Translational Research in Sjögren's Syndrome

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Abstract

Sjögren's syndrome (SS) is an autoimmune inflammatory disorder of the exocrine glands, particularly affecting lacrimal and salivary glands. Hallmark symptoms are dry mouth and dry eye, often in conjunction with general symptoms, such as malaise and fatigue. Lymphomas could develop in 5–10% of the patients. As SS is a rather complex syndrome with many features, the one patient being diagnosed with SS may suffer from a different complex of complaints than another SS patient and may thus be in need of a different treatment approach. To better classify SS patients and to personalize their treatment, many clinicians and researchers are currently working on efforts (1) to refine classification of SS patients, (2) to ease the diagnostic work-up of SS, and (3) to better understand the etiopathogenesis of SS. Latter knowledge is essential to understand the course of the disease. This way clinicians will be able to identify patients who are at risk of developing SS or lymphomas; can intervene at an early stage of the disease to prevent damage to, e.g., the glands; as well as can personalize treatment with, e.g., biologicals. In this chapter, current major achievements are discussed, and promising new directions are indicated.

10.1 Introduction

Sjögren's syndrome (SS) is an autoimmune inflammatory disorder of the exocrine glands, particularly affecting lacrimal and salivary glands. Hallmark symptoms are dry mouth and dry eyes, often in conjunction with general symptoms, such as malaise and fatigue. Lymphomas could develop in 5–10% of the SS patients.

Recent studies indicate that the prevalence of SS in the general population is about 7 per 100,000 person-years [1], which shares SS among the most common systemic autoimmune diseases. SS is nine times more frequent in women than in men. SS is mostly recognized after the age of 40 but can already present in childhood. SS can be a primary condition (dry eyes, dry mouth, recurrent swellings of salivary glands: primary SS (pSS)) or co-occur with another autoimmune disease, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and scleroderma (pSS combined with

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another autoimmune disease: secondary SS (sSS)).

With regard to SS, there are currently a lot of efforts with a translational character, e.g., (1) to better classify SS patients so that results from studies can be better compared with each other and the outcome can be better generalized, (2) to ease the diagnostic work-up of SS by either applying biomarker assays that are specific for SS or by applying (more) simple diagnostic clinical tools, and (3) to understand the etiopathogenesis of SS. Latter knowledge is essential to understand the course of the disease. This way clinicians will be able to identify patients who are at risk of developing SS or lymphomas; can intervene at an early stage of the disease to prevent damage to, e.g., the glands; as well as can personalize treatment with, e.g., biologicals. For example, Lendrem et al. [2] identified four distinct pSS clinical phenotypes. These phenotypes were defined on the basis of hierarchical cluster analysis of patient-reported pain, fatigue, dryness, anxiety, and depression. Importantly, these four phenotypes exhibited marked differences in a variety of biologic parameters. Presumably, SS patients within these clusters will differently response to a particular treatment, both in terms of subjective and objective parameters. In this chapter, current major achievements are discussed, and promising new directions are indicated.

10.2 Characterizing SS Patients

Over the years, many classification criteria for SS have been developed of which the 2002 revised American-European Consensus Group (AECG) classification criteria are currently the most frequently used. The AECG criteria combine subjective symptoms of dry eyes and dry mouth with the objective signs of keratoconjunctivitis sicca and xerostomia [3]. In 2012, endorsed by the American College of Rheumatology (ACR), a new preliminary classification criteria set was introduced that purely focused on objective measures [4]. As the ACR criteria were not well received by the international SS community, a joint working group consisting of members from ACR and European League Against Rheumatism (EULAR) developed a new classification criteria set (Table 10.1; [5]). This criteria set was endorsed by both ACR and EULAR and was well received by the international SS community. The ACR-EULAR classification criteria for pSS are a step in the right direction. Further refinement is needed, however, to increase their utility [6].

To increase the utility and to make the criteria even more commonly applicable for clinicians, lots of effort are currently applied to test whether salivary gland ultrasonography (sGUS) is more specific and more sensitive than measuring salivary secretion or other objective tests applied or could replace more invasive tests such as salivary gland biopsies. This to show that sGUS is a good diagnostic tool to add to the ACR-AECG classification criteria and even might have the potential to replace (some of the) current objective tests in future [7, 8]. The focus is on assessing the diagnostic potential of sGUS as sGUS is a well-tolerated, noninvasive, inexpensive, non-irradiating technique that is widely available in rheumatologic outpatient clinics. Recently, it was shown that a combination of positive sGUS and presence of anti-SSA/Ro antibodies highly predicts classification according to the ACR-EULAR classification criteria [8]. When applying the ACR-EULAR criteria for monitoring disease activity and treatment evaluation, it is recommended that the same ultrasonographer follows the same patient as a function of time as the interobserver variation of ultrasonographers is rather large (Fig. 10.1; Delli et al. [10]). Furthermore, it has to be assessed whether sGUS images of SS patients are grossly dissimilar from those of patients with diseases involving the salivary glands that mimic SS, such as sarcoidosis, hepatitis C, and HIV.

Table 10.1 A comparison of the AECG [3], ACR [4], and ACR/EULAR [5] classification criteria sets

Criterion	AECG[a]	ACR[a]	ACR/EULAR[a]
Subjective			
Sicca eye	+	–	Entry[b]
Sicca mouth	+	–	Entry[b]
Objective			
Ocular signs:			
Schirmer's test	**+**	–	**+**
van Bijsterveld score (VBS)[c]	**+**	–	**+**
Ocular staining score (OSS)	–	+ (≥3)	+ (≥5)
Histopathology:			
Labial gland focus score ≥1	**+**	**+**	**+**
Salivary gland involvement:			
Unstimulated whole saliva (≤0.1 ml/min)	**+**	–	**+**
Parotid sialography	+	–	–
Salivary gland scintigraphy	+	–	–
Autoantibodies/serology:			
Anti-SSA positive	**+**	**+**	**+**
Anti-SSB positive	+	+	–
ANA	–	+	–
Rheumatoid factor	–	+	–

Criteria that are present in the ACR/EULAR and in original criteria sets are depicted in bold

[a]For patients without any potentially associated disease, pSS may be defined as follows: (1) The presence of any four of the six items is indicative of pSS, as long as either histopathology or serology is positive. (2) The presence of any three of the four objective criteria items. For patients with a potentially associated disease (for instance, another well-defined connective tissue disease), the presence of a subjective item plus any two from the four objective items may be considered as indicative of sSS. Exclusion criteria AECG: history of head and neck radiation treatment, active hepatitis C infection, acquired immunodeficiency disease, pre-existing lymphoma, sarcoidosis, graft versus host disease, and use of anticholinergic drugs (since a time shorter than fourfold the half-life of the drug). Exclusion criteria ACR: similar to AECG with exception of pre-existing lymphoma and addition of amyloidosis and IgG4-related disease. Exclusion criteria ACR/EULAR: similar to AECG with exception of pre-existing lymphoma and addition of IgG4-related disease

[b]The ACR/EULAR criteria are applicable to any patients with at least one symptom of ocular or oral dryness, defined as a positive response on the validated sicca questions as defined for the AECG criteria

[c]VBS ≥4 equals OSS ≥5

10.3 Biomarkers and SS

Although a variety of diagnostic and therapeutic biomarkers has been proposed to classify pSS and its subsets over the years [11, 12], there is still a crucial need for novel specific biomarkers to ease diagnostics, to diagnose SS at an early stage, and to predict which patient might be helped with a tailored, targeted treatment or is at risk of developing specific comorbidity [13, 14].

10.3.1 Serology

A great variety of biomarkers is known to be present in serum of SS patients. The most frequently detected and most widely used are antibodies directed against nuclear components (ANA), antibodies directed against intracellular antigens (Ro52/SSA, Ro60/SSA, La/SSB), and rheumatoid factor (RF). These autoantibodies can already be detected many years before SS has become clinically apparent, which imply their

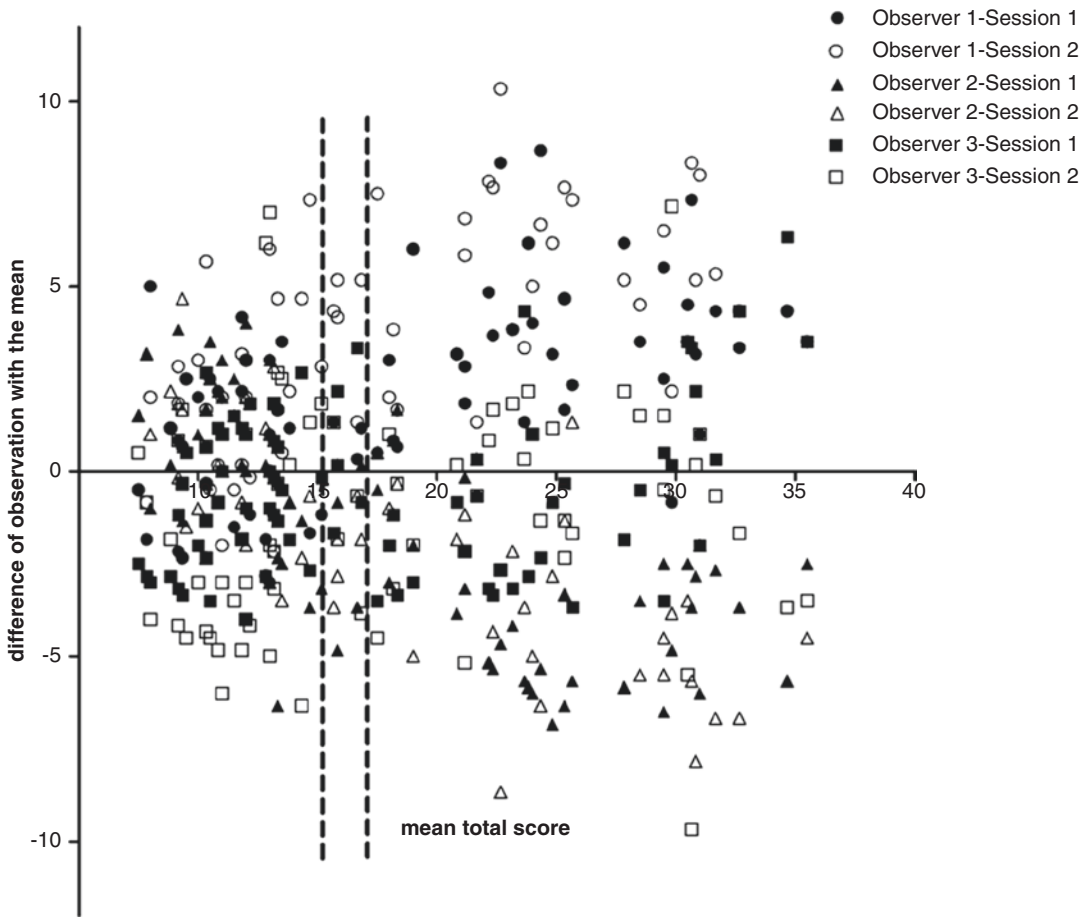

Fig. 10.1 Systematic differences in sGUS total score in pSS patients using the Hocevar scoring system [9]. For each patient, the mean of the six observations (three observers, two sessions) and the difference of these six observations with the mean were calculated and plotted against each other. The intermittent vertical lines indicate the different cutoff points applied. While the interobserver variability is rather low, the intra-observer variability is considerable. Therefore, it is recommended that the same ultrasonographer follows a patient when performing follow-up or treatment evaluation studies [10]

potential use to predict onset of pSS (Fig. 10.2; [15, 16]). It has to be mentioned, however, that it is not yet set which subject that is positive for anti-Ro/SSA and/or anti-LA/SSB will indeed develop SS in future.

Anti-Ro/SSA is found in 70–90% of SS patients and may predict the course of the disease. E.g., positivity for anti-Ro/SSA is linked with a younger age at diagnosis, a longer disease duration, a higher incidence of recurrent parotid gland swelling, a higher focus score, and a higher prevalence of extraglandular manifestations [17, 18]. Sole positivity for anti-La/SSB is rare.

Of the other antibodies present in SS patients, RF and cryoglobulins are most common, respectively, in 35–70% and 5–10% of the patients [19, 20]. Both antibodies have been linked to the development of lymphomas [21, 22]. When these antibodies are present in SS patients with parotid gland enlargement, palpable purpura, and low C4 levels, these patients are at a rather high risk of developing a lymphoma or have already developed a lymphoma. Therefore, there is currently much research ongoing to detect which biomarkers are the best markers to predict which SS patient has a high lymphoma risk. This in addition to the presumed predictive

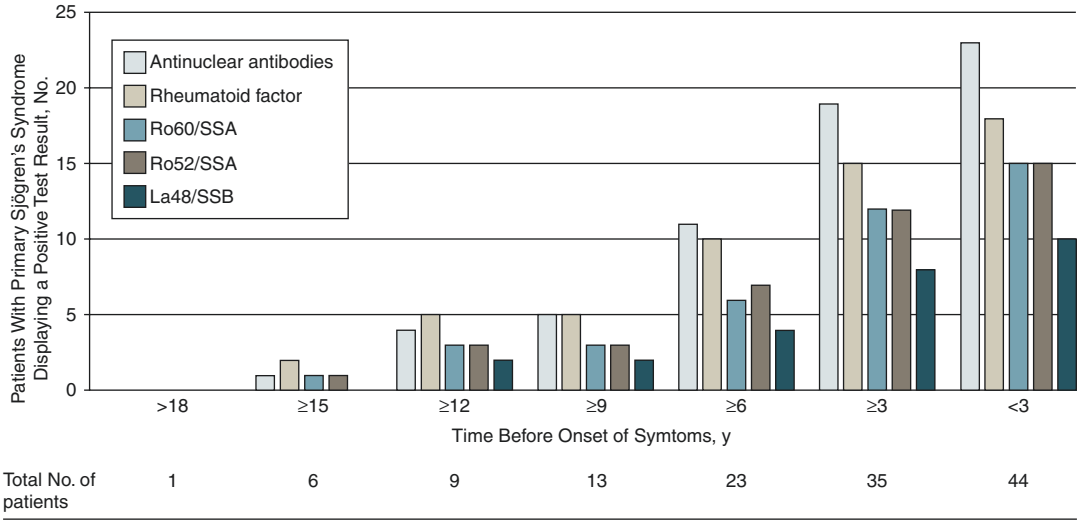

Fig. 10.2 Cumulative number of patients with pSS with autoantibodies before clinical onset (y: years). Anti-Ro/SSA and anti-La/SSB can be present many years before pSS is clinically diagnosed [15]

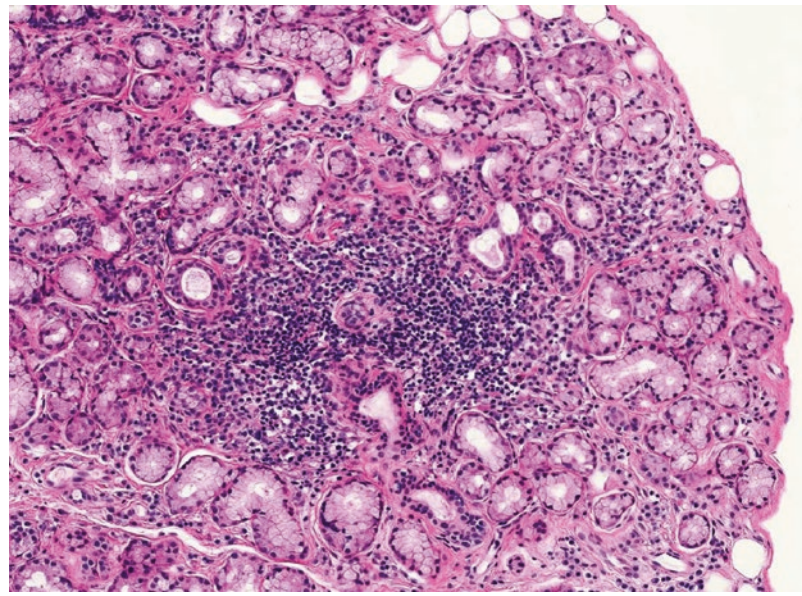

Fig. 10.3 Biopsy of a labial salivary gland (H&E stain) showing focal lymphocytic sialadenitis. Centrally in the image a focus is present, i.e., an accumulation of more than 50 lymphocytes around a duct

value of salivary gland biopsies ([23]; see section on histopathology), which is questioned by other authors [24]. It also has to be set which SS patient with a lymphoma needs treatment or just has to be closely monitored [25].

10.3.2 Histopathology

An issue that is currently heavily discussed is whether a biopsy of the labial salivary gland has to be taken to histopathologically confirm the SS diagnosis. Patients can be classified as suffering from SS syndrome when this biopsy shows focal lymphocytic sialadenitis with a focus score ≥1, i.e., the presence of at least one accumulation of 50 or more lymphocytes per 4 mm^2 (Fig. 10.3; [26]). Parotid biopsies can serve as a proper alternative to labial biopsies in the SS diagnostic work-up [27]. A major advantage of parotid biopsies is that in parotid biopsies lymphomas, mostly mucosa-associated lymphoid tissue

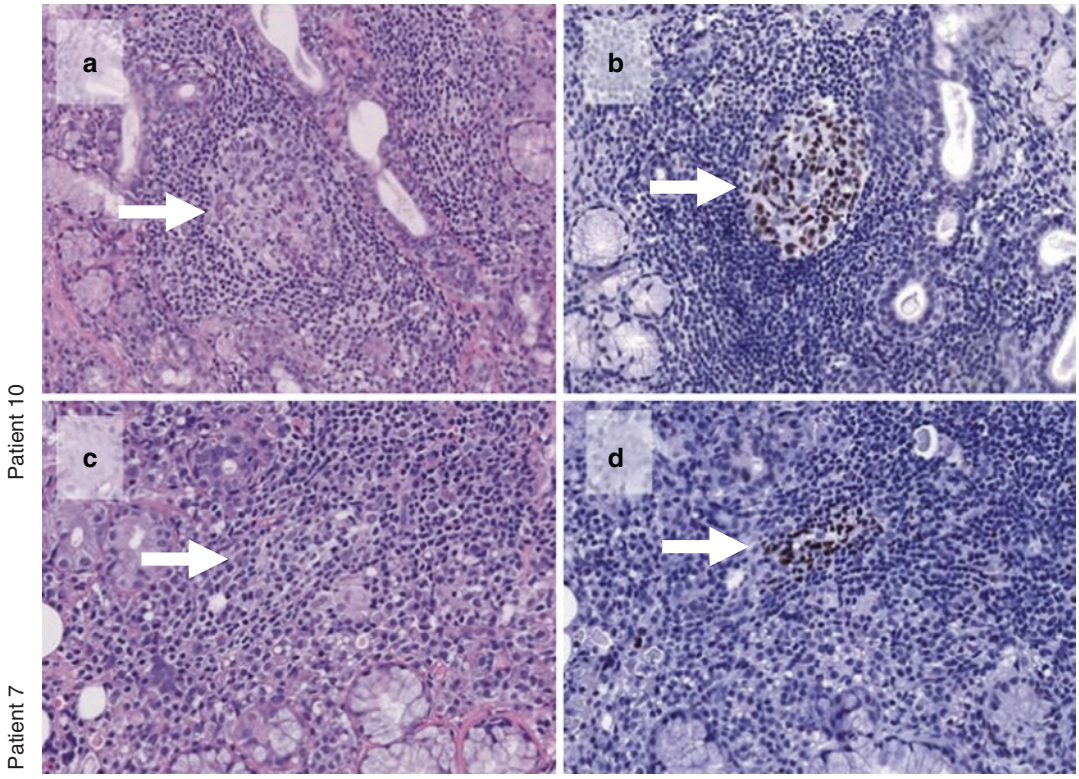

Fig. 10.4 GCs present in diagnostic labial salivary gland biopsies of pSS patients who developed a parotid MALT lymphoma later on. Arrows point to GCs. (**a**) Clearly visible GC in a periductal focus of a labial gland (H&E stain). (**b**) Bcl6 staining of the same GC as in (**a**) in a serial section. (**c**) Suspicious GC in a periductal focus of the labial gland (H&E stain). (**d**) Bcl6 staining in a serial section of (**c**) shows a small GC [24]

(MALT) lymphomas, are easier to detect as parotid glands are more commonly affected (70–80% of the lymphomas in pSS patients are MALT lymphomas in the parotid glands; Fig. 10.4). Moreover, the same parotid gland can be biopsied more often which is an important asset for treatment evaluation studies (Fig. 10.5; [28]). Theander et al. [23] and Reksten et al. [29] posed that the presences of germinal center (GC)-like structures by light microscopy in SS diagnostic salivary biopsies are also highly predictive for non-Hodgkin lymphoma development, but Haacke et al. [24] recently showed in a more extensive study that the GCs in labial gland biopsies does not differ between patients with pSS that develop parotid MALT lymphoma and patients with pSS who do not develop lymphoma (Fig. 10.6). Thus, the presence of GCs in labial gland biopsies is probably not a predictive factor for SS syndrome-associated parotid MALT lymphomas. From which cells these lymphoma's originate needs further study (see also section on lymphomas).

Recently, Delli et al. [28, 31, 32] showed that the histopathologic characteristics of parotid gland biopsies may predict which pSS patients are probably responsive to treatment with anti-CD20 therapy (rituximab) and which patient is not (Fig. 10.7). This observation brings targeted treatment within reach. In fact, the possibility of

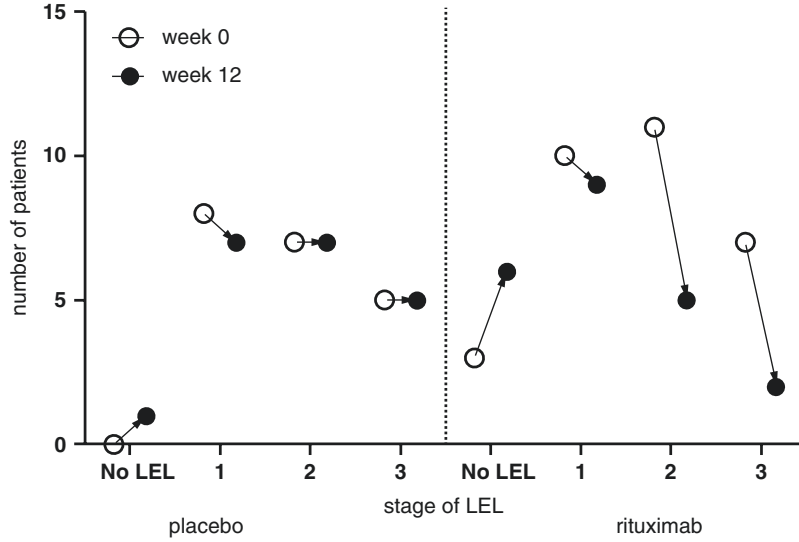

Fig. 10.5 Parotid glands allow for repeated biopsies from the same glands in pSS patients which ease comparison, e.g., for treatment evaluation studies. This figure shows the presence of three stages of LELs/mm² in placebo and anti-CD20 therapy (rituximab) treated patients at weeks 0 and 12: *Y*-axis indicates the total number of patients, while *X*-axis indicates the presence of different stages of LEL (a higher number reflects a more severe stage). Twelve weeks after anti-CD20 therapy, the number and severity of the LELs have decreased [28]

considering the composition of the inflammatory infiltrate as a tool in clinical trials to recruit homogeneous subsets of patients and to get information on the efficacy and mechanisms of action of novel drugs is challenging and very promising but still in childhood and in need of thorough studying and standardization [33]. For example, Tavoni et al. [34] showed a discrepancy among different readers in the interpretation of minor salivary gland biopsies in clinical practice. Currently, efforts are taken by a working group of EULAR to standardize histopathologic evaluation of salivary gland biopsies, including how to detect GCs [35].

10.3.3 Progress in Biomarker Research

Among the many biomarkers that are currently studied, a promising novel biomarker is the interferon (IFN) type I signature [36]. Dysregulated genes of IFN pathways, both in salivary gland tissue and peripheral blood, are considered to be an asset in diagnosing pSS and monitoring its disease activity [37–39]. E.g., presence of myxovirus resistance protein A (MxA) in cell may reflect presence of IFN type I and is correlated with EULAR SS disease activity (ESSDAI) score and levels of immunoglobulins and autoantibodies [40]. Also type II IFN seems to be involved in the pathogenesis of pSS as the focus score is higher in type II IFN pSS patients [41]. Furthermore, a higher IFNg/IFNa mRNA ratio in minor salivary gland tissue seems to be a predictor for lymphoma development [42]. However, the IFN type I signature is not specific for SS. It also could be just a biomarker for, e.g., disease activity. So to prove whether the IFN type I signature indeed is of additional value, the identification of different patient categories awaits long-term analysis of a larger cohort of patients [43].

Another key pathogenetic cytokine is B-cell-activating factor (BAFF). BAFF is present in

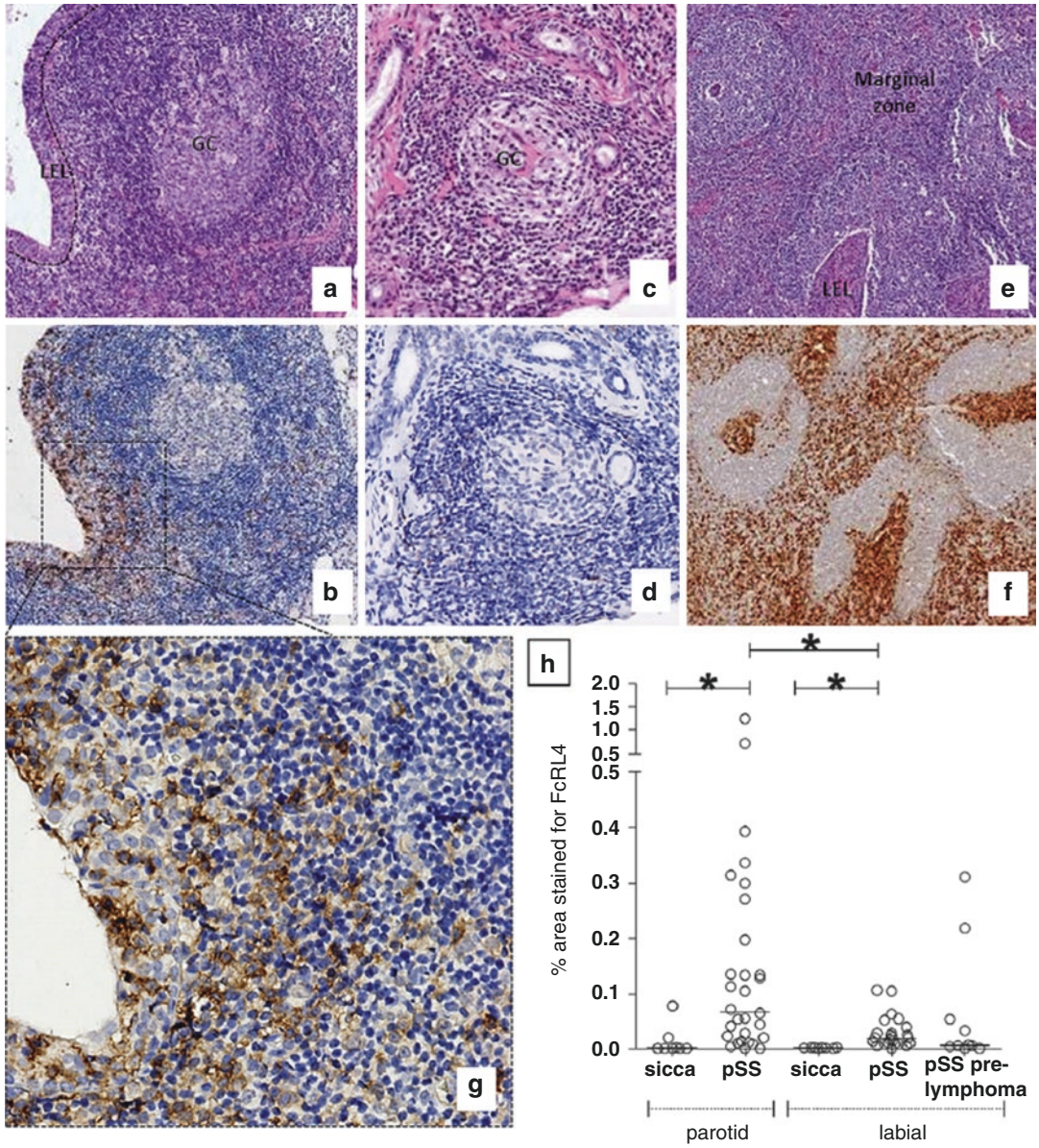

Fig. 10.6 FcRL4 B cells in parotid and labial salivary glands as well as in parotid MALT lymphomas. (**a**) Parotid gland biopsy of a pSS patient with a LEL and GC (H&E stain). (**b**) FcRL4 B cells in the same parotid gland. FcRL4 B cells are in close association with the ductal epithelium. Less FcRL4 B cells are found in the infiltrate with lower intensity of the FcRL4 stain. (**c**) Labial gland biopsy of a pSS patient (H&E stain). (**d**) FcRL4 B cells in the same labial gland. (**e**) MALT lymphoma in the parotid gland of a pSS patient (H&E stain). (**f**) FcRL4 stain same MALT lymphoma. The FcRL4 B cells cluster in and around LELs and in the marginal zone. Few FcRL4 B cells with low intensity are found despite the presence of a periductal infiltrate. (**g**) High magnification of FcRL4 B cells in the parotid gland. (**h**) Quantification of FcRL4 B cells, by measuring relative surface of FcRL4 staining. Amount of FcRL4 staining is higher in pSS patients compared to non-pSS sicca patients. In the parotid glands of pSS patients, significantly more FcRL4 positivity is present compared to labial glands of pSS patients. In diagnostic labial gland biopsies from pSS patients who developed a parotid MALT lymphoma and pSS patients who did not, FcRL4 staining was similar. *Mann-Whitney U, $p < 0.05$ [30]

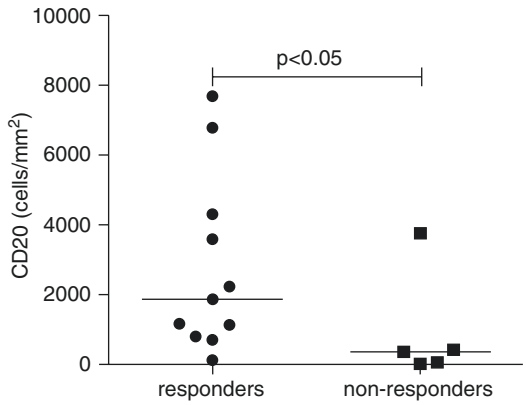

Fig. 10.7 Number of baseline $CD20^+$ B-cell/mm^2 is higher in clinical responders to anti-CD20 therapy than in non-responders [28]

peripheral blood monocytes and salivary gland tissue of SS patients. BAFF controls B-cell maturation, tolerance, and malignancy. It has been shown that BAFF levels are higher in pSS patients with higher systemic disease activity [44, 45]. It also has been shown that BAFF-driven B-cell activation may negatively affect the clinical response of pSS patients to treatment with anti-CD20 therapy [46]. Adding a BAFF blocker to anti-CD20 therapy might increase its efficacy [47].

-Omics aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms. Currently, much effort is put on how to apply genomics, proteomics, and metabolomics of serum, saliva, tears, and salivary gland tissue for diagnosing and better understanding SS as well as for patient stratification [12]. Particularly, saliva, and to a lesser extent also tears, is a very attractive biofluid for searching candidate biomarkers for pSS. Saliva is probably a more direct agent than serum as it is produced by glandular tissues that are directly affected by the disease process. Moreover, when compared to blood, saliva (as well as tears) can be collected repeatedly and noninvasively. When using glandular-specific saliva, the biomarkers detected even can be directly linked to the underlying autoimmune inflammatory deregulation and thus to mechanisms in the pathogenesis of SS.

Mass spectrometric analysis of saliva has revealed a variety of biomarkers that are preferably involved in the pathogenesis of pSS (Fig. 10.8; [48–50]). An increased expression of inflammatory and immune response-related salivary proteins is observed as well as that the secretion of other proteins is reduced, probably related to the destruction of acinar and ductal structures. Further study is needed, but it is becoming within reach that the diagnosis SS can be made on analysis of a drop of saliva. In this respect the results of a trial focusing on the application of salivary biomarkers for SS detection (NCT01807689) are eagerly awaited.

Micro-RNAs (miRNAs) are well-preserved, small non-coding RNAs of 19–25 nucleotides involved in posttranscriptional regulation of gene expression. Alevizos et al. [51] suggested that miRNAs may serve as a set of biomarkers for pSS. Research in this field is ongoing and is presumed to have a high potential. For example, it has been shown that the risk for developing pSS is related to miR-146a expression [52]. As such, miR-146a expression is a potential biomarker to be used in the diagnostic work-up of patients with a SS suspect.

Another promising approach is laser microdissection coupled with RNA-seq analysis. With this technique acini, ducts, and inflammatory foci of pSS subjects can be isolated for RNA-seq analysis. Tandon et al. [53] showed that marked differences in gene expression occur in the ductal and infiltrating cells compared to acinar cells. In particular, two chemokines involved in immune cell trafficking to secondary lymphoid tissue, viz., CCR7 and CCL21, had a markedly increased expression. The authors suggested that these chemokines may contribute to the recruitment of diverse immune cells to the salivary glands, causing inflammation and loss of secretory function that is commonly observed in SS patients.

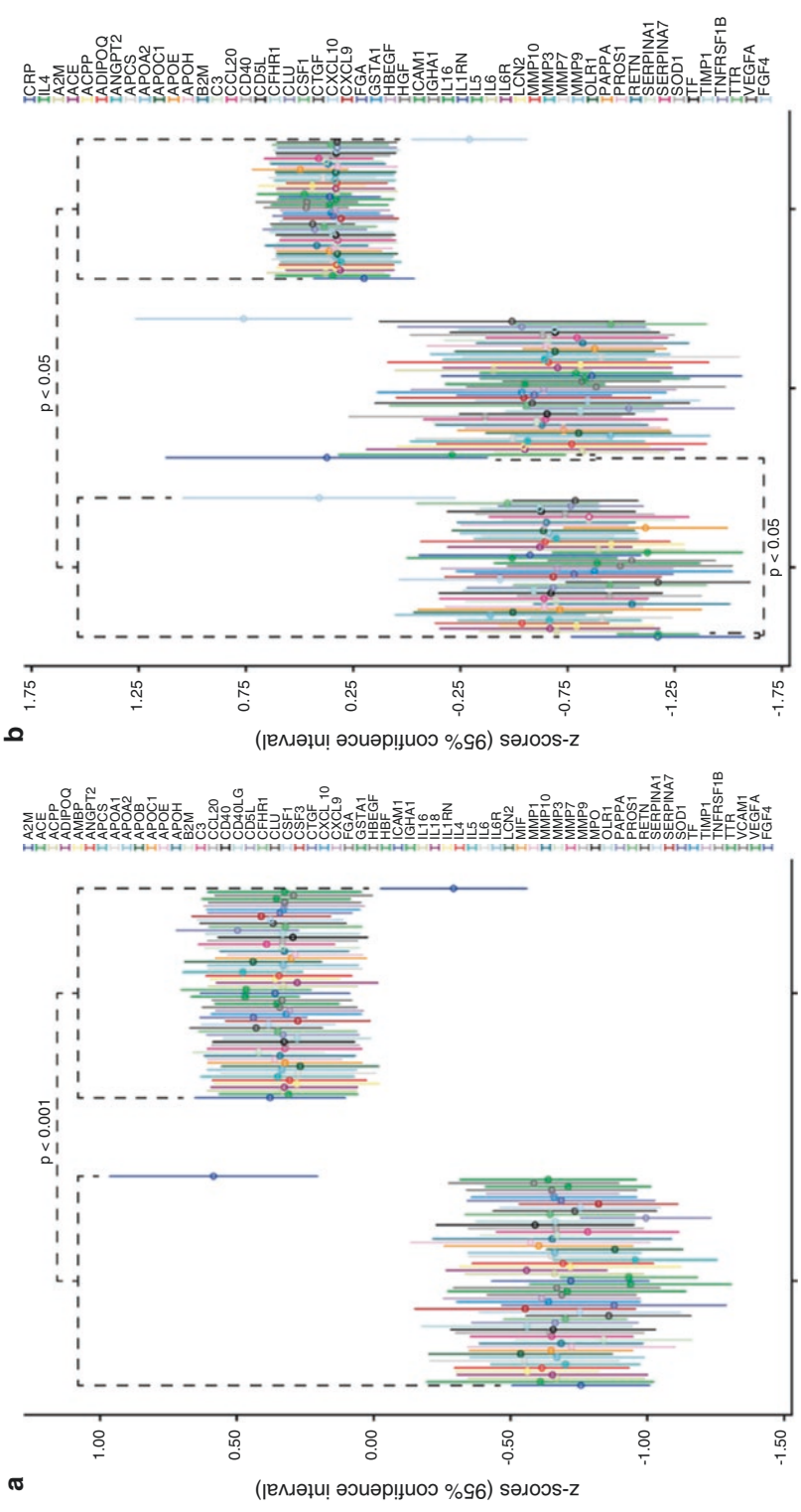

Fig. 10.8 Significant alterations in the quantity of specific proteins in saliva identified by multi-analyte profiles (MAPs). (**a**) Proteins yielding p< 0.001 by Student's two-tailed *t*-test when comparing SS patients ($n = 48$) to non-SS subjects ($n = 24$). (**b**) Proteins yielding p< 0.05 by Dunnett's two-tailed posttest (subsequent to one-way analysis of variance [two-tailed p< 0.001 were considered significant]) when comparing rheumatoid arthritis patients ($n = 12$) and SS patients ($n = 48$) to asymptomatic controls ($n = 12$). All values are represented as standardized values (Z scores); error bars indicate the 95% confidence interval for each protein. MAP proteins are annotated with their HGNC-compliant symbols [48]

10.4 Personalized Treatment: Which Biological Might Be Effective?

Presence of anti-Ro52/SSA and/or anti-La/SSB, elevated plasma levels of gamma globulins and RF, higher expression levels of Bruton's tyrosine kinase in B cells, and an increased risk of developing lymphomas, particularly mucosa-associated lymphoid tissue (MALT) lymphomas, all point toward a major role for B cells in the pathogenesis of pSS [54]. This B-cell hyperactivity seems to largely be T-cell dependent, and in particular Tfh cells play a role in SS [55].

Because of the role of B cells, anti-CD20 therapy has been considered as a potentially potent biologic disease-modifying antirheumatic drug (DMARD) to reduce disease activity. Anti-CD20 therapy results in significant depletion of $CD20^+$ B cells via several mechanisms. Open label and smaller randomized placebo-controlled trials (for a summary, see Van Nimwegen et al. [56] as well as the larger TEARS trial [57]) revealed that anti-CD20 therapy (rituximab) shows beneficial effects for pSS patients, while in a larger multicenter placebo-controlled trial, the TRACTISS trial [58], anti-CD20 therapy apparently was not effective at all. Thus, the question raises whether anti-CD20 therapy is indeed a failing therapy or whether anti-CD20 therapy is only effective in a selected category of pSS patients [59]. Probably, the same limitations apply to other biologicals that have been tested, are tested, or will be tested in SS. In other words, pSS patients that are treated with anti-CD20 therapy or other biologicals should be better selected to enhance success of a promising treatment modality. E.g., notwithstanding the negative TRACTISS trial [58], anti-CD20 therapy apparently has beneficial effects as has been reported at a clinical, biological, histological, and ultrasonographical level [56, 57, 60]. Post hoc analyses even have identified possible predictors of response, which might serve as a guide to select patients that likely will respond to a treatment with a particular biological (Fig. 10.5; [28, 31, 32]). Targeted patient inclusion will probably make, particularly when studying a disease with many manifestations as SS is, a biological to a failing or successful trial.

The central position of B cells as target for therapy is further illustrated by other recent positive trials with biologicals that are not based on the direct depletion of B cells but that do target, either directly or indirectly, these cells [56]. These biologicals comprise belimumab that binds to BAFF [47], resulting in less survival and less activation of the B cells, and abatacept [61] that blocks the co-stimulation of T cells and as a consequence the T-cell-dependent activation of the B cells [55]. Although several cytokines and chemokines decrease after anti-CD20 therapy, BAFF levels increase, likely as the result of the relative unavailability of B cells, expressing BAFF-binding receptors (Pollard et al. [62, 63]). Since high BAFF levels have been associated with humoral autoimmunity, the effect of adding belimumab to rituximab on the efficacy of rituximab is currently assessed (NCT02631538). First results are promising [47].

10.5 How to Design and Select Patients for Trials

As mentioned by Vissink and Bootsma [6], the new ACR-EULAR classification criteria for pSS do not guarantee that the proper pSS patients are selected for studies. Either these criteria need refinement or specific inclusion and exclusion criteria have to be added to the ACR-EULAR criteria for a particular study. So, when designing a trial to show efficacy of anti-CD20 therapy (rituximab) or other biologicals, the first step should be to define what specific baseline characteristics a pSS subject should have to be included in a particular trial. The information derived from previous studies with anti-CD20 therapy or other biologicals is worthwhile to identify subpopulations of SS patients that likely will respond to a particular biological.

Applying very strict criteria to include SS patients in a particular trial to make it successful also has the hazard that recruiting eligible SS patients will slowly progress. For example, Oni et al. [64] showed that when applying specific

measures of outcome, such as an EULAR SS patient-related index (ESSPRI) score ≥5 and an ESSDAI score ≥5, with requirements for unstimulated whole saliva flow >0 and anti-Ro/SSA positivity, the pool of eligible participants will greatly reduce. On the contrary, when making the inclusion to general, the result will be a failing trial unless the biological tested has such a general beneficial action that it is effective in most subcategories of SS patients.

Another critical step in trial design is to identify centers which have the tools to properly select SS patients with the required specific characteristics as well as have the experience to reliably apply the outcome parameters. For many outcome parameters, specifically trained rheumatologists (experienced in scoring ESSDAI), pathologists (targeted histologic evaluation), ophthalmologists (trained in ocular staining score), and oral and maxillofacial surgeons/specialists in oral medicine (experienced in assessing salivary gland function and taking the required type of salivary gland biopsy) are needed. It is recommended to perform trials in those expert SS centers that are able to include reasonable numbers of patients and have the needed expertise in house. This is because, in particular in multicenter trials, the inherent inter-individual variety in applying inclusion criteria and assessment tools cannot be tackled by training and calibration of clinicians when participating centers include only a few subjects. Such issues probably underlie the negative outcome of the larger randomized clinical trials performed in SS: patient selection was not sufficiently strict, and too many participating centers recruited too few patients and/or lacked the needed in-house expertise to cover all needed tests.

10.6 Lymphomas: Why Are They a Commonplace in SS Patients

Lymphomas develop in approximately 5–10% of SS patients. SS patients have an 18.8 (CI 9.5–37.3) times increased risk of developing lymphomas over the life span [65]. In most cases, these lymphomas are marginal zone B-cell lymphomas occurring in the salivary glands, in particular the parotid gland, the so-called MALT lymphomas. Lymphomas in SS patients are generally localized and follow an indolent, rather benign, clinical course, and if treatment is needed, they are very responsive to therapy [25]. In a minority of SS patients, aggressive non-Hodgkin lymphoma (NHL) is present. Even Hodgkin's disease has been described.

As mentioned before, risk factors for the development of lymphoma in SS patients include the presence of systemic activity, cytopenia, cryoglobulins, low complement C4 levels, and palpable purpura [66–68]. Whether the presence of GCs in salivary gland biopsies are predictive for the development of lymphoma is a continuing debate, but, as mentioned before, the larger study of Haacke et al. [24] could not confirm the presumption of Theander et al. [23] that GCs are indeed linked to the development of lymphomas in SS patients (see also section on histopathology).

Haacke et al. [30] tried to shed light whether MALT lymphomas preferably develop in parotid salivary glands. They showed that B cells expressing Fc receptor-like protein 4 (FcRL4), a protein that normally is expressed on a small subset of mucosa-associated B cells as well as on MALT lymphoma B cells, were present in salivary gland tissue of pSS patients where they were closely associated with ductal epithelial cells forming lymphoepithelial lesions (LELs). Remarkably, FcRL4+ B cells were far more frequent in parotid gland than in labial gland tissue (Fig. 10.6). As expected, the FcRL4 mRNA expression level in parotid MALT lymphoma was increased compared to parotid gland tissue of pSS patients without lymphoma. On the contrary, numbers of FcRL4+ B cells in labial gland biopsies taken at the time of pSS diagnosis were not predictive for later development of MALT lymphoma. The enrichment of FcRL4+ B cells in parotid gland tissue may explain why MALT lymphomas preferentially develop at this specific location pSS patients.

10.7 Etiopathogenesis

The most prominent histopathological finding in salivary gland tissue is the presence of focal mononuclear infiltrates of T and B cells and other cells, including plasma cells, macrophages, myeloid and plasmacytoid dendritic cells, and follicular dendritic cells. These infiltrates develop progressively in association with the striated ducts within glandular tissue. The result is impaired function of the glands and ultimately irreversible destruction of glandular tissue. It is also mentioned before that B cells play a central role in the immunopathogenesis and exhibit signs of hyperactivity. Hyperactivity of B cells is the consequence of the coordinated and integrated action of stimulation of the B-cell receptor, CD40, and toll-like receptors (TLR) in the presence of appropriate cytokines. The elevated levels of the B-cell receptor signaling molecule Btk, in B cells of pSS patients, illustrate the hyperactive status of B cells [69]. Overexpression of type I IFN and BAFF on one hand and IL-6 and IL-21 on the other hand is critically involved in the enhanced plasma cell formation in pSS patients. Hyperactivity of B cells results in secretion of autoantibodies and production of various cytokines [55].

As also mentioned previously, in many pSS patients, type I IFN and type I IFN-induced genes and proteins are overexpressed, resulting in the so-called type I IFN signature of pSS [70–72]. This observation also suggests involvement of viruses in the pathogenesis of SS. A variety of viruses, e.g., Epstein-Barr virus (EBV), coxsackievirus, and cytomegalovirus, are thought to play a role in onset or triggering of pSS [73]. Especially reactivation of latent EBV in genetically and hormonally susceptible individuals is presumed to play a role in the initiation and perpetuation of the chronic inflammatory autoimmune response in exocrine glands. Inoue et al. [74] postulated that binding of the exogenous ligand dioxin to the aryl hydrocarbon receptor causes lytic reactivation of EBV in B cells and salivary gland epithelial cells, resulting in immune responses in the salivary glands and possibly pSS.

10.7.1 B-Cell Hyperactivity and Role of Chemokines

Histopathologically, pSS is characterized by the presence of progressively developing focal lymphoid infiltrates around salivary gland striated ducts (lymphocytic sialadenitis; Fig. 10.3) as well as the development of LELs, in particular in parotid glands (Figs. 10.4 and 10.6). These lesions are formed by hyperplasia of the epithelium in association with lymphocytes. The histopathological features reflect the autoimmune process and manifestations of local B-cell hyperactivity. The occurrence of GCs, which are predominantly seen in the major salivary glands, is a clear sign of local activation of B cells. Another characteristic histopathological feature of pSS and witness of aberrant B-cell activity is the marked increase in the number of IgG (but not IgA)-secreting plasma cells in the exocrine glands [75]. These IgG plasma cells are predominantly present within the infiltrate, whereas IgA plasma cells dominate in the parenchyma. Chemokines are the driving force for the recruitment of lymphoid cells to sites of inflammation. As such these molecules underlie the immunopathological process in exocrine glands and contribute to B-cell hyperactivity, characteristic for pSS.

10.7.2 Germinal Centers

In approximately 25–30% of pSS patients, structures resembling GCs of secondary lymphoid organs are found within the (organized) ectopic lymphoid tissue of minor (labial) and major (parotid) salivary glands (Figs. 10.4 and 10.6) [76–80]. GCs arise after T-cell-dependent antigenic stimulation, and the presence of these structures obviously reflect local activation of B cells. In salivary glands of pSS patients, GCs are more likely to occur with increased focal infiltration and are associated with more severe disease [81].

10.7.3 B-Cell Hyperactivity and Clonal Expansion

Clonal expansions of B cells and plasma cells are increased in the salivary glands of pSS patients. These expansions are composed of IgA and/or IgG expressing cells [81]. Almost all obtained IgG and IgA sequences are somatically hypermutated, suggesting a post-GC origin of the cells. The occurrence of these clonally related cells as well as the intraclonal switching implies local activation and proliferation of B cells within the glandular tissue. Neoplastic transformations of clonally expanded cells may ultimately lead to the development of lymphoma in 5–10% of the pSS patients [82].

10.7.4 Pathogenetic Function of B Cells in pSS

The pathogenic role of autoantibodies in pSS is still largely obscure. As mentioned, the best known are anti-Ro/SSA and anti-La/SSB, both directed against ribonucleoproteins. The glandular epithelium is an important source for these autoantigens [83–85].

Besides their classical role as antibody-producing cells, activated B cells also have the ability to produce and secrete cytokines which are able to modulate immune responses [86, 87]. Herewith, B cells also play an antibody-independent role in tolerance and autoimmunity. TLR signaling appears to be critically involved in the signal required for human B cells to produce cytokine-producing cells [87]. Two subsets of cytokine secreting B cells can be identified, regulatory B cells and effector B cells. Regulatory B cells produce mainly IL-10 and TGFβ, and effector B cells produce cytokines such as IL-2, IL-4, IL-6, IL-12, IFNγ, and TNFα [86]. IL-10 producing regulatory B cells is thought to play an important role in dampening immune responses. Furuzawa-Carballeda et al. [88] showed that patients with pSS have an increased frequency of IL-10 producing circulating regulatory B cells, defined as $CD19^{+}CD38^{hi}CD24^{hi}IL\text{-}10^{+}$ cells, compared to controls. Importantly, the proportion of regulatory B cells was higher in clinically inactive pSS patients, compared to clinically active pSS patients suggesting that these cells may downregulate autoimmune inflammation to induce homeostasis.

10.8 Epilogue

The understanding of the pathogenetic mechanisms of pSS in general, and the role of B cells and plasma cells, in particular, is rapidly expanding. As discussed, -omic approaches will be another asset to elucidate further the complexity of the pathogenesis of pSS and to establish known and novel biomarkers for early diagnosis, measurement of disease activity, and definition of subgroups of pSS patients that might be susceptible to a particular treatment.

Many biological DMARDs are currently available and even more are in development to target various molecules involved in the cascade of hyperactive B cells and plasma cells including biologicals that can interfere with a large number of relevant cytokines and chemokines. In addition, non-biological drugs that inhibit B-cell receptor signaling molecules and cytokine receptors have become available. Because B-cell receptor signaling plays an important role in the autoimmune process, targeting important molecules of this pathway, such as Syk and Btk [89], is presumed to be a promising new approach for treatment of pSS too. A major potential disadvantage of all these therapies is that not only harmful autoimmune responses are affected but also beneficial humoral responses.

Besides a better understanding of the pathogenetic process and the availability of biological and synthetic DMARDs, assessment of disease activity in pSS is an essential step to rate efficacy of the treatment. With the development and validation of ESSDAI and ESSPRI, important tools have become available for rating the disease activity and patients' complaints in pSS. Both indices are complementary and should be used together in addition to objective measurements of dryness and biological markers of disease activity [90]. A change of ESSDAI of at least three

points or a change of ESSPRI with at least one point or 15% seems reasonable to show a clinically relevant effect (Seror et al. [91]). The ClinESSDAI, a modification of ESSDAI to score disease activity independent of B-cell biomarkers, should be used (1) in biological/clinical studies to avoid data collinearity, (2) in clinical trials, as secondary endpoint, to detect change independent of biological effect of the drug, and (3) in clinical practice to assess disease activity for visits where immunological tests have not been done [92]. In a real-life cohort, it was shown that ClinESSDAI is indeed a valid tool to assess clinical disease activity in pSS and may be a useful secondary endpoint in clinical trials [93].

The increased knowledge on the way how to assess patients for a particular therapy, along with the emergence of new targeted therapies, will stimulate the conduction of trials and the development of effective treatment option in SS.

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