshino T, Sato Y. Interleukin 13-positive mast cells are increased in immunoglobulin G4-related sialadenitis. Sci Rep. 2015;5:7696.
86. Mulholland GB, Jeffery CC, Satija P, Cote DWJ. Immunoglobulin G4-related disease in the head and neck: a systemic review. J Otolaryngol Head Neck Surg. 2015;44:24.
87. Stone JH, Zen Y, Deshpande V. IgG 4-related disease. N Engl J Med. 2012;366:539–51.
88. Brito-Zeron P, Kostov B, Bosch X, Acar-Denizli N, Ramos-Casals M, Stone JH. Therapeutic approach to IgG4-related disease: a sysyemic review. Medicine (Baltimore). 2016;95(26):e4002.
89. Mahajan VS, Mattoo H, Deshpande V, Pillai SS, Stone JH. IgG4-related disease. Annu Rev Pathol Mech Dis. 2014;9:315–47.
90. Geyer JT, Deshpande V. IgG4-associatted sialadenitis. Curr Opin Rheumatol. 2011;23:95–101.
91. Kamisawa T, Zen Y, Pillai S, Stone JH. IgG4-related disease. Lancet. 2015;385:1460–71.
92. Deshpande V, Zen Y, Chan JK, Yi EE, Sato Y, et al. Consensus statement in the pathology of IgG4-related disease. Mod Pathol. 2012;25(9):1181–92.
93. Okazaki K, Chari ST, Frulloni L, Lerch MM, Kamisawa T, Kawa S, Kim MH, Lévy P, Masamune A, Webster G, Shimosegawa T. Pancreatology. 2017;17(1):1–6.
94. Stone JJ. IgG4-related disease: pathophysiologic insight drive emerging treatment approaches. Clin Exp Rheumatol. 2016;34(98):66–8.
95. Seifert G. Aetiology and histological classification of sialadenitis. Pathologica. 1997;89:7–17.
96. Bradley PJ. Microbiology and management of sialadenitis. Curr Infect Dos Rep. 2002;4(3):217–2234.
97. Brook I. Aerobic and anaerobic microbiology of suppurative sialadenitis. J Med Microbiol. 2002;51:526–9.
98. Bijai LK, Jayaraman V, Austin RD. Chronic bacterial sialadenitis—a case report. Oral Surg Oral Med Oral Radiol. 2013;1(1):1–3.
99. Watanabe H, Odagiri T, Asai Y. Incidence of acute suppurative sialadenitis in end-stage cancer patients: a retrospective observational study. J Pain Symptom Manage. 2018;55:6.
100. Abdel Razek AAK, Mukherji S. Imaging of sialadenitis. Neuroradiol J. 2017;30(3):205–15.
101. Bradley PJ. Pathology and treatment of salivary gland conditions. Surgery (Oxford). 2006;24(9):304–11.
102. Madani G, Beale T. Inflammatory conditions of the salivary glands. Semin Ultrasound CT MRI. 2006;27:440–51.
103. Piyasatukit N, Awsakulsutthi S, Kintarak J. Benign lymphoepithelial cyst of parotid glands in HIV-positive patient. J Med Assoc Thai. 2015;3:141–5.
104. Zenk J, Iro H, Klintworth N, Lell M. Diagnostic imaging in sialadenitis. Oral Maxillofacial Surg Clin N Am. 2009;21:275–92.
105. Shah I. Multidrug-resistant tuberculosis in children. Pediatr Infect Dis J. 2012;31(9):970–2.
106. Bhalla AS, Gadodia A, Sharma R. Rapid diagnosis and successful drug therapy of primary parotid tuberculosis in pediatric age group: a case report and brief review of literature. Int J Infect Dis. 2010;14S:e370–1.
107. Sethi A, Sareen D, Sabherwal A, Malhotra A. Primary parotid tuberculosis: varied clinical presentations. Oral Dis. 2006;263:487–9.
108. Dixit R, Gokhroo A, Verma S, Panjabi M. Parotid gland tuberculosis. Int J Mycobacteriol. 2017;6:318–20.
109. Janmeja AK, Das SK, Kochhar S, Handa U. TB of parotid gland. Indian J Chest Dis Allied Sci. 2003;45:67–9.
110. Senbil N, Sahin F, Caglar MK, Oksal A, Yilmaz F, Tuna F. Congenital tuberculosis of the ear and parotid gland. Pediatr Infect Dis J. 1997;16:1090–1.
111. Holmes S, Gleeson MJ, Cawson RA. Mycobacterial disease of the parotid gland. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90:292–8.
112. Aygenc E, Albayrak L, Ensari S. Tuberculous parotitis. Inf Dis Clin Prac. 2002;11:555–7.

113. Birkent H, Karahatay S, Akcam T, Durmaz A, Ongoru O. Primary parotid tuberculosis mimicking parotid neoplasm: a case report. J Med Case Rep. 2008;2:62.
114. Senanayake SN. Mumps: a resurgent disease with protean manifestations. Med J Aust. 2008;189:456–9.
115. Kim JS, Oh JS, Kwon SH, Kim MS, Yoon YJ. Mumps presenting as a parotid abscess. J Craniofac Surg. 2016;27:233–5.
116. Rubin S, Eckhaus M, Rennick LJ, Bamford CG, Duprex WP. Molecular biology, pathogenesis and pathology of mumps virus. J Pathol. 2015;235:242–52.
117. Chiba Y, Horino K, Umetsu M, Wataya Y, Chiba S. Virus excretion and antibody response in saliva in natural mumps. Tohoku J Exp Med. 1973;111:229–38.
118. Gupta RK, Best J, MacMohan E. Mumps and the UL epidemic. BMJ. 2005;330:1132–5.
119. Sirmatel O, Yazgan P, Sirmatel F, Ozturk A, Ziylan Z. Radiological findings in a mumps case with multiple complications. Indian J Radiol Imaging. 2006;16:305–8.
120. Hviid A, Rubin S, Mühlemann K. Mumps. Lancet. 2008;371:932–44.
121. Krause CH, Molyneaux PJ, Ho-Yen DO, McIntyre P, Carman WF, Templeton KE. Comparison of mumps-IgM ELISA in acute infection. J Clin Virol. 2007;38:153–6.
122. Davis NF, McGuire BB, Mahon JA, Smyth AE, O'Malley KJ, Fitzpatrick JM. The increasing incidence of mumps orchitis: a comprehensive review. BJU Int. 2010;105(8):1060–5.
123. Hsu JL, Glaser SL. Epstein-Barr virus associated malignancies: epidemiologic patterns and etiologic implications. Crit Rev Oncol Hematol. 2000;34:27–53.
124. Lin FC, Chen PL, Tsao TY, Li CR, Jeng KC, Tsai SC. Prevalence of human papillomavirus and Epstein-Barr virus in salivary gland diseases. J Int Med Res. 2014;42:1093–101.
125. Labouyrie E, Merlio JPH, Beylot-Barry M, Delord B, Vergier B, Brossard G, Lacosta D, Beylot J, Leng B, Fleury H, Bloch B, de Mascarel A. Human immunodeficiency virus type 1 replication within cystic lymphoepithelial lesions of the salivary glands. Am J Clin Pathol. 1993;100:41–6.
126. Ihrler S, Zietz C, Riederer A, Diebold J, Lohrs U. HIV-related parotid lymphoepithelial cysts. Immunohistochemistry and 3-D reconstruction of surgical and autopsy material with special reference to formal pathogenesis. Virchows Arch. 1996;429:139–47.
127. Uccini S, Riva E, Antonelli G, D'Offizi G, Prozzo A, Angelici A, Faggioni A, Angekloni A, Torrisi MR, Gentile M, Baroni CD, Ruco LP. The benign cystic lymphoepithelial lesion of the parotid gland is a viral reservoir in HIV type 1-infected patients. AIDS Res Hum Retroviruses. 1999;15:1339–44.
128. Yen TL, Murr AH, Rabin J, Mhatre AN, Lalwani AK. Role of cytomegalovirus, Epstein-virus, and human herpes virus-8 in benign lymphoepithelial cysts of the parotid gland. Laryngoscope. 2004;114(8):1500–5.
129. Van Heerden WFP, Kraft K, Hemmer J, Swart TJP, Van Heerden MB, Van Rensburg EJ. Warthin's tumor is not an Epstein-Barr virus related disease. Anticancer Res. 1999;19:2881–4.
130. Aguirre JM, Echebarria MA, Martinez-Conde R, Rodriguez C, Burgos JJ, Rivera JM. Warthin tumor: a new hypothesis concerning its development. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85:60–3.
131. Sheen TS, Tsai CC, Ko JY, Chang YL, Hsu MM. Undifferentiated carcinoma of the major salivary glands. Cancer. 1997;80:357–63.
132. Lin H, Rao H, Saku. The significance of detecting BE virus and its products in benign and malignant lymphoepithelial lesion of the salivary. Zhonghu Bing Li Xue Za Zhi. 1997;26:225–8.
133. Islam NM, Bhattacharyya I, Cohen DM. Salivary gland pathology in HIV patients. Diagn Histopathol. 2012;18(9):366–72.
134. Kim JH. A case pf management of benign lymphoepithelial cysts of the parotid gland in patients infected with human immunodeficient virus. Kor J Otorhinolaryngol Head Neck Surg. 2015;58(6):417–20.
135. Kothari KS, Madiwale CV, Deshpande AA. Cystic lymphoepithelial lesion of the parotid as an early indicator of HIV infection. J Postgrad Med. 2009;55:135–6.
136. Sekikawa Y, Hongo I. HIV-associated benign lymphoepithelial cysts of the parotid glands confirmed by HIV-1 p24 antigen immunostaining. BMJ Case Rep. 2017; pii: bcr-2017-221869.
137. Michelow P, Dezube BJ, Pantanowitz L. Fine needle aspiration of salivary gland masses in HIV-infected patients. Diagn Cytopathol. 2012;40:684–90.
138. Chetty R. HIV-associated lymphoepithelial cysts and lesions: morphology and immunochemical study if the lymphoid cells. Histopathology. 1998;33:222–9.
139. Mandel L, Hong J. HIV-associated parotid lymphoepithelial cysts. J Am Dent Assoc. 1999;130:528–32.
140. Mhawej R, Richa T. Melkane AE benign lymphoepithelial cyst of unusual location: a case report. Acta Otolaryngol Case Rep. 2016;1(1):110–2.
141. Ahamed AS, Kannan VS, Velaven K, Sathyanarayanan GR, Roshni J, Elavarasi E. Lymphoepithelial cyst of the submandibular gland. J Pharm Bioallied Sci. 2014;6:185–7.
142. To VSH, Chan JYW, Tsang RKY, Wei W. Review of salivary gland neoplasms. ISRN Otolaryngol. 2012:872982, 6 pages.
143. Zarbo RJ. Salivary gland neoplasia: a review for the practicing pathologist. Mod Pathol. 2002;15(3):298–323.
144. Jee KJ, Persson M, Heikinheimo K. Genomic profiles and CRTC1-MAML2 fusion distinguishing dif-

ferent subtypes of mucoepidermoid carcinoma. Mod Pathol. 2013;26:213–22.
145. West RB, Kong C, Clarke N, et al. MYB expression and translocation in adenoid cystic carcinoma and other salivary gland tumor wit clinicopathological correlation. Am J Surg Pathol. 2011;35:92–9.
146. Haller F, Beig M, Will R, et al. Enhancer hijacking activates oncogenic transcription factor NR4A3 in acinic cell carcinoma of salivary glands. Nat Commun. 2019;10:368.
147. Fu H, Wang J, Wang L, Zhang Z, He Y. Pleomorphic adenoma of the salivary glands in children and adolescents. J Pediatr Surg. 2012;47:715–9.
148. Weng X, Guo F, Shen Z, Cheng B. Giant pleomorphic adenoma of the parotid gland with a history of more than forty years without malignant transformation. Int J Clin Exp Med. 2018;11(2):1033–7.
149. Rossi L, Giordani E, Fontana A, Di Cristofano C, Cavallaro G, Bagni O, Filippi L, Bianchi L, Rinaldi G, Congedi FP, Papa A, Caruso D, Verrico M, Silecchia G, Tomao S. An unusual case of spleen metastasis from carcinoma ex pleomorphic adenoma of the parotid gland. World J Surg Oncol. 2014;12:18.
150. Ismi O, Vayisoglu Y, Arpaci RB, Eti C, Puturgeli T, Gorur K, Ozcan C. Gland Surg. 2015;4(6):567–71.
151. Chen YK, Lin CC, Lai S, Chen CH, Wang WC, Lin YR, Hsue SS, Lin LM. Pleomorphic adenoma with extensive necrosis: report of two cases. Oral Dis. 2004;10(1):54–9.
152. Rout SK, Lath MK. Pleomorphic adenoma of the whole parotid gland: a rare clinical finding. J Craniofac Surg. 2013;24:2197–8.
153. Leurs JC, Wittekindt C, Streppel M, Guntinas-Lichius O. Carcinoma ex pleomorphic adenoma of the parotid gland. Study and implications for diagnostics and therapy. Acta Oncol. 2009;48(1):132–6.
154. Manucha V, Loffe OB. Metastasizing pleomorphic adenoma of the salivary gland. Arch Pathol Lab Med. 2008;132(9):1445–7.
155. Dhir P, David CM, Dhaduti KG. Pleomorphic adenoma of the parotid gland with cystic degeneration: a rare case. J Indian Acad Oral Med Radiol. 2014;26(4):450–3.
156. Maiorano E, Lo Muzio L, Favia G, Piattelli A. Warthin's tumour: a study of 78 cases with emphasis on bilaterality, multifocality and association with other malignancies. Oral Oncol. 2002;38(1):35–40.
157. Zhan KY, Khaja SF, Flack AB, Day TA. Benign parotid tumors. Otolaryngol Clin North Am. 2016;49(2):327–42.
158. Cope W, Naugler C, Taylor SM, Trites J, Hart RD, Bullock MJ. The association of warthin tumor with salivary ductal inclusions in intra and periparotid lymph nodes. Head Neck Pathol. 2014;8(1):73–6.
159. Kadletz L, Grasl S, Perisanidis C, Grasl MC, Erovic BM. Rising incidences of Warthin's tumors may be linked to obesity: a single-institutional experience. Eur Arch Otorhinolaryngol. 2019;276(4):1191–6.
160. Santucci M, Gallo O, Calzolari A, Bondi R. Detection of Epstein-Barr viral genome in tumor cells of Warthin's tumor of parotid gland. Am J Clin Pathol. 1993;100(6):662–5.
161. So T, Sahovaler A, Nichols A, Fung K, Yoo J, Weir MM, MacNeil SD. Utility of clinical features with fine needle aspiration biopsy for diagnosis of Warthin tumor. J Otolaryngol Head Neck Surg. 2019;48(1):41.
162. Kato H, Fujimoto K, Matsuo M, Mizuta K, Aoki M. Usefulness of diffusion-weighted MR imaging for differentiating between Warthin's tumor and oncocytoma of the parotid gland and oncocytoma. Jpn J Radiol. 2017;35:78.
163. Yuan WH, Hsu HC, Chou YH, Hsueh HC, Tseng TK, Tiu CM. Gray-scale and color Doppler ultrasonographic features of pleomorphic adenoma and Warthin's tumor in major salivary glands. Clin Imaging. 2009;33(5):348–53.
164. Lee DH, Yoon TM, Lee JK, Lim SC. Surgical treatment strategy in Warthin tumor of the parotid gland. Braz J Otorhinolaryngol. 2019;85(5):546–55.
165. Boukheris H, Curtis RE, Land CE, et al. Incidence of carcinoma of the major salivary glands according to the WHO classification, 1992 to 2006: a population-based study in the United States. Cancer Epidemiol Biomarkers Prev. 2009;18:2899–906.
166. Spiro RH. Salivary neoplasms: overview of a 35-year experience with 2,807 patients. Head Neck Surg. 1986;8:177–84.
167. Spitz MR, Tillet BC, Batsakis JG, Gibeau JM, Newell GR. Risk factors for major salivary gland carcinomas: a case-comparison study. Cancer. 1984;54:1854–9.
168. Noone AM, Howlader N, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA, editors. SEER cancer statistics review, 1975–2015. Bethesda, MD: National Cancer Institute; 2018. https://seer.cancer.gov/csr/1975_2015/, based on November 2017 SEER data submission, posted to the SEER web site.
169. Stodulski D, Mikaszewski B, Stankiewicz C. Signs and symptoms of parotid gland carcinoma and their prognostic value. Int J Oral Maxillofac Surg. 2012;41:801–6.
170. Wierzbicka M, Kopec T, Szyfter W, Kereiakes T, Bem G. The presence of facial nerve weakness on diagnosis of a parotid gland malignant process. Eur Arch Otorhinolaryngol. 2012;269:1177–82.
171. Boahene DK, Olsen KD, Lewis JE, Pinheiro AD, Pankratz VS, Bagniewski SM. Mucoepidermoid carcinoma of the parotid gland: the Mayo Clinic experience. Arch Otolaryngol Head Neck Surg. 2004;130:849–56.
172. Suzuki M, Ichimiya I, Matsushita F, et al. Histological features and prognosis of patients with mucoepidermoid carcinoma of the parotid gland. J Laryngol Otol. 1998;112(10):944–7.

173. Ho K, Lin H, Ann DK, Chu PG, Yen Y. An overview of the rare parotid gland cancer. Head Neck Oncol. 2011;3:40.
174. Al-Zahir N, Obeidi A, Al-Salam S, Al-Kayyali BS. Acinic cell carcinoma of the salivary glands: a literature review. Hematol Oncol Stem Cell Ther. 2009;2(1):259–64.
175. Miki H, Masuda E, Ohata S, Komaki K, Hirokawa M, Uehara H, Asano H, Monden Y. Late recurrence of acinic cell carcinoma of the parotid gland. J Med Invest. 1999;46(3–4):213–6.
176. Zhao J, Wang J, Yu C, Guo L, Wang K, Kiang Z, Lou J. Prognostic factors affecting the clinical outcomes of carcinoma ex pleomorphic adenoma in the major salivary glands. World J Surg Oncol. 2013;1191:180.
177. Olsen KD, Lewis JE. Carcinoma ex pleomorphic adenoma: a clinic pathologic review. Head Neck. 2001;23(9):705–12.
178. Hyman GA, Wilff M. Malignant lymphomas of the salivary glands. Am J Clin Pathol. 1976;65(4):421–38.
179. Batsakis JG. Pathology consultation primary lymphomas of the major salivary glands. Ann Otol Rhinol Laryngol. 1986;95(1):107–8.
180. Konofaos P, Spartalis E, Katsaronis P, Kouraklis G. Primary parotid gland lymphoma: a case report. J Med Case Rep. 2011;5:380.
181. Dispenza F, Cicero G, Mortellaro G, Marchese D, Kulamarva G, Dispenza C. Primary non-Hodgkins lymphoma of the parotid gland. Braz J Ortholaryngol. 2011;77(5):639–44.
182. Chhabra S, Bhutani N, Kumar M, Yadav S, Sen R. Primary non-Hodgkins lymphoma of the parotid gland: a common entity at an uncommon location. Acta Sci Cancer Biol. 2019;3(1):17–21.
183. Barnes L, Myers EN, Prokopakis EP. Primary malignant lymphoma of the parotid gland. Arch Otolaryngol Head Neck Surg. 1998;124:573–7.
184. Feinstein AJ, Ciarleglio MM, Cong X, Otremba MD, Judson BL. Parotid gland lymphomas: prognostic analysis of 2410 patients. Laryngoscope. 2013;123:1199–203.

9 Efficacy of Glandular Irrigation and Sialendoscopy in Salivary Glands Affected by Sjögren's Syndrome

K. Hakki Karagozoglu, Erik H. van der Meij, and Derk H. Jan Jager

9.1 Introduction

Sjögren's syndrome is a progressive disease characterized by a gradual and irreversible decrease in both the quantity and quality of saliva that eventually leads to xerostomia [1]. The etiology of Sjögren's syndrome involves a multifactorial process that is characterized by mononuclear infiltrates and IgG-producing plasma cells in the salivary and lacrimal glands [2–4]. This infiltration leads to the irreversible destruction of glandular tissue with a subsequent decrease in the saliva secretion rate. Hyposalivation can lead to dental caries, dental erosion, fungal and bacterial infections, digestive disorders, loss of taste, and difficulty in swallowing, which reduces the quality of life in patients [5] (Figs. 9.1, 9.2, and 9.3). Chronic salivary gland inflammation in Sjögren's syndrome patients ultimately leads to tissue fibrosis and lymphoma.

Stimulation of salivary flow is possible only if saliva-producing acinar cells are present and function in the glandular tissue or when the parenchyma recovers. Systemic treatment is

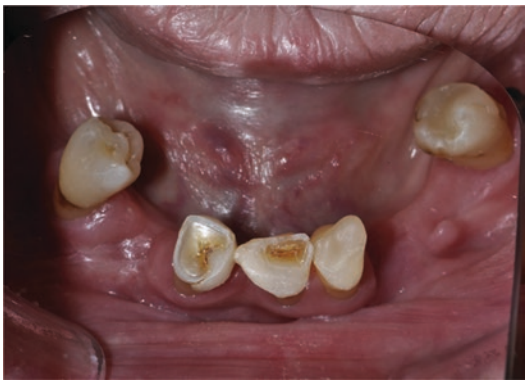

Fig. 9.1 Sjögren's syndrome patient's dentition affected by caries, demineralization, and occlusal wear

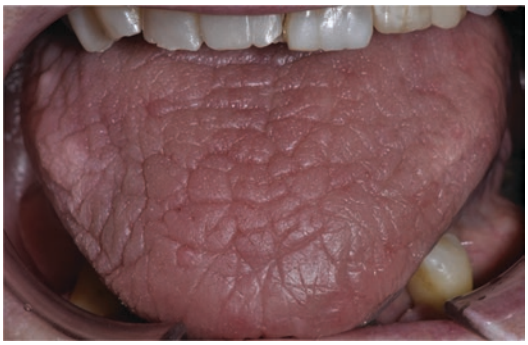

Fig. 9.2 A patient with Sjögren's syndrome, presenting severe dryness and deep tongue fissures

K. H. Karagozoglu · D. H. Jan Jager (✉)
Department of Oral and Maxillofacial Surgery and Oral Pathology, Amsterdam University Medical Center (Amsterdam UMC, Location VUmc) and Academic Centre for Dentistry Amsterdam (ACTA), Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
e-mail: d.jager@amsterdamumc.nl

E. H. van der Meij
Department of Oral and Maxillofacial Surgery, Medical Center Leeuwarden, Leeuwarden, The Netherlands

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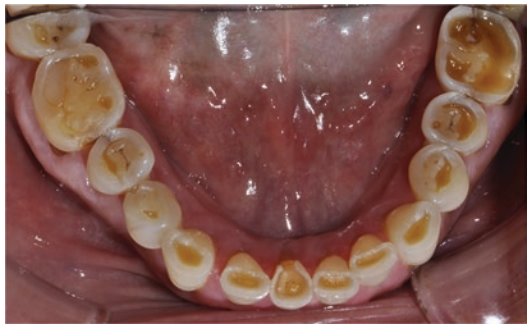

Fig. 9.3 Dentition of a patient with Sjögren's syndrome affected by dental erosion. Patients with Sjögren's syndrome lack natural protection against acids. In addition, they often consume acidic drinks with an anticipation that they can provide a relief for the dry mouth, which precipitates dental erosion

often ineffective and can result in major side effects, and no effective palliative therapy to minimize complications associated with Sjögren's syndrome is currently available [6]. At present, therapy for hyposalivation and xerostomia is limited to external hydration, artificial saliva; muscarinic receptor agonists, such as pilocarpine and cevimeline, that induce saliva secretion from residual acinar cells [7, 8]; and electrostimulation devices [9]. Until now, no ideal agent has been available to treat hyposalivation and xerostomia effectively, and consequently, there is still a need for other agents and therapeutic interventions [6]. In this chapter, we describe two techniques that are known to be effective in improving salivary flow and alleviating xerostomia in Sjögren's syndrome patients, namely, intraglandular irrigation and sialendoscopy.

9.2 Intraglandular Irrigation

Intraglandular irrigation, irrespective of the method of irrigation or irrigation fluid used, is a useful and simple method for the relief of symptoms in patients with obstructive and inflammatory disease of the salivary glands. The treatment consists of the insertion of a cannula into Stensen's or Wharton's ducts, followed by irrigation with, for example, saline or corticosteroids (Fig. 9.4). Sialography, a variant of intraglandular irrigation, is known as a useful tool for the diagnosis of obstructive and inflammatory disease of the salivary glands (Fig. 9.5).

In sialography, a contrast agent is injected intraductally after dilating the ductal orifice to image the glands radiographically. Sialography has not only been shown to be a reliable and accepted method for diagnosis, but has also shown its therapeutic value [10]. Eisdenbud and Cranin already reported in 1963 that patients with obstructive sialadenitis in the parotid and submandibular glands showed a relief of symptoms after sialography. Among patients with obstructive sialadenitis, 80% experienced reduced pain and swelling [11]. These results were explained by the therapeutic value of the irrigation procedure itself with a contrast agent during sialography [11].

Based on this outcome, several studies on the effect of intraglandular irrigation with various irrigation fluids have been conducted, such as saline, corticosteroids, and antibiotics [12, 13]. Izumi et al. suggested that the irrigation of the parotid glands with corticosteroids suspended in saline is useful in relieving xerostomia in patients with Sjögren's syndrome. In this study, 64% (18 of 28) of patients demonstrated improvement of xerostomia after four treatments of the parotid glands with a 1 mL corticosteroid solution during the period of 3 months [14]. They explained these results by the use of prednisolone sodium succinate dissolved in saline solution at a concentration of 2 mg/mL. No effect was found after rinsing with saline alone. Corticosteroids not only have anti-inflammatory effects, but also inhibit T-cell activation [14]. Salivary glands affected by Sjögren's syndrome are characterized by a focal periductal infiltrate consisting mainly of T- and B-lymphocytes [15].

More recently, it was suggested that intraglandular irrigation with saline alone may also be beneficial to relieve subjective and objective dryness of the mouth in patients with Sjögren's syndrome and other sicca patients [16]. In this study, it was reported that experienced xerostomia was improved in 81.8% of the patients, which was measured as relief of dryness on a nominal scale (yes/no). Furthermore, a significant improvement

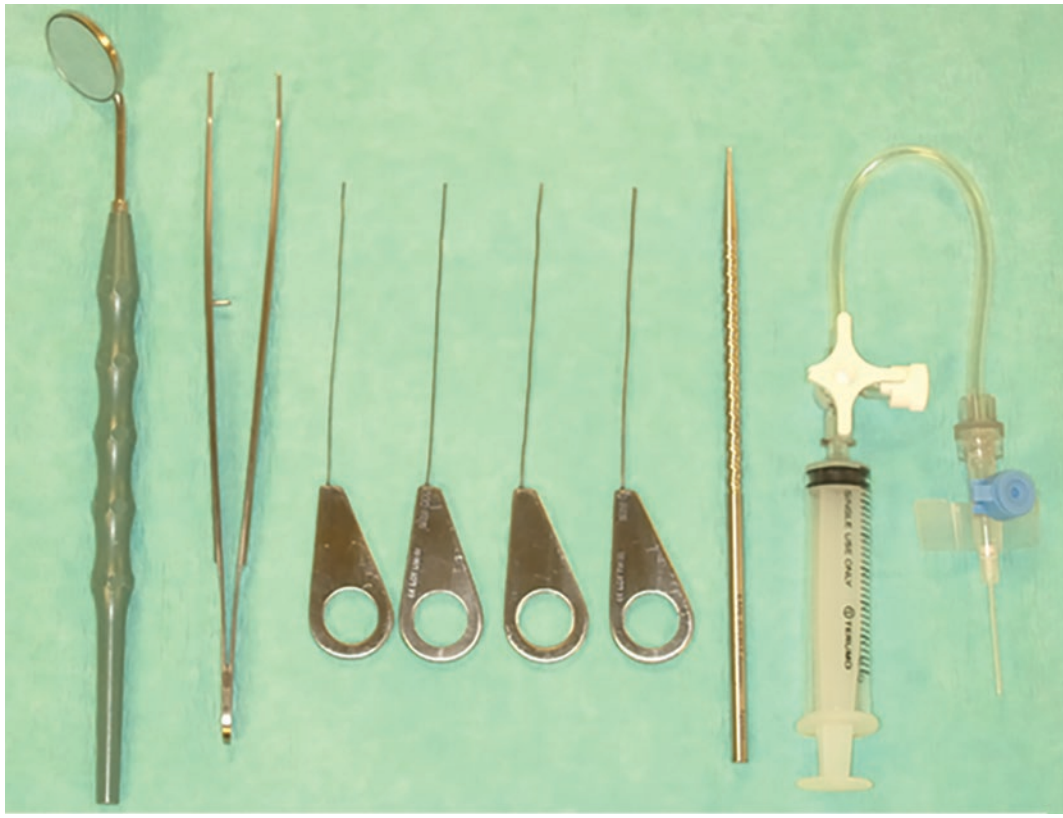

Fig. 9.4 Instruments used for the saline or corticosteroid irrigation of the major salivary glands. From left to right, dental mirror, curved forceps, salivary duct probes (size: #0000 to # 0), salivary duct dilator with tapered conical tip, and 22GA I.V. cannula connected to a 5 mL syringe

of median unstimulated whole mouth salivary secretion (UWS) was found to be from 1.40 mL/10 min (IQR:0.40–2.82) at baseline to 1.55 mL/10 min (IQR:0.40–3.90) after last treatment ($p = 0.027$).

There are several explanations for the therapeutic effect of intraglandular irrigation in Sjögren's syndrome patients. Due to an increased protein concentration in the duct, proteins may coagulate and obstruct the small intraglandular ductules. This increases the inflammatory response in the gland. The precipitated proteins also contribute to bacterial proliferation ascending from the intraoral cavity, which could cause chronic sialadenitis and sialodochitis (ductal sialadenitis) [13]. Ductal cannulation with probes and the subsequent intraglandular irrigation of the salivary gland may act to remove the blockage and reduce the concentration of proteins, microbes, and debris such as microsialoliths (small salivary stones) and mucus plugs [17, 18]. This, theoretically, could stimulate some reparative gland processes [16].

9.3 Sialendoscopy

Sialendoscopy is a minimally invasive diagnostic and interventional procedure for the management of obstructive salivary gland disorders. With this technique, endoscopes are used that are small enough to be introduced into the salivary ducts of the parotid and submandibular glands for the diagnosis and treatment of ductal pathology [19, 20]. The endoscopic approach of the major salivary glands, both diagnostic and therapeutic, was

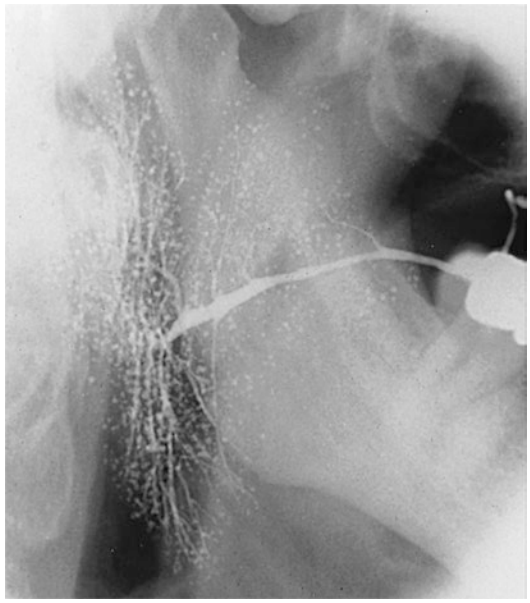

Fig. 9.5 Computed tomography (CT)-sialography of the parotid gland in an Sjögren's syndrome patient, displaying the typical features of sialectasis ("snowstorm appearance")

first described in 1990 by Dr. Philippe Katz and has become the preferred approach in the management of obstructive salivary gland disorders in the past decades [21, 22]. It allows diagnosis and treatment during the same procedure. Furthermore, sialendoscopy allows the delivery of medication for the treatment of glandular or ductal inflammation, such as corticosteroids or antibiotics [23–25].

Over the years, the development and improvement of miniaturized semirigid endoscopes and instruments have enabled the exploration of the ductal system of the parotid and submandibular glands and expanded the therapeutic possibilities. Sialendoscopy differs from ductal irrigation because it allows direct visual inspection of the ductal system during treatment and is useful in identifying and treating ductal obstructions during the procedure. It has shown its value in identifying ductal obstructions that may not be detected by imaging techniques and may, therefore, be superior to other imaging techniques for obstructive ductal pathologies. Radiolucent stones, stenoses, strictures, polyps, mucosal plugs, and foreign bodies missed by other imaging techniques can be visualized by this technique [26]. In complicated cases, sialendoscopy can be combined with other surgical interventions for the major salivary glands, such as the endoscopic-assisted transcutaneous removal of stones (Fig. 9.6).

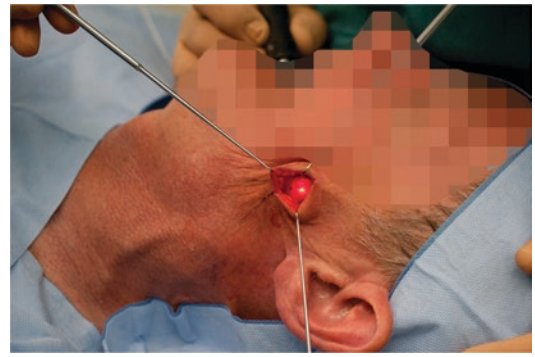

Fig. 9.6 Mini-incision above the light of the sialendoscope. The light of the sialendoscope facilitates accurate localization of the stone. Sialendoscopically assisted stone removal with preauricular mini-incision is a highly successful technique for removal of stones with minimal complications. This technique is recommended as a suitable alternative to minimally invasive treatment that saves the patient from parotidectomy. The only condition for success is the ability to reach the stone with the endoscope [27]

The introduction of this gland-sparing technique has resulted in a decreased number of excised salivary glands by 74.3 and 67.2% for submandibular and parotid glands, respectively, and related complications, such as scar formation and nerve injury [22].

Contraindications for sialendoscopy are acute suppurative sialadenitis and several anatomical conditions such as severe ductal stenosis and stones that are fixed in the duct or gland [28]. Over the years, the indications for sialendoscopy have expanded. Other indications for sialendoscopy include recurrent juvenile parotitis [29], adult chronic recurrent parotitis without sialolithiasis (salivary stone) [24], and radioiodine-induced sialadenitis and xerostomia [30]. Currently, sialendoscopy is utilized for the salivary glands affected by Sjögren's syndrome [23].

9.3.1 Instruments Used in Sialendoscopy

Sialendoscopes are available with a range of diameters. The choice of size depends on the expected diameter of the salivary duct and the nature of the procedure to be performed, namely, diagnostic or interventional. Diagnostic sialendoscopes have smaller diameters because they have an optic fiber and an irrigation channel. Interventional sialendoscopes have larger diameters because they also have a working channel enabling the insertion of instruments, such as baskets, forceps, and balloon catheters, to perform intraductal interventions (Fig. 9.7).

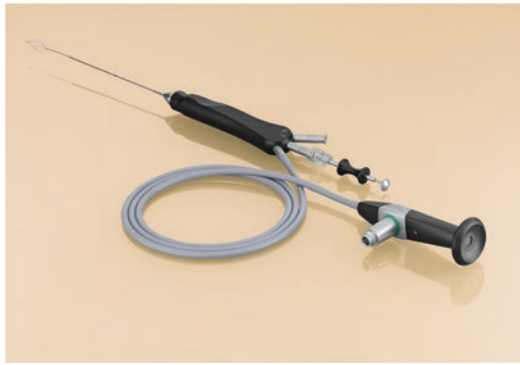

Fig. 9.7 Interventional sialendoscope with the dormia basket inserted in the working channel to perform intraductal stone extraction. (Courtesy Karl Storz SE & Co. KG)

9.3.2 Procedure

Sialendoscopy can be performed both under general anesthesia in a clinical setting and under local anesthesia in an outpatient setting [31–35]. Prerequisites for successful sialendoscopy are careful preoperative selection of patients and salivary glands, including comprehensive history, clinical examination, and preoperative imaging. It is important to have favorable anatomical conditions to perform sialendoscopy. The most challenging step in sialendoscopy is the identification and preparation of the papilla. Compared to the parotid papilla, the submandibular papilla is more difficult to identify because it is highly variable in shape, direction, and situation. The visibility and accessibility of the submandibular papilla depends on, if present, the position of the lower teeth and the depth of the floor of the mouth [28]. After the identification of the papilla, serial dilatation should be performed to enable the insertion of the sialendoscope in the ductal system and to have good access and enough space for manipulation (Fig. 9.8a, b).

The ductal system is usually in a collapsed condition. Therefore, continuous ductal irrigation through the irrigation channel is necessary to open and dilate the ductal system for atraumatic introduction of the sialendoscope. For irrigation, a clear fluid, usually saline, is used to enable optimal vision. If necessary, ductal debris can be rinsed out, and if indicated, drugs (corticoste-

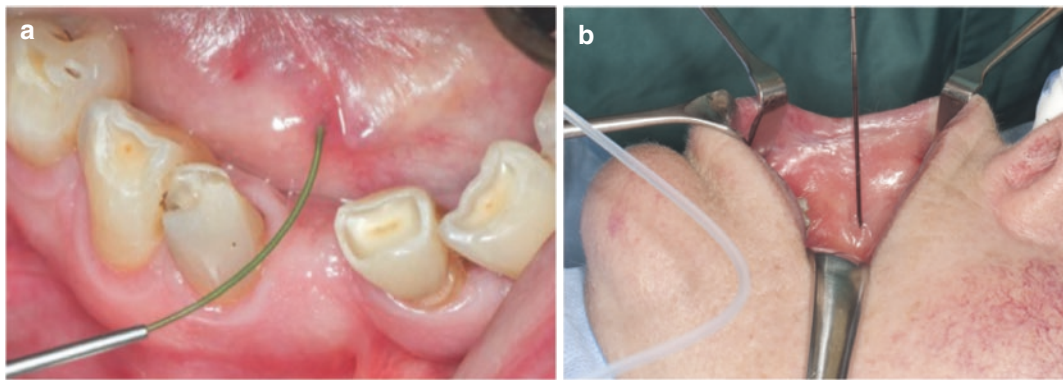

Fig. 9.8 (**a**) Approach of the orifice of Wharton's duct with the guided wire technique and hollow bougies. (**b**) Approach of the orifice of Stensen's duct

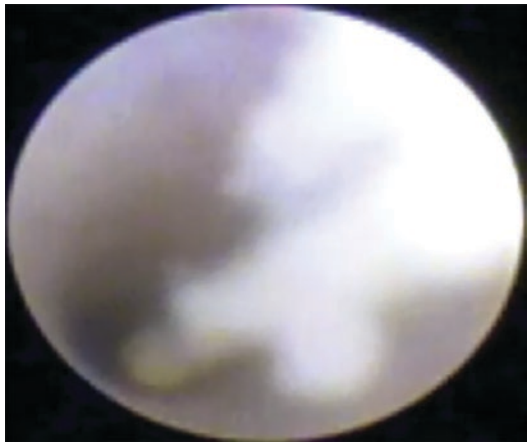

Fig. 9.9 Intraoperative appearance of a mucus plug (white area) in a parotid duct of a patient with Sjögren's syndrome

roids, antibiotics) can be instilled intraductally (Fig. 9.9) [24, 25, 36, 37].

9.3.3 Sialendoscopy in Sjögren's Syndrome Patients

Shacham et al. were the first to report that sialendoscopy in patients with Sjögren's syndrome, and systemic lupus erythematosus was able to restore salivary function and prevent recurrent infections of the salivary glands [38]. In most cases, the endoscopic examination revealed a minimal vascular supply in the lining mucosa of the ductal system, mucus plugs, and strictures. It was suggested that the major cause of recurrent sialadenitis in patients with these autoimmune diseases was stricture formation and that removal of these strictures under direct vision using sialendoscopes and thorough rinsing alleviated symptoms and improved salivary flow [38].

The presence of these strictures in the salivary glands of patients with Sjögren's syndrome has also been reported in a study by De Luca et al. [39]. In this study, it was found that strictures were present in 45% (38 of 85) and mucus plugs in 55% (47 of 85) of the glands of Sjögren's syndrome patients. In comparison to sialendoscopies performed for other etiological factors of sialadenitis, they reported more strictures, mucus plugs, mucus plugs and strictures together, kinks, ductal chromatic change from pink to white in the stricture areas, a large accumulation of mucous fibrinous material, and a thickened stiff ductal epithelium with the loss of the typical circular ridges in patients with Sjögren's syndrome [39].

The positive effects of sialendoscopy of the major salivary glands in patients with Sjögren's syndrome were also found in our studies. In a pilot study by Jager et al., a substantial effect on the dryness domain of the European League Against Rheumatism (EULAR) Sjögren's Syndrome Patient Reported Index (ESSPRI) score and an increase in the salivary MUC5B concentration was found after sialendoscopy [40]. These positive effects were also seen on the xerostomia inventory (XI) and clinical oral dryness score (CODS), but not on salivary flow rates.

In a randomized and controlled trial, Karagozoglu et al. demonstrated that sialendoscopy reduces oral dryness objectively and subjectively up to 6 months after treatment [23]. In this study, the major salivary glands of 51 patients were either treated with saline or a saline/steroid solution (40 mg/mL triamcinolone acetonide (TA) in 5 mL saline). The percentage of patients in whom improvement in salivation was observed after 24 weeks was 87.5% for UWS and 75% for stimulated whole mouth salivary secretion (SWS) in the saline group. In the steroid group, 72.2% of the participants experienced an increase in UWS and 61.1% in SWS. UWS was improved more in the saline group and SWS more in the steroid group (Fig. 9.10a, b). An explanation for the smaller increase of UWS in the steroid group could be that fewer submandibular glands were accessible and could have been irrigated compared with the saline group (38.9% vs. 56.3%).

Sialendoscopy also reduced xerostomia as measured with the XI (Fig. 9.11). Over time, xerostomia was reduced more in the saline group compared to the steroid group. This could be associated with the larger increase in UWS in the saline group.

Since the salivary gland duct directly connects to the gland, it could represent an effective route to deliver medications to the gland. Corticosteroid

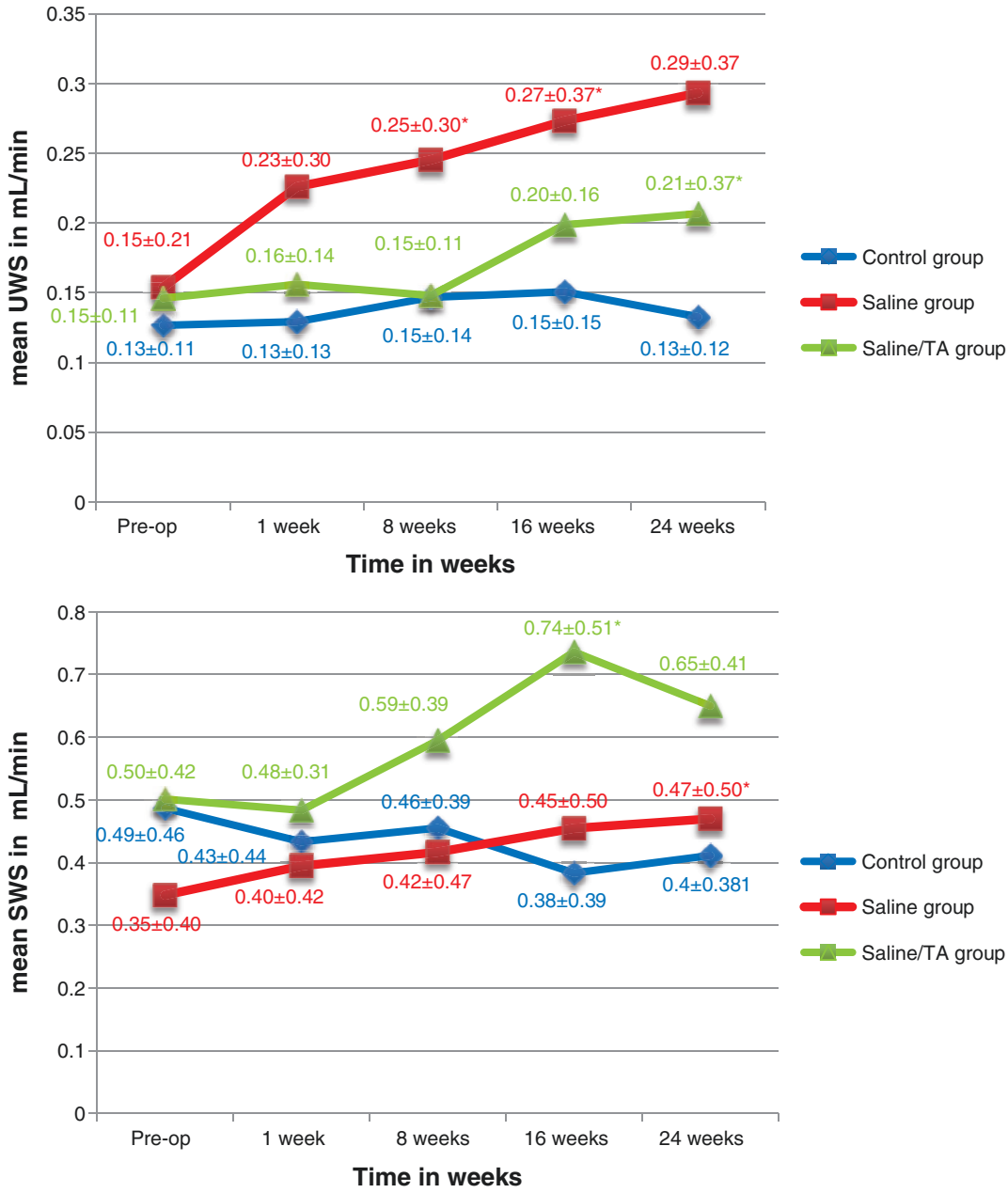

Fig. 9.10 Effect of sialendoscopy on unstimulated whole mouth salivary secretion (**a**) and stimulated whole mouth salivary secretion (**b**) up to 24 weeks after a one-time treatment of the parotid and submandibular glands. The values indicate mean ± SD. Asterisks indicate the values that significantly differ from the baseline secretion within the group ($p < 0.05$)

irrigation treatment has been shown to be effective in improving the salivary flow rate in patients with Sjögren's syndrome compared with irrigation using saline solution alone [14]. Capaccio et al. confirmed that interventional sialendoscopy with corticosteroids significantly reduced the number of painful episodes of sialadenitis and improved the subjective sensation of oral dryness

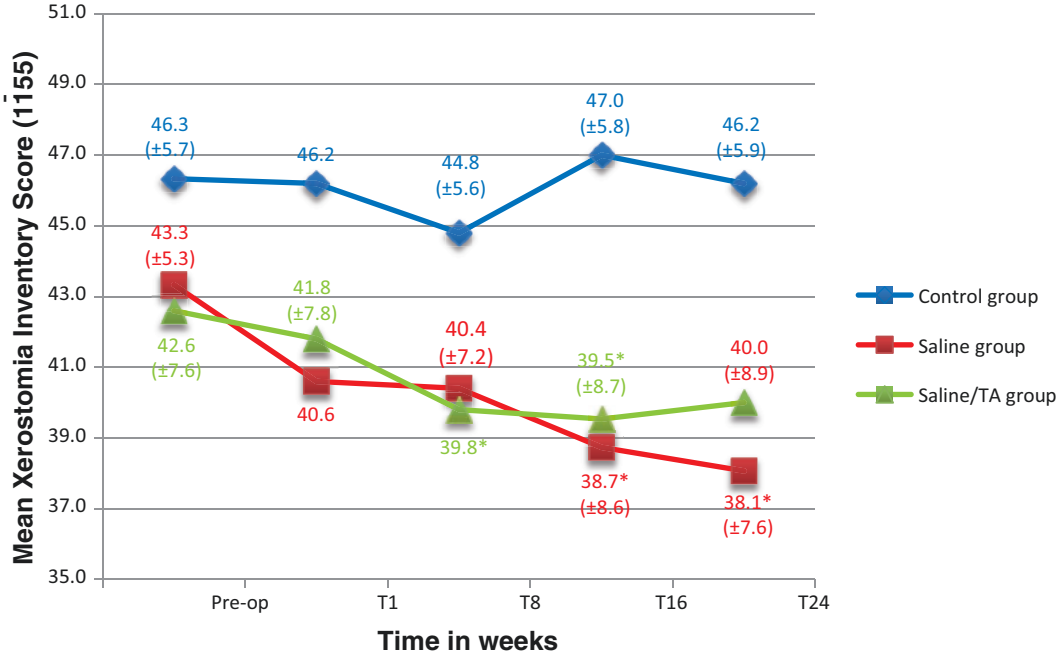

Fig. 9.11 Effect of sialendoscopy on xerostomia up to 24 weeks after treatment. Xerostomia is measured using the xerostomia inventory (mean ± SD) [41]. A lower score indicates that xerostomia is reduced. Please note that a minimal important difference for alleviation of symptoms is not yet determined for the xerostomia inventory [43]. The values marked with an asterisk differ significantly from the baseline value ($p < 0.05$)

and other disease symptoms in patients with Sjögren's syndrome [42]. The study results also suggest that the improvement in experienced xerostomia and pain, measured using the ESSPRI score, is greater when interventional sialendoscopy is combined with a cycle of six outpatient steroid ductal irrigations (one in every 3 weeks) compared to a one-time treatment (3.0 ± 0.6 vs. 4.0 ± 0.8, respectively; $p = 0.003$). XI score also improved more after a multicycle treatment, but this difference was not significant (10.6 points vs. 5.1 points, respectively).

The relief in subjective and objective dryness could be explained by the mechanical procedure, which theoretically could stimulate some reparative gland processes. The dilatation prior to the endoscopic procedure may open ductal strictures and remove debris such as microsialoliths and mucus plugs [13]. In patients with Sjogren's syndrome and other autoimmune diseases, stricture formation could be a frequent cause of salivary duct obstruction and recurrent sialadenitis [12, 17]. In the literature, it is suggested that stricture removal may improve salivary flow [12, 30] (Fig. 9.12a, b).

Additionally, Aframian et al. suggested that glandular cells may experience obstructed signaling pathways for secretion and impaired vascularization [16]. In their recent publication, possible beneficial effects of ductal irrigation through sialendoscopic treatments were explained. For example, the combination of dilatation together with retrograde sialendoscopy as a mechanical maneuver may induce stress conditioning. Based on animal models, it is suggested that the exposure of salivary glands to injuries or short heat stress resulted in the propagation of salivary gland stem cell capabilities due to cellular plasticity in the glands' parenchyma. This could promote salivary gland repair [16, 44–46].

Improvement in salivary flow is only possible if saliva-producing acinar cells are present and function in the glandular tissue or when the parenchyma recovers. Therefore, severity of the dis-

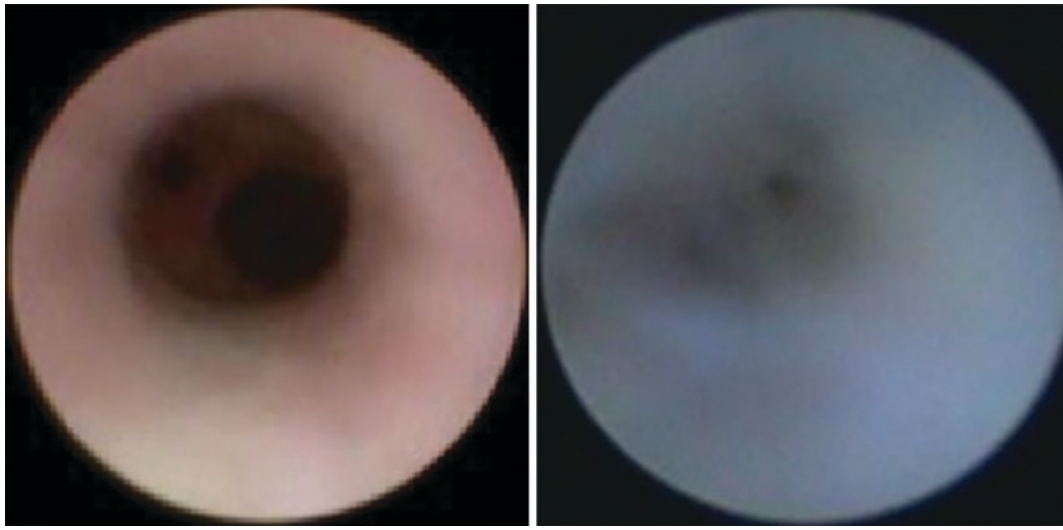

Fig. 9.12 Sialendoscopic imaging of the parotid duct. Intraoperative appearance of a normal duct (**a**) and an inflamed parotid duct that displays characteristic findings of narrow and pale passage with severe ductal stenosis (**b**)

ease, the baseline level of stimulated salivary flow, and the response of the glands to a stimulus are expected to have a significant impact on the success of this treatment. Although sialendoscopy offers new treatment possibilities, sialendoscopy in patients with Sjögren's syndrome is more complicated than other indications for sialendoscopy. Ductal orifices, especially Wharton's duct, can be harder to identify because of atrophic changes in the salivary glands, including the papilla. In patients with Sjögren's syndrome, partial or complete stenosis is a common feature caused by intraductal scar stricture formation as a result of recurrent (ascending) infections impeding sialendoscopy or ductal irrigation [47].

9.4 Conclusion

Although Sjögren's syndrome itself cannot be cured by ductal irrigation or sialendoscopy, these procedures have been shown to be a relatively simple, harmless, and palliative technique for improving xerostomia and hyposalivation. In future studies, it would be interesting to follow up patients for a longer period of time (i.e., >24 weeks) to determine the long-term sustainability of the treatment efficacy on salivary flow rates. Also, the success of retreatments would be important to investigate. Furthermore, future studies are planned for intraoperative evaluation of the therapeutic efficacy such as occlusions and saliva flow, intraoperative assessment of the delivery and penetration depth of medication in the ductal system and parenchyma, and targeted drug delivery.

References

1. Pijpe J, Kalk WWI, Bootsma H, Spijkervet FKL, Kallenberg CGM, Vissink A. Progression of salivary gland dysfunction in patients with Sjogren's syndrome. Ann Rheum Dis. 2006;66(1):107–12.
2. Mariette X, Criswell LA. Primary Sjögren's syndrome. N Engl J Med. 2018;378(10):931–9.
3. Daniels TE, Fox PC. Salivary and oral components of Sjögren's syndrome. Rheum Dis Clin N Am. 1992;18(3):571–89.
4. Fox RI. Sjögren's syndrome. Lancet. 2004;366(9482):321–31.
5. Vissink A, Bootsma H, Kroese FGM, Kallenberg CGM. How to assess treatment efficacy in Sjögren's syndrome? Curr Opin Rheumatol. 2012;24(3):281–9.
6. Ramos-Casals M, Tzioufas AG, Stone JH, Sisó A, Bosch X. Treatment of primary Sjögren syndrome: a systematic review. JAMA. 2010 Jul;304(4):452–60.
7. Aframian DJ, Helcer M, Livni D, Markitziu A. Pilocarpine for the treatment of salivary glands'

impairment caused by radioiodine therapy for thyroid cancer. Oral Dis. 2006;12(3):297–300.
8. Mercadante V, Al Hamad A, Lodi G, Porter S, Fedele S. Interventions for the management of radiotherapy-induced xerostomia and hyposalivation: a systematic review and meta-analysis. Oral Oncol. 2017;66:64–74.
9. Strietzel FP, Lafaurie GI, Mendoza GRB, Alajbeg I, Pejda S, Vuletić L, et al. Efficacy and safety of an intraoral electrostimulation device for xerostomia relief: a multicenter, randomized trial. Arthritis Rheum. 2011;63(1):180–90.
10. Yousem DM, Kraut MA, Chalian AA. Major salivary gland imaging. Radiology. 2000;216(1):19–29.
11. Eisenbud L, Cranin N. The role of sialography in the diagnosis and therapy of chronic obstructive sialadenitis. Oral Surg Oral Med Oral Pathol. 1963;16(10):1181–99.
12. Quinn JH, Graham R. Recurrent suppurative parotitis treated by intraductal antibiotics. J Oral Surg. 1973;31(1):36–9.
13. Lee C, Kim J-E, Huh K-H, Yi W-J, Heo M-S, Lee S-S, et al. Therapeutic effect of intraductal irrigation of the salivary gland: a technical report. Imaging Sci Dent. 2017;47(2):123.
14. Izumi M, Eguchi K, Nakamura H, Takagi Y, Kawabe Y, Nakamura T. Corticosteroid irrigation of parotid gland for treatment of xerostomia in patients with Sjögren's syndrome. Ann Rheum Dis. 1998;57(8):464–9.
15. Kroese FG, Abdulahad WH, Haacke E, Bos NA, Vissink A, Bootsma H. B-cell hyperactivity in primary Sjögren's syndrome. Expert Rev Clin Immunol. 2014;10(4):483–99.
16. Aframian DJ, Baaton S, Mazor S, Nadler C, Keshet N, Haviv Y, et al. Improvement of dry mouth following intraductal irrigation of salivary glands. Oral Dis. 2019;25(7):1735–43.
17. Baurmash HD. Chronic recurrent parotitis: a closer look at its origin, diagnosis, and management. J Oral Maxillofac Surg. 2004;62(8):1010–8.
18. Harrison JD, Epivatianos A, Bhatia SN. Role of microliths in the aetiology of chronic submandibular sialadenitis: a clinicopathological investigation of 154 cases. Histopathology. 1997;31(3):237–51.
19. Steck JH, Stabenow E, Volpi EM, Vasconcelos ECG. The learning progression of diagnostic sialendoscopy. Braz J Otorhinolaryngol. 2016;82(2):170–6.
20. Nahlieli O, Baruchin AM. Long-term experience with endoscopic diagnosis and treatment of salivary gland inflammatory diseases. Laryngoscope. 2000;110(6):988–93.
21. Katz P. [New method of examination of the salivary glands: the fiberscope]. Inf Dent. 1990;72(10):785–6 [article in French].
22. Rasmussen ER, Lykke E, Wagner N, Nielsen T, Waersted S, Arndal H. The introduction of sialendoscopy has significantly contributed to a decreased number of excised salivary glands in Denmark. Eur Arch Otorhinolaryngol. 2016;273(8):2223–30.
23. Karagozoglu KH, Vissink A, Forouzanfar T, Brand HS, Maarse F, Jager DHJ. Sialendoscopy enhances salivary gland function in Sjögren's syndrome: a 6-month follow-up, randomised and controlled, single blind study. Ann Rheum Dis. 2018;77(7):1025–31.
24. Jokela J, Haapaniemi A, Mäkitie A, Saarinen R. Sialendoscopy in treatment of adult chronic recurrent parotitis without sialolithiasis. Eur Arch Otorhinolaryngol. 2018;275(3):775–81.
25. Gallo A, Martellucci S, Fusconi M, Pagliuca G, Greco A, De Virgilio A, et al. Sialendoscopic management of autoimmune sialadenitis: a review of literature. Acta Otorhinolaryngol Ital. 2017;37(2):148–54.
26. Meyer A, Delas B, Hibon R, Faure F, Dehesdin D, Choussy O. Sialendoscopy: a new diagnostic and therapeutic tool. Eur Ann Otorhinolaryngol Head Neck Dis. 2013;130(2):61–5.
27. Rotnágl J, Zavázalová Š, Vorobiov O, Astl J. Sialendoscopy and combined minimally invasive treatment for large parotid stones. Biomed Res Int. 2016;2016:1354202.
28. Marchal F. The endoscopic approach to salivary gland ductal pathologies. Tuttlingen: Endo-Publishing; 2003.
29. Capaccio P, Canzi P, Gaffuri M, Occhini A, Benazzo M, Ottaviani F, et al. Modern management of paediatric obstructive salivary disorders: long-term clinical experience. Acta Otorhinolaryngol Ital. 2017;37(2):160.
30. Bhayani MK, Acharya V, Kongkiatkamon S, Farah S, Roberts DB, Sterba J, et al. Sialendoscopy for patients with radioiodine-induced sialadenitis and xerostomia. Thyroid. 2015;25(7):834–8.
31. Coniglio AJ, Deal AM, Bhate O, Hackman TG. In-office versus operating room sialendoscopy: comparison of outcomes, patient time burden, and charge analysis. Otolaryngol Head Neck Surg. 2019;160(2):255–60.
32. Bawazeer N, Carvalho J, Djennaoui I, Charpiot A. Sialendoscopy under conscious sedation versus general anesthesia. A comparative study. Am J Otolaryngol. 2018;39(6):754–8.
33. Jokela J, Haapaniemi A, Mäkitie A, Saarinen R. Sialendoscopy under local anaesthesia. Acta Otolaryngol. 2017;137(3):310–4.
34. Luers JC, Stenner M, Schinke M, Helmstaedter V, Beutner D. Tolerability of sialendoscopy under local anesthesia. Ann Otol Rhinol Laryngol. 2012;121(4):269–74.
35. Konstantinidis I, Chatziavramidis A, Tsakiropoulou E, Malliari H, Constantinidis J. Pediatric sialendoscopy under local anesthesia: limitations and potentials. Int J Pediatr Otorhinolaryngol. 2011;75(2):245–9.
36. Guo Y-F, Sun N-N, Wu C-B, Xue L, Zhou Q. Sialendoscopy-assisted treatment for chronic obstructive parotitis related to Sjogren syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;123(3):305–9.
37. Erkul E, Gillespie MB. Sialendoscopy for non-stone disorders: the current evidence. Laryngoscope Investig Otolaryngol. 2016;1(5):140–5.

38. Shacham R, Puterman MB, Ohana N, Nahlieli O. Endoscopic treatment of salivary glands affected by autoimmune diseases. J Oral Maxillofac Surg. 2011;69(2):476–81.
39. De Luca R, Trodella M, Vicidomini A, Colella G, Tartaro G. Endoscopic management of salivary gland obstructive diseases in patients with Sjögren's syndrome. J Cranio-Maxillofacial Surg. 2015;43(8):1643–9.
40. Jager DJ, Karagozoglu KH, Maarse F, Brand HS, Forouzanfar T. Sialendoscopy of salivary glands affected by Sjögren syndrome: a randomized controlled pilot study. J Oral Maxillofac Surg. 2016;74:1167–74.
41. Thomson WM, Chalmers JM, Spencer AJ, Williams SM. The Xerostomia Inventory: a multi-item approach to measuring dry mouth. Community Dent Health. 1999;16(1):12–7.
42. Capaccio P, Canzi P, Torretta S, Rossi V, Benazzo M, Bossi A, et al. Combined interventional sialendoscopy and intraductal steroid therapy for recurrent sialadenitis in Sjögren's syndrome: results of a pilot monocentric trial. Clin Otolaryngol. 2018;43(1):96–102.
43. Thomson WM. Measuring change in dry-mouth symptoms over time using the Xerostomia Inventory. Gerodontology. 2007 Mar;24(1):30–5. https://doi.org/10.1111/j.1741-2358.2007.00137.x.
44. David R, Shai E, Aframian DJ, Palmon A. Isolation and cultivation of integrin alpha(6)beta(1)-expressing salivary gland graft cells: a model for use with an artificial salivary gland. Tissue Eng Part A. 2008;14(2):331–7.
45. Okumura K, Nakamura K, Hisatomi Y, Nagano K, Tanaka Y, Terada K, et al. Salivary gland progenitor cells induced by duct ligation differentiate into hepatic and pancreatic lineages. Hepatology. 2003;38(1):104–13.
46. Weng P-L, Aure MH, Maruyama T, Ovitt CE. Limited regeneration of adult salivary glands after severe injury involves cellular plasticity. Cell Rep. 2018;24(6):1464–1470.e3.
47. Karagozoglu KH, De Visscher JG, Forouzanfar T, van der Meij EH, Jager DJ. Complications of sialendoscopy in patients with Sjögren syndrome. J Oral Maxillofac Surg. 2017;75(5):978–83.

10 Salivary Gland Ultrasonography for Primary Sjögren's Syndrome and Juvenile Sjögren's Syndrome

Akaluck Thatayatikom and Sthorn Thatayatikom

10.1 Introduction

Ultrasonography has become widely utilized as a point-of-care diagnostic tool in clinical practice because of its noninvasive and nonirradiating nature, affordable equipment, simple maneuver, high-resolution image, and rescanning at ease in any setting. Anatomical characteristics of major salivary glands (SGs) are well suited to ultrasonography since parotid glands (PGs) and submandibular glands (SMGs) are superficially located with unique echogenicity and vascularity. Understanding normal and abnormal echostructures of major SGs is an essential requirement for salivary gland ultrasonography (SGUS).

The first report of SGUS appeared in the late 1960s [1]. A homogenous pattern and high reflectivity related to multiple, evenly distributed fibrous septa in the normal SGs were described in 1970 [2]. In the 1980s, a B-mode scanning technique described a heterogeneous finding with tiny liquid-like structures, volumetric atrophy, and decreased echogenicity in primary Sjögren's syndrome (pSS) [3, 4]. Homogeneity distinguished SGUS findings of normal subjects from the adults with pSS reported in the 1990s [5]. Similar sonographic changes in juvenile Sjögren's syndrome (jSS) in pediatric patients were reported [6, 7].

Several semiquantitative scoring systems for diagnosing pSS were developed in the past two decades. Sublingual glands (SLGs), the smallest salivary gland of the three pairs of major SGs, were not included in any scoring system since SLGs are too small and varied in size for a reliable evaluation. The scoring systems' variation with different pathological definitions and inconsistency in interpreting SGUS findings yielded diverse diagnostic accuracy [8, 9]. Recently, normal and abnormal PG and SMG features in pSS were defined and validated [10]. A new standardized semiquantitative score was also developed [11] to overcome the concerns of the variation. Implementing SGUS scoring systems as one of the 2016 ACR/EULAR classification criteria for pSS increased the criteria's sensitivity [12–14].

10.2 Normal Salivary Gland Sonographic Anatomy

10.2.1 Parotid Glands (PGs)

PGs are the largest SGs located in the parotid space. The parotid space formed by the superfi-

A. Thatayatikom (✉)
Division of Pediatric Allergy/Immunology/Rheumatology, Department of Pediatrics, University of Florida Shands Children's Hospital, Gainesville, FL, USA
e-mail: atst@ufl.edu

S. Thatayatikom
Division of Pediatric Allergy, Immunology and Rheumatology, Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL, USA

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cial layer of the deep cervical fascia occupies the space lying below the external auditory canal and the mastoid process's apex and above the mandible angle. The parotid space is lateral to the parapharyngeal space and the carotid space, medial to the superficial space and the subcutaneous tissue, anterior to the carotid space, superior to the submandibular space, and posterior to the masticator space [15]. The PG fills approximately two-thirds of the parotid space. Other structures in the space are Stenson's duct, intraparotid lymph nodes, external carotid artery, retromandibular vein, facial nerve, and auriculotemporal branches of the mandibular division of the trigeminal nerve. Three structures, including the external carotid artery, retromandibular vein, and facial nerve, run parallel, in which the vein is in the middle.

The PG is anatomically divided by the intraparotid facial nerve into two-thirds or superficial lobe laterally to the nerve and one-third or deep lobe medially. The superficial lobe is the portion that overlies the preauricular region, ramus of the mandible, masseter muscle, and posterior mandibular surface. The gland extends superiorly to the zygomatic arch, inferiorly to the sternocleidomastoid muscle (SCM), and posteriorly to the superior border of SCM and mastoid tip (Fig. 10.1). The deep lobe is a small portion behind and deep to the mandibular ramus and lies within the parapharyngeal space [16]. The average gland dimensions are 5.8 and 3.4 cm in the craniocaudal and ventrodorsal axes [17]. The size of the gland is smaller in children and women. There is a high density of periglandular and intraglandular lymph nodes. The intraglandular lymph nodes are unique because of the parotid's late encapsulation during embryogenesis. Approximately 90% of the nodes are periglandular, located between the glandular tissue and capsule [16]. Most of the intraglandular lymph nodes are mainly situated within the upper and lower poles of the glands.

The normal PG parenchyma appears uniformly echotexture (Fig. 10.1b, d) comparable to normal thyroid parenchyma with a clear demarcation between the gland and the overlying tissue [11]. The parotid appears more echogenic than the surrounding muscles but less echogenic than the mandible ramus or mastoid process. In general, the echogenicity of the parenchyma depends on the amount of intraglandular fatty tissues. The gland in children appears smaller and less echogenic (darker) (Fig. 10.1e, f) than the gland in adults due to less fatty infiltration. The superficial lobe is more accessible to visualize than the deep lobe. Since the mandible and the mastoid process block the ultrasound beam, the deep lobe is partially visualized. During the scanning, the masseter muscle and mandible limit the image anteriorly; and mastoid and SCM limit the image posteriorly. Intraglandular lymph nodes are visualized as small, well-defined, and oval-shaped areas of homogenous, hypoechoic cortex with a visible hyperechoic (fatty) hilum (Fig. 10.2a) and positive Doppler's signal (Fig. 10.2b). The unique hyperechoic hilum differentiates the node from other parotid masses. The intraglandular lymph node's short axis in a normal state is usually less than 5–6 mm. The normal Stenson's duct is not visible, except in slim individuals [11]. The duct becomes visualized if it is obstructed. A Doppler ultrasound quickly identifies the two blood vessels, although in some cases, the vessels are barely visible or not visible because of the high-fat content in the gland. The retromandibular vein is typically visualized as a relatively straight vessel near the center of the parotid parenchymal. The external carotid artery is visualized as a deeper vessel in Fig. 10.1e, f. The intraglandular facial nerve is not visible.

10.2.2 Submandibular Glands (SMGs)

SMGs are the second-largest SG and occupy most of the submandibular space, a potential space corresponding to the submandibular triangle. The space is formed by the superficial layer of the deep cervical fascia and is located deep to the platysma muscle and delineated by the anterior and posterior bellies of the digastric muscle and the mandible. The anterior and floor of the space is the mylohyoid muscle, and the posterior of the space is the hyoglossus muscle. The SMG may connect to the PG since it lies

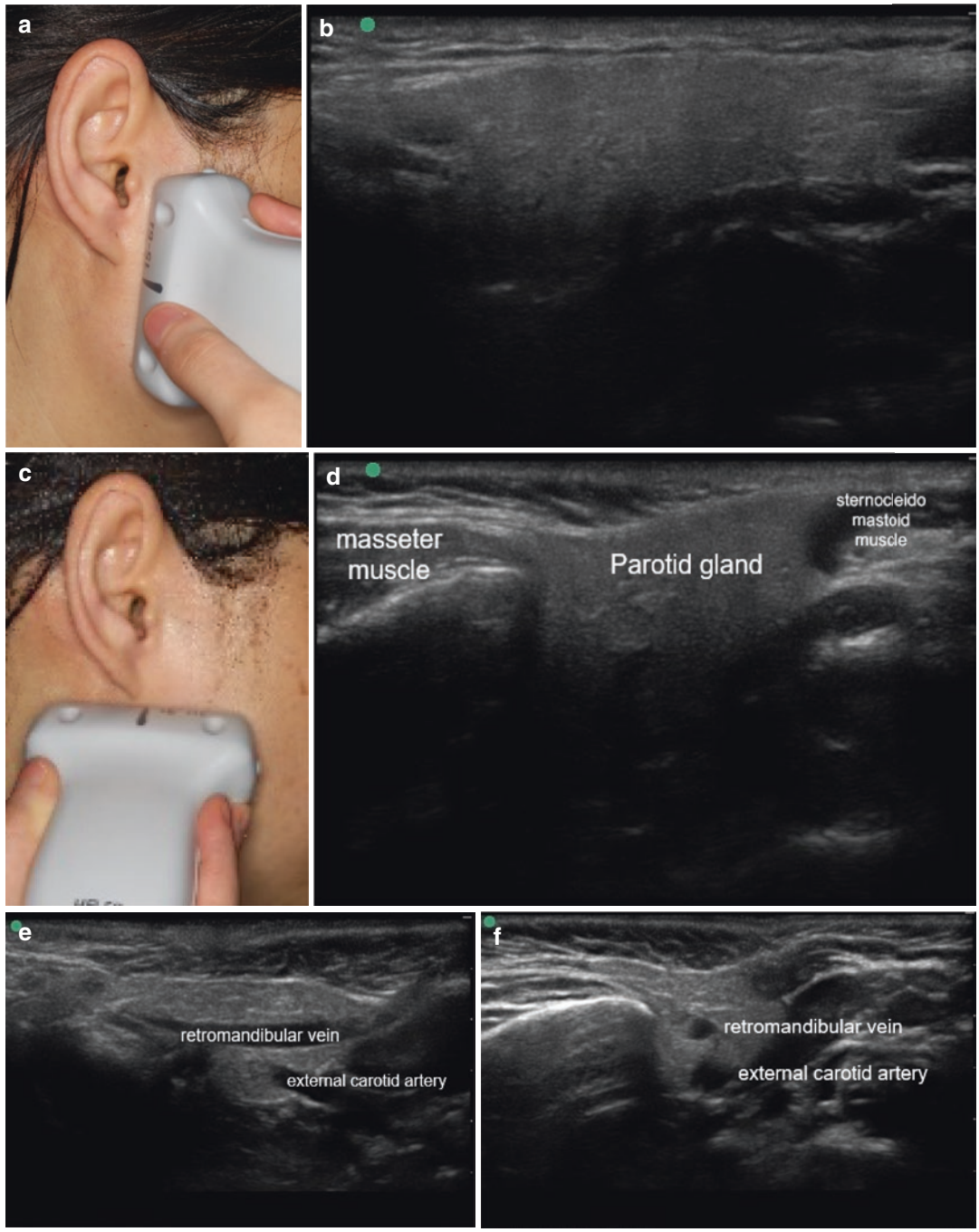

Fig. 10.1 Scanning of the normal parotid gland of a pediatric patient in the longitudinal (**a**, **b**, **e**) and transverse (**c**, **d**, **f**) planes. Children have smaller and slightly darker (less echogenic) gland than adults

anteriorly to the parotid space. The gland is folded around the mylohyoid muscle's dorsal free edge, which divides the gland to be superficial and deep lobes. Submandibular space structures include the superficial lobe of the SMG, submandibular and submental lymph nodes,

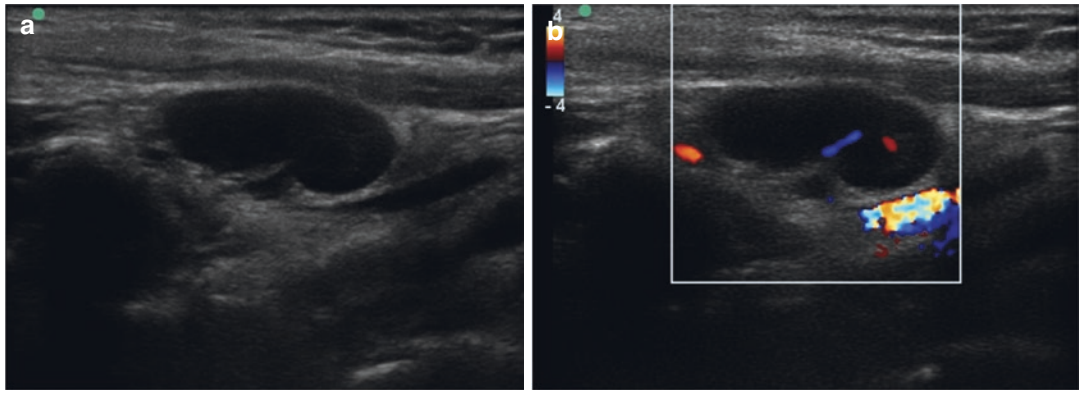

Fig. 10.2 The characteristics of the lymph node with hyperechoic hilum (**a**) and positive color Doppler's signal (**b**) visualized in a pediatric patient

facial artery, facial vein, and hypoglossal nerve's inferior segment. Both facial artery and facial vein are the SMG vessels. The facial artery, the main blood supply, may enter the gland directly. The gland produces approximately 70% of the saliva in the unstimulated state. The Wharton duct, the primary excretory duct, exits the deep lobe's medial surface and drains into the mouth's anterior floor at the sublingual caruncle.

The normal SMG parenchyma appears uniformly echotexture compared with adjacent muscles (Fig. 10.3). SMGs are hypoechoic compared to the PGs and SLGs because of less fatty tissue components. The SMGs look like a triangle in longitudinal (Fig. 10.3b) and transverse (Fig. 10.3d) views. The SMGs in children appear smaller and less echogenic (darker) (Fig. 10.3e, f) than the SMGs in adults due to less fatty infiltration and may connect to the PG (Fig. 10.4a, b). The facial vein is superficial and runs along the anterosuperior part of the gland. The facial artery may cross the SMG's parenchyma in its tortuous course and intersect the gland's posterior pole. Wharton's duct is not visible except in slim individuals. Lymph nodes of SMG are periglandular only.

10.2.3 Sublingual Glands (SLGs)

SLG is the smallest SGs in the sublingual space without a true fascial capsule. The gland lies just deep to the floor of the mouth. The SLG is superior to the mylohyoid muscle and lateral to the genioglossus and geniohyoid muscles, and medial to the mandible. The SLG size shows more significant individual variations than the PGs and SMGs. The gland has no dominant ducts and is drained by ten small ducts (the ducts of Rivinus) into the anterolateral floor of the mouth [18].

The normal SLG parenchyma appears uniformly echotexture with homogeneous hyperechogenicity compared to mylohyoid, genioglossus, and geniohyoid muscles (Fig. 10.5).

10.3 Scanning Technique

1. The patient is in a supine position or upright position. If the supine position is preferred, placing a pillow or towel under the shoulders for neck extension increases thyroid, PGs, and SMGs' accessibility and the patient/operator's comfort.
2. Putting a cotton ball or a 2″ × 2″ nonsterile sponge gauze pad in each ear to protect the ultrasound gel drop into the patient's ear canals.
3. Choosing an available highest-frequency probe such as a 7–14 MHz wideband linear probe. A lower frequency (5–7 MHz) probe may be used to visualize deep portions of the PGs and SMGs.

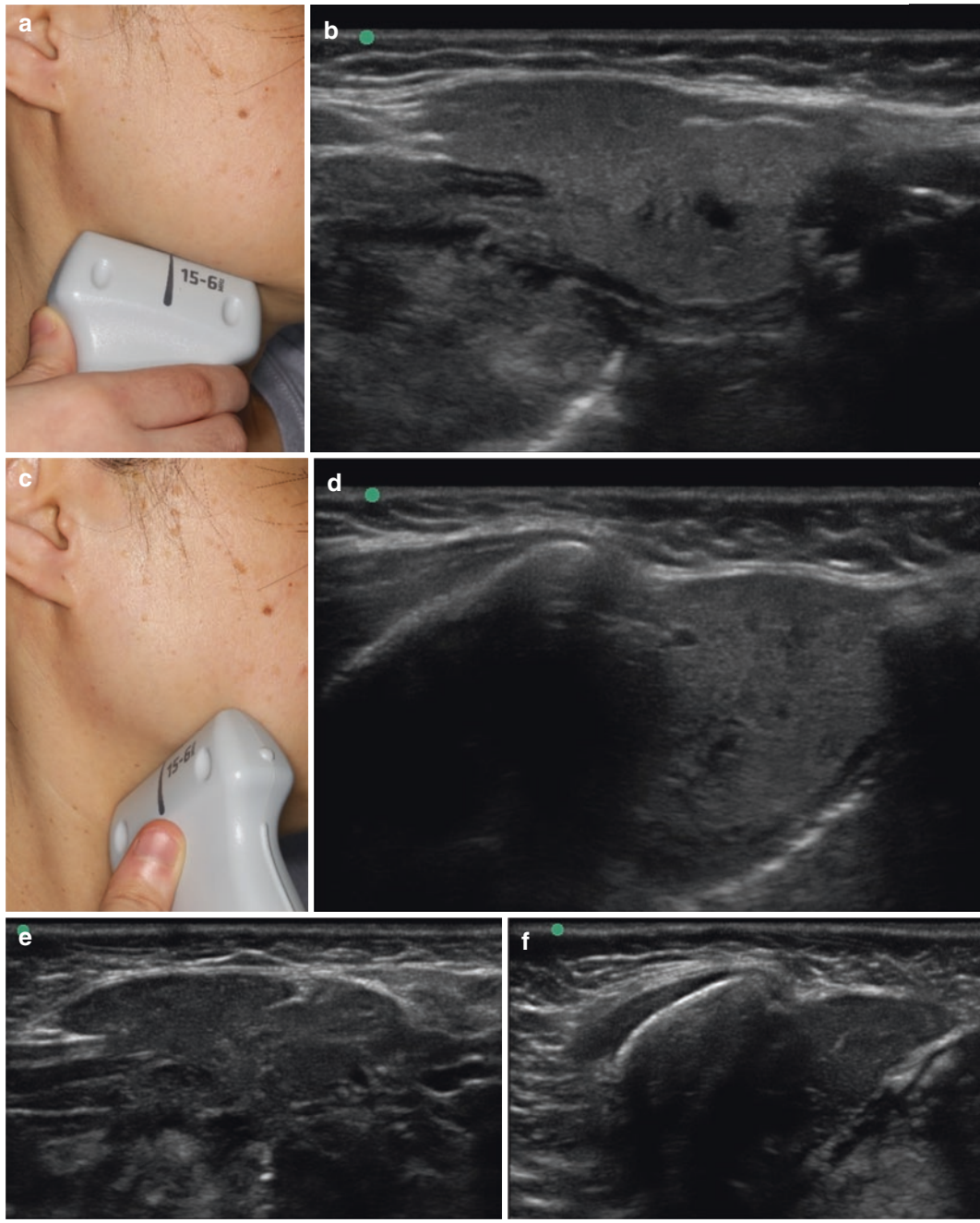

Fig. 10.3 Scanning of the normal submandibular gland of a pediatric patient in the longitudinal (**a**, **b**, **e**) and transverse (**c**, **d**, **f**) planes. Children have smaller and slightly darker (less echogenic) gland than adults

4. Scanning thyroid gland in a longitudinal (Fig. 10.6a, b) and transverse (Fig. 10.6c, d) planes. Thyroid's echogenicity and homogeneity should be reviewed. Inhomogeneous features with anechoic/hypoechoic areas suggest an underlying Hashimoto's thyroiditis (Fig. 10.6e, f).
5. Turning the patient's face away from the examined side when scanning PGs and SMGs. Both PGs and SMGs should be

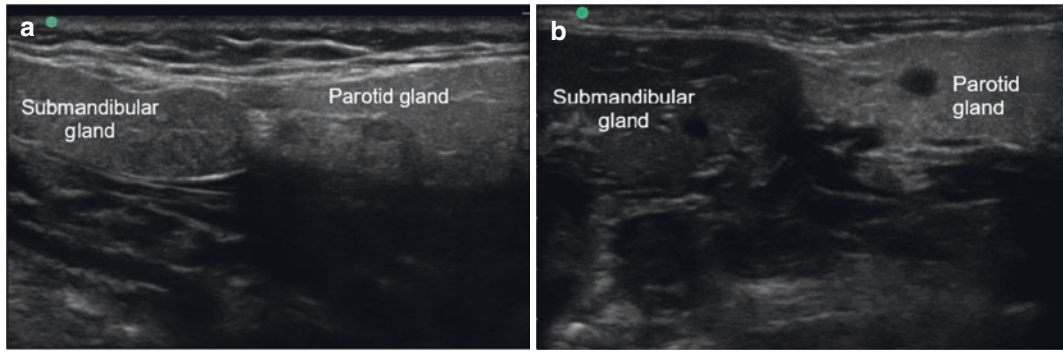

Fig. 10.4 SGUS shows the submandibular gland and parotid gland connection in an adult (**a**) and a 3-year-old boy (**b**). The submandibular glands are less echogenic than the parotid glands, especially in children

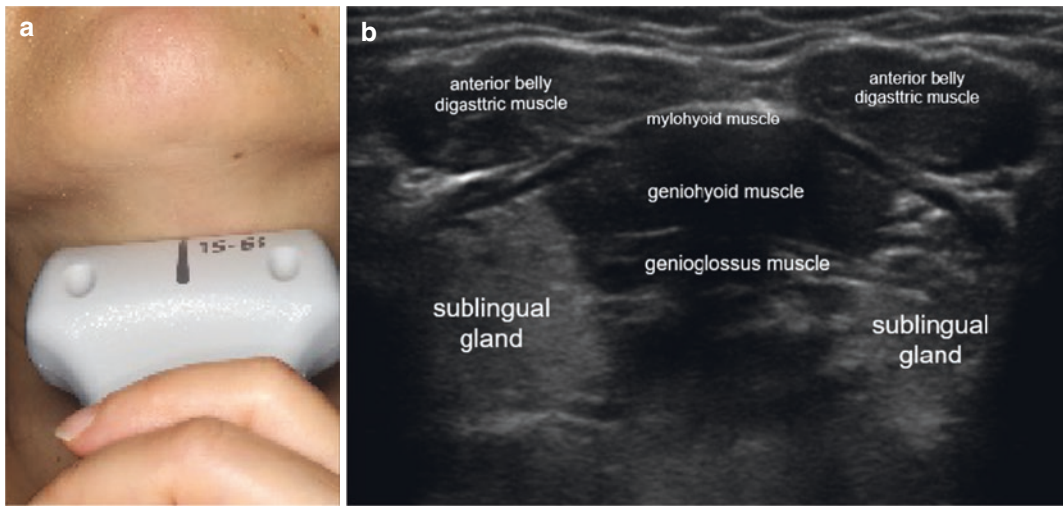

Fig. 10.5 Scanning of the normal sublingual gland of a pediatric patient in the midline transverse plane

scanned in longitudinal and transverse planes and recorded in static images and video clips.

6. Scanning the longitudinal plane of PGs by placing the probe parallel to the mandibular ramus and anterior to the ear (Fig. 10.1a) and scanning the transverse plane of PGs by placing the probe inferior to the ear and perpendicular to the mandible using the mandibular ramus and temporomandibular joint as landmarks (Fig. 10.1c).
7. Scanning the longitudinal plane of SMGs by placing the probe parallel to the mandibular body (Fig. 10.3a) and scanning the transverse plane of SMGs by placing the probe perpendicular to the body of the mandible body (Fig. 10.3c).
8. Scanning vessels and vascularity of PGs and SMGs with color or power Doppler.
9. Assessing echostructures of PGs and SMGs (Table 10.1), including echogenicity, homogeneity, hyperechoic bands, hypoechoic/anechoic areas, fatty replacement, fibrosis, calcifications, lymph nodes, posterior borders, and hypervascularity during the scanning [10].
10. If needed, SLGs can be scanned by placing the probe in the midline transverse or coronal plane of the submental area (Fig. 10.5a).

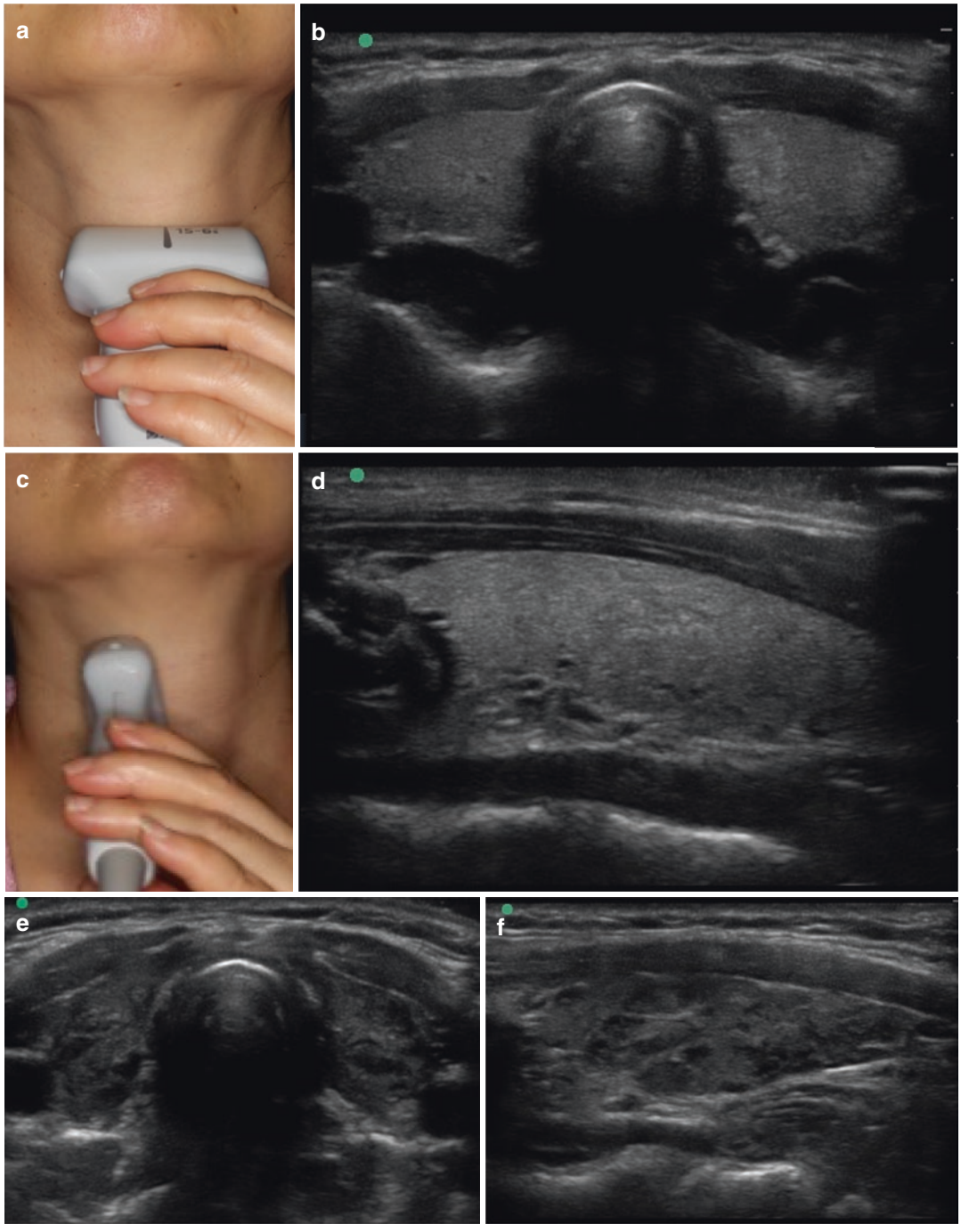

Fig. 10.6 Scanning of the normal thyroid gland of a pediatric patient in the longitudinal (**a**, **b**) and transverse (**c**, **d**) planes. Inhomogeneity with anechoic/hypoechoic findings suggests Hashimoto's thyroiditis (**e**, **f**)

10.4 Abnormal Salivary Gland Ultrasound Findings in Sjögren Syndrome

The essential echostructures in pSS and jSS by B-mode scanning are homogeneity, echogenicity, anechoic/hypoechoic areas. B-mode refers to a two-dimensional ultrasound image display showing bright dots of the ultrasound echoes. Echogenicity and homogeneity are the two SGUS items that determine the intraobserver and interobserver reliability [10]. The homogeneity is strongly correlated with anechoic/hypoechoic areas [11]. Both anechoic/hypoechoic spots and heterogeneous findings (cyst-like heterogeneity) are the most characteristic features for pSS and jSS (Fig. 10.7). The anechoic and hypoechoic areas possibly represent infiltration by lymphocytes, which destroy salivary parenchyma and dilated ducts [19].

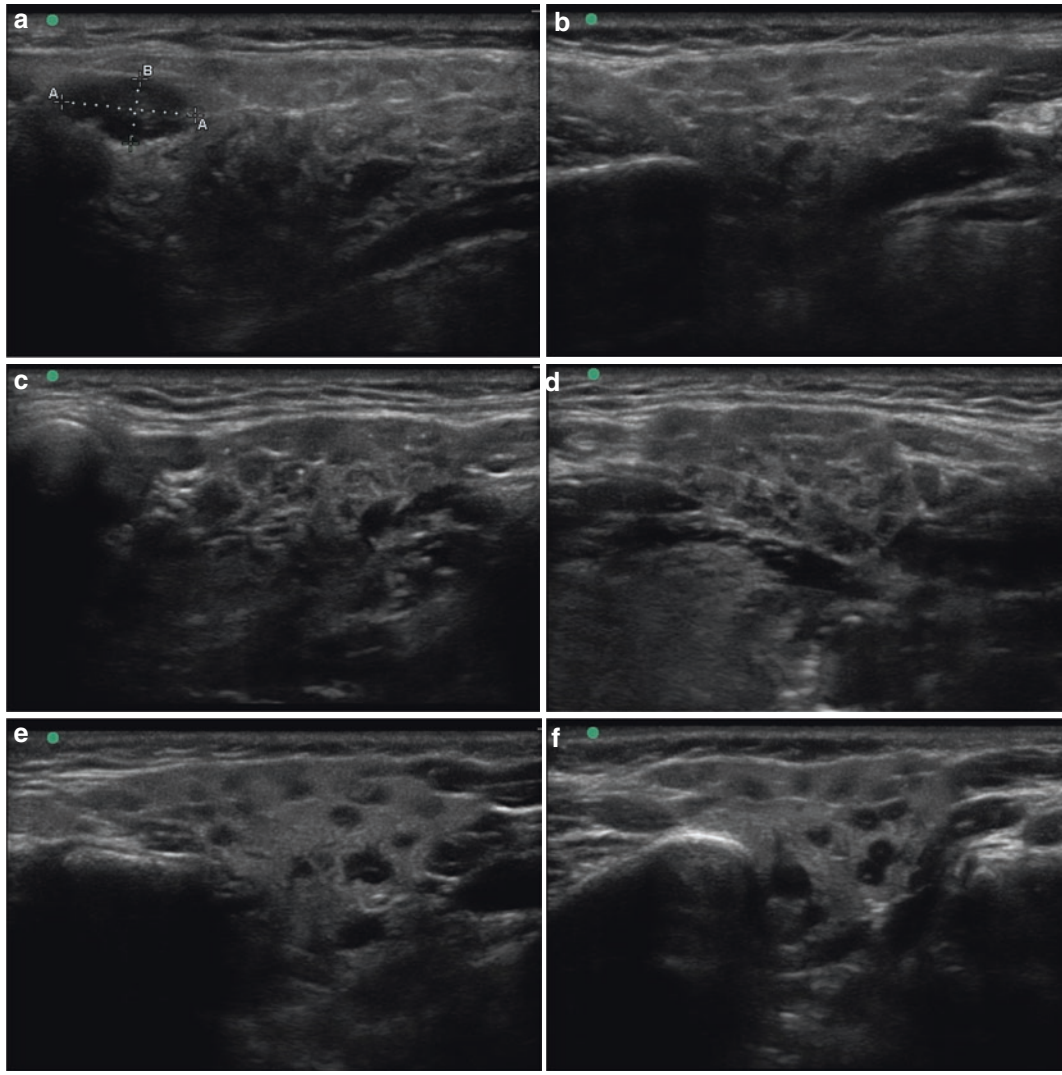

Fig. 10.7 The characteristic SGUS findings of juvenile Sjögren's syndrome. The characteristic heterogeneity and anechoic/hypoechoic areas in parotids (**a**, **b**) and submandibular glands (**c**, **d**) of a teenager boy are shown. Similar findings are found in a 7-year-old girl with juvenile Sjögren's syndrome (**e**, **f**). An enlarged intraglandular lymph node (**a**) and hyperechoic bands (**a**, **b**) are noted

Other SGUS findings (Table 10.1) are hyperechoic foci or intraglandular calcifications, hyperechoic bands, fibrosis, enlarged lymph nodes (Fig. 10.7a), fatty replacement, and changes of the posterior border and the gland size [11, 20, 21]. The hyperechoic calcified foci are typically tiny and do not demonstrate a posterior acoustic shadow. Hypervascularity assessed by color or power Doppler indicates acute flare or SG parenchyma inflammation (Fig. 10.8). The abnormal echostructures do not significantly change within the 2 years of scanning in pSS [22]. The SGUS findings are not pathognomonic for pSS and jSS. Additional clinical information is required to interpret the SGUS abnormalities.

SGUS findings of the right major SG are correlated with the left major SG in pSS. If both PG and SMG scanning cannot be completed, at least one PG and one SMG should be scanned in pSS [23]. It is unknown whether the correlation between the right and left major SG is the same as that of the SGUS findings in jSS. It is possible that the findings of PG are not correlated with those of SMGs since those two types of major SGs glands do not share the same parenchymal echostructure, and the disease severity affecting the glands can vary.

10.5 Salivary Gland Ultrasound Scoring Systems

SGUS has clinically proved its value for the evaluation of pSS and jSS. Various semiquantitative SGUS scoring systems for the diagnosis of pSS were proposed based upon the B-mode scanning.

Table 10.1 Echostructures of parotid and submandibular glands [11, 21]

	Normal	Abnormal
Echogenicity	• Identical to the normal thyroid gland	• Hypoechogenicity: the presence of hypo/anechoic areas • Hyperechogenicity: the presence of fatty infiltration or fibrosis with hyperechoic bands
Homogeneity	• Identical to the normal thyroid gland	• Heterogeneity: the presence of hypo/anechoic areas, numerous hyperechoic bands
Anechoic/hypoechoic area	• Not detected	• Anechoic/hypoechoic areas: small areas located anywhere, not compressible, no blood flow detected by color Doppler, not hyperechoic in the center
Fatty replacement	• Homogenously hyperechoic parenchyma compared with adjacent tissue in healthy elderly individuals	• Fatty replacement is detected in a small group of pSS
Fibrosis	• Not detected except for mild hyperechoic bands	• Hyperechoic bands develop into fibrotic tissue indistinguishable from adjacent soft tissue in early-stage SS and end-stage SS
Lymph nodes	• An anechoic, round, or oval area, less than 1 cm in diameter with or without an echogenic hilus and with or without blood flow by color Doppler • Intraglandular lymph nodes located in the upper and lower poles or the middle of the PG's superficial portion • Intraglandular lymph node not present in SMGs	• Lymph nodes with a diameter greater than 1 cm
Calcifications	• Not detected	• Calcifications: hyperechoic structures with or without acoustic shadowing, located in the parenchyma or ducts
Posterior border	• The posterior border or the deep gland boundary visualized and identified by a hyperechoic line between and adjacent tissues on the transverse and longitudinal views	

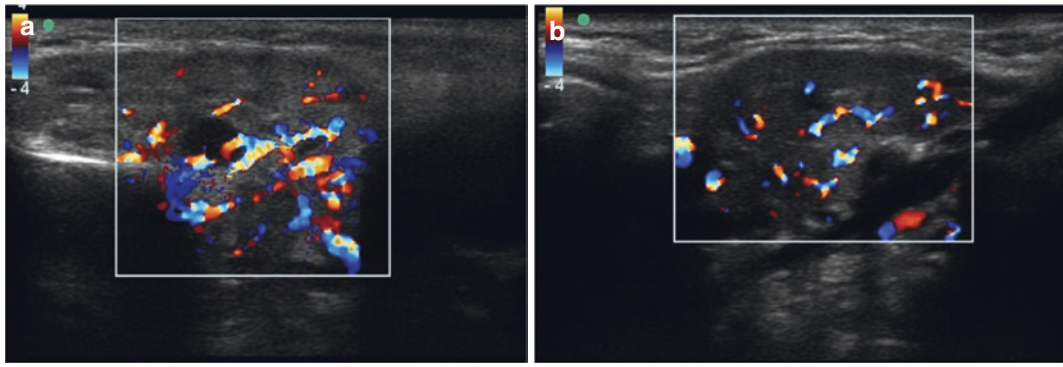

Fig. 10.8 Hypervascularity of active inflammation of the parotid gland (**a**) and submandibular gland (**b**) assessed by color Doppler

The two most reliable and consistent scanning results, echogenicity and homogeneity, are incorporated in most scoring systems. Hyperechoic bands, calcifications, glandular borders, and lymph nodes are unreliable and difficult to assess; however, these findings are applied to certain scoring systems [11, 21].

The first semiquantitative scoring system of SGUS was developed in 1992 [24]. Over 20 scoring systems were subsequently developed in the past two decades. The scoring systems are classified into three systems based upon their total scores: 0–4, 0–16, and 0–48. The Hocevar 0–48 scoring system is the most comprehensive and was applied to several pSS studies [25]. The 0–4 scoring systems show higher specificity and less heterogeneity than others. The 0–4 scoring system is recommended as a universal diagnostic standard [9].

10.5.1 Salivary Gland Ultrasound Scoring System and the Diagnosis of pSS/jSS

A meta-analysis of SGUS scoring systems in diagnosing pSS showed a pooled sensitivity of 69% and specificity of 92% [8]. A negative SGUS result does not exclude a diagnosis of pSS. Table 10.2 shows a few scoring systems with details and the cutoff. Implementing the scoring systems into the 2016 ACR/EULAR classification criteria for the diagnosis of pSS increases the criteria's sensitivity without changing its specificity (Table 10.3). Recently, a novel, reliable, semiquantitative scoring system of SGUS was developed as a consensual scoring system [11]. The usefulness of the novel scoring system in diagnosing pSS and jSS requires further studies.

10.5.2 Salivary Gland Ultrasound Scoring System and Salivary Gland Biopsy

Abnormal SGUS is correlated with minor salivary gland biopsy (MSGB) and PG biopsies in pSS [26–30]. Although a positive SGUS is highly predictive of positive biopsies, they are not interchangeable. The SGUS result cannot replace MSGB in the criteria. Approximately, 16 and 22% of positive SGUS have negative MSGB and PG biopsies, and 26 and 14% of negative SGUS have positive MSGB and PG biopsies accordingly [31]. MSGB remains necessary to perform if SGUS is negative. If SGUS replaces MSGB in the 2016 ACR/ EULAR classification criteria, the criteria's sensitivity is substantially reduced.

10.5.3 Salivary Gland Ultrasound Scoring System and Autoantibodies

Abnormal SGUS may predict SS antibodies' positivity in pSS because of the good agreement of the SGUS and SS antibody results [7, 27, 31–

Table 10.2 Semiquantitative salivary gland ultrasound scoring systems

Authors			
Hocevar [25]	Cornec [61]	Theander [62]	Jousse-Joulin [11]
Score = 0 (each item) • Echogenicity comparable to thyroid • Normal homogeneous • No hypoechogenic area • No hyperechogenic reflections[a] • Clearly defined border of MSG	**Grade = 0** • Normal homogeneous glands	**Score = 0** • Completely homogeneous	**Grade = 0** • Normal parenchyma
Score = 1 (each item) • Decreased echogenicity • Mild inhomogeneity • A few hypoechogenic areas • A few hyperechogenic reflections[a] • Partly defined borders	**Grade = 1** • Small hypoechogenic areas measuring <2 mm with echogenic bands	**Score = 1** • Mildly inhomogeneous	**Grade = 1** • Mild inhomogeneity without anechoic/hypoechoic areas • Diffuse homogeneity with a hyperechoic gland compared with adjacent tissue (fatty echostructure)
Score = 2 (each item) • Inhomogeneity • Several hypoechogenic areas • Several hyperechogenic reflections[a] • Ill-defined borders	**Grade = 2** • Multiple hypoechogenic areas measuring <2 mm with echogenic bands	**Score = 2** • Several rounded hypoechoic lesions	**Grade = 2** • Moderate inhomogeneity with focal anechoic/hypoechoic areas surrounded by normal tissue
Score = 3 (each item) • Grossly inhomogeneous gland • Numerous hypoechogenic areas • Numerous hyperechogenic reflections[a] • Border not visible	**Grade = 3** • Multiple hypoechogenic areas measuring 2–6 mm with hyperechogenic bands	**Score = 3** • Numerous or confluent rounded hypoechoic lesions	**Grade = 3** • Diffuse inhomogeneity with anechoic/hypoechoic areas occupying the entire gland, no normal tissue • Hyperechoic bands of fibrotic tissue indistinguishable from the adjacent soft tissues
NA	**Grade = 4** • Multiple hypoechogenic areas measuring >6 mm • Multiple calcifications with echogenic bands	NA	NA
58.8%[b] 98.7%[c]	62.8%[b] 95%[c]	52%[b] 98%[c]	NA
The cutoff score = 17 (The summation of the scores from the 4 MSGs is calculated)	The cutoff grade = 2 or more (The highest grade of the 4 MSGs is applied)	The cutoff score = 2 or more (The highest score of the 4 MSGs is applied)	NA

[a]The scores are only applied for the parotid glands. The scores of the hyperechogenic reflections of the submandibular glands are 0 (absent) and 1 (present)
[b]Sensitivity
[c]Specificity of SGUS findings of the scoring system

33]. The majority of cases with positive SGUS and anti-SSA fulfill the 2016 ACR/EULAR classification criteria [7, 31]. However, more studies are needed to support whether the combination of the positive SGUS and anti-SSA can replace the MSGB result. More studies of the positive SGUS and anti-SSA are needed to support the findings.

10.5.4 Salivary Gland Ultrasound Scoring System and Sialometry

Abnormal SGUS show an inversed correlation with unstimulated whole salivary flow studies [7, 26–28, 30]. However, the accuracy of abnormal

Table 10.3 Incorporation of salivary gland ultrasonography into the 2016 ACR/EULAR classification criteria

	Items of grayscale (score)	
Author	van Nimwegen [63]	Jousse-Joulin [13]
Grade 0	No hypoechogenic area (0)	No anechoic/hypoechoic area (0)
Grade 1	A few scattered hypoechogenic areas (1)	Hypoechoic areas occupying less than 25% of the gland surface areas (1)
Grade 2	Several hypoechogenic areas (2)	Anechoic/hypoechoic areas occupying 25%–50% of the gland surface areas (2)
Grade 3	Numerous hypoechogenic areas (3)	Anechoic/hypoechoic areas occupying more than 50% of the gland surface areas (3)
Grade 4	NA	Anechoic/hypoechoic areas occupying the entire gland (4)
Sensitivity Specificity	97.3%[a] 90.2%[a]	95.6%[a] 82.6%[a]
Final score and the cutoff	• The average score for hypoechogenic areas of one PG and one SMG • Cutoff score = 1.5 or more for pSS	• The highest MSG score of anechoic/hypoechoic area • Cutoff score = 2 or more for pSS

[a]The sensitivity and specificity of the 2016 ACR/EULAR classification criteria with the addition of SGUS

SGUS to predict salivary gland dysfunction is known to be poor [31].

10.6 SGUS Mimicking Sjögren's Syndrome

10.6.1 Juvenile Recurrent Parotitis (JRP)

Juvenile recurrent parotitis (JRP) is an unknown cause of recurrent nonobstructive, nonsuppurative parotitis in children with unilateral or bilateral PG swellings. The typical presentation is pain, swelling, redness of skin overlying the gland, and fever (Fig. 10.9a). Chronic recurrent parotitis or chronic sialadenitis in adults with the same clinical and sonographic features is less common [34]. SGUS findings in JRP are multiple anechoic/hypoechoic areas, heterogeneous parenchyma echostructures, and hypervascularization with increased Doppler's signal [35]. The hypoechoic areas in JRP correspond to either sialectasis or lymphocytic infiltration [36]. Both JRP and jSS share the same clinical and PG sonographic findings (Figs. 10.9 and 10.10), especially in young children with parotitis and negative SSA/SSB antibodies. Since JRP does not involve minor SGs and SMGs, the positive MSGB and abnormal SMG ultrasound may distinguish these two conditions. Magnetic resonance imaging (MRI) findings of PGs without fat degeneration and architecture destruction possibly suggest JRP [37].

10.6.2 Mumps

Mumps, a paramyxovirus infection, is characterized by painful and inflamed PGs and constitutional symptoms. Bilateral PG involvement is shown in 70% of cases, PG and SMG involvement is presented in 11% of cases, and isolated SMG involvement is noted in 1–2% of cases. Although 30% of infected cases are asymptomatic, severe complications including orchitis, oophoritis, mastitis, pancreatitis, aseptic meningitis, encephalitis, and deafness were reported. SGUS findings in mump are enlarged SG, homogenous echogenicity, heterogeneously hypoechoic intraglandular lymph nodes, and hypervascularity [35, 38].

10.6.3 Chronic Bacterial Infection

An incomplete resolution of an acute infection caused by *Staphylococcus aureus*, *Streptococcus* spp., anaerobic bacteria, and aerobic and facultative Gram-negative bacilli leads to chronic bacterial infection [39]. SGUS shows multiple oval

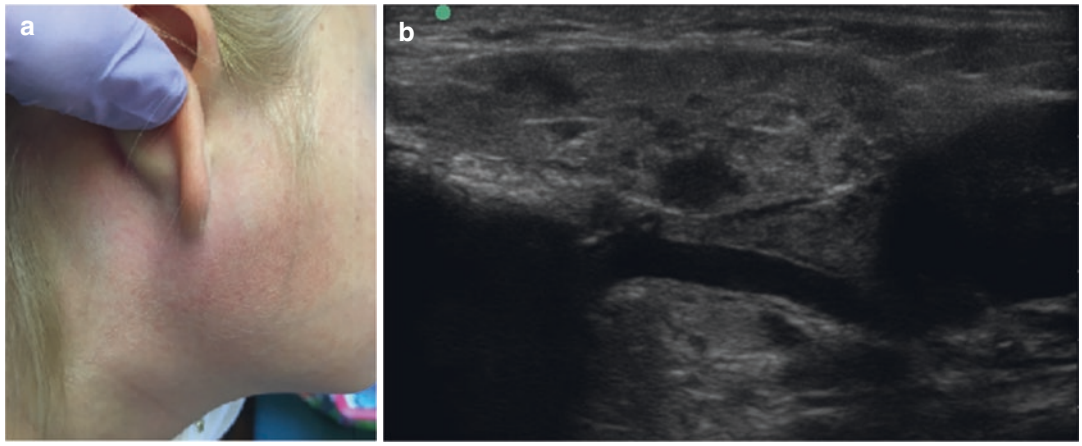

Fig. 10.9 A 6-year-old girl presented with recurrent pain, swelling, and redness of the right parotid gland (**a**) and SGUS revealed heterogeneous parenchyma with multiple anechoic/hypoechoic areas (**b**). Both juvenile Sjögren's syndrome and juvenile recurrent parotitis are common differential diagnoses in young children

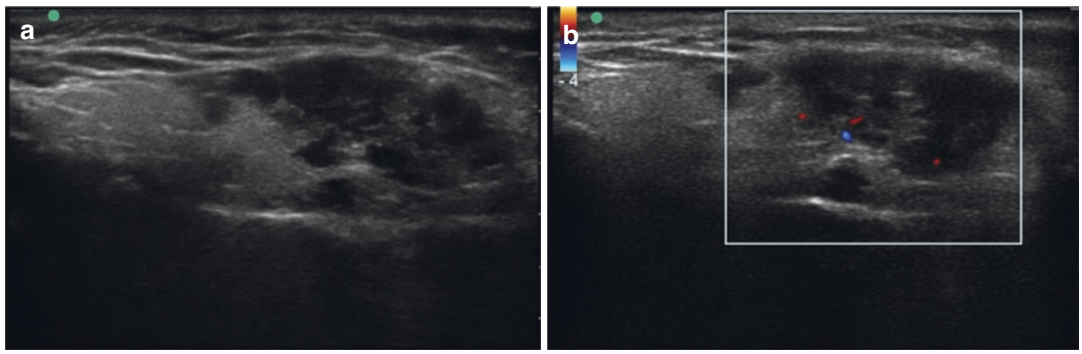

Fig. 10.10 SGUS findings of chronic bacterial infection in a teenager with recurrent pain and swelling of the parotid gland

hypoechoic areas, varying degrees of sialectasis, and hyperechoic septations with hypervascularization (Fig. 10.10) [40].

10.6.4 IgG4-Related Disease

IgG4-related disease (IgG4-RD), a fibroinflammatory disorder, commonly involves SGs [41]. Classic findings of IgG4-RD are bilateral symmetric diffuse swelling of SGs and lacrimal glands. Ultrasonography is considered the most useful imaging modality for IgG4-RD. The characteristic of ultrasound findings in IgG4-RD is a nodal pattern predominantly affecting SMGs (92%) with less-affecting PGs (33%) [42]. The nodal pattern is described as hypoechoic areas with high vascularization and bulging from normal surface commonly detected in SMGs. Both SMGs and PGs are typically homogenous with normal parenchymal echogenicity. The changes are not observed in pSS. The nodal pattern detected by SGUS may not be seen on CT and MRI [43].

10.6.5 Sarcoidosis

Sarcoidosis, a chronic multisystem inflammatory granulomatous disease with unknown etiology, affecting the SGs, involves mostly PGs. Both sarcoidosis and pSS may have similar signs and

symptoms with persistent asymptomatic or painful PG swelling. SGUS findings of sarcoidosis are multiple hypoechoic areas and numerous echogenic septa with the hypervascular area and heterogeneous parenchyma with or without enlarged intraglandular lymph nodes [44]. The findings in sarcoidosis cannot distinguish from the findings in pSS [45].

10.6.6 Systemic Immunoglobulin Light-Chain Amyloidosis (AL Amyloidosis)

AL amyloidosis is the most common form of systemic amyloidosis characterized by the proliferation of clonal plasma cells and immunoglobulin light-chain production, leading to SG enlargement and other organ damage [46]. SGUS findings in AL amyloidosis are hypoechoic lesions and hyperechoic septa, similar to the findings in pSS [45].

10.6.7 HIV Infections

HIV-associated SG disease commonly involves bilateral PGs. Typically, the parotitis is asymmetrical, painless, slow-growing with xerostomia, and salivary gland hypofunction. The PG ultrasound findings in HIV-infected patients can be normal and abnormal. There are four distinct ultrasound patterns including (1) lymphocytic aggregations (diffuse small hypoechoic or anechoic areas with moderate to ill-defined margins interspersed within normal isoechoic areas, not associated with posterior acoustic enhancement), (2) lymphoepithelial cysts, a pathognomonic of HIV infection (well-circumscribed round hypoechoic area with size 5 mm or larger and posterior acoustic enhancement), (3) fatty infiltration (whole hypoechoic gland), and (4) lymphadenopathy (oval-shaped hypoechoic areas or nodules with echogenic hilum and hilar blood flow). Both lymphocytic aggregations and lymphoepithelial cysts may be similar to the findings in SS [47].

10.6.8 Lymphoma

Lymphomas are the second most common malignancies in the head and neck. SG lymphomas can be primary or secondary, and extranodal (arising outside a lymphoid organ) or nodal (appearing inside an intraglandular lymph node or periparotid lymph node) [48]. Primary SG lymphomas commonly involve unilateral or bilateral PGs, and most are classified as extranodal marginal-zone B-cell non-Hodgkin's lymphoma originating from mucosa-associated lymphoid tissue (MALT) [49]. SS is a well-known risk factor for primary SG lymphoma. There are two defined sonographic patterns of SG lymphomas, including linear echogenic strands pattern and segmental pattern. The linear echogenic strands pattern is a marked hypoechoic area with interspersed linear echogenic strands, and the segmental pattern is multiple, relatively large hypoechoic segments. The SGUS findings represent the histopathologic findings of expanding lymphoma cells demarcated by narrow or wide fibrous bands [50, 51].

10.6.9 Postradiotherapy

Radiotherapy alters and damages PG parenchyma and SG acinar cells leading to xerostomia. SGUS of PG postradiotherapy appears isoechoic or hypoechoic echogenicity, small gland size, heterogeneous echotexture with multiple hypoechoic areas, and hyperechoic fibrotic streaks [52].

10.7 Other Imaging for Primary Sjögren's Syndrome and Juvenile Sjögren's Syndrome

10.7.1 Plain Radiograph

A plain radiograph is an inexpensive and simple study for evaluating salivary stones, especially SMG stones since the SMG stones are relatively large and radiopaque [53]. It has no diagnostic role for pSS and jSS.

10.7.2 Sialography

Sialography is an old diagnostic procedure based on the injection of a dye into the salivary duct orifice (Stenson and Wharton duct) to visualize the entire ductal architecture and identify salivary calculi, ductal anomaly, and stricture. Abnormal sialography with diffuse sialectasis, including punctate, cavity, or destructive pattern, is a criterion in the 2002 American-European Consensus Group (AECG) classification. The abnormal findings result from cystic ductal dilatations or extravasation of the contrast material into the parenchyma. The extravasation of the contrast material is possibly related to the dysfunction of tight junctions between striated ductal cells caused by inflammatory cytokines. Its sensitivity and specificity are 80 and 89% based upon the 2002 AECG criteria [54].

Sialography is excluded from the 2016 ACR/EULAR criteria because of its invasiveness, potential complications including ductal rupture or activation of dormant infection, irradiating nature, and contraindications such as acute infection, acute inflammation, and contrast allergy. SGUS has replaced sialography since it provides similar information while being considerably less invasive.

10.7.3 Salivary Gland Scintigraphy

Salivary gland scintigraphy, a nuclear imaging technique with radioactive tracer infusion (Technetium-99m pertechnetate), demonstrates the glandular function by evaluating the distribution and speed of eliminating the radiotracer after a secretive stimulation. It reveals both physiologic and pathologic secretory functions. The potential benefit of scintigraphy is to monitor SG functioning over time.

Abnormal scintigraphy results are delayed uptake, reduced concentration, and delayed excretion of tracer described in the 2002 AECG criteria. Its sensitivity and specificity are 89 and 50% [55]. Although it is noninvasive, safe, well-tolerated, reproducible, and easy to perform, the test results cannot distinguish the secretive dysfunction of pSS from other diseases [55]. In addition, there are no guidelines or consensus on how the test should be performed and interpreted [56].

10.7.4 Computed Tomography (CT)

Computed tomography (CT) and MRI identify the homogeneity and inhomogeneity of adipose tissue distribution and accumulation related to destruction and shrinkage from chronic inflammation. CT is more readily available and lower-cost with a shorter scan time than MRI. Non-contrast-enhanced CT is useful in identifying small calculi in the gland or duct. Contrast-enhanced CT is useful where MRI is contraindicated, or the access to MRI is limited.

Normal fatty tissue inside the PGs is homogeneously distributed and contributes to the low CT attenuation regardless of the subject's age. Abnormal CT findings of the PGs in SS include heterogeneity of attenuation caused by fatty degeneration, abnormal fat tissue deposition, diffuse punctate calcification, atrophy or swelling of the gland, nodularity, and cystic lesions. The diffuse punctate calcification of bilateral PGs is highly specific for SS and found in 30% of cases [57]. The drawback of CT scan is radiation exposure, artifacts from metal dental fillings, and its contraindications, including impaired kidney function and contrast hypersensitivity.

10.7.5 Magnetic Resonance Imaging (MRI) and Magnetic Resonance Sialography (MR Sialography)

MRI is noninvasive, nonionizing imaging, providing pathologic changes of major SG in SS. T1-weighted image and fat-suppressed T2-weighted image are recommended. Intravenous injection of a contrast medium increases the detection of abnormalities, but it is not mandatory for SS diagnosis. Normal PGs are intermediate to high single intensity on T1- and T2-weighted images due to high intraglandular fat content. The submandibular and sublingual

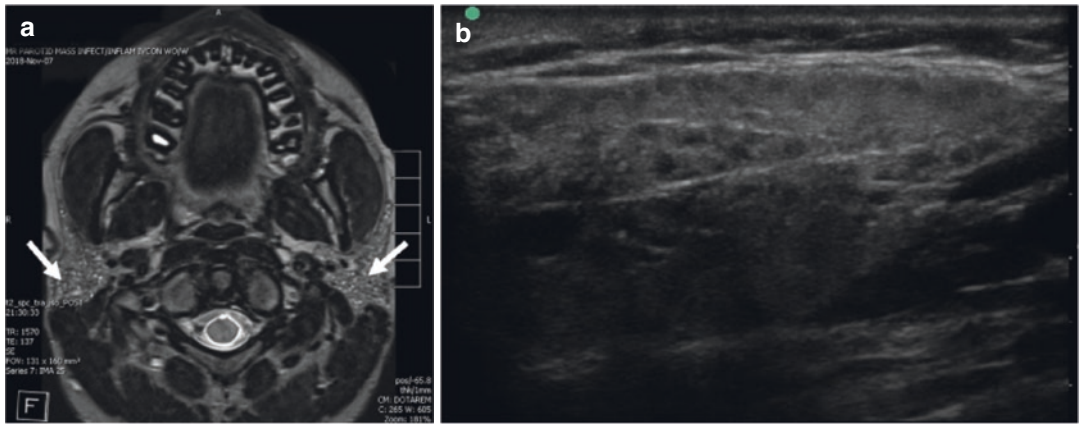

Fig. 10.11 A characteristic MRI finding of the parotid gland of a teenager with juvenile Sjögren's syndrome shows salt-and-pepper appearance (**a**, white arrow) compared to SGUS (**b**)

glands demonstrate a more intermediate signal on T1-weighted image [58]. MRI can identify early SS with edema and gland enlargement, lobular destruction with diffuse micro and/or macrocystis changes associated with deposition of fibrous tissue and fat [55]. Both high-signal intensity on T1- and T2-weighted images and low-signal intensity in fat-suppressed T2-weighted images indicate abnormal adipose tissue deposition. The characteristic MRI of PGs and SMGs is heterogeneous signal intensity (mixed low- and high-signal intensity) distribution on T1- and T2-weighted images with a salt-and-pepper appearance or a honeycomb appearance (Fig. 10.11).

MR sialography, a new modification of MRI, reveals early diseases of PG without the injection of contrast material. MR sialography is obtained with a heavily T2-weighted 3D-Fast spin-echo appearance. MR sialography's abnormal findings are multiple high-signal-intensity spots with apple tree appearance and enlargement or reduction of the gland's size. A combination of MRI and MR sialography provides high sensitivity and specificity for observing parenchymal abnormalities and differentiation of SS [59, 60].

The significant disadvantages of MRI and MR sialography are higher cost, length of the examination, patient intolerance, sedation requirement in young children, and its contraindications, including retained metallic foreign bodies such as dental braces, cardiac pacemakers, or insulin pumps.

10.8 Conclusion

SGUS has been increasingly used as a first-line imaging tool to evaluate and diagnose pSS and jSS with reasonable accuracy. It offers a noninvasive assessment of typical morphological changes in the major SGs. There is growing evidence to support the abnormal sonographic features as a criterion for the next disease classification. The potential roles of SGUS in reducing the need for MSGB and monitoring treatment response require further studies.

References

1. Kitamura T, et al. Ultrasonic diagnosis in oto-rhinolaryngology. Eye Ear Nose Throat Mon. 1969;48(5):329–37.
2. Baker S, Ossoinig KC. Ultrasonic evaluation of salivary glands. Trans Sect Otolaryngol Am Acad Ophthalmol Otolaryngol. 1977;84(4 Pt 1):Orl-750-62.
3. de Clerck LS, et al. Ultrasonography and computer tomography of the salivary glands in the evaluation of Sjögren's syndrome. Comparison with parotid sialography. J Rheumatol. 1988;15(12):1777–81.
4. Bruneton JN, et al. Indications for ultrasonography in parotid pathologies. Rofo. 1983;138(1):22–4.

5. Kawamura H, et al. Salivary gland echography in patients with Sjögren's syndrome. Arthritis Rheum. 1990;33(4):505–10.
6. Civilibal M, et al. A child with primary Sjogren syndrome and a review of the literature. Clin Pediatr (Phila). 2007;46(8):738–42.
7. Hammenfors DS, et al. Juvenile Sjögren's syndrome: clinical characteristics with focus on salivary gland ultrasonography. Arthritis Care Res (Hoboken). 2020;72(1):78–87.
8. Delli K, et al. Diagnostic properties of ultrasound of major salivary glands in Sjogren's syndrome: a meta-analysis. Oral Dis. 2015;21(6):792–800.
9. Zhou M, et al. Diagnostic accuracy of salivary gland ultrasonography with different scoring systems in Sjogren's syndrome: a systematic review and meta-analysis. Sci Rep. 2018;8(1):17128.
10. Jousse-Joulin S, et al. Salivary gland ultrasound abnormalities in primary Sjogren's syndrome: consensual US-SG core items definition and reliability. RMD Open. 2017;3(1):e000364.
11. Jousse-Joulin S, et al. Video clip assessment of a salivary gland ultrasound scoring system in Sjogren's syndrome using consensual definitions: an OMERACT Ultrasound Working Group reliability exercise. Ann Rheum Dis. 2019;78:967–73.
12. Geng Y, et al. Salivary gland ultrasound integrated with 2016 ACR/EULAR classification criteria improves the diagnosis of primary Sjogren's syndrome. Clin Exp Rheumatol. 2020;38(2):322–8.
13. Jousse-Joulin S, et al. Weight of salivary gland ultrasonography compared to other items of the 2016 ACR/EULAR classification criteria for primary Sjogren's syndrome. J Intern Med. 2020;287(2):180–8.
14. van Nimwegen JF, et al. Incorporation of salivary gland ultrasonography into the American College of Rheumatology/European League Against Rheumatism criteria for primary Sjogren's syndrome. Arthritis Care Res (Hoboken). 2020;72(4):583–90.
15. Gervasio A, et al. Sonographic anatomy of the neck: the suprahyoid region. J Ultrasound. 2011;14(3):130–5.
16. Kochhar A, Larian B, Azizzadeh B. Facial nerve and parotid gland anatomy. Otolaryngol Clin N Am. 2016;49(2):273–84.
17. Silvers AR, Som PM. Salivary glands. Radiol Clin N Am. 1998;36(5):941–66, vi.
18. Abdullah A, Rivas FF, Srinivasan A. Imaging of the salivary glands. Semin Roentgenol. 2013;48(1):65–74.
19. Bialek EJ, et al. US of the major salivary glands: anatomy and spatial relationships, pathologic conditions, and pitfalls. Radiographics. 2006;26(3):745–63.
20. Cornec D, et al. Salivary gland ultrasound to diagnose Sjögren's syndrome: a claim to standardize the procedure. Rheumatology. 2014;54(1):199–200.
21. Jousse-Joulin S, et al. Salivary gland ultrasound abnormalities in primary Sjögren's syndrome: consensual US-SG core items definition and reliability. RMD Open. 2017;3(1):e000364.
22. Gazeau P, et al. Time-course of ultrasound abnormalities of major salivary glands in suspected Sjögren's syndrome. Joint Bone Spine. 2018;85(2):227–32.
23. Mossel E, et al. Scoring hypoechogenic areas in one parotid and one submandibular gland increases feasibility of ultrasound in primary Sjogren's syndrome. Ann Rheum Dis. 2018;77(4):556–62.
24. De Vita S, et al. Salivary gland echography in primary and secondary Sjögren's syndrome. Clin Exp Rheumatol. 1992;10(4):351–6.
25. Hocevar A, et al. Ultrasonographic changes of major salivary glands in primary Sjogren's syndrome. Diagnostic value of a novel scoring system. Rheumatology (Oxford). 2005;44(6):768–72.
26. Baldini C, et al. Salivary gland ultrasonography: a highly specific tool for the early diagnosis of primary Sjogren's syndrome. Arthritis Res Ther. 2015;17:146.
27. Hammenfors DS, et al. Diagnostic utility of major salivary gland ultrasonography in primary Sjogren's syndrome. Clin Exp Rheumatol. 2015;33(1):56–62.
28. Cornec D, et al. High-grade salivary-gland involvement, assessed by histology or ultrasonography, is associated with a poor response to a single rituximab course in primary Sjögren's syndrome: data from the TEARS randomized trial. PLoS One. 2016;11(9):e0162787.
29. Astorri E, et al. Ultrasound of the salivary glands is a strong predictor of labial gland biopsy histopathology in patients with Sicca symptoms. J Oral Pathol Med. 2016;45(6):450–4.
30. Kim JW, et al. Salivary gland ultrasonography findings are associated with clinical, histological, and serologic features of Sjogren's syndrome. Scand J Rheumatol. 2018;47(4):303–10.
31. Mossel E, et al. Ultrasonography of major salivary glands compared with parotid and labial gland biopsy and classification criteria in patients with clinically suspected primary Sjogren's syndrome. Ann Rheum Dis. 2017;76(11):1883–9.
32. Nieto-Gonzalez JC, et al. Salivary gland ultrasound is linked to the autoimmunity profile in patients with primary Sjogren's syndrome. J Int Med Res. 2018;48:300060518767031.
33. Lee KA, Lee SH, Kim HR. Diagnostic and predictive evaluation using salivary gland ultrasonography in primary Sjögren's syndrome. Clin Exp Rheumatol. 2018;36 Suppl 112(3):165–72.
34. Abdel Razek AAK, Mukherji S. Imaging of sialadenitis. Neuroradiol J. 2017;30(3):205–15.
35. Sitheeque M, et al. Juvenile recurrent parotitis: clinical, sialographic and ultrasonographic features. Int J Paediatr Dent. 2007;17(2):98–104.
36. Papadopoulou-Alataki E, et al. Juvenile recurrent parotitis: the role of sialendoscopy. Int J Inflamm. 2019;2019:7278907.
37. Kimura Y, et al. Magnetic resonance imaging-based differentiation between juvenile recurrent parotitis and juvenile Sjögren's syndrome. Oral Radiol. 2011;27(1):73–7.

38. Sirmatel O, et al. Radiological findings in a mumps case with multiple complications. Indian J Radiol Imaging. 2006;16(3):305–8.
39. Brook I. The bacteriology of salivary gland infections. Oral Maxillofac Surg Clin North Am. 2009;21(3):269–74.
40. Orlandi MA, Pistorio V, Guerra PA. Ultrasound in sialadenitis. J Ultrasound. 2013;16(1):3–9.
41. Maritati F, Peyronel F, Vaglio A. IgG4-related disease: a clinical perspective. Rheumatology (Oxford). 2020;59(Suppl 3):iii123–31.
42. James-Goulbourne T, Murugesan V, Kissin EY. Sonographic features of salivary glands in Sjögren's syndrome and its mimics. Curr Rheumatol Rep. 2020;22(8):36.
43. Shimizu M, et al. Effectiveness of imaging modalities for screening IgG4-related dacryoadenitis and sialadenitis (Mikulicz's disease) and for differentiating it from Sjögren's syndrome (SS), with an emphasis on sonography. Arthritis Res Ther. 2015;17(1):223.
44. Teymoortash A, Werner JA. Parotid gland involvement in sarcoidosis: sonographic features. J Clin Ultrasound. 2009;37(9):507–10.
45. Law ST, et al. Comparison of ultrasound features of major salivary glands in sarcoidosis, amyloidosis, and Sjögren's syndrome. Arthritis Care Res (Hoboken). 2020;72(10):1466–73.
46. Merlini G, et al. Systemic immunoglobulin light chain amyloidosis. Nat Rev Dis Primers. 2018;4(1):38.
47. Kabenge C, et al. Diagnostic ultrasound patterns of parotid glands in human immunodeficiency virus-positive patients in Mulago, Uganda. Dentomaxillofac Radiol. 2010;39(7):389–99.
48. Cabeçadas J, et al. Lymphomas of the head and neck region: an update. Virchows Arch. 2019;474(6):649–65.
49. Paliga A, et al. Salivary gland lymphoproliferative disorders: a Canadian tertiary center experience. Head Neck Pathol. 2013;7(4):381–8.
50. Bahn YE, et al. Sonographic appearances of mucosa-associated lymphoid tissue lymphoma of the submandibular gland confirmed with sonographically guided core needle biopsy. J Clin Ultrasound. 2011;39(4):228–32.
51. Orita Y, et al. Characteristic ultrasound features of mucosa-associated lymphoid tissue lymphoma of the salivary and thyroid gland. Acta Otolaryngol. 2014;134(1):93–9.
52. Ying M, Wu VW, Kwong DL. Comparison of sonographic appearance of normal and postradiotherapy parotid glands: a preliminary study. Ultrasound Med Biol. 2007;33(8):1244–50.
53. Bag AK, et al. Imaging of inflammatory disorders of salivary glands. Neuroimaging Clin N Am. 2018;28(2):255–72.
54. Song GG, Lee YH. Diagnostic accuracies of sialography and salivary ultrasonography in Sjogren's syndrome patients: a meta-analysis. Clin Exp Rheumatol. 2014;32(4):516–22.
55. Baldini C, et al. Imaging in primary Sjogren's syndrome: the 'obsolete and the new'. Clin Exp Rheumatol. 2018;36 Suppl 112(3):215–21.
56. Afzelius P, et al. Imaging of the major salivary glands. Clin Physiol Funct Imaging. 2016;36(1):1–10.
57. Sun Z, et al. Diagnostic accuracy of parotid CT for identifying Sjögren's syndrome. Eur J Radiol. 2012;81(10):2702–9.
58. Nayak GK, Hagiwara M. Imaging of salivary gland pathology. Oper Tech Otolaryngol Head Neck Surg. 2018;29(3):116–28.
59. Niemela RK, et al. Magnetic resonance imaging and magnetic resonance sialography of parotid glands in primary Sjogren's syndrome. Arthritis Rheum. 2001;45(6):512–8.
60. Tomiita M, et al. Usefulness of magnetic resonance sialography in patients with juvenile Sjogren's syndrome. Clin Exp Rheumatol. 2005;23(4):540–4.
61. Cornec D, et al. Contribution of salivary gland ultrasonography to the diagnosis of Sjögren's syndrome: toward new diagnostic criteria? Arthritis Rheum. 2013;65(1):216–25.
62. Theander E, Mandl T. Primary Sjogren's syndrome: diagnostic and prognostic value of salivary gland ultrasonography using a simplified scoring system. Arthritis Care Res (Hoboken). 2014;66(7):1102–7.
63. van Nimwegen JF, et al. Incorporation of salivary gland ultrasonography into the American College of Rheumatology/European League Against Rheumatism criteria for primary Sjogren's syndrome. Arthritis Care Res. 2020;72(4):583–90.

11 Pharmacological Management of Sjögren's Syndrome

You-Jung Ha and Yun Jong Lee

11.1 Introduction

Sjögren's syndrome (SS) is a chronic systematic disorder characterized histologically by a focal lymphocytic infiltration in exocrine glands and immunologically by B cell hyperactivity. Although its major target organs include salivary and lacrimal glands, SS has diverse extraglandular involvements through different pathogenetic mechanisms [1, 2]. Generally, most SS patients have a stable course of the disease, and flares in SS are less easily detectable than those in rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) [3]. However, a significant portion of patients suffer from intractable fatigue and musculoskeletal pain, and about 2–5% of patients are complicated by lymphoma [1, 2, 4]. Such clinical heterogenicity often makes it challenging to evaluate disease activity and determine treatment targets in SS patients.

Y.-J. Ha
Division of Rheumatology, Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea
e-mail: hayouya@snubh.org

Y. J. Lee (✉)
Division of Rheumatology, Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea
e-mail: yn35@snu.ac.kr

Several placebo-controlled trials confirmed the therapeutic effect of oral cholinergic agonists such as pilocarpine and cevimeline on sicca symptoms [5]. However, there has been no treatment that can reverse glandular hypofunction or completely resolve sicca symptoms to date. Additionally, clinical trials of various biologic agents have failed to improve fatigue significantly [6]. Furthermore, recent controlled trials reported no improvement of SS patient symptoms with hydroxychloroquine (HCQ) that has been widely prescribed as an immunomodulatory drug for SS [7, 8]. SS patients have reported having a relatively stable oral and ocular dryness, fatigue, and health-related quality of life over 5–7 years [9–11]. Moreover, the whole salivary flow rate and salivary gland ultrasound abnormalities were unchanged for 2–5 years [12, 13].

The pharmacological management of SS is generally based on symptomatic treatment of sicca and nonspecific broad-spectrum immunosuppressive agents of systemic manifestations [5]. Because treatment decisions vary depending on the individual physician, some groups have recently published SS management guidelines [14–17]. The recent European League Against Rheumatism (EULAR) recommendations emphasize the importance of a multidisciplinary approach involving various health professionals and primary care physicians [17]. Additionally, since SS patients have a chronic course without complete remission, it stresses that a proper balance between therapeutic and adverse effects

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should be considered in long-term SS treatment [17]. In this chapter, we summarize the efficacy of drugs used to treat each SS manifestation systematically and provide an overall perspective of the pharmacological management of SS.

11.2 Treatment of Salivary Gland Involvement

Because sicca symptoms are presented in more than 95% of SS patients [18], the relief of sicca is an important and common issue in the therapeutic approach in SS. Before deciding a specific treatment for salivary gland involvement, salivary gland function is recommended to be evaluated using whole salivary flow rates or salivary scintigraphy. Subjective xerostomia levels are significantly affected by environmental and emotional factors and often are not associated with residual salivary gland function [19]. Also, a meticulous history taking and examination are mandatory to rule out non-SS-related conditions such as sarcoidosis, candidiasis, or burning mouth syndrome. In the case of persistent salivary gland swelling, non-Hodgkin's lymphoma and IgG4-related disease should be considered. Current treatment options are summarized in Table 11.1.

Table 11.1 Pharmacologic treatment according to the glandular manifestations of SS

Manifestations	Treatment options
Dry mouth	Saliva substitutions
	Topical saliva stimulants
	Oral muscarinic agonists (pilocarpine or cevimeline)
	Mucolytic agents (bromhexine, *N*-acetylcysteine, or ambroxol)
	Salivary gland irrigation/ sialendoscopic intervention
Dry eye	Artificial tears
	Ophthalmic ointments
	Topical corticosteroid
	Topical nonsteroidal anti-inflammatory drugs
	Topical cyclosporine
	Autologous serum eye drops
	Oral muscarinic agonists (pilocarpine or cevimeline)
	Punctal occlusion, temporal or permanent
Salivary gland swelling	Nonsteroidal anti-inflammatory drugs
	Glucocorticoids
	Anti-B cell agents (rituximab or belimumab)
	Sialendoscopic intervention
	Antibiotics if infectious parotitis
Friction oral ulcer	Oral gel
	Oral muscarinic agonists
Candidiasis	Topical antifungal agents (nystatin or miconazole)
	Oral antifungal agents (fluconazole)
	Oral muscarinic agonists
Dental caries	Remineralizing agents, fluoride or non-fluoride
	Topical salivary stimulants
	Local chlorhexidine gargle
	Oral muscarinic agonists
Dry cough	According to the underlying causes, for example, acid suppression for gastroesophageal reflux
	Nebulized saline solution
	Oral muscarinic agonists
	Mucolytic agents
Vaginal dryness	Estrogen-containing or non-containing vaginal moisturizers
	Topical estrogen cream

11.2.1 Dry Mouth

The first approach to dry mouth is topical therapies and environmental humidification, especially in SS patients with mild glandular dysfunction. There is limited evidence for topical therapies for dry mouth in systematic reviews [20, 21]; various trials showed symptomatic improvement without significant adverse events [5]. Saliva substitutions are referred to Chap. 1 in this book. Also, there has been no study available on the efficacy of topical saliva stimulants in SS patients. Nonetheless, many patients use sugar-free gum or sweets and frequently report their benefits. The British Society for Rheumatology (BSR) recommends that SS patients should be advised to chew xylitol-containing sugar-free gums [15]. If patients with moderate dysfunction do not improve dry mouth with local therapies, oral muscarinic agonists are considered.

Pilocarpine and cevimeline have been approved for dry mouth in SS. Although they target the same family of receptor, cevimeline may have

fewer side effects than pilocarpine due to less selectivity for the receptor subtype in the heart. However, it is not available worldwide [22].

11.2.1.1 Pilocarpine Hydrochloride

Three randomized controlled trials (RCTs) reported that oral pilocarpine leads to a significant increase in whole salivary flow rates and dry mouth improvement compared to placebo [23–25]. They used a regimen consisting of pilocarpine (20–30 mg/day) in four divided doses for 12 weeks. However, pilocarpine-induced sweating has been reported in 10–34% when taking pilocarpine 7.5–15 mg/day [26]. This adverse effect can limit patients to reach an effective therapeutic dose and lead to decreased patient compliance. Therefore, in the BSR guideline, a lower starting dose of pilocarpine (5 mg once daily) is recommended [15]. If the response is not enough, the dose can be progressively escalated by 5 mg/day every week, up to the maximum tolerated dose. In the case of patients who are sensitive to oral pilocarpine, pilocarpine hydrochloride eye drops may be applied sublingually as an alternative option to deliver a smaller dose although it is off-labeled [15]. One drop of 2 or 4% pilocarpine ophthalmic solution is equivalent to 1 or 2 mg of oral pilocarpine, respectively. Other adverse effects include chilling, rhinorrhea, blurred vision, nausea, dyspepsia, diarrhea, abdominal pain, and frequent urination. Additionally, oral pilocarpine is contraindicated in patients with uncontrolled asthma and chronic obstructive pulmonary disease, unstable heart disease, closed-angle glaucoma, and acute iritis, due to its cholinergic action.

In RCTs, an improvement in dry mouth and the salivary flow rate was reported 6–12 weeks after starting oral pilocarpine. Regular administration of pilocarpine, even at lower doses than recommended, could continuously increase salivation through enhanced expression of muscarinic acetylcholine receptor 3 (M3) [27]. Therefore, the therapeutic efficacy of pilocarpine is recommended to be assessed after 2–3 months of therapy [15]. Three RCTs and two single-arm studies reported that oral pilocarpine improves dry eye symptoms over 3 months in SS patients [24, 25, 28–30]. Moreover, pilocarpine can improve objective measures for ocular sicca, such as rose bengal or fluorescein staining score and tear breakup time (BUT), via an increase in the number of goblet cells and mucin production [28–30]. However, in these studies, oral pilocarpine does not significantly enhance the lacrimal flow rate measured by a Schirmer's test. Oral doses of pilocarpine varied from 7.5 to 30 mg/day according to different studies. However, compared between doses for symptomatic improvement, one trial showed that dry mouth symptom was significantly improved at 20 mg/day of pilocarpine, but significant relief in ocular symptoms was attained at 30 mg/day [24].

There have been no well-designed studies on the efficacy or safety of pilocarpine in juvenile-onset SS. An observation study reported that five girls (9–16 years of age) increased salivary production and improved dry mouth symptoms without serious adverse events at 5–10 mg/day of oral pilocarpine for 1 month [31]. Therefore, M3R agonist is considered to improve salivary dysfunction and relatively safe in pediatric SS patients [16].

There is limited information on the predictive factor on the efficacy of daily pilocarpine in patients with SS. However, it was reported in a previous study that when salivary flow rate was measured at 1, 2, and 3 h after stimulation with 0.1 ml administered sublingually of an ophthalmic 5% pilocarpine solution (5 mg pilocarpine), stimulated salivary flow rate was greater than 0.1 mL/min in a group of SS patients with a shorter disease duration, milder grades in salivary scintigraphic findings and focal lymphocyte infiltration in salivary gland biopsies, and lower frequency of positive immunological markers [32]. These factors could be associated with a residual salivary capacity or non-severe disease.

11.2.1.2 Cevimeline Hydrochloride

Compared to pilocarpine hydrochloride, cevimeline hydrochloride is a muscarinic agonist with a longer half-life (3–6 h vs. 0.8 h) and higher specificity for M1R and M3R [33]. Although profiles of adverse effects are similar between pilocarpine and cevimeline, patients taking cevimeline are less likely to have adverse events than those taking pilocarpine because of high affinity for M1R and M3R in the salivary/lacrimal glands instead of M2R in the heart [22, 34]. The efficacy of the

two drugs in salivation in patients with hyposalivation is similar [34, 35].

The most commonly used and studied cevimeline dose is 60–90 mg/day divided into two or three times daily. Among three RCTs with SS patients, two studies showed that 90 mg/day of cevimeline significantly improved dry mouth symptoms and increased salivary flow rates [36, 37]. There was a significant improvement in xerostomia-related questionnaire scores observed in another crossover study, but no change was observed in salivary flow rate [38]. Additionally, it was reported that cevimeline treatment significantly improves subjective and objective assessments for dry eye and increases lacrimal flow rate [36, 39]. Like pilocarpine, cevimeline responsive patients were more likely to have normal sialographic findings, negative minor salivary gland biopsy, negative anti-La/SS-B, or absence of hypergammaglobulinemia [40, 41].

11.2.1.3 Other Strategies

Some SS patients are too sensitive or nonresponsive to oral muscarinic agonists, especially when glandular tissues are severely damaged. Although we have limited therapeutic options in such cases, other reagents may help relieve their sicca symptoms. Several mucolytic agents (bromhexine, *N*-acetylcysteine, or ambroxol) were studied in SS patients in the 1970s–1980s. Five clinical trials of bromhexine have been published [42–46]. When 32–64 mg/day of bromhexine was administered for 2–3 weeks, four studies reported that bromhexine significantly stimulated tear production or increased BUT. One study reported improved dry mouth symptoms [45] whereas no improvement in salivary flow rate in SS patients and healthy controls by bromhexine reported in another study [43]. A crossover trial of *N*-acetylcysteine and one single-arm study of ambroxol (135 mg/day for 8 weeks) showed an improvement of dry eye and/or mouth with no change in objective measures [47, 48]. Nizatidine, an H2 histamine receptor antagonist, at 300 mg/day for 8 weeks significantly increased the salivary secretion and improved xerostomia-related clinical conditions by 20% over baseline [49]. These medications have good safety and tolerability profiles. However, the results in the studies need to be interpreted carefully because of marginal benefits and study limitations including a small sample size.

Several open trials of methotrexate and mizoribine showed improved sicca symptoms or increase in the salivary flow rate, especially in SS patients free of minor salivary gland fibrosis [50–52]. However, to treat sicca symptoms, immunomodulating or immunosuppressive agents, including HCQ, glucocorticoids (GCs), or rituximab, are not recommended because of a lack of evidence on their efficacy on salivation [16, 17].

Two randomized controlled studies and four cohort studies described the effect of salivary gland irrigation with 2 mg/mL of prednisolone, 100 mg of hydrocortisone, 4 mg/mL of betamethasone, or normal saline with/without sialendoscopic intervention [53–58]. The intervention significantly increased salivary flow and improved dry mouth, and its effects remained in a period of up to 3 years. Although sialoendoscopy is not widely available and there are no well-designed long-term studies published, sialendoscopic intervention with irrigation may be useful in SS patients with chronic obstructive sialadenitis unresponsive to medical therapy [15, 16].

11.2.2 Salivary Gland Swelling

Salivary gland swelling in a well-recognized manifestation in SS patients and about 25–66% of patients have recurrent parotid or submandibular enlargement over the course of the disease [59]. In patients with SS, salivary gland swelling results from disease activity, duct obstruction secondary to stricture, stone, or thick secretion, retrograde bacterial infection, and non-Hodgkin's lymphoma. Especially in children and young adults, salivary gland swelling is often a presenting feature preceding sicca symptom and hyposalivation. In a case with acute inflammatory parotitis without infection or stones, BSR recommends a short course of oral prednisolone or a single intramuscular injection of methylprednisone [15]. EULAR recommends using a stepwise approach: first, nonsteroidal anti-inflammatory

drugs (NSAIDs) for 3–5 days; second, GCs (0.3 mg/kg/day); third, B cell-targeted agents such as rituximab and belimumab [17].

If a patient has chronically persistent parotid swelling, the medical management is limited. An open-label study of HCQ showed that several salivary inflammatory markers were decreased, and the improvement of salivary gland swelling was observed in 2/14 patients with SS [60]. However, HCQ is generally considered ineffective in salivary gland swelling. Since sialendoscopic intervention can dilate a ductal stricture and remove mucus plugs and debris with irrigation or with forceps, the recurrence of glandular swelling can be reduced in SS patients with chronic salivary gland swelling [57].

Because parotid enlargement tends to decrease over time, surgical treatment such as partial parotidectomy is not indicated. Infectious parotitis is sometimes difficult to distinguish from salivary gland swelling by SS because of the absence of constitutional symptoms and normal levels of acute-phase reactants [61]. If a patient has pus discharge from the parotid duct, infectious parotitis can be diagnosed. Because *Staphylococcus aureus* is the most common etiologic microorganism in community-acquired parotitis, first-line antibiotics should include antistaphylococcal antibiotics such as nafcillin, oxacillin, and cefazolin [62]. Since lymphoma is developed in 2–5% and can present as progressive, persistent, or nodular enlargement of the salivary glands, progressive enlargement or newly developed nodular lesion in the parotid gland can be a clue to malignant lymphoma. If lymphoma is suspected, a tissue biopsy is mandatory.

11.2.3 Salivary Dysfunction-Related Problems

11.2.3.1 Frictional Oral Ulcer

Because of the loss of salivary lubrication, SS patients often suffer from oral mucosal friability and frictional oral ulcers. Oral gel-like formulations such as polyvinylpyrrolidone and sodium hyaluronate oral gel may be useful [15]. Previous studies on oral M3R agonists did not evaluate oral mucosal function or integrity. However, since a cevimeline trial reported a significant improvement in the morphological appearance of the tongue [38], pilocarpine or cevimeline could relief oral mucosal friability.

11.2.3.2 Candidiasis

Low saliva production leads to a reduction of antimicrobial proteins or peptides levels and an increase in the incidence of oral infection. Oral candidiasis can be observed in about 40% of SS patients [63]. Candidiasis usually presents as plaques, pseudomembranous or erythematous mucosal lesions, fissured tongue, filiform papillae atrophy, and angular cheilitis. Also, it can present with oral or tongue pain without typical mucosal change [61]. Topical nystatin and miconazole are useful for simple intraoral infection and angular cheilitis, respectively [63]. However, if a patient has an erythematous infection or is resistant to topical antifungal agents, oral fluconazole (50 mg/day for 10 days) is indicated [15]. A small size trial reported that 1-year administration of pilocarpine decreased *Candida* carriage rate from 75 to 25% [64]. Therefore, oral M3R agonists can reduce *Candida* colonization in the oral cavity in patients with SS.

11.2.3.3 Dental Caries

SS patients have increased risk of caries development and subsequent tooth loss secondary to salivary hypofunction. A systematic review showed that SS patients have significantly higher values of decayed, missing, and filled teeth and surfaces (DMFT and DMFS) than controls [65]. However, a recent large cohort study did not find the protective effect of pilocarpine on dental caries in patients with SS during the mean follow-up of 2.6 years [66]. Thus, a more active multidisciplinary approach is required to prevent dental caries in SS patients. The recent guidelines for rheumatologists or dentists describe evidence-based management strategies for caries prevention in SS patients [15, 67]. It is strongly recommended to use topical fluoride (or non-fluoride remineralizing agents), local or systemic salivary stimulants, and local chlorhexidine and general approaches to improve oral health.

11.3 Treatment of Lacrimal Gland Involvement

Current guidelines recommend using artificial tears and gels/ointments as the first-line treatment for dry eye. Various artificial tears and ocular gels/ointments include lubricants with a polymer base or viscosity agent (methylcellulose or hyaluronate). Because preservatives in artificial tears may damage and irritate the ocular surface, single-use and preservative-free formulations are generally recommended in SS patients requiring frequent (≥4/day) applications [15, 17]. Ocular gels/ointments could be beneficial to control nocturnal ocular symptom, but eyelid hygiene must be performed to prevent blepharitis.

If severe or refractory to the first-line approach, a short-term course of topical corticosteroids or a long-term use of cyclosporine ophthalmic emulsion is recommended under ophthalmic supervision [15–17]. These drugs act as an anti-inflammatory agent for chronic corneal inflammation. Additionally, according to the EULAR recommendation, autologous serum eye drops (20% solution) can be considered in severe cases refractory or intolerant to topical cyclosporine [17]. There is limited evidence supporting its efficacy, and there are some disadvantages including the infection risk and the need to refrigerate. But autologous serum is expected to be helpful in ocular dryness because serum contains growth factors, anti-inflammatory proteins, and matrix-degrading enzyme inhibitors.

In case of failure of the abovementioned topical therapies, the EULAR recommends oral muscarinic agonists or punctal occlusion as a rescue therapy [17]. Pilocarpine or cevimeline has been reported to improve subjective ocular dryness (but not objective outcomes). More detailed information on the management and treatment of dry eye is available in Chap. 9. Clinical Management of Dry Eye in SS.

11.4 Treatment of Other Xeroses

Major target organs of SS are the salivary and lacrimal glands. However, the involvement of the other exocrine glands, including the upper airways and vagina, occurs in SS patients.

11.4.1 Dry Cough

Dry cough is observed in 54% of patients with SS [68]. It may result from airway dryness, abnormal mucociliary clearance, bronchial inflammation or hyperresponsiveness, and gastroesophageal reflux [69]. Additionally, chronic dry cough is a sign of interstitial lung disease associated with SS. Although there is no study on the treatment of dry cough in SS, humidification, or nebulized saline solution, mucolytic agents and oral M3R agonists are recommended by BSR [15].

11.4.2 Vaginal Dryness

Vaginal dryness and dyspareunia were reported in 55 and 61% of SS patients, respectively [70]. Vaginal change affects their poor sexual function and high sexual distress [71]. SS patients with vaginal dryness can use several products designed for vaginal dryness, including vaginal moisturizers [15]. If postmenopausal women with vaginal atrophy complain of vaginal dryness, topical estrogen cream or estrogen-containing vaginal moisturizers may be indicated.

11.5 Treatment of Extraglandular Involvement

Systemic involvement in SS is a crucial prognostic factor because it can be severe in about 15% and leads to irreversible organ dysfunction [72]. However, not all systemic involvement essentially requires systemic treatment, and its treatment depends on the affected organ and its severity. There are insufficient data supporting certain drugs in a specific manifestation, hence the management of systemic involvement may vary depending on individual physicians in clinical practice. As mentioned earlier, several societies have recently published the recommendations for SS management [14–17], which largely relied on the expert consensus. The assessment of disease activity is essential to determine treatment strategy, and EULAR Sjögren's syndrome dis-

ease activity index (ESSDAI) [73, 74] has been increasingly used clinically and for research. The most recently published EULAR recommendation [17] suggested the tailored treatment according to organ-specific severity using ESSDAI definitions.

The current treatment options are summarized in Table 11.2.

Table 11.2 Pharmacologic treatment according to the extraglandular manifestations of SS

Manifestations	Treatment options
Arthralgia/arthritis	Acetaminophen
	Nonsteroidal anti-inflammatory drugs
	Hydroxychloroquine
	Glucocorticoids, short-term or intra-articular
	Methotrexate
	Other antirheumatic drugs
	Rituximab or abatacept
Purpura/skin ulcers	Systemic glucocorticoids, if moderate or high activity
	Immunosuppressants
	Rituximab for cryoglobulinemic vasculitis
	Cyclophosphamide or plasma exchange for refractory vasculitis
Annular erythema	Topical glucocorticoids or tacrolimus
	Systemic glucocorticoids, if diffuse involvement
	Hydroxychloroquine
	Immunosuppressants
Interstitial lung disease	Glucocorticoids, if moderate to high activity
	Immunosuppressants
	Rituximab for pulmonary hemorrhage
Pulmonary arterial hypertension	Endothelin receptor antagonists (bosentan, ambrisentan, macitentan)
	Phosphodiesterase inhibitor (sildenafil, tadalafil, vardenafil)
	Soluble guanylate cyclase inhibitors (riociguat)
	Prostacyclin analogues (epoprostenol, iloprost, treprostinil beraprost)
	Selective prostacyclin receptor antagonist (selexipag)
Renal involvement	Bicarbonate supplementation for renal tubular acidosis
	Glucocorticoids, if moderate to high activity
	Immunosuppressants
	Rituximab and/or plasma exchange for cryoglobulinemic membranous proliferative glomerulonephritis
Interstitial cystitis	Glucocorticoids with calcineurin inhibitors
	Local bladder treatment including hydraulic distension
Peripheral nerve involvement	Gabapentin or pregabalin
	Fludrocortisone acetate or midodrine, if autonomic dysfunction
	Glucocorticoids
	Intravenous immunoglobulin
	Immunosuppressants
	Rituximab
	Plasma exchange
Central nerve involvement	High-dose or pulsed glucocorticoids, especially vasculitis or neuromyelitis optica spectrum disorders (NMOSD)
	Immunosuppressants such as cyclophosphamide
	Rituximab and/or plasma exchange if refractory
	Eculizumab for recurrent NMOSD
Neutropenia	G-CSF if infectious condition in severe neutropenia
Severe hemolytic anemia or	Glucocorticoids
Immune thrombocytopenia	Intravenous immunoglobulin
	Rituximab
Inflammatory myositis	Glucocorticoids
	Intravenous immunoglobulin
	Immunosuppressants

11.5.1 Evidence for the Use of Antirheumatic Drugs and Biologics

Non-biologic anti-inflammatory drugs, such as GCs, antimalarials, immunosuppressive agents, and intravenous immunoglobulin (IVIg), are commonly used in treating systemic symptoms associated with SS, even though strong evidence is lacking. Their use to treat SS is based on limited data from uncontrolled trials or its effectiveness in managing other autoimmune diseases. EULAR recommends that systemic therapies may be considered for the treatment of active systemic disease (clinical ESSDAI ≥1).

GCs are frequently used for managing the active systemic disease. In potential life-threatening manifestations, pulse GC therapy (≥250 mg prednisone equivalent a day for one or a few days) or high-dose GCs (>30 mg, but ≤100 mg prednisone equivalent a day) [75] can be considered. However, to avoid adverse effects, the dosage and treatment duration of GCs should be minimized [17]. Concomitant synthetic immunosuppressive agents are often given for refractory cases or GC-sparing purposes.

HCQ is a commonly used drug in SS, but evidence of its effectiveness is variable. Several studies reported improved symptoms such as joint pain, fatigue, and serologic parameters in SS [60, 76, 77]. However, the JOQUER study [7], a pivotal RCT of HCQ daily 400 mg in SS with mild disease activity, failed to prove its efficacy.

Considering the pathophysiology of SS and successful treatment outcomes in other autoimmune diseases, the use of biologic agents in SS may be a promising approach. However, their role in the treatment of extraglandular involvements in SS remains controversial. Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown a beneficial effect on the systemic involvement of SS [78, 79]. Meanwhile, subsequent two key randomized trials [80, 81] and a recent systematic review [82] on rituximab did not demonstrate its efficacy in SS. Another B cell-targeted agent, belimumab, demonstrated a significant reduction in ESSDAI in open-label trials [83, 84]. The efficacy of belimumab needs to be confirmed in further large, well-designed RCTs. The RCTs of antitumor necrosis factor (TNF) agents, etanercept and infliximab, failed to show clinical and serologic improvement [85, 86]. An open-label study using abatacept suggests its efficacy in improving disease activity [87], but recently published result of a RCT found no statistically significant placebo differences in improving clinical measures [88]. Taken together, the role of biologics in SS is still debatable, but B cell-targeted therapies may be considered in SS patients with severe, refractory systemic disease, as recommended by EULAR [17]. We discuss the treatment of extraglandular involvements according to the involved organ herein.

11.5.2 Articular Manifestations

Articular manifestations occurred in 30–60% of patients with SS [89]. For the management of musculoskeletal symptoms of SS, arthralgia (subjective feeling) should be distinguished from arthritis (objective inflammatory signs suggesting joint inflammation in the physical examination or imaging studies). The musculoskeletal pain associated with other non-inflammatory conditions such as osteoarthritis or fibromyalgia syndrome should also be separately managed. The ESSDAI score classified the presence of arthralgia in hands, wrists, ankles, and feet accompanied by morning stiffness (>30 min) as low activity, 1–5 synovitis (of 28 total counts) as moderate activity, and ≥6 synovitis as high activity [74]. Acetaminophen or NSAIDs are used for the symptomatic relief of acute musculoskeletal pain [89]. When arthralgia does not respond to NSAIDs, HCQ can be considered based on several uncontrolled studies and a recent meta-analysis [76, 90, 91]. Two RCTs investigating the role of HCQ in SS failed to demonstrate its significant efficacy, but there was a positive trend in the improvement of joint pain [7, 77].

The main drugs used to treat inflammatory arthritis are also NSAIDs and HCQ, especially in moderate activity. In severe cases (moderate-

activity refractory to NSAIDs plus HCQ or high-activity articular involvement), short-term GCs therapy is considered effective [89]. Antirheumatic agents, including methotrexate, leflunomide, sulfasalazine, cyclosporine A, or azathioprine, may be considered [92]. Although head-to-head comparisons between these drugs have never been conducted in SS, methotrexate is the most frequently used drug to treat inflammatory arthritis in clinical practice. It is recommended as a second-line drug following HCQ in the American College of Rheumatology (ACR) guidelines [93]. Biologic drugs such as rituximab and abatacept may be considered as a therapeutic option for SS patients with inflammatory arthritis refractory to conventional drugs, extrapolating from the use of these drugs in RA.

11.5.3 Cutaneous Purpura/Ulcers

The prevalence of cutaneous vasculitis in SS is 10% [68]. Based on clinical experience, GCs are the mainstay of cutaneous vasculitis treatment. The ESSDAI score classifies the activity of cutaneous vasculitis according to the extent of cutaneous involvement and the presence of ulcers; moderate activity, limited cutaneous vasculitis including urticarial vasculitis or purpura limited to feet and ankle; high activity, diffuse cutaneous vasculitis, including urticarial vasculitis, or diffuse purpura, or ulcers related to vasculitis [74]. Moderate-dose GCs alone are usually used in moderate activity. To treat high ESSDAI cutaneous involvement, moderate- to high-dose GCs may be considered as the first-line option, and immunosuppressive agents such as azathioprine, methotrexate, or mycophenolate mofetil are the second options to be used [17, 94]. In the case of cryoglobulinemic vasculitis, rituximab is preferred [95], with no evidence supporting its superiority over classic immunosuppressive drugs in clinical trials. Cylclophosphamide or plasma exchange options for patients with refractory cutaneous vasculitis, especially in life-threatening cryoglobulinemic vasculitis.

11.5.4 Annular Erythema

Annular erythema can occur in approximately 10% of SS patients [96]. In limited cases, topical GCs are primarily used. Systemic treatment might be considered in SS patients with diffuse annular erythema. HCQ and/or low-dose GCs are first-line drugs, and in refractory cases, other antimalarials and/or moderate- to high-dose GCs are considered [17, 97]. Also, there have been small studies documenting the efficacy of cyclosporine A, methotrexate, and topical tacrolimus [98–100].

11.5.5 Interstitial Lung Disease

The reported prevalence of clinically significant lung involvement in patients with SS ranges from 9 to 24%. Though the entire respiratory system can be affected in SS, interstitial lung disease (ILD) is a major problem because it is associated with impaired pulmonary function and increased mortality [101]. Nonspecific interstitial pneumonitis (NSIP) is the most common subtype (45%) observed in SS, followed by usual interstitial pneumonia (UIP, 17%), lymphocytic interstitial pneumonia (LIP, 15%), and organizing pneumonia (OP, 8%) [69].

Since RCTs for only SS-ILD are unavailable, the treatment of SS-ILD usually follows the treatment strategy of connective tissue disease (CTD)-ILD. Not all SS patients with ILD are progressive, so those who have mild activity (no respiratory symptoms and abnormal findings in the HRCT <10%) might be monitored regularly. In moderately to highly active pulmonary involvement, GCs are the mainstay of ILD treatment, especially indicated for LIP and OP, less in NSIP and even less in UIP [69]. Immunosuppressant (azathioprine, mycophenolate mofetil, cyclosporine A, chlorambucil, and cyclophosphamide) can be combined when SS patients with ILD suffer from shortness of breath at rest (NYHA III or IV) or had an abnormal lung function test with DLco <40% or FVC <60% [17]. Regarding biologic drugs, there have been case reports describing the efficacy of tocilizumab, abatacept, and rituximab

in SS patients with refractory ILD [102–104]. Rituximab would be useful in SS patients with acute pulmonary hemorrhage associated with cryoglobulinemia [105].

11.5.6 Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH, mean pulmonary arterial pressure of ≥25 mmHg with a pulmonary capillary wedge pressure ≤15 mmHg) is a serious complication of SS with high mortality. The prevalence of PAH in SS is relatively rare compared to other CTD, such as systemic sclerosis or systemic lupus erythematosus [106]. The British registry data have shown that SS-PAH accounts for only 1% of CTD-PAH [107], whereas the data in Asian populations have reported a higher distribution (15%) [108].

There is no specific recommendation for the treatment of SS-PAH, and the therapeutic approach of SS-PAH itself usually follows that of idiopathic PAH or CTD-PAH. Supportive care, including diuretics for preventing fluid overload, oxygen supplementation, or respiratory rehabilitation, is beneficial. Several drugs targeting the prostacyclin, endothelin-1, and nitric oxide pathways have shown efficacy for PAH treatment in RCTs over the past two decades; (1) endothelin receptor antagonists (bosentan, ambrisentan, macitentan), (2) phosphodiesterase inhibitor (sildenafil, tadalafil, vardenafil), (3) soluble guanylate cyclase inhibitors (riociguat), (4) prostacyclin analogues (epoprostenol, iloprost, treprostinil beraprost), and (5) oral selective IP prostacyclin receptor antagonist (selexipag) [109]. The role of immunosuppression in SS-PAH is questionable, though a small cohort study revealed a favorable outcome in SS-PAH treated with immunosuppressants such as cyclophosphamide, methotrexate, leflunomide, and tacrolimus [108].

11.5.7 Renal Involvement

The characteristic renal involvements in SS are tubulointerstitial nephritis (TIN)/renal tubular acidosis (RTA) and glomerulonephritis (GN). The reported prevalence rates of TIN/RTA and GN in SS patients were 9% and 4%, respectively [68]. Concerning tubulointerstitial involvement, type 1 distal RTA comprises the majority (97%), and it can accompany hypokalemic weakness/paralysis (69%), nephrocalcinosis (17%), and rarely renal failure (24%) at clinical presentation. The typical form of GN in SS is membranous proliferative GN (MPGN), sometimes associated with cryoglobulinemia.

As a symptomatic treatment, a long-term bicarbonate and/or electrolyte supplementation may be needed to prevent life-threatening electrolyte imbalance. GCs are the cornerstone in patients with moderate- (tubular acidosis with renal failure [GFR <60 m/min], GN with proteinuria [1–1.5 g/day] without hematuria or renal failure, or histologic evidence of extramembranous GN or important lymphoid infiltrate) to high- (GN with proteinuria >1.5 g/day or hematuria or renal failure, or histologic evidence of proliferative GN or cryoglobulinemia-related renal involvement) activity renal involvement [17]. Several immunosuppressive agents, including azathioprine, cyclosporine, mycophenolate mofetil, and cyclophosphamide, are often combined to achieve and maintain remission, although cyclophosphamide is no longer recommended for TIN due to its adverse effects [110]. In SS patients with MPGN secondary to cryoglobulinemia, GC pulse therapy combined with rituximab and/or plasma exchange is usually considered, despite weak evidence based on an open-label and retrospective study [79].

11.5.8 Interstitial Cystitis

There are published case reports and series reporting successful treatment of SS-associated interstitial cystitis with combination therapy of GCs and cyclosporine or tacrolimus [111, 112]. Local bladder treatment, such as hydraulic distension, dimethyl sulfoxide instillation, and urinary catheter insertion in case of obstructive renal failure, can be performed if needed [111].

11.5.9 Peripheral Nerve Involvement

Neurologic manifestation is reported in approximately 20% of SS patients, comprising 15% of peripheral and 5% of central nervous system (CNS) involvement [113]. Recent recommendations are based on limited evidence of small, uncontrolled studies or extrapolated from the treatment of other systemic involvements of SS or neurologic involvement of other autoimmune diseases.

Patients with SS may exhibit various peripheral neuropathies, including pure sensory polyneuropathy, dorsal root ganglionopathy, trigeminal neuralgia, sensorimotor polyneuropathy, mononeuritis multiplex, polyradiculopathies, and autonomic neuropathy. Management of the peripheral nervous system is complicated due to its heterogeneity and should be approached toward the underlying pathogenic mechanism.

Treatment for pure sensory neuropathy or small-fiber neuropathy is mainly symptomatic, including control of neuropathic pain with gabapentin or pregabalin and correction of cardiovascular risk factors [17, 114]. The use of tricyclic antidepressants such as amitriptyline and nortriptyline are commonly avoided due to their anticholinergic effect. Trigeminal neuropathy/neuralgia does not usually respond to GCs and is generally managed similarly to the treatment of trigeminal neuralgia. Autonomic dysfunction associated with SS is conservatively treated with fludrocortisone acetate or midodrine [115].

Other peripheral neuropathies are generally treated with GCs, with dosages depending on disease severity. IVIg, immunosuppressive drugs, such as azathioprine, mycophenolate mofetil and cyclophosphamide, or rituximab, are frequently combined if necessary, with variable responses [94, 115]. Plasma exchange can be considered in refractory disease or life-threatening conditions (e.g., severe neuropathy associated with cryoglobulinemic vasculitis) [95]. SS patients with mononeuritis multiplex should be evaluated for underlying cryoglobulinemic vasculitis. In the case of mononeuritis multiplex or vasculitis-related polyneuropathy, EULAR recommends a sequential approach as follows: first, moderate-to high-dose GCs; second, oral immunosuppressive agent or rituximab; and rescue, cyclophosphamide and/or plasma exchange [17]. IVIg may be beneficial for SS patients with sensorimotor polyneuropathies, ataxic sensory neuropathy, and peripheral demyelinating disorder [116–118]. Also, EULAR recommends IVIg as the first-line treatment in SS-associated ganglionopathy and chronic inflammatory demyelinating polyneuropathy [17].

11.5.10 Central Nerve Involvement

The CNS manifestations of SS encompass CNS vasculitis, neuromyelitis optica spectrum disorders (NMOSD), aseptic meningitis, and multiple sclerosis mimetic [115]. Aseptic meningitis may be managed by symptomatic therapy. Abnormal hyperintense lesions on brain magnetic resonance imaging have been reported in SS patients, and its underlying mechanisms seem to be immunologically mediated small vessel vasculopathy/vasculitis [119].There have been increasing reports of coexistence of SS and NMOSD, an autoimmune inflammatory demyelinating CNS disease, which are often associated with the presence of anti-aquaporin-4 antibodies [120]. SS patients with NMOSD warrant aggressive immunosuppressive therapy because of the progressive and relapsing nature of NMOSD, and both BSR and EULAR encourage aggressive immunosuppression [15, 17]. In SS patients with CNS vasculitis, NMOSD, or meningitis not responding symptomatic therapy, high-dose GCs or pulsed steroids are the first-line treatments. Regarding immunosuppressive drugs, cyclophosphamide may be used as second-line induction therapy for cases refractory to GCs, and mycophenolate mofetil or azathioprine may be used as a maintenance regimen. In severe or life-threatening cases, rituximab and/or plasma exchange is considered as rescue therapy [17]. Eculizumab, a monoclonal antibody against terminal complement activation of C5, has shown benefit for patients with anti-aquaporin 4 antibody-positive relapsing NMOSD in an open-label trial [121].

11.5.11 Fatigue

Fatigue is reported in up to 70% of the SS patients leading to reduced health-related quality of life [122]. Some small studies have demonstrated reductions in fatigue levels in SS patients treated with HCQ [60, 76, 77], whereas the same effect was not observed in a large RCT of HCQ [7]. Although evidence supporting the use of HCQ in treating fatigue is weak, the recommendation from ACR comments that HCQ may be considered in selected situations to treat fatigue [93]. Of the several non-biologic compounds that have been evaluated in RCTs, dehydroepiandrosterone [123, 124], omega-6 fatty acid gamma-linolenic acid [125] and low-dose doxycycline [126] failed to improve fatigue in SS. In terms of biologic agents, improvement in fatigue by rituximab is observed in a small prospective open-label study and earlier RCTs [80, 127–129]. In contrast, another larger RCT [81] and recent meta-analyses [82, 130] did not confirm its efficacy to improve fatigue.

As non-pharmacological interventions, aerobic exercise has shown to improve fatigue in a single, relatively small ($n = 19$), non-randomized control trial [131]; therefore, the BSR guidelines recommended regular exercise and a graded exercise program for SS patients presenting with fatigue.

11.5.12 Hematologic Involvement

Hematologic abnormalities are frequently encountered in SS. Anemia, especially anemia of chronic disease, is the most common finding (30%), followed by leukopenia (14%), thrombocytopenia (11%), and neutropenia (7%) [132, 133]. Because severe hematologic abnormalities are infrequent in SS, treatment is not mandatory in most cases. Treatment of severe hematologic abnormalities in SS is empirical. In the case of severe neutropenia (absolute neutrophil count <500/mm^3), the use of human granulocyte colony-stimulating factor (G-CSF) is considered, especially in patients with recurrent/severe infections [17]. Patients with severe immune thrombocytopenia (platelet count <50,000/mm^3) and severe hemolytic anemia (hemoglobin <8 g/dL) receive GCs as the first-line drug, and IVIg or rituximab is considered in refractory or symptomatic cases. Plasma exchange and cyclophosphamide are the other options as rescue therapy [17].

SS is known to have the highest risk of lymphoma development among autoimmune diseases: a standardized incidence ratio of 5–44 when compared to general population [2, 17]. Especially, salivary gland swelling, cutaneous vasculitis, low-serum complement levels, ectopic germinal center-like structures in minor salivary gland biopsy, and high disease activity (ESSDAI score ≥14) are risk factors for the development of malignant lymphoma [2, 4]. Also, several hematologic findings, including neutropenia and lymphopenia at baseline, are associated with the increased risk of lymphoma development [132].

Most cases with SS-related lymphoma present marginal zone lymphoma, including mucosa-associated lymphoid tissue (MALT) lymphoma and nodal marginal zone lymphoma [4, 16]. The most frequent site of MALT lymphoma is the salivary glands with a primary site being the parotid glands. However, it can occur in other mucosal sites such as the lung and stomach. The treatment of B cell lymphoma must be individualized based on the pathologic subtype and current therapeutic guidelines and should be managed by hematologist/oncologists [17]. The detailed information on lymphoma diagnosis and treatment is available in Chap. 8.

11.5.13 Muscular Involvement

Muscle pain without muscle weakness and muscle enzyme elevation corresponds to low ESSDAI domain, and analgesics may be prescribed to relieve myalgia. This symptom needs to be differentiated from chronic widespread pain associated with fibromyalgia, which coexists in 35–50% of patients with SS [134]. Occasionally, inflammatory myositis with muscle weakness or elevated muscle enzyme can be present in SS [135, 136], and it should be confirmed by electromyog-

raphy or biopsy and distinguished from steroid-induced myopathy. GCs are used as the first-line treatment of inflammatory myositis with favorable outcomes [135]. Other conventional immunosuppressive drugs (methotrexate, azathioprine, mycophenolate mofetil, and calcineurin inhibitors) and IVIg can be used alone or combined, as used in patients with inflammatory myopathy [94].

11.6 Conclusion

Currently, the overall evidence about therapeutic agents in SS patients is at low level, and there are still many unmet clinical needs in the management SS. However, several studies have recently reported the treatment guidelines that may be applicable to manage SS patients, based on recent evidence. The role of biologic agents in SS has been extensively investigated, revealing inconclusive results. Several new potential therapeutics targeting SS pathogenesis are actively being studied [137]. In the future, high-quality scientific evidence focusing on the superiority of certain drugs or treatment strategies and biomarkers to predict treatment response in SS will be needed.

References

1. Ramos-Casals M, Tzioufas AG, Font J. Primary Sjögren's syndrome: new clinical and therapeutic concepts. Ann Rheum Dis. 2005;64:347–54. https://doi.org/10.1136/ard.2004.025676.
2. Brito-Zerón P, Baldini C, Bootsma H, Bowman SJ, Jonsson R, Mariette X, et al. Sjögren syndrome. Nat Rev Dis Primers. 2016;2:16047. https://doi.org/10.1038/nrdp.2016.47.
3. Vitali C, Palombi G, Baldini C, Benucci M, Bombardieri S, Covelli M, et al. Sjögren's Syndrome Disease Damage Index and disease activity index: scoring systems for the assessment of disease damage and disease activity in Sjögren's syndrome, derived from an analysis of a cohort of Italian patients. Arthritis Rheum. 2007;56:2223–31. https://doi.org/10.1002/art.22658.
4. Nocturne G, Mariette X. Sjögren syndrome-associated lymphomas: an update on pathogenesis and management. Br J Haematol. 2015;168:317–27. https://doi.org/10.1111/bjh.13192.
5. Ramos-Casals M, Tzioufas AG, Stone JH, Sisó A, Bosch X. Treatment of primary Sjögren syndrome: a systematic review. JAMA. 2010;304:452–60. https://doi.org/10.1001/jama.2010.1014.
6. Miyamoto ST, Lendrem DW, Ng WF, Hackett KL, Valim V. Managing fatigue in patients with primary Sjögren's syndrome: challenges and solutions. Open Access Rheumatol. 2019;11:77–88. https://doi.org/10.2147/oarrr.S167990.
7. Gottenberg JE, Ravaud P, Puechal X, Le Guern V, Sibilia J, Goeb V, et al. Effects of hydroxychloroquine on symptomatic improvement in primary Sjogren syndrome: the JOQUER randomized clinical trial. JAMA. 2014;312:249–58. https://doi.org/10.1001/jama.2014.7682.
8. Yoon CH, Lee HJ, Lee EY, Lee EB, Lee WW, Kim MK, et al. Effect of hydroxychloroquine treatment on dry eyes in subjects with primary Sjögren's syndrome: a double-blind randomized control study. J Korean Med Sci. 2016;31:1127–35. https://doi.org/10.3346/jkms.2016.31.7.1127.
9. Haldorsen K, Bjelland I, Bolstad AI, Jonsson R, Brun JG. A five-year prospective study of fatigue in primary Sjögren's syndrome. Arthritis Res Ther. 2011;13:R167. https://doi.org/10.1186/ar3487.
10. Gannot G, Lancaster HE, Fox PC. Clinical course of primary Sjögren's syndrome: salivary, oral, and serologic aspects. J Rheumatol. 2000;27:1905–9.
11. Theander E, Andersson SI, Manthorpe R, Jacobsson LT. Proposed core set of outcome measures in patients with primary Sjögren's syndrome: 5 year follow up. J Rheumatol. 2005;32:1495–502.
12. Haldorsen K, Moen K, Jacobsen H, Jonsson R, Brun JG. Exocrine function in primary Sjögren syndrome: natural course and prognostic factors. Ann Rheum Dis. 2008;67:949–54. https://doi.org/10.1136/ard.2007.074203.
13. Gazeau P, Cornec D, Jousse-Joulin S, Guellec D, Saraux A, Devauchelle-Pensec V. Time-course of ultrasound abnormalities of major salivary glands in suspected Sjögren's syndrome. Joint Bone Spine. 2018;85:227–32. https://doi.org/10.1016/j.jbspin.2017.02.007.
14. Vivino FB, Carsons SE, Foulks G, Daniels TE, Parke A, Brennan MT, et al. New treatment guidelines for Sjogren's disease. Rheum Dis Clin N Am. 2016;42:531–51. https://doi.org/10.1016/j.rdc.2016.03.010.
15. Price EJ, Rauz S, Tappuni AR, Sutcliffe N, Hackett KL, Barone F, et al. The British Society for Rheumatology guideline for the management of adults with primary Sjogren's syndrome. Rheumatology (Oxford). 2017;56:e24–48. https://doi.org/10.1093/rheumatology/kex166.
16. Sumida T, Azuma N, Moriyama M, Takahashi H, Asashima H, Honda F, et al. Clinical practice guideline for Sjogren's syndrome 2017. Mod Rheumatol. 2018;28:383–408. https://doi.org/10.1080/14397595.2018.1438093.

17. Ramos-Casals M, Brito-Zeron P, Bombardieri S, Bootsma H, De Vita S, Dorner T, et al. EULAR recommendations for the management of Sjogren's syndrome with topical and systemic therapies. Ann Rheum Dis. 2020;79:3–18. https://doi.org/10.1136/annrheumdis-2019-216114.
18. Brito-Zerón P, Acar-Denizli N, Zeher M, Rasmussen A, Seror R, Theander E, et al. Influence of geolocation and ethnicity on the phenotypic expression of primary Sjögren's syndrome at diagnosis in 8310 patients: a cross-sectional study from the Big Data Sjögren Project Consortium. Ann Rheum Dis. 2017;76:1042–50. https://doi.org/10.1136/annrheumdis-2016-209952.
19. Cho HJ, Yoo JJ, Yun CY, Kang EH, Lee HJ, Hyon JY, et al. The EULAR Sjogren's syndrome patient reported index as an independent determinant of health-related quality of life in primary Sjogren's syndrome patients: in comparison with non-Sjogren's sicca patients. Rheumatology (Oxford). 2013;52:2208–17. https://doi.org/10.1093/rheumatology/ket270.
20. Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R. Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev. 2011;(12):CD008934. https://doi.org/10.1002/14651858.CD008934.pub2.
21. Bakarman EO, Keenan AV. Limited evidence for non-pharmacological interventions for the relief of dry mouth. Evid Based Dent. 2014;15:25–6. https://doi.org/10.1038/sj.ebd.6400989.
22. Noaiseh G, Baker JF, Vivino FB. Comparison of the discontinuation rates and side-effect profiles of pilocarpine and cevimeline for xerostomia in primary Sjögren's syndrome. Clin Exp Rheumatol. 2014;32:575–7.
23. Wu CH, Hsieh SC, Lee KL, Li KJ, Lu MC, Yu CL. Pilocarpine hydrochloride for the treatment of xerostomia in patients with Sjögren's syndrome in Taiwan--a double-blind, placebo-controlled trial. J Formos Med Assoc. 2006;105:796–803. https://doi.org/10.1016/s0929-6646(09)60266-7.
24. Papas AS, Sherrer YS, Charney M, Golden HE, Medsger TA Jr, Walsh BT, et al. Successful treatment of dry mouth and dry eye symptoms in Sjögren's syndrome patients with oral pilocarpine: a randomized, placebo-controlled, dose-adjustment study. J Clin Rheumatol. 2004;10:169–77. https://doi.org/10.1097/01.rhu.0000135553.08057.21.
25. Vivino FB, Al-Hashimi I, Khan Z, LeVeque FG, Salisbury PL 3rd, Tran-Johnson TK, et al. Pilocarpine tablets for the treatment of dry mouth and dry eye symptoms in patients with Sjögren syndrome: a randomized, placebo-controlled, fixed-dose, multicenter trial. P92-01 Study Group. Arch Intern Med. 1999;159:174–81. https://doi.org/10.1001/archinte.159.2.174.
26. Hendrickson RG, Morocco AP, Greenberg MI. Pilocarpine toxicity and the treatment of xerostomia. J Emerg Med. 2004;26:429–32. https://doi.org/10.1016/j.jemermed.2003.09.013.
27. Minagi HO, Ikai K, Araie T, Sakai M, Sakai T. Benefits of long-term pilocarpine due to increased muscarinic acetylcholine receptor 3 in salivary glands. Biochem Biophys Res Commun. 2018;503:1098–102. https://doi.org/10.1016/j.bbrc.2018.06.125.
28. Tsifetaki N, Kitsos G, Paschides CA, Alamanos Y, Eftaxias V, Voulgari PV, et al. Oral pilocarpine for the treatment of ocular symptoms in patients with Sjögren's syndrome: a randomised 12 week controlled study. Ann Rheum Dis. 2003;62:1204–7. https://doi.org/10.1136/ard.2002.003889.
29. Aragona P, Di Pietro R, Spinella R, Mobrici M. Conjunctival epithelium improvement after systemic pilocarpine in patients with Sjogren's syndrome. Br J Ophthalmol. 2006;90:166–70. https://doi.org/10.1136/bjo.2005.078865.
30. Kawakita T, Shimmura S, Tsubota K. Effect of oral pilocarpine in treating severe dry eye in patients with Sjögren syndrome. Asia Pac J Ophthalmol (Phila). 2015;4:101–5. https://doi.org/10.1097/apo.0000000000000040.
31. Tomiita M, Takei S, Kuwada N, Nonaka Y, Saito K, Shimojo N, et al. Efficacy and safety of orally administered pilocarpine hydrochloride for patients with juvenile-onset Sjögren's syndrome. Mod Rheumatol. 2010;20:486–90. https://doi.org/10.1007/s10165-010-0313-7.
32. Rosas J, Ramos-Casals M, Ena J, García-Carrasco M, Verdu J, Cervera R, et al. Usefulness of basal and pilocarpine-stimulated salivary flow in primary Sjögren's syndrome. Correlation with clinical, immunological and histological features. Rheumatology (Oxford). 2002;41:670–5. https://doi.org/10.1093/rheumatology/41.6.670.
33. Yasuda H, Niki H. Review of the pharmacological properties and clinical usefulness of muscarinic agonists for xerostomia in patients with Sjögren's syndrome. Clin Drug Investig. 2002;22:67–73. https://doi.org/10.2165/00044011-200222020-00001.
34. Farag AM, Holliday C, Cimmino J, Roomian T, Papas A. Comparing the effectiveness and adverse effects of pilocarpine and cevimeline in patients with hyposalivation. Oral Dis. 2019;25:1937–44. https://doi.org/10.1111/odi.13192.
35. Brimhall J, Jhaveri MA, Yepes JF. Efficacy of cevimeline vs. pilocarpine in the secretion of saliva: a pilot study. Spec Care Dentist. 2013;33:123–7. https://doi.org/10.1111/scd.12010.
36. Petrone D, Condemi JJ, Fife R, Gluck O, Cohen S, Dalgin P. A double-blind, randomized, placebo-controlled study of cevimeline in Sjögren's syndrome patients with xerostomia and keratoconjunctivitis sicca. Arthritis Rheum. 2002;46:748–54. https://doi.org/10.1002/art.510.
37. Fife RS, Chase WF, Dore RK, Wiesenhutter CW, Lockhart PB, Tindall E, et al. Cevimeline for the treatment of xerostomia in patients with Sjögren

syndrome: a randomized trial. Arch Intern Med. 2002;162:1293–300. https://doi.org/10.1001/archinte.162.11.1293.
38. Leung KC, McMillan AS, Wong MC, Leung WK, Mok MY, Lau CS. The efficacy of cevimeline hydrochloride in the treatment of xerostomia in Sjögren's syndrome in southern Chinese patients: a randomised double-blind, placebo-controlled crossover study. Clin Rheumatol. 2008;27:429–36. https://doi.org/10.1007/s10067-007-0723-x.
39. Ono M, Takamura E, Shinozaki K, Tsumura T, Hamano T, Yagi Y, et al. Therapeutic effect of cevimeline on dry eye in patients with Sjögren's syndrome: a randomized, double-blind clinical study. Am J Ophthalmol. 2004;138:6–17. https://doi.org/10.1016/j.ajo.2004.02.010.
40. Komai K, Shiozawa K, Tanaka Y, Yoshihara R, Tanaka C, Sakai H, et al. Sjögren's syndrome patients presenting with hypergammaglobulinemia are relatively unresponsive to cevimeline treatment. Mod Rheumatol. 2009;19:416–9. https://doi.org/10.1007/s10165-009-0176-y.
41. Yamada H, Nakagawa Y, Wakamatsu E, Sumida T, Yamachika S, Nomura Y, et al. Efficacy prediction of cevimeline in patients with Sjögren's syndrome. Clin Rheumatol. 2007;26:1320–7. https://doi.org/10.1007/s10067-006-0507-8.
42. Frost-Larsen K, Isager H, Manthorpe R. Sjörgren's syndrome treated with bromhexine: a randomised clinical study. Br Med J. 1978;1:1579–81. https://doi.org/10.1136/bmj.1.6127.1579.
43. Tapper-Jones LM, Aldred MJ, Cadogan SJ, Walker DM, Dolby AE, Hopkins R, et al. Sjögren's syndrome treated with bromhexine: a reassessment. Br Med J. 1980;280:1356. https://doi.org/10.1136/bmj.280.6228.1356.
44. Manthorpe R, Frost-Larsen K, Høj L, Isager H, Prause JU. Bromhexine treatment of Sjögren's syndrome: effect on lacrimal and salivary secretion, and on proteins in tear fluid and saliva. Scand J Rheumatol. 1981;10:177–80. https://doi.org/10.3109/03009748109095294.
45. Avisar R, Savir H, Machtey I, Ovaknin L, Shaked P, Menache R, et al. Clinical trial of bromhexine in Sjögren's syndrome. Ann Ophthalmol. 1981;13:971–3.
46. Prause JU, Frost-Larsen K, Høj L, Isager H, Manthorpe R. Lacrimal and salivary secretion in Sjögren's syndrome: the effect of systemic treatment with bromhexine. Acta Ophthalmol. 1984;62:489–97. https://doi.org/10.1111/j.1755-3768.1984.tb08430.x.
47. Walters MT, Rubin CE, Keightley SJ, Ward CD, Cawley MI. A double-blind, cross-over, study of oral N-acetylcysteine in Sjögren's syndrome. Scand J Rheumatol Suppl. 1986;61:253–8.
48. Ichikawa Y, Tokunaga M, Shimizu H, Moriuchi J, Takaya M, Arimori S. Clinical trial of ambroxol (Mucosolvan) in Sjögren's syndrome. Tokai J Exp Clin Med. 1988;13:165–9.
49. Kasama T, Shiozawa F, Isozaki T, Matsunawa M, Wakabayashi K, Odai T, et al. Effect of the H2 receptor antagonist nizatidine on xerostomia in patients with primary Sjögren's syndrome. Mod Rheumatol. 2008;18:455–9. https://doi.org/10.1007/s10165-008-0078-4.
50. Skopouli FN, Jagiello P, Tsifetaki N, Moutsopoulos HM. Methotrexate in primary Sjögren's syndrome. Clin Exp Rheumatol. 1996;14:555–8.
51. Nakayamada S, Saito K, Umehara H, Ogawa N, Sumida T, Ito S, et al. Efficacy and safety of mizoribine for the treatment of Sjögren's syndrome: a multicenter open-label clinical trial. Mod Rheumatol. 2007;17:464–9. https://doi.org/10.1007/s10165-007-0627-2.
52. Nakayamada S, Fujimoto T, Nonomura A, Saito K, Nakamura S, Tanaka Y. Usefulness of initial histological features for stratifying Sjogren's syndrome responders to mizoribine therapy. Rheumatology (Oxford). 2009;48:1279–82. https://doi.org/10.1093/rheumatology/kep228.
53. Takagi Y, Katayama I, Tashiro S, Nakamura T. Parotid irrigation and cevimeline gargle for treatment of xerostomia in Sjögren's syndrome. J Rheumatol. 2008;35:2289–91. https://doi.org/10.3899/jrheum.080370.
54. Izumi M, Eguchi K, Nakamura H, Takagi Y, Kawabe Y, Nakamura T. Corticosteroid irrigation of parotid gland for treatment of xerostomia in patients with Sjögren's syndrome. Ann Rheum Dis. 1998;57:464–9. https://doi.org/10.1136/ard.57.8.464.
55. Shacham R, Puterman MB, Ohana N, Nahlieli O. Endoscopic treatment of salivary glands affected by autoimmune diseases. J Oral Maxillofac Surg. 2011;69:476–81. https://doi.org/10.1016/j.joms.2010.10.002.
56. De Luca R, Trodella M, Vicidomini A, Colella G, Tartaro G. Endoscopic management of salivary gland obstructive diseases in patients with Sjögren's syndrome. J Craniomaxillofac Surg. 2015;43:1643–9. https://doi.org/10.1016/j.jcms.2015.06.036.
57. Capaccio P, Canzi P, Torretta S, Rossi V, Benazzo M, Bossi A, et al. Combined interventional sialendoscopy and intraductal steroid therapy for recurrent sialadenitis in Sjögren's syndrome: results of a pilot monocentric trial. Clin Otolaryngol. 2018;43:96–102. https://doi.org/10.1111/coa.12911.
58. Karagozoglu KH, Vissink A, Forouzanfar T, Brand HS, Maarse F, Jager DHJ. Sialendoscopy enhances salivary gland function in Sjögren's syndrome: a 6-month follow-up, randomised and controlled, single blind study. Ann Rheum Dis. 2018;77:1025–31. https://doi.org/10.1136/annrheumdis-2017-212672.
59. Mignogna MD, Fedele S, Lo Russo L, Lo Muzio L, Wolff A. Sjögren's syndrome: the diagnostic potential of early oral manifestations preceding hyposalivation/xerostomia. J Oral Pathol Med. 2005;34:1–6. https://doi.org/10.1111/j.1600-0714.2004.00264.x.

60. Tishler M, Yaron I, Shirazi I, Yaron M. Hydroxychloroquine treatment for primary Sjogren's syndrome: its effect on salivary and serum inflammatory markers. Ann Rheum Dis. 1999;58:253–6. https://doi.org/10.1136/ard.58.4.253.
61. Venables PJ. Management of patients presenting with Sjogren's syndrome. Best Pract Res Clin Rheumatol. 2006;20:791–807. https://doi.org/10.1016/j.berh.2006.05.003.
62. Fattahi TT, Lyu PE, Van Sickels JE. Management of acute suppurative parotitis. J Oral Maxillofac Surg. 2002;60:446–8. https://doi.org/10.1053/joms.2002.31234.
63. Hernandez YL, Daniels TE. Oral candidiasis in Sjögren's syndrome: prevalence, clinical correlations, and treatment. Oral Surg Oral Med Oral Pathol. 1989;68:324–9. https://doi.org/10.1016/0030-4220(89)90218-1.
64. Rhodus NL, Liljemark W, Bloomquist C, Bereuter J. Candida albicans levels in patients with Sjögren's syndrome before and after long-term use of pilocarpine hydrochloride: a pilot study. Quintessence Int. 1998;29:705–10.
65. Maarse F, Jager DHJ, Alterch S, Korfage A, Forouzanfar T, Vissink A, et al. Sjögren's syndrome is not a risk factor for periodontal disease: a systematic review. Clin Exp Rheumatol. 2019;37(Suppl 118):225–33.
66. Hsu CY, Hung KC, Lin MS, Ko CH, Lin YS, Chen TH, et al. The effect of pilocarpine on dental caries in patients with primary Sjögren's syndrome: a database prospective cohort study. Arthritis Res Ther. 2019;21:251. https://doi.org/10.1186/s13075-019-2031-7.
67. Zero DT, Brennan MT, Daniels TE, Papas A, Stewart C, Pinto A, et al. Clinical practice guidelines for oral management of Sjögren disease: dental caries prevention. J Am Dent Assoc. 2016;147:295–305. https://doi.org/10.1016/j.adaj.2015.11.008.
68. Ramos-Casals M, Brito-Zerón P, Seror R, Bootsma H, Bowman SJ, Dörner T, et al. Characterization of systemic disease in primary Sjögren's syndrome: EULAR-SS Task Force recommendations for articular, cutaneous, pulmonary and renal involvements. Rheumatology (Oxford). 2015;54:2230–8. https://doi.org/10.1093/rheumatology/kev200.
69. Flament T, Bigot A, Chaigne B, Henique H, Diot E, Marchand-Adam S. Pulmonary manifestations of Sjögren's syndrome. Eur Respir Rev. 2016;25:110–23. https://doi.org/10.1183/16000617.0011-2016.
70. Marchesoni D, Mozzanega B, De Sandre P, Romagnolo C, Gambari PF, Maggino T. Gynaecological aspects of primary Sjogren's syndrome. Eur J Obstet Gynecol Reprod Biol. 1995;63:49–53. https://doi.org/10.1016/0301-2115(95)02224-u.
71. van Nimwegen JF, Arends S, van Zuiden GS, Vissink A, Kroese FG, Bootsma H. The impact of primary Sjögren's syndrome on female sexual function. Rheumatology (Oxford). 2015;54:1286–93. https://doi.org/10.1093/rheumatology/keu522.
72. Baldini C, Pepe P, Quartuccio L, Priori R, Bartoloni E, Alunno A, et al. Primary Sjogren's syndrome as a multi-organ disease: impact of the serological profile on the clinical presentation of the disease in a large cohort of Italian patients. Rheumatology (Oxford). 2014;53:839–44. https://doi.org/10.1093/rheumatology/ket427.
73. Seror R, Ravaud P, Bowman SJ, Baron G, Tzioufas A, Theander E, et al. EULAR Sjogren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogren's syndrome. Ann Rheum Dis. 2010;69:1103–9. https://doi.org/10.1136/ard.2009.110619.
74. Seror R, Theander E, Brun JG, Ramos-Casals M, Valim V, Dörner T, et al. Validation of EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient indexes (ESSPRI). Ann Rheum Dis. 2015;74:859–66. https://doi.org/10.1136/annrheumdis-2013-204615.
75. Buttgereit F, da Silva JA, Boers M, Burmester GR, Cutolo M, Jacobs J, et al. Standardised nomenclature for glucocorticoid dosages and glucocorticoid treatment regimens: current questions and tentative answers in rheumatology. Ann Rheum Dis. 2002;61:718–22. https://doi.org/10.1136/ard.61.8.718.
76. Fox RI, Dixon R, Guarrasi V, Krubel S. Treatment of primary Sjogren's syndrome with hydroxychloroquine: a retrospective, open-label study. Lupus. 1996;5(Suppl 1):S31–6.
77. Kruize AA, Hené RJ, Kallenberg CG, van Bijsterveld OP, van der Heide A, Kater L, et al. Hydroxychloroquine treatment for primary Sjögren's syndrome: a two year double blind cross-over trial. Ann Rheum Dis. 1993;52:360–4. https://doi.org/10.1136/ard.52.5.360.
78. Carubbi F, Cipriani P, Marrelli A, Benedetto P, Ruscitti P, Berardicurti O, et al. Efficacy and safety of rituximab treatment in early primary Sjögren's syndrome: a prospective, multi-center, follow-up study. Arthritis Res Ther. 2013;15:R172. https://doi.org/10.1186/ar4359.
79. Gottenberg JE, Cinquetti G, Larroche C, Combe B, Hachulla E, Meyer O, et al. Efficacy of rituximab in systemic manifestations of primary Sjogren's syndrome: results in 78 patients of the AutoImmune and Rituximab registry. Ann Rheum Dis. 2013;72:1026–31. https://doi.org/10.1136/annrheumdis-2012-202293.
80. Devauchelle-Pensec V, Mariette X, Jousse-Joulin S, Berthelot JM, Perdriger A, Puéchal X, et al. Treatment of primary Sjögren syndrome with rituximab: a randomized trial. Ann Intern Med. 2014;160:233–42. https://doi.org/10.7326/m13-1085.
81. Bowman SJ, Everett CC, O'Dwyer JL, Emery P, Pitzalis C, Ng WF, et al. Randomized controlled trial of rituximab and cost-effectiveness analysis in treating fatigue and oral dryness in primary Sjögren's

syndrome. Arthritis Rheumatol. 2017;69:1440–50. https://doi.org/10.1002/art.40093.
82. Letaief H, Lukas C, Barnetche T, Gaujoux-Viala C, Combe B, Morel J. Efficacy and safety of biological DMARDs modulating B cells in primary Sjögren's syndrome: systematic review and meta-analysis. Joint Bone Spine. 2018;85:15–22. https://doi.org/10.1016/j.jbspin.2017.06.004.
83. De Vita S, Quartuccio L, Seror R, Salvin S, Ravaud P, Fabris M, et al. Efficacy and safety of belimumab given for 12 months in primary Sjögren's syndrome: the BELISS open-label phase II study. Rheumatology (Oxford). 2015;54:2249–56. https://doi.org/10.1093/rheumatology/kev257.
84. Mariette X, Seror R, Quartuccio L, Baron G, Salvin S, Fabris M, et al. Efficacy and safety of belimumab in primary Sjögren's syndrome: results of the BELISS open-label phase II study. Ann Rheum Dis. 2015;74:526–31. https://doi.org/10.1136/annrheumdis-2013-203991.
85. Mariette X, Ravaud P, Steinfeld S, Baron G, Goetz J, Hachulla E, et al. Inefficacy of infliximab in primary Sjögren's syndrome: results of the randomized, controlled trial of Remicade in primary Sjögren's syndrome (TRIPSS). Arthritis Rheum. 2004;50:1270–6. https://doi.org/10.1002/art.20146.
86. Sankar V, Brennan MT, Kok MR, Leakan RA, Smith JA, Manny J, et al. Etanercept in Sjögren's syndrome: a twelve-week randomized, double-blind, placebo-controlled pilot clinical trial. Arthritis Rheum. 2004;50:2240–5. https://doi.org/10.1002/art.20299.
87. Meiners PM, Vissink A, Kroese FG, Spijkervet FK, Smitt-Kamminga NS, Abdulahad WH, et al. Abatacept treatment reduces disease activity in early primary Sjögren's syndrome (open-label proof of concept ASAP study). Ann Rheum Dis. 2014;73:1393–6. https://doi.org/10.1136/annrheumdis-2013-204653.
88. Baer A, Gottenberg J-E, WSC E. OP0039 Efficacy and safety of abatacept in active primary Sjogren's syndrome: results of a randomised placebo-controlled phase III trial. Ann Rheum Dis. 2019;78:89 LP–90.
89. Fauchais AL, Ouattara B, Gondran G, Lalloue F, Petit D, Ly K, et al. Articular manifestations in primary Sjogren's syndrome: clinical significance and prognosis of 188 patients. Rheumatology (Oxford). 2010;49:1164–72. https://doi.org/10.1093/rheumatology/keq047.
90. Fox RI, Chan E, Benton L, Fong S, Friedlaender M, Howell FV. Treatment of primary Sjögren's syndrome with hydroxychloroquine. Am J Med. 1988;85:62–7. https://doi.org/10.1016/0002-9343(88)90365-8.
91. Wang SQ, Zhang LW, Wei P, Hua H. Is hydroxychloroquine effective in treating primary Sjogren's syndrome: a systematic review and meta-analysis. BMC Musculoskelet Disord. 2017;18:186. https://doi.org/10.1186/s12891-017-1543-z.
92. van Woerkom JM, Kruize AA, Geenen R, van Roon EN, Goldschmeding R, Verstappen SM, et al. Safety and efficacy of leflunomide in primary Sjögren's syndrome: a phase II pilot study. Ann Rheum Dis. 2007;66:1026–32. https://doi.org/10.1136/ard.2006.060905.
93. Carsons SE, Vivino FB, Parke A, Carteron N, Sankar V, Brasington R, et al. Treatment guidelines for rheumatologic manifestations of Sjogren's syndrome: use of biologic agents, management of fatigue, and inflammatory musculoskeletal pain. Arthritis Care Res (Hoboken). 2017;69:517–27. https://doi.org/10.1002/acr.22968.
94. Saraux A, Pers JO, Devauchelle-Pensec V. Treatment of primary Sjogren syndrome. Nat Rev Rheumatol. 2016;12:456–71. https://doi.org/10.1038/nrrheum.2016.100.
95. Roccatello D, Saadoun D, Ramos-Casals M, Tzioufas AG, Fervenza FC, Cacoub P, et al. Cryoglobulinaemia. Nat Rev Dis Primers. 2018;4:11. https://doi.org/10.1038/s41572-018-0009-4.
96. Brito-Zerón P, Retamozo S, Akasbi M, Gandía M, Perez-De-Lis M, Soto-Cardenas MJ, et al. Annular erythema in primary Sjogren's syndrome: description of 43 non-Asian cases. Lupus. 2014;23:166–75. https://doi.org/10.1177/0961203313515764.
97. Katayama I, Kotobuki Y, Kiyohara E, Murota H. Annular erythema associated with Sjögren's syndrome: review of the literature on the management and clinical analysis of skin lesions. Mod Rheumatol. 2010;20:123–9. https://doi.org/10.1007/s10165-009-0257-y.
98. Kuhn A, Ruland V, Bonsmann G. Cutaneous lupus erythematosus: update of therapeutic options part I. J Am Acad Dermatol. 2011;65:e179–93. https://doi.org/10.1016/j.jaad.2010.06.018.
99. Nobeyama Y, Matsuzaki H, Nakagawa H. Annular erythema of Sjögren's syndrome treated successfully with low-dose cyclosporine. J Dermatol. 2014;41:463–4. https://doi.org/10.1111/1346-8138.12475.
100. Yokota K, Shichinohe R, Hayasaka T. Topical tacrolimus in the treatment of annular erythema associated with Sjögren's syndrome. Clin Exp Dermatol. 2005;30:450–1. https://doi.org/10.1111/j.1365-2230.2005.01803.x.
101. Nannini C, Jebakumar AJ, Crowson CS, Ryu JH, Matteson EL. Primary Sjogren's syndrome 1976-2005 and associated interstitial lung disease: a population-based study of incidence and mortality. BMJ Open. 2013;3:e003569. https://doi.org/10.1136/bmjopen-2013-003569.
102. Justet A, Ottaviani S, Dieudé P, Taillé C. Tocilizumab for refractory organising pneumonia associated with Sjögren's disease. BMJ Case Rep. 2015;2015 https://doi.org/10.1136/bcr-2014-209076.
103. Thompson G, McLean-Tooke A, Wrobel J, Lavender M, Lucas M. Sjögren syndrome with associated lymphocytic interstitial pneumonia successfully treated with tacrolimus and abatacept as an alternative to

rituximab. Chest. 2018;153:e41–e3. https://doi.org/10.1016/j.chest.2017.12.010.
104. Chen MH, Chen CK, Chou HP, Chen MH, Tsai CY, Chang DM. Rituximab therapy in primary Sjögren's syndrome with interstitial lung disease: a retrospective cohort study. Clin Exp Rheumatol. 2016;34:1077–84.
105. Johnston SL, Dudley CR, Unsworth DJ, Lock RJ. Life-threatening acute pulmonary haemorrhage in primary Sjögren's syndrome with cryoglobulinaemia. Scand J Rheumatol. 2005;34:404–7. https://doi.org/10.1080/03009740510018741.
106. Shahane A. Pulmonary hypertension in rheumatic diseases: epidemiology and pathogenesis. Rheumatol Int. 2013;33:1655–67. https://doi.org/10.1007/s00296-012-2659-y.
107. Condliffe R, Kiely DG, Peacock AJ, Corris PA, Gibbs JS, Vrapi F, et al. Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. Am J Respir Crit Care Med. 2009;179:151–7. https://doi.org/10.1164/rccm.200806-953OC.
108. Liu Z, Yang X, Tian Z, Qian J, Wang Q, Zhao J, et al. The prognosis of pulmonary arterial hypertension associated with primary Sjögren's syndrome: a cohort study. Lupus. 2018;27:1072–80. https://doi.org/10.1177/0961203318756287.
109. Thakkar V, Lau EM. Connective tissue disease-related pulmonary arterial hypertension. Best Pract Res Clin Rheumatol. 2016;30:22–38. https://doi.org/10.1016/j.berh.2016.03.004.
110. François H, Mariette X. Renal involvement in primary Sjögren syndrome. Nat Rev Nephrol. 2016;12:82–93. https://doi.org/10.1038/nrneph.2015.174.
111. Darrieutort-Laffite C, André V, Hayem G, Saraux A, Le Guern V, Le Jeunne C, et al. Sjögren's syndrome complicated by interstitial cystitis: a case series and literature review. Joint Bone Spine. 2015;82:245–50. https://doi.org/10.1016/j.jbspin.2014.12.007.
112. Ueda Y, Tomoe H, Takahashi H, Takahashi Y, Yamashita H, Kaneko H, et al. Interstitial cystitis associated with primary Sjögren's syndrome successfully treated with a combination of tacrolimus and corticosteroid: a case report and literature review. Mod Rheumatol. 2016;26:445–9. https://doi.org/10.3109/14397595.2014.895283.
113. Carvajal Alegria G, Guellec D, Mariette X, Gottenberg JE, Dernis E, Dubost JJ, et al. Epidemiology of neurological manifestations in Sjögren's syndrome: data from the French ASSESS Cohort. RMD Open. 2016;2:e000179. https://doi.org/10.1136/rmdopen-2015-000179.
114. Martinez AR, Nunes MB, Nucci A, França MC Jr. Sensory neuronopathy and autoimmune diseases. Autoimmune Dis. 2012;2012:873587. https://doi.org/10.1155/2012/873587.
115. Margaretten M. Neurologic manifestations of primary Sjögren syndrome. Rheum Dis Clin N Am. 2017;43:519–29. https://doi.org/10.1016/j.rdc.2017.06.002.
116. Rist S, Sellam J, Hachulla E, Sordet C, Puéchal X, Hatron PY, et al. Experience of intravenous immunoglobulin therapy in neuropathy associated with primary Sjögren's syndrome: a national multicentric retrospective study. Arthritis Care Res (Hoboken). 2011;63:1339–44. https://doi.org/10.1002/acr.20495.
117. Takahashi Y, Takata T, Hoshino M, Sakurai M, Kanazawa I. Benefit of IVIG for long-standing ataxic sensory neuronopathy with Sjögren's syndrome. IV immunoglobulin. Neurology. 2003;60:503–5. https://doi.org/10.1212/01.wnl.0000046680.47883.7d.
118. Nobile-Orazio E, Cocito D, Jann S, Uncini A, Beghi E, Messina P, et al. Intravenous immunoglobulin versus intravenous methylprednisolone for chronic inflammatory demyelinating polyradiculoneuropathy: a randomised controlled trial. Lancet Neurol. 2012;11:493–502. https://doi.org/10.1016/s1474-4422(12)70093-5.
119. Alexander EL. Neurologic disease in Sjögren's syndrome: mononuclear inflammatory vasculopathy affecting central/peripheral nervous system and muscle. A clinical review and update of immunopathogenesis. Rheum Dis Clin N Am. 1993;19:869–908.
120. Shahmohammadi S, Doosti R, Shahmohammadi A, Mohammadianinejad SE, Sahraian MA, Azimi AR, et al. Autoimmune diseases associated with neuromyelitis optica spectrum disorders: a literature review. Mult Scler Relat Disord. 2019;27:350–63. https://doi.org/10.1016/j.msard.2018.11.008.
121. Pittock SJ, Lennon VA, McKeon A, Mandrekar J, Weinshenker BG, Lucchinetti CF, et al. Eculizumab in AQP4-IgG-positive relapsing neuromyelitis optica spectrum disorders: an open-label pilot study. Lancet Neurol. 2013;12:554–62. https://doi.org/10.1016/s1474-4422(13)70076-0.
122. Ng WF, Bowman SJ. Primary Sjogren's syndrome: too dry and too tired. Rheumatology (Oxford). 2010;49:844–53. https://doi.org/10.1093/rheumatology/keq009.
123. Virkki LM, Porola P, Forsblad-d'Elia H, Valtysdottir S, Solovieva SA, Konttinen YT. Dehydroepiandrosterone (DHEA) substitution treatment for severe fatigue in DHEA-deficient patients with primary Sjögren's syndrome. Arthritis Care Res (Hoboken). 2010;62:118–24. https://doi.org/10.1002/acr.20022.
124. Hartkamp A, Geenen R, Godaert GL, Bootsma H, Kruize AA, Bijlsma JW, et al. Effect of dehydroepiandrosterone administration on fatigue, well-being, and functioning in women with primary Sjögren syndrome: a randomised controlled trial. Ann Rheum Dis. 2008;67:91–7. https://doi.org/10.1136/ard.2007.071563.
125. Theander E, Horrobin DF, Jacobsson LT, Manthorpe R. Gamma-linolenic acid treatment of fatigue associated with primary Sjögren's syndrome. Scand J Rheumatol. 2002;31:72–9. https://doi.org/10.1080/03009740252937577.

126. Seitsalo H, Niemelä RK, Marinescu-Gava M, Vuotila T, Tjäderhane L, Salo T. Effectiveness of low-dose doxycycline (LDD) on clinical symptoms of Sjögren's syndrome: a randomized, double-blind, placebo controlled cross-over study. J Negat Results Biomed. 2007;6:11. https://doi.org/10.1186/1477-5751-6-11.
127. Dass S, Bowman SJ, Vital EM, Ikeda K, Pease CT, Hamburger J, et al. Reduction of fatigue in Sjögren syndrome with rituximab: results of a randomised, double-blind, placebo-controlled pilot study. Ann Rheum Dis. 2008;67:1541–4. https://doi.org/10.1136/ard.2007.083865.
128. Meijer JM, Meiners PM, Vissink A, Spijkervet FK, Abdulahad W, Kamminga N, et al. Effectiveness of rituximab treatment in primary Sjögren's syndrome: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 2010;62:960–8. https://doi.org/10.1002/art.27314.
129. Devauchelle-Pensec V, Pennec Y, Morvan J, Pers JO, Daridon C, Jousse-Joulin S, et al. Improvement of Sjögren's syndrome after two infusions of rituximab (anti-CD20). Arthritis Rheum. 2007;57:310–7. https://doi.org/10.1002/art.22536.
130. Souza FB, Porfírio GJ, Andriolo BN, Albuquerque JV, Trevisani VF. Rituximab effectiveness and safety for treating primary Sjögren's syndrome (pSS): systematic review and meta-analysis. PLoS One. 2016;11:e0150749. https://doi.org/10.1371/journal.pone.0150749.
131. Strömbeck BE, Theander E, Jacobsson LT. Effects of exercise on aerobic capacity and fatigue in women with primary Sjogren's syndrome. Rheumatology (Oxford). 2007;46:868–71. https://doi.org/10.1093/rheumatology/kem004.
132. Baimpa E, Dahabreh IJ, Voulgarelis M, Moutsopoulos HM. Hematologic manifestations and predictors of lymphoma development in primary Sjögren syndrome: clinical and pathophysiologic aspects. Medicine (Baltimore). 2009;88:284–93. https://doi.org/10.1097/MD.0b013e3181b76ab5.
133. Fietta P, Delsante G, Quaini F. Hematologic manifestations of connective autoimmune diseases. Clin Exp Rheumatol. 2009;27:140–54.
134. Atzeni F, Cazzola M, Benucci M, Di Franco M, Salaffi F, Sarzi-Puttini P. Chronic widespread pain in the spectrum of rheumatological diseases. Best Pract Res Clin Rheumatol. 2011;25:165–71. https://doi.org/10.1016/j.berh.2010.01.011.
135. Espitia-Thibault A, Masseau A, Néel A, Espitia O, Toquet C, Mussini JM, et al. Sjögren's syndrome-associated myositis with germinal centre-like structures. Autoimmun Rev. 2017;16:154–8. https://doi.org/10.1016/j.autrev.2016.12.006.
136. Colafrancesco S, Priori R, Gattamelata A, Picarelli G, Minniti A, Brancatisano F, et al. Myositis in primary Sjögren's syndrome: data from a multicentre cohort. Clin Exp Rheumatol. 2015;33:457–64.
137. Mavragani CP, Moutsopoulos HM. Sjögren's syndrome: old and new therapeutic targets. J Autoimmun. 2020;110:102364. https://doi.org/10.1016/j.jaut.2019.102364.

12 Gene Therapy for Sjögren's Syndrome

Hongen Yin and John A. Chiorini

12.1 Why Gene Therapy

Sjögren's syndrome (SS) represents a complex autoimmune disease with limited treatment options. Although patients can have systemic disease, the majority of affected individuals have loss of salivary or lacrimal glandular function. The introduction of biologic-based therapies has advanced the field of treatment. New therapies using recombinant proteins or small molecules, which target B cells, T cells, and other cells of the immune system, are being developed and many biologics are in clinical trials. Many of these drugs have been successful in treating other autoimmune diseases and they may be beneficial for Sjögren's patients as well. In general, these therapies have several limitations. A major limitation is the need for repeated injections of the drug to maintain stable concentration of therapeutic protein in vivo. As some biologics have short half-lives in vivo, higher than therapeutic levels of drug may be injected, which can result in adverse effects.

Gene therapy allows for constitutive expression of therapeutic proteins in a precise manner and offers the possibility to go beyond conventional protein replacement approaches. It can reengineer cells to serve as a depot organ for expression of a recombinant protein or to employ a gain-of-function approach to express intracellular factors, which is not usually possible with conventional protein replacement therapy. In this chapter, we will review the current state of gene therapy for SS.

12.2 Gene Delivery Strategies

There are both in vivo and ex vivo strategies for gene therapy. Ex vivo strategies are being explored to use genetically engineered cells that are injected directly into the affected tissue. This approach is currently used in the treatment of cancer with engineered T cells, but is at an early stage of development for other diseases. On the other hand, the field of in vivo delivery where the gene is directly introduced into the patients affected organ has advanced rapidly in the last 10 years. From preclinical studies to applications of approved drugs, gene therapy has been explored in a diverse set of conditions including blindness, neurologic disease, and hemophilia.

12.2.1 Nonviral Gene Therapy

Gene therapy involves the introduction of nucleic acid into a cell with the goal of achieving a thera-

H. Yin · J. A. Chiorini (✉)
Adeno-Associated Virus Biology Section, Epithelial and Salivary Gland Biology and Dysfunction Immunology and Inflammation, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA
e-mail: yinho@nih.gov; jchiorini@dir.nidcr.nih.gov

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peutic effect. In general, this can be accomplished by either nonviral or viral-based approaches. Nonviral gene therapy can involve the use of carrier molecules, such as liposomes, polymers, or physical methods like ultrasonic waves or electricity to facilitate the movement of the nucleic acid across the plasma membrane and into the cell. This approach has been used to deliver genes into a diverse set of organs ranged from muscles to the liver. Nonviral gene therapy was successful in the salivary glands (SGs) but only yielded low-level expression [1]. More advanced technology utilizing ultrasound-induced microbubbles to deliver plasmids to mouse SG showed that ultrasound-assisted reporter gene transfer with the 15% microbubble solution was stable for up to 28 days [2]. The duration could be extended by the use of alternative forms of DNA that lacked a plasmid-based backbone [3]. In general, a limitation to this approach is the transient nature of the expression. In most studies, expression is limited to less than a week. Much of this limitation to this approach is due to innate immune responses to the plasmid backbone of the DNA that is introduced or the degradation of the siRNA molecules in the case of RNA-based drugs. Ongoing studies to overcome these limitations include but are not limited to: modification of the siRNA oligos to improve stability, removal of the bacterial elements from the plasmids, production of the nucleic acid enzymatically, or use of viral-derived forms of DNA. The future prospects of nonviral nucleic acid delivery are exciting, and the technology could play an important role in the future as the tools evolve.

12.2.2 Viral Gene Therapy

Viral-based approaches are rapidly maturing into drug delivery systems. As a vector for gene transfer, viruses represent a natural vehicle for introducing nucleic acid into a cell since they have specifically evolved to deliver viral genes into host cells. Through the use of molecular biology, viruses can be modified to serve as molecular trucks for the delivery of therapeutic genes into cells. Over the years, many viral-based platforms have been developed, and their utility depends largely on the specific application. For example, lentiviral and retroviral vectors have immerged as a top method for engineering immune cells. They have been extensively used to engineer immune cells for cancer therapy through ex vivo manipulation of a patient's cells, which are then reinfused into the patient. These infused cells could then circulate and alter the course of the patient's condition (for review, see [4]). Viral-based vectors might be more useful in localized therapy including adenoviruses (Ad) or adeno-associated viruses (AAV). For both systems, extensive studies of the biology of the virus have resulted in robust methods of production and purification, making those viruses attractive tools to utilize.

One of the first viral vectors, based on a DNA virus, are those derived from the adenovirus family of viruses. Adenoviruses (Ad) are non-enveloped viruses with a double-stranded genome, approximately 35 kb in length. They represent a diverse family of more than 50 human viruses. Most Ad-based vectors are derived from adenovirus type 5 (Ad5), and early vectors were made replication incompetent by the deletion of one group of genes (E1), leaving room for approximately 8 kb of new DNA. Production of recombinant vectors is achieved by using a cell line that stably expresses the E1 genes to allow trans-complementation of the deleted genome.

Improvements in the generation of recombinant particles have resulted in the development of high-capacity adenoviral vectors that are able to package up to 36 kb of DNA. Removal of more viral DNA reduced the host immune response to the vector but also resulted in the addition of low-level contamination with the helper virus that is used to produce high-capacity vectors [5]. However, despite these improvements, the particles can still induce an immune response, and particles can be neutralized by host antibodies to the particle.

First-generation Ad-based vectors are reported to transduce many cell types in the SG, resulting in robust and transient expression of recombinant proteins. The transient expression is largely due to the immune response to the vector. However, low doses of vector that are not sufficient to

induce an immune response can result in long-term transgene expression in human SG [6].

Compared to Ad, AAV vectors can induce a lower level of immune activation. AAV vectors are based on an expanded family of helper-dependent parvoviruses with unique cell tropisms. Although the vast majority of vectors that will enter the cell remain episomal and do not integrate in the host genome, transduction with these vectors can be long-term. For example, the SG represents a slowly dividing epithelia. In vivo transduction experiments in mouse SG have shown persistent transgene expression for the life of the animal [7]. Many vectors are currently available for the targeting of ductal and acinar cells within the gland [8, 9], and new vectors are currently being developed [10]. In addition to naturally derived vectors based on reported isolates of AAV, design of synthetic vector particles or rational mutagenesis of the vector capsid is rapidly advancing and will provide additional tools to more precisely target distinct cell population and increase the potential of gene therapy to be used in targeted tissue engineering.

12.3 Biosafety for Current Gene Therapy

The overall safety of gene therapy has been reported elsewhere as has more specific reviews on safety of AAV vectors [11]. Over 100 trials worldwide have been conducted with AAV vectors, and no long-term adverse events linked to the vector have been reported. Inflammation in response to AAV vectors has been reported and typically has been associated with the development neutralizing antibodies to the capsid that could limit the ability to repeat the dosing with the AAV vector. Doses given intravenously greater than 1×10^{12} vp/kg body weight are also reported to initiate a T cell response [12]. More recently, neuroinflammation in CNS-targeted applications has been reported in some animal models [13–15]. Few studies have looked at gene therapy vectors in the SG. Studies with Ad-based vectors in a number of species have shown they can be delivered to SG without lasting negative effects [16–20]. AAV vectors in mice showed minimal effects on the gland [21]. For AAV vectors, vector DNA could be detected in blood in a dose-dependent manner only on day 3, with no detectable levels on later days. No vector DNA was detected in saliva. Of the 15 different organs collected from each mouse and tested for vector DNA, only the targeted SG and draining lymph node had vector DNA at >50 copies/mg tissue on day 92. Adenoviral-mediated transfer of the cDNA encoding human water channel aquaporin-1, hAQP1 (AdhAQP1), to human SG resulted in improved gland activity. The trial design was a phase 1 clinical trial using a single previously irradiated parotid gland in 11 patients. The study was using an open-label, single-dose, dose-escalation design [22]. Analysis of the first 42 days following vector delivery showed all patients tolerated vector delivery and study procedures well, and positive objective and subjective responses were seen in five patients, all at doses $<5.8\times10^{9}$ vp/gland. At higher doses, the patients likely initiated an immune response to the vector, and no improvement in gland function was observed. These findings have encouraged the use of AAV-based vectors, which have demonstrated lower immunogenicity and more stable expression compared with adenoviral vectors.

12.4 What to Deliver

Although the etiology of SS is unclear, the disease is considered to be a slowly progressing condition with likely initiating events occurring years prior to the development of symptoms. Proposed triggers include a number of pathogens, mainly viruses, but could also be due to bacterial infection. Although the triggering event may initiate an immune response that fails to resolve and leads to the development of autoimmunity, it is also possible that the infection may not be completely eliminated, resulting in the establishment of low-level latent infection that acts as a chronic source of immune stimulus/activation. Indeed, SGs are long reported to act as a reservoir for latent viruses [23]. It is this initiating event in the context of a genetic predisposition that likely

results in the initiation of the pathogenesis, which can include the formation of autoantibodies, infiltrating cells within the gland, release of pro-inflammatory cytokines, and the loss of secretory epithelial function. All of these represent potential targets for intervention in gene therapy. As SS presents as an autoimmune disease, immunomodulation represents an attractive target point for intervention, and there are many ongoing trials that target the immune system (see [24] for review).

As a therapeutic intervention, gene therapy offers the possibility to either better target the expression of recombinant proteins to the affected tissue or deliver intracellular molecules that can alter cellular function. In general, targets for gene therapy can be divided into four categories: (1) cytokines, (2) receptors, (3) intracellular regulators, and (4) intracellular proteins. Immune and nonimmune pathways contributing to SS are summarized in Fig. 12.1.

In the context of a permissive genetic environment SS secretory gland epithelial cell (SGEC) can initiate an autoimmune epithelitis triggered by environmental change such as virus infection or loss of SG epithelial integrity. This event activates the innate immune response dominated by natural killer (NK) cells and macrophages (Mϕ). NK cells produce interferon-gamma (IFN-γ), which contributes to the IFN signature and acts as a link between the innate and adaptive immune response. The NK cells, SGEC, and Mϕ produce a large number of pro-inflammatory cytokines and chemokines such as tumor necrosis factor alpha (TNF-α), IL-6, IL-1, chemokine (C-X-C motif) ligand 13 (CXCL13), which are responsible for SG tissue damage, activation of adaptive immune response, and facilitation of lymphocyte homing. Apoptosis of SGEC or exocytosis induces production of autoantigens such as Ro, La, and M3R. SGEC acts as nonprofessional antigen-presenting cells (APC) and together with the professional APC dendritic cells (DC) presents autoantigens to T helper cells. Antigen presentation requires co-stimulatory signaling molecule interactions, including CD40: CD40L, B7:CD28, intercellular adhesion molecule-1 (ICAM-1), and lymphocyte function-associated antigen-1 (LFA-1) (ICAM-1:LFA1), to activate the T cells and produce pro-inflammatory Th1 and Th17 cells. These cells can be stimulated by IL-12 and IL-23, to produce their signature cytokines, IFN-γ and IL-17, respectively, leading to further SG tissue damage, cell apoptosis, and glandular dysfunction. Cytotoxic T lymphocyte antigen-4 (CTLA4), an immune checkpoint molecule, can inhibit the activation of T cells via competition with CD28 to bind B7. TGF-β producing Treg cells, which can be activated by CTLA4, suppress Th1 and Th17 activation. The cytokine IL-27 also blocks the Th17 activation by suppressing IL-17 production. Another IFN cytokine, IFN-α, is produced by plasmacytoid dendritic cells (pDCs), which are activated by virus or endogenous nucleic acid containing immune complexes bound to TLRs. High level of IFN-α results in an activation of B cells via cytokines such as IL-14, B cell-activating factor (BAFF), and a proliferation-inducing ligand (APRIL). BAFF and APRIL combine with their specific B cell receptors, BAFF-R and proteoglycans, respectively, and common receptors transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor (TACI) and B-cell maturation antigen (BCMA) to activate the B cells. B cell activation is mainly through T cell-dependent pathways. In a subset of patients, follicular helper T cells (Tfh) provide help to B cells in the germinal center (GC)-like structure. Activation of B cells leads to the production of autoantibodies such as antinuclear antibody (ANA), anti-Ro (SSA), anti-La (SSB), rheumatoid factor (RF), and anti-M3 muscarinic receptors (anti-M3R). Nonimmune cytokines such as BMP6 can lead to a change in acinar cells through alteration of water channel AQP5, which also decreases SG function.

12.4.1 Cytokines

SS presents as a heterogeneous disease in patients and is often associated with an increase in a number of cytokines. These pro-inflammatory cytokines include IFN (-α, β, and γ), TNF-α, and interleukins such as IL-1, IL-2, 12, 18, 17, and 23. In contrast, the expression of anti-

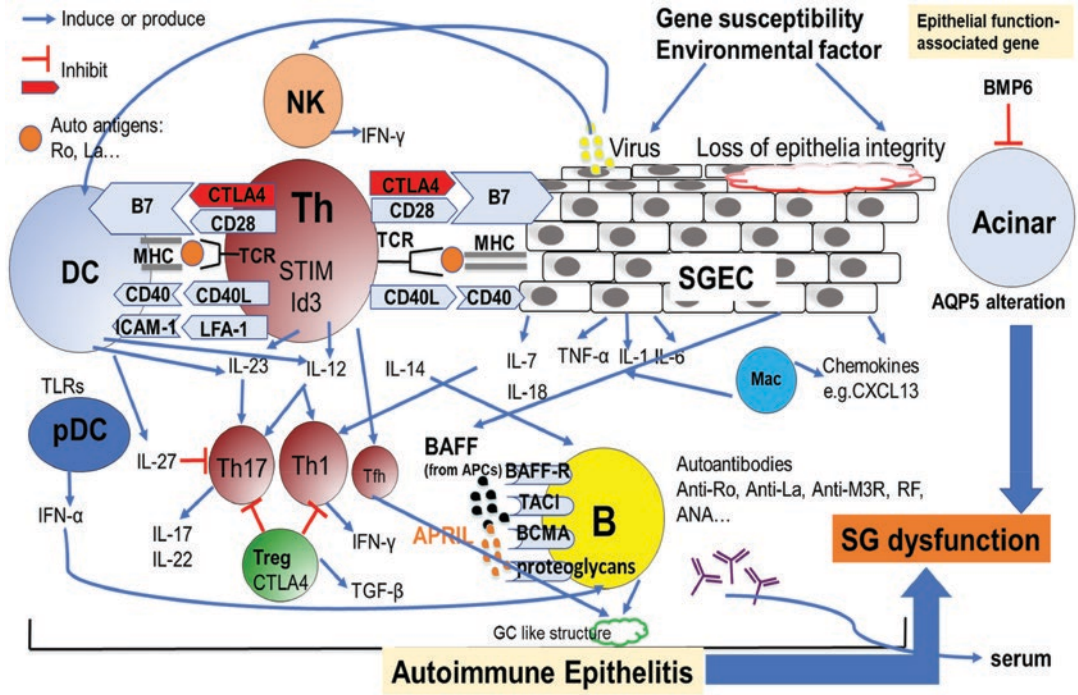

Fig. 12.1 Immune and nonimmune pathways contributing to Sjögren's syndrome

inflammatory cytokines such as IL-10 tends to be suppressed. Therefore, inhibiting the production of pro-inflammatory cytokines or increasing the expression of anti-inflammatory cytokines represents a potential therapeutic route for the treatment of SS. An advantage with gene therapy in this context would be the possibility of producing the therapeutic cytokine locally at the source, limiting systemic off-target effects (Fig. 12.1, see "Autoimmune Epithelitis").

12.4.2 Receptors

Modulation of the immune system has been an important point of therapeutic intervention in several autoimmune diseases. An alternative to directly affecting the level of the cytokine itself is to introduce molecules that act to block the effect of existing cytokines and in this way modulate their activity. For example, antibodies or soluble forms of native receptors have been developed to many cytokines involved in autoimmunity including ustekinumab and etanercept, which bind IL-23 and TNF-α, respectively. A limitation to these approaches is the high level of systemic side effects often associated with infusion of recombinant proteins, including increased risk of infection. Other complications of protein therapy include the generation of antibodies to the therapeutic protein. Expression by gene therapy-based approaches limits this through localized expression and the relatively slower kinetics of expression, which may lead to tolerance to the protein.

12.4.3 Intracellular Regulators

Like small molecules, gene therapy offers the possibility of delivering drugs that can enter the cell and alter expression of key pathways. While these molecules could be proteins like transcription factors that could activate or repress specific pathways, exciting work is being done with RNA-based therapeutic molecules. Thus, rather than the therapeutic being the translated product of the vector, the transcribed message is the drug. MicroRNAs (miRNA) are emerging as a

broad mechanism for the control of RNA expression, and their expression is altered in many disease states. In contrast, short hairpin RNA (shRNA), which ultimately forms siRNAs, is generally more specific to a gene or specific sequence. Vectors encoding these noncoding regulatory RNA molecules could be developed to suppress the production of cytokines or cytokine receptors, as shown in the case of BAFF, where its splicing variant (ΔBAFF) functioned as a natural inhibitor [25]. Off-target effects or general interference with the RNA-induced silencing complex (RISC) machinery that processes RNA can act to limit this approach or produce toxicity.

12.4.4 Intracellular Proteins

Xerostomia is a hallmark of SS. Salivation is a complex process involving the coordinated interaction of many proteins. Following a neurologic or mechanical stimuli, salivary acinar cells are stimulated to release intracellular calcium that triggers the release of chloride ions into the lumen of the gland. Water then follows this osmotic gradient into the lumen of the gland via specific water channels called aquaporins (AQPs). The study of knockout mouse models as well as the molecular study of specific proteins in this pathway have confirmed the importance of many of the key proteins in this process such as AQP5, Na-K-Cl cotransporter1 (NKCC), inositol triphosphate receptor (IP3R) types 2 and 3, matriptase, and M3 and M1 muscarinic receptors [26–30]. Gene therapy offers the possibility to increase the expression of these proteins if they are downregulated in the disease or substitute another protein with complementary function to restore activity. Traditionally, this approach has been used to replace intracellular enzymes involved in metabolic pathway defects. As our understanding of the physiologic blocks to saliva production has become clear, this approach is being evaluated as a therapeutic option in the treatment of SS (Fig. 12.1, see "Loss of epithelia integrity" and "AQP5 alteration").

12.5 How to Control Expression

In addition to the vehicle used to transfer the gene into a cell, an important consideration in the design of a gene therapy treatment is the regulation of expression of the vector. This is critical to the overall success of the gene transfer as the regulatory elements incorporated into the expression cassette determine the expression level as well as the duration and can limit off-target effect. The elements that regulate expression consist of several components including the core promoter, enhancers, intron and splice sites, and polyadenylation signals. Much work has focused on the promoter and enhancer combination to dictate the regulation of expression. Often viral promoters such as the immediate early (IE) promoter from the cytomegalovirus (CMV) have been used in many applications or as parts in chimeric promoter such as the CAG promoter (a combination of the CMV early enhancer, promoter, and first exon of the chicken beta-actin gene and a splice acceptor from the rabbit beta-globin gene) [31].

For autoimmune diseases, the incorporation of inducible regulatory elements would be advantageous to allow the transgene expression to respond to flares in inflammation that can occur. Many natural promoters contain regulatory elements that can respond to endogenous signals and modulate their expression. These promoters typically have a low basal level of expression that can respond to increase in ligands such as steroid hormone or interferon expression.

In addition to promoter elements that control gene expression at the transcriptional level, expression can be regulated posttranscriptionally. miRNA-dependent regulation of gene expression is presented as an alternative level of regulation (for review, see [32]). Typically, miRNAs are being developed to prevent off-target expression of the transgene. As a regulatory element, they are typically added to the 3′-UTR of the transgene, where they direct the degradation of the transgene mRNA in the presence of a matching miRNA. Advances in this technology are now allowing miRNAs to be used to enhance gene expression. By placing miRNA binding sites

between a translational inhibitory signal in the mRNA and the transgene, induction of expression is possible [33].

The SG presents a unique environment for expression and regulation of gene expression. In addition to viral promoters, many tissue-specific promoters have also been explored [34]. For local gene therapy, the use of tissue-specific promoters may have some advantages as it would limit off-target expression. In the SG, tissue-specific promoters targeting ductal or acinar cells have been explored in the context of both nonviral vectors and adenoviral vectors. One promoter that showed good expression in the SG of both rat parotid cell line as well as in vivo animal studies, although weaker than more universal promoters such as CMV, RSV, and EF1a, was derived from the AQP5 promoter, which is an acinar cell-specific gene [34].

In consideration of the environment of a SG in Sjögren's patients, the use of a CMV promoter has a number of advantages. Many studies have reported that SG is the preferred site of human CMV infection and that both acinar and ductal cells can be infected. Historically, CMV was referred to as the SG virus. Studies of this promoter in context of human and rodent cell lines show that the human CMV promoter is not methylated in human cell lines, promoting long-term expression [35]. Finally, CMV is known to be further activated in an IFN-stimulated environment that likely exists in the SG of Sjögren's patients due to the presence of NF-kB responsive elements in the promoter [36].

12.6 SS-like Animal Models Used in Preclinical Studies

The current understanding of SS identifies the disease as an autoimmune epithelitis of the SG (for review, see [37, 38]). Although genetics is likely important, it is thought to be induced by environmental factors such as virus infection, followed by stimulation of the innate and adaptive immune response with NK cells, DCs, Mϕ, and T and B lymphocytes. The outcome of the autoimmune epithelitis is exocrinopathy, local inflammation, and autoantibody production (for review, see [38]). In line with the multifactorial aspect of the disease that is associated with the etiology and pathogenesis of SS, numerous mouse models have been developed to mimic specific characteristics common to the human disease (for review, see [39, 40]). Existing mouse models that could be used for preclinical efficacy testing can be divided into two groups: inducers of epithelitis and spontaneous animal models.

12.6.1 Inducers of Autoimmune Epithelitis in SS

12.6.1.1 Loss of Epithelial Hemeostasis

Loss of epithelial homeostasis caused by tight junction deficiency is one of the critical factors to trigger the onset of autoimmune epithelitis in SS (for review, see [37]). Matriptase is a protease that is critical to maintain and regulate tight junctions in salivary gland epithelia (Fig. 12.1, see "Autoimmune Epithelitis/Environmental Factors/Loss of epithelial integrity"). In embryonic mice, ablation of matriptase resulted in the loss of secretory epithelial cell function and induction of autoimmunity similar to that observed in human SS, including lymphocytic infiltrates, production of SS-specific autoantibodies, and overall activation of the immune system. Acute ablation of matriptase expression locally in the SG resulted in significant SG dysfunction in the absence of immune activation [30]. Although all the phenotypes associated with SS were observed in the matriptase-deficient model, the poor long-term survival of the mice makes it difficult to test the mice for a SS gene therapy study.

12.6.1.2 Autoimmune Epithelitis by Virus Infection

Extrinsic factors, especially viral infection, TLR activation of the innate immune response, in concert with the activation of IFN pathway are another important trigger of autoimmune epithelitis of SS (for review, see [37, 38]). Hepatitis delta virus (HDV) has been associated with the occurrence of SS [41] (Fig. 12.1, see "Autoimmune Epithelitis/Environmental Factors/

Virus"). Expression of HDV antigens in mouse SG resulted in reduced stimulated saliva flow, an increase in focal lymphocytic infiltrates, and the development of autoantibodies [41]. In addition, Ad virus and CMV infections are reported to develop SS-like profiles [42, 43]. All these models could be useful in preclinical studies of the induction of SS or testing gene therapy applications.

12.6.1.3 Autoimmune Epithelitis by Immune Cells Activation/ Deletion

12.6.1.3.1 T Cell-Targeted

In CBA or SJL mice, thyroid-targeted expression of IL-12 in transgenic mice resulted in the development of a SS-like disease with SG dysfunction, progressively increased gland infiltration over time and a consistent upregulation of ANA, and an age-dependent increase in autoantibodies against SSB/La [44]. In addition to its therapeutic potential, gene therapy vectors offer excellent tools for functional genomic studies. Ad-mediated expression of IL-17 locally in the SG of mice can be used to activate critical autoimmune T cells, Th17 cells, and induced a SS-like phenotype in C57BL/6 mice [45] that develops lymphocytic foci, increased cytokine levels, changes in ANA profiles, and temporal loss of saliva flow. This model was used to study anti-IL17-targeted gene therapy [46] (see below "Preclinical gene therapies studies in mice"). Id3 is an important gene regulating in the regulation of T cell differentiation. Id3 conditional knockout in mice caused a disorder of T cell development, and the mice exhibited gland dysfunction and lymphocytic infiltration SG and LG [47]. T cell-targeted deletion of stromal interaction molecule (STIM) 1 and STIM2 displays a SS-like phenotype [48] in the double-knockout (DKO) mouse.

12.6.1.3.2 Dendritic Cell (DC)-Targeted

DC immunoreceptor (DCIR) knockout mice spontaneously develop sialadenitis and epithelitis associated with elevated serum autoantibodies along with rheumatoid arthritis (RA) [49].

12.6.1.3.3 B Cell-Targeted

B cell activating cytokines, IL-14 and BAFF overexpression, induce not only a SS-like exocrinopathy, but also lymphoma, which is highly associated to abnormal B cell activation in SS [50].

12.6.1.3.4 Autoantigens

Several models have shown that immunization with a self-antigen in vivo may result in autoimmune inflammation in mice, which can be used as an efficient induction mechanism for the development of SS-like phenotype in mice. Autoantigens, such as Ro60 or Ro52 peptides [51] [52], muscarinic acetylcholine type-3 (M3R) receptor [53], and carbonic anhydrase-II (CA-II) peptides [54], induce exocrinopathy, SG/LG lymphocytic infiltration, and autoantibodies, which are similar to human SS disease. Further research has suggested that a possible key regulator for the induction of SS in Ro peptide antigen-immunized mice might be IFN-γ producing Th1 cells [55].

The strategy to mimic autoimmune epithelitis is through triggering one aspect of the autoimmune disorder (Fig. 12.1, see "Autoimmune Epithelitis"). Although these models may show SS-like disease, they are limited representations of a heterogeneous disease. Therefore, a more comprehensive model that presents a complete SS profile is needed to conduct gene therapy studies. Nevertheless, these models shed light on new avenues and help explore novel gene therapies targeting various aspects of autoimmune epithelitis.

12.6.1.4 Epithelial Function-Associated Gene

Through the analysis of differentially expressed genes in the SG transcriptome, a previously unreported increase in bone morphogenetic protein 6 (BMP6) expression was observed [56]. BMP6 is an important cytokine in TGF-β super family. BMP6 has a role in autoimmunity as its overexpression in the skin of mice can induce a psoriasis-like condition, and its increased expression is observed in the SG of the nonobese diabetic (NOD) mouse model of autoimmunity [56, 57]. Expression of BMP6 in the salivary or lacrimal gland of healthy mice resulted in gland dys-

function, which is independent of the autoantibodies and immune activation associated with the disease [56]. A key finding for the loss of SG function in this model is that the overexpression of BMP6 altered AQP5 expression, a water channel protein that is critical for the salivation process. A recent study using inhibitors of BMP6 signaling showed recovery of AQP5 in a mouse model of SS [58], which further supported targeting therapies for the treatment of SS to BMP6 or AQP5 as promising approaches in treating salivary hypofunction [9] (Fig. 12.1, see "Epithelial Function-Associated Gene").

12.6.2 Spontaneous Models

Over the past decade, scientists have developed inbred strains of mice that spontaneously develop a SS-like characteristics, including MRL/lpr mice, NZB/NZW mice, NOD, and NOD-derived strains such as NOD.B10-H2^b mice [59] and C57BL/6.NOD-Aec1Aec2 mice [60], etc. (for review, see [40]). A complication in some of these models is the development of diabetes or other autoimmune diseases. The development of diabetes in NOD mice is problematic as it limits the life expectancy of the mice and can require insulin treatment to control blood sugar. Even then, variability in the development of disease is reported [61]. The NOD.B10-H2^b [59] and C57BL/6.NOD-Aec1Aec2 [60] are two strains derived from the NOD background that only develop a SS-like phenotype.

The C57BL/6.NOD-Aec1Aec2 mice contain two loci from NOD mice in a C57BL/6 background and exhibit the full SS-like phenotype. In general, C57BL/6.NOD-Aec1Ace2 mice present a rapid SS-like disease progression and the development of the hallmarks, exocrine gland lymphocytic infiltration, and dysfunction. In NOD mice, small salivary and lacrimal gland infiltration can occur by 8 weeks and are clearly identifiable by 12–16 weeks of age, followed by secretory dysfunction by 16–20 weeks of age. Autoantibodies can also be detected in NOD mice during the 8–16 weeks period. Disease progression is similar to the C57BL/6.NOD-Aec1Ace2 mice and can be divided into three phases: phase 1 (preclinical stage, 0–8 weeks of age), phase 2 (clinical stage, 8–16 weeks of age), and phase 3 (full-blown disease stage, 16 weeks and older). The mouse strain exhibits pronounced secretory dysfunctions of the SG and LG during the phase 2 and phase 3 [60, 62].

While the disease remains progressive, the survival and general condition of the C57BL/6.NOD-Aec1Aec2 mice is stable, which makes it more ideal to be used as a SS model. Both sexes of C57BL/6.NOD-Aec1Aec2 mice develop SS-like disease with different features [60, 62, 63]. The induction of SS disease in the C57BL/6.NOD-Aec1Aec2 has been investigated in many aspects, including apoptotic events, genetic susceptibility, epigenetic changes, adaptive immune, and altered glandular homeostasis prior to innate and adaptive immune responses.

12.7 Preclinical Gene Therapies Studies in Mice

Several preclinical gene therapy studies have been undertaken to establish efficacy in the SS animal models. Many of these studies involve Ad or AAV vectors, which can direct localized sustained expression in the submandibular galnd (SMG) of mice by retroductal cannulation [8]. Often a control group of mice is cannulated with a vector encoding LacZ or GFP. In both SG and systemic (serum) expression and secretion, the recombinant protein can be observed after retroductal cannulation [64, 65]. SG function is measured by pilocarpine-stimulated saliva flow volume before gene delivery as baseline, and every 4–6 weeks during the study [8, 9, 46, 65]. The treatments and effects of these studies are summarized in Table 12.1.

12.7.1 Immunomodulatory Gene Therapies

Immunomodulatory gene therapies were designed to block pro-inflammatory cytokines or enhance protective cytokines to correct the auto-

Table 12.1 Gene therapy preclinical trials for SS

Therapeutic vector	Animal model (gender)	Vector delivery				Therapeutic effect					Ref #
		dose (particles)	Site	Time[a]	End point[a]	↓LF	↓Cytokines	↑Saliva	↑Tear	↓Abs	
Immunomodulatory											
AAV2-sTNFR1IgG	NOD (F)	1×10^{10}/gland	SMG	8	24	N	Y	N	N	N	[66]
rAAVhIL-10	NOD	2.5×10^{10}/gland	SMG / IM	8, 16	20	Y (SMG)	Y	Y(SMG, IM late)	N/A	N/A	[8]
rAAV2-CTLA4IgG	NOD (M+F)	1×10^{10}/gland	SMG	6	30	N	Y	Y	N	N	[65]
rAAV2-sCD40:Fc	NOD (F)	1×10^{11}/gland	SMG	8, 10, 16	16/20	N	N	N	N/A	N/A	[67]
rAAV2-ICAM1:Fc	NOD (F)	1×10^{11}/gland	SMG	8, 12, 16, 20	20	Y (8 weeks)	N	N	N	N	[68]
Ad5-IL17R:Fc	C57BL/6.NOD-Aec1Aec2 (M&F)	1×10^{7}/gland	SMG	6–8, 15–17	26, 27	Y	Y	Y	N/A	Y	[46]
rAAV2-IL27	C57BL/6.NOD-Aec1Aec2 (M&F)	2×10^{10}/mouse	IV	6, 14	20	N	Y	Y	N/A	Y	[69]
AAV-U7ΔBAFF	NOD (F)	1×10^{11}/gland	SMG	8	20	Y	N/A	Y	N/A	Y	[70]
rAAV2-TACI-Fc	NOD (F)	1×10^{10}/gland	SMG	10	20	Y	N	N	N	N/A	[71]
AAV2-VIP	NOD (F)	1×10^{10}/gland	SMG	8	16	N	Y	Y	N/A	N/A	[72]
Non-immunomodulatory											
AAV2-AQP1	BMP6 SG overexpression (F)	1×10^{11}/gland	SMG	12	16	N/A	N/A	Y	N/A	N/A	[9]
	C57BL/6.NOD-Aec1Aec2 (M&F)			36	46	N	Y	Y	Y	N	

[a]Weeks old; *LF* lymphocytic foci

immune epithelitis in SS (Fig. 12.1, see "Autoimmune Epithelitis").

12.7.1.1 Targeting Cytokines

12.7.1.1.1 Blocking Pro-inflammatory Cytokine: Tumor Necrosis Factor-α (TNF-α)

TNF-α is mainly produced by NK cells and Mϕ and is a master pro-inflammatory cytokine involved in the development, tissue damage, and dysfunction in a variety of autoimmune diseases such as SS, RA, and systemic lupus erythematosus (SLE) [73, 74]. The revolutionary benefit seen in RA with inhibitors of TNF-α inspired several clinical trials in SS patients with mixed results [75, 76]. AAV2 vectors encoding soluble TNF-α receptor 1-IgG (AAV2-sTNFR1IgG) administered locally in the submandibular glands (SMG) of 8 weeks old female NOD mice resulted in decreased saliva flow, suggesting a negative effect on the protection of SG function. Although the local delivery to the SG showed a protective immune response with increased levels of TGF-β1 and decreased levels of Th1, Th2, and Th17 cytokines, decreased TGF-β and increased T cell cytokine expressions were also detected in plasma [66].

12.7.1.1.2 Overexpressing Protective Cytokine: IL-10

Immunomodulatory gene therapy in SS can be achieved by the expression of protective cytokines such as IL-10 and IL-27 (see below "Targeting Th17 cells"), which are important as enhancers of Treg cells and suppress Th17 cells in autoimmune diseases [77–80]. AAV vectors encoding human IL-10 (rAAVhIL-10) were delivered to female NOD by retrograde SMG cannulation or IM injection at age 8 weeks (early) or at 16 weeks (late). After submandibular administration of rAAVhIL-10, salivary flow rates at 20 weeks for both the early and late treatment groups were significantly higher than for control vector treated and untreated mice. Systemic delivery of rAAVhIL-10 led to improved salivary flow in the late treatment group. Lymphocytic infiltrates in SG, however, were significantly reduced only in mice receiving rAAVhIL-10 locally in the SMG. In addition, improvement in the blood sugar levels was also observed in the NOD mice when the IL-10 expression was targeted to the SG, indicating some systemic benefit of this treatment [8]. This study shows that expression of hIL-10 by rAAV vectors can have disease-modifying effects in the SG of NOD mice.

12.7.1.2 Targeting Co-Stimulatory Molecules

12.7.1.2.1 Cytotoxic T Lymphocyte Antigen-4 (CTLA-4)

The activation of autoimmune T cells and B cells requires co-stimulatory molecules. CTLA4 is one of the critical checkpoints that negatively regulates B7: CD28 co-stimulatory pathway between DC and T cells interaction. The negative regulation of CTLA4 is the result of competition with CD28 to bind B7–1/2 (CD80/ CD86) [79, 81–83]. The recombinant fusion protein of CTLA4 and a constant immunoglobulin region (CTLA4IgG, Abatacept, Orencia) is a prescription therapy for RA [84]. AAV2 vectors encoding a CTLA4IgG fusion protein was delivered to SMG of C57BL/6.NOD-Aec1Aec2 mice with early-stage disease at 8 weeks. Overexpression and secretion of CTLA4IgG both locally in SMG and systemically in the serum were observed. While the mice receiving control vector had a continuous decline of saliva, AAV2-CTLA4IgG-treated mice had a recovery of salivary flow to baseline level, which was sustained until the end point of the study at 30 weeks. Decreased T, B, DC, and Mϕ cells were also seen in the glands of AAV-CTLA4IgG-treated mice compared with controls. In addition, decreased pro-inflammatory cytokines and increased TGF-β1 expression were observed in the serum of CTLA4IgG-treated mice compared with controls [65]. In clinical trials of abatacept, a mild disease stage-dependent improvement was reported [85].

12.7.1.2.2 CD40

CD40-CD154 (CD40 ligand) is another co-stimulatory pathway that is upregulated in the SG of SS patients [86]. Studies in NOD mice treated with an AAV vector encoding a soluble CD40

fused with human Fc (sCD40:Fc) and delivered to SMG reported a number of gene expression changes that were associated with immune regulation. However, systemic and local markers of inflammation and exocrine gland function were not improved. These results would suggest that the level of expression of sCD40:Fc was not sufficient or that targeting CD40 co-stimulation alone may not be sufficient to alter the progression of disease in NOD mice [87]. In a phase II clinical trial of the anti-CD40 monoclonal antibody CFZ533, a significant decrease in disease activity score and the germinal center-related chemokine, CXCL-13, was reported [67].

12.7.1.2.3 Intercellular Adhesion Molecule-1 (ICAM-1)

Another important co-stimulatory pathway in T and B cell communication is the intercellular adhesion molecule 1 (ICAM) and lymphocyte function-associated antigen-1 (LFA-1) interaction. ICAM-1 expression was increased in the SG epithelial cells of SS patients [88]. SMG retroductal cannulation of AAV vectors encoding a soluble ICAM-1:Fc fusion molecule was able to compete for binding of LFA-1 with cell-associated ICAM in young NOD mice and resulted in a decrease in lymphocytic foci and IgM in the SG. However, the same treatment in older mice with more advanced disease resulted in increased T cells in the gland and increase levels of immunoglobulins, which is a marker of B cell activation. Stimulated salivary flow was unchanged in both early and late treatment groups [89]. The finding in older mice corresponded to the findings in a small phase 1 clinical trial of efalizumab, a monoclonal antibody that blocks the binding of LFA-1 to ICAM-1 in SS. Patients developed an increase in inflammation and worsened SG function [68].

12.7.1.3 Targeting Th17 Cells

12.7.1.3.1 IL-17

IL-17 producing Th17 cells are one of the major pro-inflammatory cells involved in SS development in patients and animal models. Functional genomics studies using AAV vectors to locally express IL-17 in the SG of healthy mice induced a SS-like disease [45, 90]. Two preclinical gene therapy studies have investigated IL-17 modulation as an intervention for SS: (1) soluble IL-17 receptor (IL17R:Fc) [46] and (2) expression of IL-27, a regulatory cytokine for IL-17 signaling [91].

Time course studies in the C57BL/6.NODAec1Aec2 mouse model of SS showed expression of IL-17 suggesting its expression served as a key point in the progression of the disease [45]. SG gene therapy using Ad-5 vectors encoding IL17R:Fc delivered in SMG of C57BL/6.NODAec1Aec2 mice between 6 and 8 weeks of age (a pre-disease stage) or 15–17 weeks of age (a diseased stage) resulted in approximately 50% decrease of IL-17 and Th17 cells in serum and spleen, respectively. These immunologic changes were accompanied by a significant amelioration of the SS-like phenotype in the mice, including decreased SG focus score (FS), recovery of SG function, and normalization of ANA levels. Ad-5 IL17R:Fc delivered in pre-disease mice maintained baseline saliva flow level till the end point of the study at 19 weeks post vector delivery. Ad-5 IL17R:Fc gene therapy in disease stage C57BL/6.NODAec1Aec2 mice resulted a 50% increase of salivary flow than baseline after 12 weeks post vector delivery, while the control group showed no change [46]. However, a phase II clinical trial to assess the effects of IL-17 antibody (secukinumab) in dry eye has been completed (NCT01250171), and it was shown that systemic administration of IL-17 antibody does not influence the severity of dry eye sign and symptoms.

12.7.1.3.2 IL-27

A function of IL-27 is to inhibit the expression of IL-17A (commonly referred to as IL-17), and its expression reduces the severity of Th17-mediated autoimmune diseases [69, 92]. Gene therapy to decrease IL-17 activity by increasing expression of IL-27 has been tested in C57BL/6.NOD-Aec1Aec2 mice. Systemic expression of IL-27 by IV injection in either 6 weeks old (pre-disease) or 14 weeks old (clinical disease) mice resulted in decreased IL-17 in the serum and moderation

of the disease phenotype in the mice. While mice treated with control vector or saline at 6 or 14 weeks of age exhibited a relatively rapid loss in SFRs and remained low, the rAAV2-IL27-treated mice at 6 weeks of age showed a slow recovery starting at 4 weeks post cannulation and returned to baseline level by the end of the study at 20 weeks post vector delivery. Mice treated with rAAV2-IL27 at 14 weeks of age also showed minor improvements in their saliva flow starting at 8 weeks post cannulation. These suggest that IL-27 gene therapy might prevent the continuous decrease of salivary function when treatment is done prior to the onset of disease and may partially restore glandular function at a later stage. rAAV2-IL27 injections at different disease stages had little effect on FS but resulted in structural changes in LF, lower titers of ANAs with changes in staining patterns [69].

12.7.1.4 Targeting B Cells

APRIL and BAFF (BAFF/APRIL system) are members of TNF family and play pivotal roles in B cell activation, including augmentation of B cell antigen presentation, co-stimulation of B cells, B cell survival, regulation of B cell tolerance, and germinal center maintenance. BAFF binds to three receptors: BAFF-R, TACI, and BCMA, whereas APRIL interacts with TACI, BCMA, and proteoglycans [93]. BAFF expression was found in serum of SG epithelial cells from SS patients, and expression in transgenic mice induces SS-like phenotype [94–96].

12.7.1.4.1 B Cell-Activating Factor

The inhibition of BAFF in SS patients has been investigated in two large clinical studies and yielded promising results [97]. Although belimumab, an antibody to BAFF, did not show clinical benefits on glandular inflammation or function, there was an improvement in patient outcome and disease activity score. Gene therapy locally targeting BAFF expression has also yielded promising results. Rather than blocking the effect of circulating BAFF protein, the approach used in local gene therapy was to block BAFF expression intracellularly by changing the splicing of the natural message for BAFF to produce a truncated form (ΔBAFF), which is a physiological inhibitor of BAFF. An AAV vector containing a modified U7 promoter was able to induce exon skipping in the mouse BAFF mRNA. When this vector was retroductally cannulated into the SMG of 8 weeks old female NOD mice, by week 20, BAFF protein expression was significantly reduced along with a decrease in FS, B lymphocytes, and plasma cells in the gland. The ΔBAFF local overexpression mice had more than 60% increase in salivary flow compared with the control vector group. These preclinical studies further support local blockage of BAFF as a therapeutic approach to the treatment of SS [98].

12.7.1.4.2 Transmembrane Activator and CAML Interactor (TACI)

TACI acts as a shared receptor for both BAFF and APRIL signaling. Blockade of this receptor with a recombinant form of the receptor was shown to delay the onset of disease in mouse models of SLE [70]. Therefore, it would appear to be an attractive target for intervention in SS. Preclinical studies in NOD mice treated with AAV vectors that encoded a soluble form of the TACI receptor fusion protein showed a significant reduction in FS as well as changes in IgD+ cells and CD138+ B cells with decreased production of IgG and IgM compared to control mice. However, SG function was not improved in the TACI-treated mice. Further studies are needed to evaluate dual blockade of APRIL and BAFF by sTACI:Fc as a treatment for SS [99].

12.7.1.5 Targeting Neuroendocrine-Immune Regulator

12.7.1.5.1 Vasoactive Intestinal Peptide (VIP)

Vasoactive intestinal peptide (VIP) is a neuroendocrine-immune regulator and was reported to ameliorate RA in animal models via a shift from Th1 to Th2 response or by downregulating NF-kB activity [71, 100]. 10^{10} particles/gland of rAAV2hVIPAAV2-VIP was delivered to SMG of female NOD mice at 8 weeks of age. Eight weeks after vector delivery, the salivary flow rates for the rAAV2LacZ and rAAV2hVIP

group were about two times higher than the control vector group. Although FS did not change, a decrease in pro-inflammatory cytokines and full recovery of gland function suggest that VIP is a promising therapeutics in SS [101].

12.7.2 Non-immunomodulatory Gene Therapies

12.7.2.1 AQP1

In addition to gene therapy as an approach to modulating the immune imbalance in SS, gene therapy could also be used to directly restore secretory function in the glands by expression of rate-limiting proteins in the secretion of saliva that are inhibited during the course of disease. In SG, the water channel molecule, AQP5, on acinar cells is crucial in the initiation of fluid movement that leads to saliva secretion. Altered AQP5 expression has been reported in many studies of SS patients and mouse models of the disease [29, 56] (Fig. 12.1, see "Epithelial Function-Associated Gene"). AQP5 allows the movement of water across the membrane in response to the osmotic gradient created by the release of chloride ions into the lumen of the gland. AQP gene therapy to repair membrane water permeability defects has been tested in animal models of SG hypofunction induced by radiation damage [17, 72, 102] to the gland and also in a clinical trial for radiation-induced SG hypofunction with positive results reported [22, 103, 104]. In these studies, the expression and function of AQP5 is replaced by the expression of AQP1, another member of the AQP family, which shows membrane polarization-independent distribution. Furthermore, unlike AQP5, which is regulated by the transient receptor potential 4, (TRP4) in the SG, AQP1 is not reported to be regulated [105]. Thus, expression of AQP1 would create a facilitated pathway for water to move through a cell in response to an osmotic gradient.

Positive effect of SG-targeted AQP gene therapy was observed in both BMP6 SG overexpressing mice and C57BL/6.NOD-Aec1Aec2 mice with established late-stage disease [9]. At a dose of 10^{11} particles/mouse, secretory function was improved by more than 70% at 4 weeks post cannulation in BMP6 SG overexpressing mice. In the C57BL/6.NOD-Aec1Aec2 mice treated with AQP1 SG gene therapy, a 400% increase in saliva flow was observed with significant downregulation of overall inflammation in SG and serum, including decreased Th1 and Th17 cells and increased Treg cells. This local gene therapy with AQP1 also improved systemic inflammation. Although the mechanism is not clear, restoration of flow could lead to decreased antigen exposure, lowering glandular inflammation and inducing a return to immune homeostasis. Interestingly, SG-targeted AQP gene therapy also significantly increased secretory function at a distal site, the LG [9].

12.8 Future Therapeutic Targets in Gene Therapy

Gene therapy is rapidly advancing from concept to preclinical to drugs for treating a number of diseases. A key aspect of future development for the field is the vectors used to deliver the genes and the regulation of the therapeutics expression. Although AAV is showing promise as a vector platform, it has limitations in packaging size, immunogenicity, and persistence of expression in dividing cells.

With respect to gene therapy for SS, new vectors with enhanced transduction activity for acinar cells will be a benefit. This will both improve the therapeutic index and reduce the potential for an immune response to the vector. However, the future will largely be dictated by developing a better understanding of the underlying pathology of the disease so that therapies can target underlying causes rather than simply treating symptoms. Understanding environmental triggers responsible for the initiation of the disease would open several new avenues for therapy. In gene therapy, this could result in developing vectors that initiate the expression of neutralizing antibodies to a contributing pathogen or siRNA producing vectors that silence the pathogen or the

inflammatory pathway. Likewise, future treatments will rely on understanding the elements of the genetic predisposition that allows the development of SS upon the introduction of an environmental trigger.

By understanding epigenetic regulators of SS, key drivers for the disease can be identified, and regulation of autoreactive immune cells can be achieved. Initial steps for this approach are to understand the regulatory network of the SG gene expression [106] and to improve glandular dysfunction by turning on silent genes in acinar cells, such as AQP1, instead of adding a new gene [107]. Future work may involve utilization of targeted CRISPR/Cas-based activators or repressors to regulate specific pathways rather than introducing a single gene. In the near future, gene therapy with AQPs promises a good likelihood of success in addressing salivary hypofunction, which is a chief complaint of SS patients. Resolution of markers of inflammation by AQPs will need a close consideration and evaluation. Given the overall advances in the field and the promising preclinical results with animal models of SS, rapid progress in gene therapy is warranted.

12.9 Concluding Thoughts

In summary, the variety of preclinical studies in gene therapies provide both useful information and directions for future clinical application as well as advance an understanding of the basic imbalance associated with SS. The results also provide valuable reference for other autoimmune diseases. Although it has not been reported yet, advances in siRNA, CRISPR, and CAR-T combined with gene therapy may present new opportunities for the treatment of this disease.

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References

1. Baccaglini L, Shamsul Hoque AT, Wellner RB, Goldsmith CM, Redman RS, Sankar V, et al. Cationic liposome-mediated gene transfer to rat salivary epithelial cells in vitro and in vivo. J Gene Med. 2001;3(1):82–90.
2. Passineau MJ, Zourelias L, Machen L, Edwards PC, Benza RL. Ultrasound-assisted non-viral gene transfer to the salivary glands. Gene Ther. 2010;17(11):1318–24.
3. Geguchadze R, Wang Z, Zourelias L, Perez-Riveros P, Edwards PC, Machen L, et al. Proteomic profiling of salivary gland after nonviral gene transfer mediated by conventional plasmids and minicircles. Mol Ther Methods Clin Dev. 2014;1:14007.
4. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science (New York, NY). 2015;348(6230):62–8.
5. Lee CS, Bishop ES, Zhang R, Yu X, Farina EM, Yan S, et al. Adenovirus-mediated gene delivery: potential applications for gene and cell-based therapies in the new era of personalized medicine. Genes Dis. 2017;4(2):43–63.
6. Alevizos I, Zheng C, Cotrim AP, Liu S, McCullagh L, Billings ME, et al. Late responses to adenoviral-mediated transfer of the aquaporin-1 gene for radiation-induced salivary hypofunction. Gene Ther. 2017;24(3):176–86.
7. Voutetakis A, Kok MR, Zheng C, Bossis I, Wang J, Cotrim AP, et al. Reengineered salivary glands are stable endogenous bioreactors for systemic gene therapeutics. Proc Natl Acad Sci U S A. 2004;101(9):3053–8.
8. Kok MR, Yamano S, Lodde BM, Wang J, Couwenhoven RI, Yakar S, et al. Local adeno-associated virus-mediated interleukin 10 gene transfer has disease-modifying effects in a murine model of Sjogren's syndrome. Hum Gene Ther. 2003;14(17):1605–18.
9. Lai Z, Yin H, Cabrera-Perez J, Guimaro MC, Afione S, Michael DG, et al. Aquaporin gene therapy corrects Sjogren's syndrome phenotype in mice. Proc Natl Acad Sci U S A. 2016;113(20):5694–9.
10. Di Pasquale G, Rivero P, Tora M, Swaim W, Sheikh T, Teos L, et al. Transduction of salivary gland acinar cells in rodents with adeno associated viral vectors results in persistent exocrine and endocrine release of recombinant proteins [abstract]. Mol Ther. 2015;23:S221.
11. Taghian T, Marosfoi MG, Puri AS, Cataltepe OI, King RM, Diffie EB, et al. A safe and reliable technique for CNS delivery of AAV vectors in the cisterna magna. Mol Ther. 2020;28(2):411–21.
12. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, et al. Successful transduction of liver in hemophilia by AAV-factor IX and limitations imposed by the host immune response. Nat Med. 2006;12(3):342–7.
13. Hinderer C, Katz N, Buza EL, Dyer C, Goode T, Bell P, et al. Severe toxicity in nonhuman primates and piglets following high-dose intravenous administra-

tion of an adeno-associated virus vector expressing human SMN. Hum Gene Ther. 2018;29(3):285–98.
14. Hordeaux J, Hinderer C, Goode T, Buza EL, Bell P, Calcedo R, et al. Toxicology study of intra-cisterna magna adeno-associated virus 9 expressing iduronate-2-sulfatase in rhesus macaques. Mol Ther Methods Clin Dev. 2018;10:68–78.
15. Hordeaux J, Hinderer C, Goode T, Katz N, Buza EL, Bell P, et al. Toxicology study of intra-cisterna magna adeno-associated virus 9 expressing human alpha-L-iduronidase in rhesus macaques. Mol Ther Methods Clin Dev. 2018;10:79–88.
16. Delporte C, Miller G, Kagami H, Lillibridge CD, O'Connell BC, Atkinson JC, et al. Safety of salivary gland-administered replication-deficient recombinant adenovirus in rats. J Oral Pathol Med. 1998;27(1):34–8.
17. Shan Z, Li J, Zheng C, Liu X, Fan Z, Zhang C, et al. Increased fluid secretion after adenoviral-mediated transfer of the human aquaporin-1 cDNA to irradiated miniature pig parotid glands. Mol Ther. 2005;11(3):444–51.
18. O'Connell AC, Baccaglini L, Fox PC, O'Connell BC, Kenshalo D, Oweisy H, et al. Safety and efficacy of adenovirus-mediated transfer of the human aquaporin-1 cDNA to irradiated parotid glands of non-human primates. Cancer Gene Ther. 1999;6(6):505–13.
19. Adesanya MR, Redman RS, Baum BJ, O'Connell BC. Immediate inflammatory responses to adenovirus-mediated gene transfer in rat salivary glands. Hum Gene Ther. 1996;7(9):1085–93.
20. O'Connell BC, Zheng C, Jacobson-Kram D, Baum BJ. Distribution and toxicity resulting from adenoviral vector administration to a single salivary gland in adult rats. J Oral Pathol Med. 2003;32(7):414–21.
21. Momot D, Zheng C, Yin H, Elbekai RH, Vallant M, Chiorini JA. Toxicity and biodistribution of the serotype 2 recombinant adeno-associated viral vector, encoding Aquaporin-1, after retroductal delivery to a single mouse parotid gland. PLoS One. 2014;9(3):e92832.
22. Baum BJ, Alevizos I, Zheng C, Cotrim AP, Liu S, McCullagh L, et al. Early responses to adenoviral-mediated transfer of the aquaporin-1 cDNA for radiation-induced salivary hypofunction. Proc Natl Acad Sci U S A. 2012;109(47):19403–7.
23. Zheng C, Nikolov NP, Alevizos I, Cotrim AP, Liu S, McCullagh L, et al. Transient detection of E1-containing adenovirus in saliva after the delivery of a first-generation adenoviral vector to human parotid gland. J Gene Med. 2010;12(1):3–10.
24. Odani T, Chiorini JA. Targeting primary Sjogren's syndrome. Mod Rheumatol. 2019;29(1):70–86.
25. Gavin AL, Ait-Azzouzene D, Ware CF, Nemazee D. DeltaBAFF, an alternate splice isoform that regulates receptor binding and biopresentation of the B cell survival cytokine, BAFF. J Biol Chem. 2003;278(40):38220–8.
26. Evans RL, Park K, Turner RJ, Watson GE, Nguyen HV, Dennett MR, et al. Severe impairment of salivation in Na+/K+/2Cl- cotransporter (NKCC1)-deficient mice. J Biol Chem. 2000;275(35):26720–6.
27. Futatsugi A, Nakamura T, Yamada MK, Ebisui E, Nakamura K, Uchida K, et al. IP3 receptor types 2 and 3 mediate exocrine secretion underlying energy metabolism. Science (New York, NY). 2005;309(5744):2232–4.
28. Gautam D, Duttaroy A, Cui Y, Han SJ, Deng C, Seeger T, et al. M1-M3 muscarinic acetylcholine receptor-deficient mice: novel phenotypes. J Mol Neurosci. 2006;30(1–2):157–60.
29. Krane CM, Melvin JE, Nguyen HV, Richardson L, Towne JE, Doetschman T, et al. Salivary acinar cells from aquaporin 5-deficient mice have decreased membrane water permeability and altered cell volume regulation. J Biol Chem. 2001;276(26):23413–20.
30. Yin H, Kosa P, Liu X, Swaim WD, Lai Z, Cabrera-Perez J, et al. Matriptase deletion initiates a Sjogren's syndrome-like disease in mice. PLoS One. 2014;9(2):e82852.
31. Miyazaki J, Takaki S, Araki K, Tashiro F, Tominaga A, Takatsu K, et al. Expression vector system based on the chicken beta-actin promoter directs efficient production of interleukin-5. Gene. 1989;79(2):269–77.
32. Dhungel B, Ramlogan-Steel CA, Steel JC. MicroRNA-regulated gene delivery systems for research and therapeutic purposes. Molecules. 2018;23(7):1500.
33. Harel L, Gefen N, Carmi O, Orbach P, Einat P, Abitbol G. Novel expression vectors enabling induction of gene expression by small-interfering RNAs and microRNAs. PLoS One. 2014;9(12):e115327.
34. Zheng C, Baum BJ. Evaluation of viral and mammalian promoters for use in gene delivery to salivary glands. Mol Ther. 2005;12(3):528–36.
35. Zheng C, Baum BJ, Liu X, Goldsmith CM, Perez P, Jang SI, et al. Persistence of hAQP1 expression in human salivary gland cells following AdhAQP1 transduction is associated with a lack of methylation of hCMV promoter. Gene Ther. 2015;22(9):758–66.
36. Pan RY, Xiao X, Chen SL, Li J, Lin LC, Wang HJ, et al. Disease-inducible transgene expression from a recombinant adeno-associated virus vector in a rat arthritis model. J Virol. 1999;73(4):3410–7.
37. Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjogren's syndrome. Nat Rev Rheumatol. 2010;6(9):529–37.
38. Sandhya P, Kurien BT, Danda D, Scofield RH. Update on pathogenesis of Sjogren's syndrome. Curr Rheumatol Rev. 2017;13(1):5–22.
39. Chiorini JA, Cihakova D, Ouellette CE, Caturegli P. Sjogren syndrome: advances in the pathogenesis from animal models. J Autoimmun. 2009;33(3–4):190–6.
40. Peck AB, Nguyen CQ. What can Sjogren's syndrome-like disease in mice contribute to

human Sjogren's syndrome? Clin Immunol. 2017;182:14–23.
41. Weller ML, Gardener MR, Bogus ZC, Smith MA, Astorri E, Michael DG, et al. Hepatitis delta virus detected in salivary glands of Sjogren's syndrome patients and recapitulates a Sjogren's syndrome-like phenotype in vivo. Pathog Immun. 2016;1(1):12–40.
42. Bombardieri M, Barone F, Lucchesi D, Nayar S, van den Berg WB, Proctor G, et al. Inducible tertiary lymphoid structures, autoimmunity, and exocrine dysfunction in a novel model of salivary gland inflammation in C57BL/6 mice. J Immunol. 2012;189(7):3767–76.
43. Fleck M, Kern ER, Zhou T, Lang B, Mountz JD. Murine cytomegalovirus induces a Sjogren's syndrome-like disease in C57Bl/6-lpr/lpr mice. Arthritis Rheum. 1998;41(12):2175–84.
44. Vosters JL, Landek-Salgado MA, Yin H, Swaim WD, Kimura H, Tak PP, et al. Interleukin-12 induces salivary gland dysfunction in transgenic mice, providing a new model of Sjogren's syndrome. Arthritis Rheum. 2009;60(12):3633–41.
45. Nguyen CQ, Yin H, Lee BH, Carcamo WC, Chiorini JA, Peck AB. Pathogenic effect of interleukin-17A in induction of Sjogren's syndrome-like disease using adenovirus-mediated gene transfer. Arthritis Res Ther. 2010;12(6):R220.
46. Nguyen CQ, Yin H, Lee BH, Chiorini JA, Peck AB. IL17: potential therapeutic target in Sjogren's syndrome using adenovirus-mediated gene transfer. Lab Investig. 2011;91(1):54–62.
47. Guo Z, Li H, Han M, Xu T, Wu X, Zhuang Y. Modeling Sjogren's syndrome with Id3 conditional knockout mice. Immunol Lett. 2011;135(1–2):34–42.
48. Cheng KT, Alevizos I, Liu X, Swaim WD, Yin H, Feske S, et al. STIM1 and STIM2 protein deficiency in T lymphocytes underlies development of the exocrine gland autoimmune disease, Sjogren's syndrome. Proc Natl Acad Sci U S A. 2012;109(36):14544–9.
49. Fujikado N, Saijo S, Yonezawa T, Shimamori K, Ishii A, Sugai S, et al. Dcir deficiency causes development of autoimmune diseases in mice due to excess expansion of dendritic cells. Nat Med. 2008;14(2):176–80.
50. Shen L, Suresh L, Li H, Zhang C, Kumar V, Pankewycz O, et al. IL-14 alpha, the nexus for primary Sjogren's disease in mice and humans. Clin Immunol. 2009;130(3):304–12.
51. Scofield RH, Henry WE, Kurien BT, James JA, Harley JB. Immunization with short peptides from the sequence of the systemic lupus erythematosus-associated 60-kDa Ro autoantigen results in anti-Ro ribonucleoprotein autoimmunity. J Immunol. 1996;156(10):4059–66.
52. Szczerba BM, Kaplonek P, Wolska N, Podsiadlowska A, Rybakowska PD, Dey P, et al. Interaction between innate immunity and Ro52-induced antibody causes Sjogren's syndrome-like disorder in mice. Ann Rheum Dis. 2016;75(3):617–22.
53. Iizuka M, Wakamatsu E, Tsuboi H, Nakamura Y, Hayashi T, Matsui M, et al. Pathogenic role of immune response to M3 muscarinic acetylcholine receptor in Sjogren's syndrome-like sialadenitis. J Autoimmun. 2010;35(4):383–9.
54. Nishimori I, Bratanova T, Toshkov I, Caffrey T, Mogaki M, Shibata Y, et al. Induction of experimental autoimmune sialoadenitis by immunization of PL/J mice with carbonic anhydrase II. J Immunol. 1995;154(9):4865–73.
55. Yin H, Vosters JL, Roescher N, D'Souza A, Kurien BT, Tak PP, et al. Location of immunization and interferon-gamma are central to induction of salivary gland dysfunction in Ro60 peptide immunized model of Sjogren's syndrome. PLoS One. 2011;6(3):e18003.
56. Yin H, Cabrera-Perez J, Lai Z, Michael D, Weller M, Swaim WD, et al. Association of bone morphogenetic protein 6 with exocrine gland dysfunction in patients with Sjogren's syndrome and in mice. Arthritis Rheum. 2013;65(12):3228–38.
57. Blessing M, Schirmacher P, Kaiser S. Overexpression of bone morphogenetic protein-6 (BMP-6) in the epidermis of transgenic mice: inhibition or stimulation of proliferation depending on the pattern of transgene expression and formation of psoriatic lesions. J Cell Biol. 1996;135(1):227–39.
58. Yin H, Kalra L, Lai Z, Guimaro MC, Aber L, Warner BM, et al. Inhibition of bone morphogenetic protein 6 receptors ameliorates Sjogren's syndrome in mice. Sci Rep. 2020;10(1):2967.
59. Nguyen C, Cornelius J, Singson E, Killedar S, Cha S, Peck AB. Role of complement and B lymphocytes in Sjogren's syndrome-like autoimmune exocrinopathy of NOD.B10-H2b mice. Mol Immunol. 2006;43(9):1332–9.
60. Cha S, Nagashima H, Brown VB, Peck AB, Humphreys-Beher MG. Two NOD Idd-associated intervals contribute synergistically to the development of autoimmune exocrinopathy (Sjogren's syndrome) on a healthy murine background. Arthritis Rheum. 2002;46(5):1390–8.
61. Lodde BM, Mineshiba F, Kok MR, Wang J, Zheng C, Schmidt M, et al. NOD mouse model for Sjogren's syndrome: lack of longitudinal stability. Oral Dis. 2006;12(6):566–72.
62. Nguyen C, Singson E, Kim JY, Cornelius JG, Attia R, Doyle ME, et al. Sjogren's syndrome-like disease of C57BL/6.NOD-Aec1 Aec2 mice: gender differences in keratoconjunctivitis sicca defined by a cross-over in the chromosome 3 Aec1 locus. Scand J Immunol. 2006;64(3):295–307.
63. Nguyen CQ, Sharma A, Lee BH, She JX, McIndoe RA, Peck AB. Differential gene expression in the salivary gland during development and onset of xerostomia in Sjogren's syndrome-like disease of the C57BL/6.NOD-Aec1Aec2 mouse. Arthritis Res Ther. 2009;11(2):R56.
64. Yamano S, Huang LY, Ding C, Chiorini JA, Goldsmith CM, Wellner RB, et al. Recombinant

adeno-associated virus serotype 2 vectors mediate stable interleukin 10 secretion from salivary glands into the bloodstream. Hum Gene Ther. 2002;13(2):287–98.
65. Yin H, Nguyen CQ, Samuni Y, Uede T, Peck AB, Chiorini JA. Local delivery of AAV2-CTLA4IgG decreases sialadenitis and improves gland function in the C57BL/6.NOD-Aec1Aec2 mouse model of Sjogren's syndrome. Arthritis Res Ther. 2012;14(1):R40.
66. Vosters JL, Yin H, Roescher N, Kok MR, Tak PP, Chiorini JA. Local expression of tumor necrosis factor-receptor 1:immunoglobulin G can induce salivary gland dysfunction in a murine model of Sjogren's syndrome. Arthritis Res Ther. 2009;11(6):R189.
67. Fisher BZM, Ng W, Bombardieri M, Posch M, Papas A, et al. The novel anti-CD40 monoclonal antibody CFZ533 shows beneficial effects in patients with primary Sjögren's syndrome: a phase IIa double-blind, placebo-controlled randomized trial [abstract]. Arthritis Rheumatol. 2017;69:10.
68. Illei G. Clinical investigations of Sjogren's syndrome [Grant application]; 2009.
69. Batten M, Li J, Yi S, Kljavin NM, Danilenko DM, Lucas S, et al. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. Nat Immunol. 2006;7(9):929–36.
70. Gross JA, Dillon SR, Mudri S, Johnston J, Littau A, Roque R, et al. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. Impaired B cell maturation in mice lacking BLyS. Immunity. 2001;15(2):289–302.
71. Vervoordeldonk MJ, Tak PP. Cytokines in rheumatoid arthritis. Curr Rheumatol Rep. 2002;4(3):208–17.
72. Delporte C, O'Connell BC, He X, Lancaster HE, O'Connell AC, Agre P, et al. Increased fluid secretion after adenoviral-mediated transfer of the aquaporin-1 cDNA to irradiated rat salivary glands. Proc Natl Acad Sci U S A. 1997;94(7):3268–73.
73. Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjogren's syndrome. J Immunol. 1994;152(11):5532–9.
74. Mariette X, Ravaud P, Steinfeld S, Baron G, Goetz J, Hachulla E, et al. Inefficacy of infliximab in primary Sjogren's syndrome: results of the randomized, controlled trial of remicade in primary Sjogren's syndrome (TRIPSS). Arthritis Rheum. 2004;50(4):1270–6.
75. Zandbelt MM, de Wilde P, van Damme P, Hoyng CB, van de Putte L, van den Hoogen F. Etanercept in the treatment of patients with primary Sjogren's syndrome: a pilot study. J Rheumatol. 2004;31(1):96–101.
76. Sankar V, Brennan MT, Kok MR, Leakan RA, Smith JA, Manny J, et al. Etanercept in Sjogren's syndrome: a twelve-week randomized, double-blind, placebo-controlled pilot clinical trial. Arthritis Rheum. 2004;50(7):2240–5.
77. Cutolo M, Soldano S, Montagna P, Sulli A, Seriolo B, Villaggio B, et al. CTLA4-Ig interacts with cultured synovial macrophages from rheumatoid arthritis patients and downregulates cytokine production. Arthritis Res Ther. 2009;11(6):R176.
78. Downie-Doyle S, Bayat N, Rischmueller M, Lester S. Influence of CTLA4 haplotypes on susceptibility and some extraglandular manifestations in primary Sjogren's syndrome. Arthritis Rheum. 2006;54(8):2434–40.
79. Perkins D, Wang Z, Donovan C, He H, Mark D, Guan G, et al. Regulation of CTLA-4 expression during T cell activation. J Immunol. 1996;156(11):4154–9.
80. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature. 2003;423(6939):506–11.
81. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med. 2000;192(2):303–10.
82. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev. 2006;212:8–27.
83. Matsumura R, Umemiya K, Goto T, Nakazawa T, Kagami M, Tomioka H, et al. Glandular and extraglandular expression of costimulatory molecules in patients with Sjogren's syndrome. Ann Rheum Dis. 2001;60(5):473–82.
84. Genovese MC, Becker JC, Schiff M, Luggen M, Sherrer Y, Kremer J, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med. 2005;353(11):1114–23.
85. Adler S, Korner M, Forger F, Huscher D, Caversaccio MD, Villiger PM. Evaluation of histologic, serologic, and clinical changes in response to abatacept treatment of primary Sjogren's syndrome: a pilot study. Arthritis Care Res. 2013;65(11):1862–8.
86. Roescher N, Lodde BM, Vosters JL, Tak PP, Catalan MA, Illei GG, et al. Temporal changes in salivary glands of non-obese diabetic mice as a model for Sjogren's syndrome. Oral Dis. 2012;18(1):96–106.
87. Roescher N, Vosters JL, Lai Z, Uede T, Tak PP, Chiorini JA. Local administration of soluble CD40:Fc to the salivary glands of non-obese diabetic mice does not ameliorate autoimmune inflammation. PLoS One. 2012;7(12):e51375.
88. Kapsogeorgou EK, Manoussakis MN. Salivary gland epithelial cells (SGEC): carriers of exquisite B7-2 (CD86) costimulatory molecules. J Autoimmun. 2010;35(3):188–91.
89. Roescher N, Vosters JL, Yin H, Illei GG, Tak PP, Chiorini JA. Effect of soluble ICAM-1 on a Sjogren's

syndrome-like phenotype in NOD mice is disease stage dependent. PLoS One. 2011;6(5):e19962.
90. Nguyen CQ, Hu MH, Li Y, Stewart C, Peck AB. Salivary gland tissue expression of interleukin-23 and interleukin-17 in Sjogren's syndrome: findings in humans and mice. Arthritis Rheum. 2008;58(3):734–43.
91. Lee BH, Carcamo WC, Chiorini JA, Peck AB, Nguyen CQ. Gene therapy using IL-27 ameliorates Sjogren's syndrome-like autoimmune exocrinopathy. Arthritis Res Ther. 2012;14(4):R172.
92. Yang J, Yang M, Htut TM, Ouyang X, Hanidu A, Li X, et al. Epstein-Barr virus-induced gene 3 negatively regulates IL-17, IL-22 and RORgamma t. Eur J Immunol. 2008;38(5):1204–14.
93. Dillon SR, Gross JA, Ansell SM, Novak AJ. An APRIL to remember: novel TNF ligands as therapeutic targets. Nat Rev. 2006;5(3):235–46.
94. Ittah M, Miceli-Richard C, Gottenberg JE, Sellam J, Eid P, Lebon P, et al. Viruses induce high expression of BAFF by salivary gland epithelial cells through TLR- and type-I IFN-dependent and -independent pathways. Eur J Immunol. 2008;38(4):1058–64.
95. Mariette X, Roux S, Zhang J, Bengoufa D, Lavie F, Zhou T, et al. The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjogren's syndrome. Ann Rheum Dis. 2003;62(2):168–71.
96. Lavie F, Miceli-Richard C, Ittah M, Sellam J, Gottenberg JE, Mariette X. Increase of B cell-activating factor of the TNF family (BAFF) after rituximab treatment: insights into a new regulating system of BAFF production. Ann Rheum Dis. 2007;66(5):700–3.
97. Mariette X, Seror R, Quartuccio L, Baron G, Salvin S, Fabris M, et al. Efficacy and safety of belimumab in primary Sjogren's syndrome: results of the BELISS open-label phase II study. Ann Rheum Dis. 2015;74(3):526–31.
98. Roescher N, Vosters JL, Alsaleh G, Dreyfus P, Jacques S, Chiocchia G, et al. Targeting the splicing of mRNA in autoimmune diseases: BAFF inhibition in Sjogren's syndrome as a proof of concept. Mol Ther. 2014;22(4):821–7.
99. Vosters JL, Roescher N, Illei GG, Chiorini JA, Tak PP. TACI-Fc gene therapy improves autoimmune sialadenitis but not salivary gland function in non-obese diabetic mice. Oral Dis. 2012;18(4):365–74.
100. Yin H, Cheng H, Yu M, Zhang F, Lin J, Gao Y, et al. Vasoactive intestinal peptide ameliorates synovial cell functions of collagen-induced arthritis rats by down-regulating NF-kappaB activity. Immunol Investig. 2005;34(2):153–69.
101. Lodde BM, Mineshiba F, Wang J, Cotrim AP, Afione S, Tak PP, et al. Effect of human vasoactive intestinal peptide gene transfer in a murine model of Sjogren's syndrome. Ann Rheum Dis. 2006;65(2):195–200.
102. Gao R, Yan X, Zheng C, Goldsmith CM, Afione S, Hai B, et al. AAV2-mediated transfer of the human aquaporin-1 cDNA restores fluid secretion from irradiated miniature pig parotid glands. Gene Ther. 2011;18(1):38–42.
103. Baum BJ, Zheng C, Cotrim AP, Goldsmith CM, Atkinson JC, Brahim JS, et al. Transfer of the AQP1 cDNA for the correction of radiation-induced salivary hypofunction. Biochim Biophys Acta. 2006;1758(8):1071–7.
104. Alevizos I, Zheng C, Cotrim AP, Goldsmith CM, McCullagh L, Berkowitz T, et al. Immune reactivity after adenoviral-mediated aquaporin-1 cDNA transfer to human parotid glands. Oral Dis. 2017;23(3):337–46.
105. Liu X, Bandyopadhyay BC, Nakamoto T, Singh B, Liedtke W, Melvin JE, et al. A role for AQP5 in activation of TRPV4 by hypotonicity: concerted involvement of AQP5 and TRPV4 in regulation of cell volume recovery. J Biol Chem. 2006;281(22):15485–95.
106. Michael DG, Pranzatelli TJF, Warner BM, Yin H, Chiorini JA. Integrated epigenetic mapping of human and mouse salivary gene regulation. J Dent Res. 2019;98(2):209–17.
107. Wang Z, Pradhan-Bhatt S, Farach-Carson MC, Passineau MJ. Artificial induction of native Aquaporin-1 expression in human salivary cells. J Dent Res. 2017;96(4):444–9.

Clinical Management of Dry Eye in Sjögren's Syndrome

13

Joon Young Hyon

Dry eye is defined as a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, which is accompanied by ocular symptoms [1]. Dry eye is classified into two primary categories, aqueous deficient or evaporative, and Sjögren's syndrome is major subcategory of aqueous deficient dry eye [2].

Dry eye or keratoconjunctivitis sicca, along with dry mouth, is one of the hallmarks of clinical manifestations in Sjögren's syndrome. Dry eye in Sjögren's syndrome typically accompanies with decreased tear secretion. Symptoms of dry eye vary from lack of ocular symptoms to devastating ocular soreness and decreased visual acuity. However, it is well known that ocular symptom does not correlate well with signs of keratoconjunctivitis sicca, such as corneal/conjunctival epithelial erosion and corneal filaments [3]. Thus, thorough ocular evaluation should be performed to assess ocular surface damage and residual lacrimal gland function irrespective of ocular symptoms.

J. Y. Hyon (✉)
Department of Ophthalmology, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea
e-mail: jyhyon@snu.ac.kr

13.1 Diagnosis of Dry Eye

Four aspects should be evaluated for the diagnosis of dry eye: (1) tear production, (2) tear film stability, (3) ocular surface damage, and (4) patient symptom.

Schirmer's test is a conventional method to measure tear production by placing a strip of paper onto the lateral one-third of lower lid margin, and the length of wetting after 5 min is measured as a score. It is performed without topical anesthesia to assess basic tear secretion and reflex tearing as well. The cutoff value of ≤5 mm/5 min [4] or ≤10 mm/5 min [5] is proposed for the diagnosis of dry eye. However, tear secretion ≤5 mm/5 min is typically used for the classification criteria for Sjögren's syndrome.

Strip meniscometry, a variation of Schirmer's test, is to measure tear volume from tear meniscus. It takes only 5 s to measure and is reported to have 81% sensitivity and 99% specificity when combined with tear breakup time (TBUT) in the diagnosis of dry eye [6]. However, diagnostic efficacy of strip meniscometry for Sjögren's syndrome is not established well.

TBUT is the time interval between a blink and the first appearance of a break spot in the tear film. The most frequently employed test is fluorescein breakup time, in which 1.0–2.0% sodium fluorescein solution is used to stain tear film, and the cutoff value of ≤5 or ≤10 s is used. The cutoff

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value of TBUT ≤10 s is generally used, and cutoff value of ≤5 s was suggested when small volumes of fluorescein are installed [7]. The sensitivity and specificity of the test have been reported to be 72.2% and 61.6% in Sjögren's syndrome [8]. Noninvasive TBUT can be measured without using fluorescein solution using interferometry [9] or through observation of reflection from the ocular surface.

Punctate or patchy patterns of ocular surface staining is a key feature of many ocular surface conditions, and ocular surface staining with topical dyes is routinely used in the diagnosis and management of dry eye. Rose bengal has been used to stain ocular surface epithelial cells that are not protected by mucin. However, Rose bengal has been replaced by 1% lissamine green due to its cytotoxicity. Recently, double dye using fluorescein and lissamine green is employed to evaluate ocular surface damage from dryness and inflammation [10]. Various scoring systems of ocular surface staining are used in an office setting and for clinical studies. Recently, the Sjögren's International Collaborative Clinical Alliance (SICCA) grading system for corneal fluorescein and conjunctival lissamine staining pattern (SICCA ocular staining score [OSS]) is used as the key ocular diagnostic parameter for diagnosis of Sjögren's syndrome (Table 13.1). The OSS is based on lissamine green and fluorescein staining, and an abnormal OSS of ≥5 (or van Bijsterveld score of ≥4) is considered to be positive. Grading of cornea, nasal conjunctiva, and temporal conjunctiva is performed with points from 0 to 3, and extra points can be added up to 3 according to corneal fluorescein pattern (1 point each for patches of confluent staining, staining in pupillary area, and one or more filaments) [11] (Fig. 13.1).

Ocular symptoms of dry eye in Sjögren's syndrome include dryness, itching, eye fatigue, foreign body sensation, discharge, injection, or soreness. Patients also complain visual symptoms, such as decreased vision, visual fluctuation, decrease in contrast, or blurry vision. 2016 ACR-EULAR classification criteria adopted the definition of ocular symptoms from 2002 AECG definition [12, 13]. Having ocular symptoms were defined as a positive response to at least one of the following questions:

1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than three times a day?

Various other questionnaires are available to assess patients' symptoms and provide an opportunity for screening patients with potential dry eye [14–18]. Ocular Surface Disease Index (OSDI) is the most frequently used tool that consists of 12 questions regarding ocular symptoms, triggering factors, and impact on visual function [16]. OSDI has been validated for discriminating between normal, mild to moderate, and severe dry eye. Subjective symptom and objective sign

Table 13.1 Ocular SICCA grading

Score	Cornea	Conjunctiva
0	No PEE	0–9 dots
1	1–5 PEE	10–32 dots
2	6–30 PEE	33–100 dots
3	>30 PEE	>100 dots (or confluent staining ≥4 mm^2)

An additional point is added if:
1. PEE in the central 4 mm of the cornea
2. One or more filaments
3. One or more patches of confluent staining

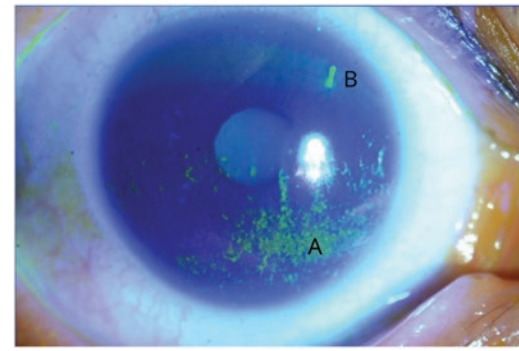

Fig. 13.1 Fluorescein staining of the cornea in a Sjögren's syndrome patient shows patches of confluent staining **(a)** and filament (**b**)

poorly correlated each other in dry eye. In addition, it has been reported that corneal sensitivity is often decreased in patients with Sjögren's syndrome [19, 20]. It is speculated that chronic irritation on the ocular surface results in neuropathic condition following earlier hyperesthesia phase. It is not uncommon that Sjögren's syndrome patient with severe ocular surface damage often lacks ocular symptom and complains visual symptom only.

One of the main indicators of dry eye is increased tear osmolarity. Hyperosmolarity results in cascade of inflammatory events involving mitogen-activated protein (MAP) kinases and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling pathway and is considered as one of the key "core mechanisms" in the pathogenesis of dry eye [21, 22]. Tear hyperosmolarity is reported to be a hallmark of dry eye disease [23] although there have been several publications disputing the efficacy of tear osmometer in the diagnosis dry eye [24–27]. High tear osmolarity in Sjögren's syndrome patients is reported to be correlated with low Schirmer score and low OSDI score [28]. However, measuring tear osmolarity is not always feasible for Sjögren's syndrome patients in the clinical setting because of difficulty in acquiring enough amount of tear from Sjögren's syndrome patients.

Dry eye often has comorbid meibomian gland dysfunction (MGD) to which tear film instability and ocular surface change may be attributed. It has been reported that destruction of meibomian glands is also associated with change in the ocular surface in patient with Sjögren's syndrome [29]. Meibomian glands are holocrine glands embedded in tarsal plates along the upper and lower rims of eyelid [30]. They produce meibum and contribute to formation of lipid layer of tear film which prevents tear film from evaporation. MGD is the major cause of evaporative dry eye, and loss or alteration of the meibomian glands results in hyposecretion of meibum, and evaporation of aqueous tear film [31]. The frequency of MGD is also higher in Sjögren's syndrome patients than in the normal population. Meibomian gland is known to be androgen target organ, and insufficiency of androgen can increase the risk of MGD [32]. Women with Sjögren's syndrome were proven to be androgen-deficient, which is thought to contribute to higher incidence of MGD in Sjögren's syndrome than in normal population [33]. Recently introduced meibography is a useful procedure for visualizing meibomian gland morphology. It can assess the severity of gland destruction and identify evaporative component of disease. A diagnostic device that provides meibography image using near-infrared illumination and measures lipid layer thickness of tear film using interferometer is available as well. Sometimes, feasibility of this test can be limited because Sjögren's syndrome patients have very little aqueous component in their tear film, which confound the interpretation of lipid layer thickness measurement.

13.2 Treatment of Dry Eye in Sjögren's Syndrome

Tear film is composed of three layers, and each layer performs its own function to maintain and stabilize tear film. The innermost mucus layer which has a direct contact with corneal epithelium contains mucin, immunoglobulins, glucose, and enzymes [34]. The mucus layer anchors aqueous layer to the cornea and provides hydrophilic environment enabling even spread of aqueous layer. The aqueous layer is between mucus and lipid layer, and tear film liquid is continuously secreted by lacrimal gland, then drained into nasolacrimal duct eliminating dirty particles. And it also lubricates the cornea during blinking and prevents dehydration of tear film as well. The outermost layer is lipid layer, and it keeps tear film stability by reducing surface tension and by providing tear film re-spreading [35]. Imbalances or dysfunction of any of three layers above can induce tear film instability, and specific type of dry eye can occur according to which layer is invaded.

Dry eye has two major categories, aqueous deficient and evaporative. However, these two

components are mixed and overlapped in a certain clinical case. Proper diagnosis and classification to determine which component is dominant in a specific patient is important to establish treatment strategy. Sjögren's syndrome is well-known as a typical example of aqueous deficient dry eye. However, evaporative component still may exist in Sjögren's syndrome dry eye patient. Main treatment of target of dry eye in Sjögren's syndrome is aqueous component of tear film. Treatment scheme includes tear replacement, tear retention, and stimulation of tear secretion. Treatment is also aimed at controlling ocular surface inflammation and improving ocular surface homeostasis.

13.2.1 Lubricant

Topical lubricants, or artificial tears, are first-line therapies of dry eye. Basic rationale is to replenish tear film and lubricate the ocular surface. Artificial tear products vary in their active ingredients, viscosity, osmolarity, and preservatives. Common active ingredients are carboxymethyl cellulose, hydroxypropyl methylcellulose, hyaluronic acid, hydroxypropyl-guar, and hydroxypropyl cellulose. There have been a few publications reporting that hyaluronic acid-based artificial tear has advantage over other polymer-based lubricants in promoting epithelial healing [36, 37]. Sjögren's syndrome patients often instill artificial tears with very frequent dose for almost lifelong period, thus preservative-free formulation is recommended to prevent potential ocular toxicity by preservatives. Artificial tear with high viscosity tends to stay longer on the ocular surface and enhances lubrication, but also causes blurry vision after instillation of eye drops. Very high viscous artificial tears or gel-type polymer may be used before bedtime. Several hypotonic artificial tears have been introduced to compensate hyperosmolarity of tear film and, thus, to subside osmolarity-induced inflammation. Although some reports suggest that hypotonic artificial tear can be beneficial to corneo-conjunctival epithelium [38, 39], impact of intermittent instillation of hypotonic artificial tear on long-term effect of tear osmolarity remains in question.

13.2.2 Secretagogues

Oral cholinergic agonists, pilocarpine and cevimeline, may have beneficial effects on symptoms and reduction in OSS, while they have failed to show improvement in tear production [40–42]. Oral secretagogues are generally considered to have greater effects on dry mouth than dry eye. Diquafosol is a P2Y2 receptor agonist that stimulates water transportation across conjunctival epithelial cells and mucin secretion from goblet cells. Commercial product as 3% diquafosol sodium ophthalmic solution is available in some country such as Japan and South Korea. It has not been approved by FDA in the United States. Several clinical trials suggest that diquafosol is useful in various dry eye condition, including aqueous deficient dry eye, MGD, Sjögren's syndrome, and dry eye following ocular surgery [43–47]. Yokoi et al. reported that topical installation of 3% diquafosol sodium significantly increased tear meniscus radius from 0.16 ± 0.07 to 0.21 ± 0.08 mm in Sjögren's syndrome patients [43]. Rebamipide is a mucin secretagogue, and 2% rebamipide ophthalmic solution has been approved for the treatment dry eye. It has been reported that fluorescein score was decreased from 0.5 ± 1.1 to 0.2 ± 0.4, and TBUT was increased from 2.4 ± 1.0 to 4.1 ± 1.0 after 2 weeks treatment with topical rebamipide in short TBUT type of dry eye. This study also showed that dry eye symptoms including foreign body sensation, dryness, photophobia, eye pain, and blurred vision improved or stayed unchanged without worsening after 4 weeks [48].

13.2.3 Topical Corticosteroid

Inflammation plays major role in the development and progression of dry eye. Infiltration of activated T cells is found in the lacrimal gland of Sjögren's syndrome, which results in the destruction of acinar and ductular tissue and hypoproduction of the tear. Hyperosmolarity of tear film induces inflammation on the ocular surface cells, which leads to expression of inflammatory cytokines and matrix metalloproteinases (MMPs).

Chronic inflammation in the ocular surface results in the death of surface epithelial cells and goblet cells.

Corticosteroid is the potent anti-inflammatory agent, and topical corticosteroid effectively improves symptoms, corneal staining, and filamentary keratitis in Sjögren's syndrome patients. While topical corticosteroids are indispensable in the management of ocular surface inflammation in Sjögren's syndrome dry eye, there is a risk of developing steroid-induced cataract, increased intraocular pressure, and ocular surface infection. Topical corticosteroids are usually employed as short-term use for moderate-to-severe disease.

13.2.4 Cyclosporine A

Cyclosporine A is an immunomodulatory agent with anti-inflammatory activities, which inhibits IL-2 expression and subsequent activation of CD4+/CD8+ T lymphocytes. Ophthalmic solution 0.05% is available as a commercial formulation and approved for the treatment of moderate-to-severe dry eye. In phase III study, topical cyclosporine A improved corneal staining, Schirmer values, and subjective symptoms in Sjögren's syndrome and non-Sjögren's syndrome dry eye patients. Cyclosporine A also decreased the number of activated conjunctival lymphocyte, increased conjunctival goblet cells, and reduced expression of pre-apoptotic marker such as CD40, CD40 ligand, and Fas in dry eye patients with or without Sjögren's syndrome [49]. Randomized controlled trial showed that subjective symptoms such as burning and pricking sensation, light sensitivity and pain as well as objective signs including Schirmer's test, TBUT, and redness were significantly improved after 1 week and 1 month of treatment with 0.05% cyclosporine A in Sjögren's syndrome patients when compared to placebo treatment [50].

13.2.5 Lifitegrast

Lifitegrast downregulates T cell-mediated inflammation by blocking the interaction of intracellular adhesion molecule 1 (ICAM-1) and integrin, lymphocyte function-associated antigen (LFA-1). Lifitegrast 5% ophthalmic solution achieved significant improvement of inferior corneal staining and eye dryness symptoms score after 12 weeks of treatment in phase 2 and phase 3 clinical trials, then received FDA approval for the treatment of signs and symptoms of dry eye [51, 52]. Animal study showed that lifitegrast inhibited desiccation stress-induced interferon gamma (IFN-r) expression and Sjögren's syndrome-like keratoconjunctivitis sicca in the dry eye mouse model [53].

13.2.6 Blood Derivatives

Serum contains biologically active molecules, such as nutrient content, vitamins, and growth factors, which are similar to those of tear fluid. Autologous serum has been used to treat severe ocular surface disease including Sjögren's syndrome since 1980s [54, 55]. Various concentration of serum from 20 to 50% have been reported, and there are many Level I studies that have shown beneficial effects on symptoms, TBUT, OSS, and epithelial healing in Sjögren's syndrome [55–60]. Platelet also contains several growth factors with regenerative capabilities. Platelet-rich plasma, plasma rich in growth factors, and plasma lysate with different preparation protocol have been reported the efficacy of platelet preparation in reducing symptoms and improving ocular surface staining [61].

13.2.7 Bandage Contact Lens

Therapeutic soft contact lens provides mechanical protection and retention of tear film. Bandage contact lens reduces pain and discomfort, and also promotes epithelial healing by preventing desiccation of ocular surface. However, use of contact lens on the already compromised ocular surface would increase the risk of microbial keratitis. Bandage contact lens is usually prescribed for short-term use. It may be worn for long-term with careful follow-up and concomi-

tant use of prophylactic topical antibiotics. Silicone hydrogel is high oxygen permeable soft contact lens material and is suitable for the patients with ocular surface disease, which is susceptible to hypoxic damage [62]. A prospective, randomized study showed that bandage contact lens has more beneficial effects on OSDI scores and corneal staining scores than autologous serum in Sjögren's syndrome-associate dry eye patients [56].

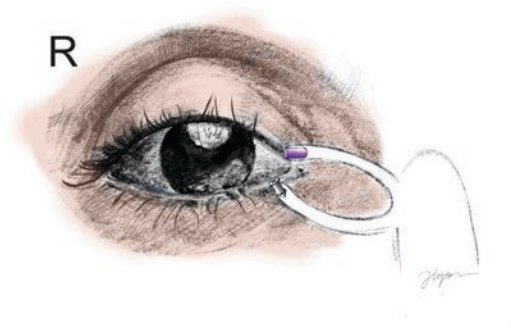

Fig. 13.2 Illustration of the right eye shows inserted temporary punctal plug (upper canaliculus, in purple) and permanent punctal plug (lower canaliculus, in gray)

13.2.8 Tear Retention

Sjögren's syndrome patients typically have very low Schirmer score, and it is not uncommon that Schirmer score is zero in these patients. Treatment strategies to accumulate as much tears as possible on the ocular surface include punctal plugs or occlusion goggles. Occlusion goggles creates moisture chamber by providing tight sealing of periocular space and protects the cornea from wind, dust, and other irritants. By doing so, symptoms of dry eye are alleviated and patients feel more comfortable during and after wearing occlusion goggles. Secreted tears on the ocular surface drain to the lacrimal sac through the upper and lower punctum in the medial eyelid. Punctal plugs are either absorbable/"temporary" or nonabsorbable/"permanent" (Fig. 13.2). Temporary punctal plugs reside in the canaliculus for 1–4 months and dissolve afterward. It is used to relieve symptoms and signs when there is aggravation from seasonal or environmental triggers, or as a "test" procedure before permanent punctal plugs or punctal occlusion. Punctal occlusion is the surgical procedure including cauterization, suturing, or canalicular ligation. It is reserved for the patients who have benefit from the punctal plugs but are unable to maintain the device.

Various inflammatory cytokines in the tear film and from the surface epithelial cells can be also accumulated on the ocular surface with tear retention procedure. Earlier or concurrent use of topical corticosteroid or other anti-inflammatory agents is needed to prevent increased inflammatory response after tear retention. Randomized controlled trials have shown that punctal plugs resulted in improvement of OSDI, corneal fluorescein staining, Schirmer score, TBUT, rose bengal score, and symptoms in Sjögren's syndrome dry eye patients [63, 64].

13.2.9 Essential Fatty Acids

Essential fatty acids are based on linoleic acid (LA) [omega-6 group], and alpha-linoleic acid (ALA) [omega-3 fatty group]. As they can be not synthesized in the body, it should be provided from dietary sources. The long chain omega-3, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can be synthesized from ALA, but with low conversion efficiency. Therefore, dietary intake oral supplementation of the long chain omega-3 is recommended for its anti-inflammatory effects in dry eye patients.

Several randomized controlled trials have shown that oral n-3 fatty acids, in the form of EPA and DHA, result in improvement in symptoms, Schirmer score, or TBUT [65–67]. However, recent a large multicenter, double-blind clinical study failed to show the benefit of omega-3 supplementation for the treatment of dry eye [68]. There is little evidence that essential fatty acids supplement is beneficial for the ocular complications of Sjögren's syndrome. One double-masked randomized controlled trial compared the oral daily dose of LA 112 mg and

gamma-linolenic acid (GLA) 15 mg and placebo for 1 month in Sjögren's syndrome and showed improvement of symptoms and corneal staining scores [69].

13.3 Summary

Impact of proper tear secretion on visual function in daily life is profound. Comprehensive ocular evaluation should be performed to assess the severity of dry eye condition and its etiologic factors in patients. Sjögren's syndrome patients frequently presented with dry eye symptoms in the eye clinic. Careful history taking of the patients would identify the possibility of autoimmune Sjögren's syndrome in those patients. Treatment strategy usually follows a stepwise approach as ocular lubricant alone is not usually sufficient to relieve symptoms and manage inflammatory process in Sjögren's syndrome patients. There are new attempts to relieve dry eye symptoms of Sjögren's syndrome by using biological agents such as rituximab and abatacept as well as interferon-based therapeutic strategies, but long-term clinical studies are necessary to confirm their efficacy in Sjögren's syndrome [70]. Timely application of anti-inflammatory treatment is critical in the long-term management of Sjögren's syndrome-related dry eye.

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References

1. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, et al. TFOS DEWS II definition and classification report. Ocul Surf. 2017;15(3):276–83.
2. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf. 2007;5(2):75–92.
3. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. Cornea. 2004;23(8):762–70.
4. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf. 2007;5(2):108–52.
5. de Monchy I, Gendron G, Miceli C, Pogorzalek N, Mariette X, Labetoulle M. Combination of the Schirmer I and phenol red thread tests as a rescue strategy for diagnosis of ocular dryness associated with Sjogren's syndrome. Invest Ophthalmol Vis Sci. 2011;52(8):5167–73.
6. Ibrahim OM, Dogru M, Ward SK, Matsumoto Y, Wakamatsu TH, Ishida K, et al. The efficacy, sensitivity, and specificity of strip meniscometry in conjunction with tear function tests in the assessment of tear meniscus. Invest Ophthalmol Vis Sci. 2011;52(5):2194–8.
7. Lemp MA, Hamill JR Jr. Factors affecting tear film breakup in normal eyes. Arch Ophthalmol. 1973;89(2):103–5.
8. Vitali C, Moutsopoulos HM, Bombardieri S. The European Community Study Group on diagnostic criteria for Sjogren's syndrome. Sensitivity and specificity of tests for ocular and oral involvement in Sjogren's syndrome. Ann Rheum Dis. 1994;53(10):637–47.
9. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. Optom Vis Sci. 1997;74(1):8–13.
10. Yoon KC, Im SK, Kim HG, You IC. Usefulness of double vital staining with 1% fluorescein and 1% lissamine green in patients with dry eye syndrome. Cornea. 2011;30(9):972–6.
11. Whitcher JP, Shiboski CH, Shiboski SC, Heidenreich AM, Kitagawa K, Zhang S, et al. A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjogren's Syndrome International Registry. Am J Ophthalmol. 2010;149(3):405–15.
12. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League against rheumatism classification criteria for primary Sjogren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. Arthritis Rheumatol. 2017;69(1):35–45.
13. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61(6):554–8.
14. Begley CG, Caffery B, Chalmers RL, Mitchell GL, Dry Eye Investigation Study Group. Use of the dry eye questionnaire to measure symptoms of ocular irritation in patients with aqueous tear deficient dry eye. Cornea. 2002;21(7):664–70.
15. Abetz L, Rajagopalan K, Mertzanis P, Begley C, Barnes R, Chalmers R, et al. Development and validation of the impact of dry eye on everyday life (IDEEL) questionnaire, a patient-reported outcomes (PRO) measure for the assessment of the burden of dry eye on patients. Health Qual Life Outcomes. 2011;9:111.
16. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol. 2000;118(5):615–21.

17. Schaumberg DA, Gulati A, Mathers WD, Clinch T, Lemp MA, Nelson JD, et al. Development and validation of a short global dry eye symptom index. Ocul Surf. 2007;5(1):50–7.
18. Blackie CA, Solomon JD, Scaffidi RC, Greiner JV, Lemp MA, Korb DR. The relationship between dry eye symptoms and lipid layer thickness. Cornea. 2009;28(7):789–94.
19. Han SB, Hyon JY, Wee WR, Lee JH, Lee YJ, Yun PY. Reduced corneal sensitivity in patients with primary Sjogren's syndrome. Acta Ophthalmol. 2010;88(7):e277–8.
20. Bourcier T, Acosta MC, Borderie V, Borras F, Gallar J, Bury T, et al. Decreased corneal sensitivity in patients with dry eye. Invest Ophthalmol Vis Sci. 2005;46(7):2341–5.
21. Li DQ, Chen Z, Song XJ, Luo L, Pflugfelder SC. Stimulation of matrix metalloproteinases by hyperosmolarity via a JNK pathway in human corneal epithelial cells. Invest Ophthalmol Vis Sci. 2004;45(12):4302–11.
22. Luo L, Li DQ, Corrales RM, Pflugfelder SC. Hyperosmolar saline is a proinflammatory stress on the mouse ocular surface. Eye Contact Lens. 2005;31(5):186–93.
23. Lemp MA, Bron AJ, Baudouin C, Benitez Del Castillo JM, Geffen D, Tauber J, et al. Tear osmolarity in the diagnosis and management of dry eye disease. Am J Ophthalmol. 2011;151(5):792–8.e1.
24. Tashbayev B, Utheim TP, Utheim OA, Raeder S, Jensen JL, Yazdani M, et al. Utility of tear Osmolarity measurement in diagnosis of dry eye disease. Sci Rep. 2020;10(1):5542.
25. Bunya VY, Fuerst NM, Pistilli M, McCabe BE, Salvo R, Macchi I, et al. Variability of tear osmolarity in patients with dry eye. JAMA Ophthalmol. 2015;133(6):662–7.
26. Amparo F, Jin Y, Hamrah P, Schaumberg DA, Dana R. What is the value of incorporating tear osmolarity measurement in assessing patient response to therapy in dry eye disease? Am J Ophthalmol. 2014;157(1):69–77.e2.
27. Szalai E, Berta A, Szekanecz Z, Szucs G, Modis L Jr. Evaluation of tear osmolarity in non-Sjogren and Sjogren syndrome dry eye patients with the TearLab system. Cornea. 2012;31(8):867–71.
28. Bunya VY, Langelier N, Chen S, Pistilli M, Vivino FB, Massaro-Giordano G. Tear osmolarity in Sjogren syndrome. Cornea. 2013;32(7):922–7.
29. Menzies KL, Srinivasan S, Prokopich CL, Jones L. Infrared imaging of meibomian glands and evaluation of the lipid layer in Sjogren's syndrome patients and nondry eye controls. Invest Ophthalmol Vis Sci. 2015;56(2):836–41.
30. Jester JV, Nicolaides N, Smith RE. Meibomian gland studies: histologic and ultrastructural investigations. Invest Ophthalmol Vis Sci. 1981;20(4):537–47.
31. Mishima S, Maurice DM. The oily layer of the tear film and evaporation from the corneal surface. Exp Eye Res. 1961;1:39–45.
32. Sullivan DA, Sullivan BD, Evans JE, Schirra F, Yamagami H, Liu M, et al. Androgen deficiency, meibomian gland dysfunction, and evaporative dry eye. Ann N Y Acad Sci. 2002;966:211–22.
33. Chen X, Utheim OA, Xiao J, Adil MY, Stojanovic A, Tashbayev B, et al. Meibomian gland features in a Norwegian cohort of patients with primary Sjogren s syndrome. PLoS One. 2017;12(9):e0184284.
34. Nichols BA, Chiappino ML, Dawson CR. Demonstration of the mucous layer of the tear film by electron microscopy. Invest Ophthalmol Vis Sci. 1985;26(4):464–73.
35. Driver PJ, Lemp MA. Meibomian gland dysfunction. Surv Ophthalmol. 1996;40(5):343–67.
36. Gomes JA, Amankwah R, Powell-Richards A, Dua HS. Sodium hyaluronate (hyaluronic acid) promotes migration of human corneal epithelial cells in vitro. Br J Ophthalmol. 2004;88(6):821–5.
37. Ho WT, Chiang TH, Chang SW, Chen YH, Hu FR, Wang IJ. Enhanced corneal wound healing with hyaluronic acid and high-potassium artificial tears. Clin Exp Optom. 2013;96(6):536–41.
38. Baeyens V, Bron A, Baudouin C, Vismed/Hylovis Study Group. Efficacy of 0.18% hypotonic sodium hyaluronate ophthalmic solution in the treatment of signs and symptoms of dry eye disease. J Fr Ophtalmol. 2012;35(6):412–9.
39. Troiano P, Monaco G. Effect of hypotonic 0.4% hyaluronic acid drops in dry eye patients: a cross-over study. Cornea. 2008;27(10):1126–30.
40. Papas AS, Sherrer YS, Charney M, Golden HE, Medsger TA Jr, Walsh BT, et al. Successful treatment of dry mouth and dry eye symptoms in Sjogren's syndrome patients with oral pilocarpine: a randomized, placebo-controlled, dose-adjustment study. J Clin Rheumatol. 2004;10(4):169–77.
41. Tsifetaki N, Kitsos G, Paschides CA, Alamanos Y, Eftaxias V, Voulgari PV, et al. Oral pilocarpine for the treatment of ocular symptoms in patients with Sjogren's syndrome: a randomised 12 week controlled study. Ann Rheum Dis. 2003;62(12):1204–7.
42. Vivino FB, Al-Hashimi I, Khan Z, LeVeque FG, Salisbury PL 3rd, Tran-Johnson TK, et al. Pilocarpine tablets for the treatment of dry mouth and dry eye symptoms in patients with Sjogren syndrome: a randomized, placebo-controlled, fixed-dose, multicenter trial. P92-01 Study Group. Arch Intern Med. 1999;159(2):174–81.
43. Yokoi N, Kato H, Kinoshita S. The increase of aqueous tear volume by diquafosol sodium in dry-eye patients with Sjogren's syndrome: a pilot study. Eye (Lond). 2016;30(6):857–64.
44. Bremond-Gignac D, Gicquel JJ, Chiambaretta F. Pharmacokinetic evaluation of diquafosol tetrasodium for the treatment of Sjogren's syndrome. Expert Opin Drug Metab Toxicol. 2014;10(6):905–13.
45. Koh S, Ikeda C, Takai Y, Watanabe H, Maeda N, Nishida K. Long-term results of treatment with diquafosol ophthalmic solution for aqueous-deficient dry eye. Jpn J Ophthalmol. 2013;57(5):440–6.

46. Mori Y, Nejima R, Masuda A, Maruyama Y, Minami K, Miyata K, et al. Effect of diquafosol tetrasodium eye drop for persistent dry eye after laser in situ keratomileusis. Cornea. 2014;33(7):659–62.
47. Park DH, Chung JK, Seo DR, Lee SJ. Clinical effects and safety of 3% diquafosol ophthalmic solution for patients with dry eye after cataract surgery: a randomized controlled trial. Am J Ophthalmol. 2016;163:122–31.e2.
48. Koh S, Inoue Y, Sugmimoto T, Maeda N, Nishida K. Effect of rebamipide ophthalmic suspension on optical quality in the short break-up time type of dry eye. Cornea. 2013;32(9):1219–23.
49. Brignole F, Pisella PJ, De Saint JM, Goldschild M, Goguel A, Baudouin C. Flow cytometric analysis of inflammatory markers in KCS: 6-month treatment with topical cyclosporin A. Invest Ophthalmol Vis Sci. 2001;42(1):90–5.
50. Deveci H, Kobak S. The efficacy of topical 0.05% cyclosporine A in patients with dry eye disease associated with Sjogren's syndrome. Int Ophthalmol. 2014;34(5):1043–8.
51. Tauber J, Karpecki P, Latkany R, Luchs J, Martel J, Sall K, et al. Lifitegrast ophthalmic solution 5.0% versus placebo for treatment of dry eye disease: results of the randomized phase III OPUS-2 study. Ophthalmology. 2015;122(12):2423–31.
52. Sheppard JD, Torkildsen GL, Lonsdale JD, D'Ambrosio FA Jr, McLaurin EB, Eiferman RA, et al. Lifitegrast ophthalmic solution 5.0% for treatment of dry eye disease: results of the OPUS-1 phase 3 study. Ophthalmology. 2014;121(2):475–83.
53. Guimaraes de Souza R, Yu Z, Stern ME, Pflugfelder SC, de Paiva CS. Suppression of Th1-mediated Keratoconjunctivitis Sicca by Lifitegrast. J Ocul Pharmacol Ther. 2018;34(7):543–9.
54. Fox RI, Chan R, Michelson JB, Belmont JB, Michelson PE. Beneficial effect of artificial tears made with autologous serum in patients with keratoconjunctivitis sicca. Arthritis Rheum. 1984;27(4):459–61.
55. Tsubota K, Goto E, Fujita H, Ono M, Inoue H, Saito I, et al. Treatment of dry eye by autologous serum application in Sjogren's syndrome. Br J Ophthalmol. 1999;83(4):390–5.
56. Li J, Zhang X, Zheng Q, Zhu Y, Wang H, Ma H, et al. Comparative evaluation of silicone hydrogel contact lenses and autologous serum for management of Sjogren syndrome-associated dry eye. Cornea. 2015;34(9):1072–8.
57. Lopez-Garcia JS, Garcia-Lozano I, Rivas L, Ramirez N, Raposo R, Mendez MT. Autologous serum eye drops diluted with sodium hyaluronate: clinical and experimental comparative study. Acta Ophthalmol. 2014;92(1):e22–9.
58. Noble BA, Loh RS, MacLennan S, Pesudovs K, Reynolds A, Bridges LR, et al. Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular surface disease. Br J Ophthalmol. 2004;88(5):647–52.
59. Hwang J, Chung SH, Jeon S, Kwok SK, Park SH, Kim MS. Comparison of clinical efficacies of autologous serum eye drops in patients with primary and secondary Sjogren syndrome. Cornea. 2014;33(7):663–7.
60. Yoon KC, Heo H, Im SK, You IC, Kim YH, Park YG. Comparison of autologous serum and umbilical cord serum eye drops for dry eye syndrome. Am J Ophthalmol. 2007;144(1):86–92.
61. Avila MY, Igua AM, Mora AM. Randomised, prospective clinical trial of platelet-rich plasma injection in the management of severe dry eye. Br J Ophthalmol. 2018. Advance online publication. http://dx.doi.org/10.1136/bjophthalmol-2018-312072.
62. Yucekul B, Mocan MC, Kocabeyoglu S, Tan C, Irkec M. Evaluation of long-term silicone hydrogel use on ocular surface inflammation and tear function in patients with and without meibomian gland dysfunction. Eye Contact Lens. 2019;45(1):61–6.
63. Mansour K, Leonhardt CJ, Kalk WW, Bootsma H, Bruin KJ, Blanksma LJ, et al. Lacrimal punctum occlusion in the treatment of severe keratoconjunctivitis Sicca caused by Sjogren syndrome: a uniocular evaluation. Cornea. 2007;26(2):147–50.
64. Qiu W, Liu Z, Ao M, Li X, Wang W. Punctal plugs versus artificial tears for treating primary Sjogren's syndrome with keratoconjunctivitis SICCA: a comparative observation of their effects on visual function. Rheumatol Int. 2013;33(10):2543–8.
65. Bhargava R, Kumar P, Arora Y. Short-term omega 3 fatty acids treatment for dry eye in young and middle-aged visual display terminal users. Eye Contact Lens. 2016;42(4):231–6.
66. Deinema LA, Vingrys AJ, Wong CY, Jackson DC, Chinnery HR, Downie LE. A randomized, double-masked, placebo-controlled clinical trial of two forms of omega-3 supplements for treating dry eye disease. Ophthalmology. 2017;124(1):43–52.
67. Epitropoulos AT, Donnenfeld ED, Shah ZA, Holland EJ, Gross M, Faulkner WJ, et al. Effect of oral re-esterified omega-3 nutritional supplementation on dry eyes. Cornea. 2016;35(9):1185–91.
68. Dry Eye Assesment and Management Study Research Group, Asbell PA, Maguire MG, Pistilli M, Ying GS, et al. n-3 Fatty acid supplementation for the treatment of dry eye disease. N Engl J Med. 2018;378(18):1681–90.
69. Aragona P, Bucolo C, Spinella R, Giuffrida S, Ferreri G. Systemic omega-6 essential fatty acid treatment and pge1 tear content in Sjogren's syndrome patients. Invest Ophthalmol Vis Sci. 2005;46(12):4474–9.
70. Kuklinski E, Asbell PA. Sjogren's syndrome from the perspective of ophthalmology. Clin Immunol. 2017;182:55–61.