

REVIEW

Sjögren's syndrome: studying the disease in mice

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Abstract

Sjögren's syndrome (SS), a systemic autoimmune disease, is characterized by inflammation of exocrine tissues accompanied by a significant loss of their secretory function. Clinical symptoms develop late and there are no diagnostic tests enabling early diagnosis of SS. Thus, particularly to study these covert stages, researchers turn to studying animal models where mice provide great freedom for genetic manipulation and testing the effect of experimental intervention. The present review summarizes current literature pertaining to both spontaneous and extrinsic-factor induced SS-like diseases in mouse models, discussing advantages and disadvantages related to the use of murine models in SS research.

Introduction

Assuming that studying a model organism will provide us with relevant information about the organism of our primary interest, investigation of nonhuman animals represents an important pillar in today's biomedical research. Over the past decades, the most popular experimental model to emerge is the common house mouse, irrespective of different living environments, the evolutionary distance and some well-recognized discrepancies in innate and adaptive immune responses between mice and men. Despite such concerns, researchers generally accept these limitations in order to circumnavigate technological and ethical issues related to research conducted in humans. Indeed, immunology has embraced the study of mice as a model organism and has accumulated tremendous insight into the intricacies of the human immune system and its involvement in both preventing and effecting disease.

In the present article, the murine models for Sjögren's syndrome (SS) are presented along the lines of spontaneous and extrinsic-factor induced models of SS-like

disease and are discussed with special focus on disease phenotype and alterations induced in association with genetic modification and experimental intervention. We also highlight common biological themes reported in context with both the etiology and the underlying pathogenic mechanisms of experimental SS and address their potential relevance for SS in humans.

Sjögren's syndrome: a summary

SS is a chronic autoimmune disease, which mainly affects the exocrine glands. Nearly all patients complain of a persistent feeling of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca) [1,2]. These symptoms can be confirmed by multiple objective tests indicating significant functional impairment of the salivary and lacrimal glands. Histological evaluation of minor salivary glands obtained from patients with SS usually shows large and persistent focal infiltrates of mononuclear cells, often referred to as lymphocytic foci. These foci consist of mainly T cells, fewer B cells and smaller numbers of natural killer cells and dendritic cells. Often associated with such inflammation are acinar epithelial cell atrophy, progressive fibrosis and the presence of adipocytes in the salivary glands. Since approximately 60 to 80% of patients with SS produce anti-Ro antibodies and 40 to 60% produce anti-La autoantibodies [2], SS diagnosis also relies on the presence of these two biomarkers [3].

Affecting approximately 0.1 to 0.6% of the total population, SS is considered a relatively common rheumatic disease. In addition, SS is estimated to be 10 times more common in women compared with men. SS may extend from an autoimmune exocrinopathy to effect diverse extraglandular manifestations in the musculoskeletal, pulmonary, gastrointestinal, hepatobiliary, hematologic, vascular, dermatologic, renal and nervous systems. In contrast to systemic lupus erythematosus (SLE), where increased mortality has been reported as a consequence of the disease, the overall mortality in SS is comparable with the rate in the general population [4]. The risk of developing non-Hodgkin's lymphoma, however, is reported to be increased 16-fold in patients with SS compared with a control population [5]. Unfortunately, all therapies tested to date have proven ineffective in reversing the course of SS. Regrettably, relatively few studies in the field of rheumatology address SS specifically.

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With the possible exceptions of a few autoimmune diseases (for example, rheumatic heart disease), the etiology of most autoimmune diseases remains a mystery. The latter is also true for SS, despite multiple attempts to identify factors that might trigger the onset of a pathogenic immune response specifically directed against the exocrine glands. With a subset of SS patients exhibiting strong type 1 and type 2 interferon signatures [6,7], there is reason to believe that a viral agent is involved – but why some individuals are susceptible and others are not most probably resides in the individual's genetic background. An activated type 1 interferon system has also been described in other autoimmune diseases (for example, SLE) [8]. Much is known about the exogenous and endogenous inducers of type 1 interferons and the molecular pathways that may mediate a continuous interferon production involving Toll-like receptor-dependent amplification and propagation of the immune response [9]. Less is known, however, about the functional role of specific gene variants in the regulation of the type 1 interferon system. Complicating this picture, unfortunately, is the fact that an important share of patients with SS suffers from secondary SS, defined as SS manifested in individuals diagnosed with other autoimmune diseases such as SLE, rheumatoid arthritis or scleroderma.

Another confounding factor in SS that has surfaced is the increasing recognition that the severity of secretory dysfunction does not necessarily correlate with the degree of leukocytic infiltration or the loss of acinar tissue. This raises the distinct possibility that immune process-related alterations within the glandular tissues, disturbing saliva production and/or secretion, are involved in the impairment of exocrine gland secretion observed in patients with SS [10,11].

The aim of the present review is to provide the reader with an overview and specific information about murine strains that have been proposed as models of SS. The review also highlights findings and hypotheses regarding the etiology and pathogenesis of SS that arose from research conducted in animal models [12,13].

Model organisms: a summary

In principle, due to the common descent of all living organisms, discoveries made in one species might provide scientists with valuable information about another species. For this promise to be fulfilled, researchers depend on a critical level of conservation between the species studied and the species of primary interest. As stated earlier in the manuscript, the mouse is the organism of choice for the majority of immunologists and has also become an integral element of the bed-to-bedside drug development strategy. As species, humans and mice diverged approximately 70 million years ago;

and from an immunologist's point of view of special importance, they evolved in two different ecological niches. Nevertheless, the major paradigms about the working principles of the immune system appear to translate particularly well between the two species [14]. There is a need, however, for further delineation of species-specific differences in order to increase predictability of how findings from a murine strain may translate to a human population [14].

Direct comparison of the human and mouse genomes has confirmed the close relationship of these two mammalian species, as there are only about 300 genes that are unique to either humans or mice [15]. Because of differences in the development and lifespan between humans and mice, one may certainly argue that significant differences exist in the timing of gene expressions, but the basic workings of molecular and biological pathways have been shown to be similar, if not identical. One must remember, however, the aspects of very distinct differences in innate and adaptive immunity that exist between mice and men [14]. Nonetheless, one can only be impressed by how relevant information from mouse studies is to humans. To researchers, perhaps the most compelling feature in the context of research using animal models is the mouse lifespan and fertility. In addition, most societies grant scientists considerable liberties in testing new hypotheses in mice by allowing genetic manipulation and the strict control of an animal's living environment. As a consequence, resources related to research in mice became highly accessible – including thousands of inbred and genetically modified strains, detailed experimental protocols, elaborated research-related reagents and databases containing extensive data collections [16].

Experimental research and Sjögren's syndrome

Several aspects either directly related to the nature of SS or associated with current technical limitations underscore the necessity for research involving animal models. Prior to the onset of overt SS, physiological and structural changes are thought to take place in the exocrine glands, but due to the covert nature of the early stages of autoimmune diseases, studying these events in patients is virtually impossible. Similarly, collection of human specimens that represent a time course of the disease is difficult.

Ideally, a mouse model of SS mimics several clinical, histopathological and immunological features of the human disease combined with a high incidence of disease. In any cohort of SS patients, however, individuals are genetically diverse and the exhibited disease profiles are heterogeneous. There is thus significant reason to develop multiple murine strains, which manifest, to different extents, SS-like disease manifestations. This is

also important as, obviously, each mouse only represents one genetic background. Unsuccessful clinical trials, based on seemingly promising results of treating autoimmune diseases in mice, might be due to the fact that the trial's design relied too heavily on a single mouse model; for example, nonobese diabetic (NOD) mice for the study of type 1 diabetes (T1D) [17].

Regarding mechanisms underlying the different aspects of the pathogenesis of SS, studying genetically altered strains allows the testing of more specific hypotheses with regard to, for example, a specific protein, cell type or functional pathway. Nevertheless, the overall functioning principles of a system can often not be predicted by studying the properties of its isolated parts as its state may strongly rely on the interaction of multiple components.

Murine models of spontaneous disease

In outbred populations of higher organisms such as rodents, cats, dogs and horses, the occurrence of inflammatory conditions reminiscent of autoimmune diseases in humans is not uncommon. Also comparable with the situation in humans is heterogeneity in terms of genetic backgrounds, disease activity and clinical manifestations displayed under outbred conditions. A potential explanation for this phenomenon might be related to a possible null correlation between some autoimmune diseases and reproductive success. Hence, model organisms that develop a SS-like disease spontaneously might well, as a group, represent the assumed multifactorial etiology and complex pathogenesis of SS in humans. As mostly inbred strains are used for research purposes, the conclusions drawn from an experimental study may translate well to a subpopulation of patients while being only partly valid or invalid for other groups of patients with SS.

The earliest murine models of SS identified were strains that develop SS-like disease manifestations spontaneously. A pertinent fact is that these models mimic in part as a group the complex genetics and diverse disease phenotypes found in patients with SS. The disease phenotypes of these strains are discussed below (Tables 1 and 2).

NZB, NZW and (NZB/NZW) F_1 mice

In (NZB/NZW) F_1 mice, histopathological manifestations of SS coincide with features reminiscent of SLE [18,19]. In NZW mice, exocrine gland inflammation is more pronounced in females compared with males, whereas this phenomenon is generally less apparent in NZB mice [20,21].

Although other SS-related disease manifestations are not very pronounced in (NZB/NZW) F_1 mice, a more recent study demonstrated that an unspecific inflammatory stimulus, evoked by Freund's incomplete adjuvant,

can trigger a significant drop in salivary gland function already in an early phase of the disease, while this intervention protocol affected anti-Ro levels at a latter disease stage [22]. Thought to alter the sizes of T-cell subsets, administration of anti-CD25 monoclonal antibodies shortly after birth also exacerbates sialoadenitis and auto-antibody production in this strain [23]. Lastly, Toll-like receptor 3 engagement through polyinosinic:polycytidylic acid has been studied in an attempt to recapitulate the effect of a dsRNA virus infection on the SS-like disease manifested in these mice [24]. As a result, inflammatory mediators downstream of Toll-like receptor 3, such as type-1 interferon, were transcribed and a concomitant transient loss in salivary gland secretory function was observed [24].

MRL and MRL/*lpr* mice

In 1982 the MRL strain, at the time already established as a model of SLE, was reported to develop periductal lymphoid infiltrates in the salivary glands [25]. MRL/*lpr* mice differ from MRL mice with respect to a mutation involving the *Fas* gene [26]; however, negative selection in the thymus does not seem to be impaired in either strain [27]. In addition, irrespective of the *lpr* mutation in the *Fas* gene, MRL/*lpr* mice express a detectable amount of apoptosis-related FAS protein on lymphoid cells [28]. Nevertheless, defective apoptosis associated with the *lpr* mutation results in increased susceptibility and severity of the disease, most probably through acceleration of the disease course [26,28].

Immunohistochemical analyses of the organs targeted by the inflammation show the presence of activated T cells [29,30], whose importance was further confirmed in T-cell transfer experiments [31]. Inflammatory lesions in the salivary glands of MRL/*lpr* mice contain B cells producing IgA and IgM rheumatoid factor [32] and were, in addition, identified to be sites of IFN γ production [30]. Of potential concern, despite female predominance and the rare occurrence of anti-Ro autoantibodies, the clinical hallmarks of SS – hyposalivation and keratoconjunctivitis sicca – are absent in this model.

NFS/*sld* mice

The NFS/*sld* mouse provides a model in which aberrant immune responses against α -fodrin are elicited [33]. A defect in salivary gland development leads to aberrant enzymatic proteolysis of the structural protein fodrin by caspase [33]. Indeed, some patients with SS produce antibodies specific to the 125 kDa subunit of α -fodrin [34]. However, the association between antibodies to α -fodrin and SS does not seem to be as strong as originally thought [35]. Thymectomy performed in NFS/*sld* mice 3 days after birth results in development of T-cell dominated infiltrates in the salivary and lacrimal glands,

Table 1. Spontaneous models of Sjögren's syndrome

Strain/ modification ^a	SS-like disease manifestations ^b	Remarks ^b	Reference
(NZB/NZW)F ₁	SG and LG infl.	SLE-like disease	[19]
MRL	SG and LG infl.	SLE-like disease	[29]
<i>lpr</i>	↑ SG and LG infl.	<i>lpr</i> exacerbates the MRL disease phenotype	[30]
NFS/ <i>sld</i>	None	Aberrant proteolysis of α-fodrin	[33]
3d-Tx	SG and LG infl.	Inflammatory lesions in multiple organs	[34]
IQI/Jic	SG and LG infl.	Inflammatory lesions in multiple organs	[37]
NOD	SG and LG infl., ↓ SG and LG function	T1D, multiple immune system-related alterations	[12]
<i>H2^b</i>	Similar to original NOD strain	No T1D	[42]
C57BL/6	May develop SG infl. at an old age	Widely used recipient strain	
NOD- <i>Aec1Aec2</i>	SG and LG infl., ↓ SG and LG function	Increased applicability compared with NOD mice	[41]
<i>Il2^{-/-}</i>	SG and LG infl., ↓ SG function	Generalized lymphoproliferative disease	[91]
<i>Il2Rα^{-/-}</i>	SG and LG infl., ↓ SG function	Generalized lymphoproliferative disease	[91]
<i>Foxp3^{-/-}</i>	None	More susceptible to SS-like disease	[94]
<i>Il10</i> Tg	SG and LG infl., ↓ SG and LG function	↑ apoptosis in the SG	[95]
<i>Il14a</i> Tg	SG infl., ↓ SG function	High incidence of CD5 ⁺ lymphoma, nephritis	[101]
<i>Baff</i> Tg	SG and LG infl., ↓ SG function	MZ B-cell dominated infl., SLE-like disease	[106]
<i>Tgfb1^{-/-}</i>	SG and LG infl., ↓ SG function	Poor viability, mixed inflammatory cell response	[112]
<i>Tgfb1</i> Tg	↓ SG function	Impaired SG architecture, SG tissue fibrosis	[113]
<i>Thbs1^{-/-}</i>	LG infl., anti-Ro and anti-La	Detailed assessment of the eye component	[116]
<i>Ar^{-/-}</i>	SG infl.	Estrogen-dependent disease	[119]
<i>RbAp48</i> Tg	SG and LG infl., ↓ SG function, anti-Ro and anti-La	Assessment of the inflammatory milieu in the SG	[122]
<i>Id3^{-/-}</i>	SG and LG infl., ↓ SG function, anti-Ro and anti-La	Exocrine gland dysfunction precedes SG and LG infl.	[125]
<i>Id3^{-/-};LckCre</i>	SG and LG infl., ↓ SG function, anti-Ro and anti-La	T-cell lineage specific deletion of <i>Id3</i>	[126]
<i>r1ΔT/r2n</i>	SG and LG infl., anti-Ro	↓ T _{reg} ⁺ inflammatory lesions in multiple organs	[130]
<i>IkBa^{MM}</i>	SG and LG infl., anti-Ro and anti-La	Defective feedback regulation of NF-κB	[133]
C57BL/10	May develop SG infl. at an old age	C57BL/6 and C57BL/10 diverged prior to 1937	
NOD- <i>Nss1/Idd5</i>	SG infl.	SG function has not been assessed	[82]
SJL	SG and LG infl., anti-Ro and anti-La	Susceptible to multiple autoimmune diseases	[98]
<i>Il12</i> Tg	↑ SG and LG infl., ↓ SG function, ↑ anti-Ro and anti-La	IL-12 exacerbates the SJL disease phenotype	[98]
BALB/c	None	Often used as control strain	
<i>Act1^{-/-}</i>	SG and LG infl., anti-Ro and anti-La	MZ B-cell dominated infl., SLE-like disease	[115]

Selection of murine strains in which Sjögren's syndrome (SS)-like disease manifestations develop spontaneously. ↑, increased; ↓, decreased; Ar, aromatase; 3d-Tz, 3 days after birth thymectomy; infl., inflammation; LG, lacrimal gland; MZ, marginal zone; NOD, nonobese diabetic mice; SG, salivary gland; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; Tg, transgenic; T_{reg}⁺, regulatory T cell. ^aSpecific modification of the original strain. ^bFor modified strains, listings refer to relative changes compared with the original, not indented, strain listed above.

and – secondary to the SS-like disease – the NFS/*sld* mice undergoing thymectomy 3 days after birth also tend to develop inflammatory lesions in other organs [36].

IQI/Jic mice

The IQI/Jic strain was developed from the same stock that gave rise to the NOD mouse. Selection, however, was for mice that exhibited a SS-like disease comparable with NOD mice but in the absence of T1D. IQI/Jic mice develop focal inflammation in the salivary and lacrimal glands, accompanied by parenchymal destruction [37]. Sialoadenitis progresses over time and becomes more prominent in females compared with males. IQI/Jic mice also develop inflammatory lesions in several other organs, including the lung, pancreas and kidneys [38].

Interestingly, kallikrein-13 has recently been suggested to play a role in the etiology of the SS-like disease manifested in IQI/Jic mice [39]. Kallikreins, together with other proteases, were found to be part of the salivary proteome characteristic for patients with SS [40].

Nonobese diabetic mice and related strains

The NOD strain descends from a cataract-prone strain of outbred Jcl/ICR mice and is today the most extensively characterized model of SS and T1D. Although some genetic loci related to diabetes (*idd^s* loci) contribute to the inflammatory changes in the exocrine glands, it seems that diabetes and SS develop independently of each other [41-43]. T1D in NOD mice is restricted to the expression of the class II major histocompatibility

Table 2. Alterations in disease phenotype observed in association with genetic modification and experimental intervention

Strain/ modification/ intervention	SS-like phenotype ^a	Remarks ^a	Reference
(NZB/NZW)F ₁	SG and LG infl.	SLE-like disease	[19]
IFA	↑ SG infl., ↓ SG function, anti-Ro	↑ DC numbers early in the disease process	[22]
Anti-CD25	↑ SG infl., anti-Ro	↑ ANA	[23]
Poly(I:C)	↓ SG function (transient)	Role of TLR3 engagement	[24]
NOD	SG and LG infl., ↓ SG and LG function	T1D, multiple immune system-related alterations	[12]
<i>scid</i>	No SG infl., ↑ SG function	Abnormal salivary gland physiology remains	[60]
<i>Igμ^{null}</i>	↑ SG function	Insulinitis but no progression to overt T1D	[64]
<i>Il4^{-/-}</i>	↑ SG function	Absence of anti-M3R IgG ₁	[77]
<i>Ifny^{-/-}</i>	No SG infl., ↑ SG function, ↑ LG infl.	Retained LG infl.	[78]
<i>IfnyR^{-/-}</i>	No SG infl., ↑ SG function, ↑ LG infl.	Retained LG infl.	[78]
<i>Tnfr1-FcI_g3</i> , Tg	↓ SG and LG infl.	Insulinitis but no progression to overt T1D	[75]
<i>Ica69^{-/-}</i>	↓ SG and LG infl.	Unchanged incidence rate of T1D	[61]
<i>Aire^{-/-}</i>	↑ LG infl., ↓ LG function	Role of OB1a and central tolerance	[63]
NZW- <i>Ssial3</i>	↓ SG infl., SG function unchanged	Unchanged insulinitis score	[55]
<i>E2f1^{-/-}</i>	↑ SG infl., ↓ SG function	↑ T1D, ↓ T _{regs}	[58]
Hsp60	↓ SG infl., ↑ SG function	Biomarkers in saliva predict treatment success	[57]
Hsp60 amino acids 437 to 460	↓ SG infl., ↑ SG function	Biomarkers in saliva indicate SG function	[57]
LTβR-Ig	Arrested progression of SG infl.	Changes in cellular composition of SG infl.	[48]
Anti-VCAM1	↓ LG infl.	LG function, SG infl. and SG function not assessed	[76]
Anti-α _v -integrin	↓ LG infl.	LG function, SG infl. and SG function not assessed	[76]
Anti-PNAd	↓ LG infl.	LG function, SG infl. and SG function not assessed	[76]
Anti-L-selectin	↓ LG infl.	LG function, SG infl. and SG function not assessed	[76]
Anti-LFA1	↓ LG infl.	LG function, SG infl. and SG function not assessed	[76]
<i>Il10</i>	↓ SG infl., ↑ SG function	Retrograde gene delivery through SG ducts	[96]
<i>Tnfr1-Ig</i>	↓ SG function	Retrograde gene delivery through SG ducts	[74]
NOD-H2 ^b	SG and LG infl., ↓ SG and LG function	No T1D	[42]
<i>Il4^{-/-}</i>	↑ SG function	Absence of anti-M3R IgG ₁	[65]
<i>C.Stat6^{-/-}</i>	↑ SG function	Absence of anti-M3R IgG ₁	[66]
NOD- <i>scid</i>	No SG infl., normal SG function	Abnormal salivary gland physiology remains	[60]
<i>Ifny^{-/-}</i>	No SG and LG infl., ↑ SG function	Improved salivary gland physiology	[78]
<i>E2f1^{-/-}</i>	No SG infl., retained ↓ SG function	Effect of E2F1 deficiency on SG development	[59]
C57BL/6.NOD- <i>Aec1Aec2</i>	SG and LG infl., ↓ SG and LG function	Increased applicability compared with NOD mice	[41]
<i>C3^{-/-}</i>	No SG and LG infl., ↑ SG function	Assessing the role of C3	[88]
<i>Il17r:Fc</i>	↓ SG infl., ↑ SG function	Retrograde gene delivery through SG ducts	[81]
C57BL/6	May develop SG infl. at an old age	Widely used recipient strain	
<i>Il17a</i>	SG infl., ↓ SG function	Retrograde gene delivery through SG ducts	[80]
C57BL/6- <i>Il14a</i> Tg	SG infl., ↓ SG function	High incidence of CD5 ⁺ lymphoma, nephritis	[101]
<i>Lta^{-/-}</i>	↓ SG infl., ↑ SG function	LTa-dependent disease phenotype	[104]
C57BL/6- <i>Id3^{-/-}</i>	SG and LG infl., ↓ SG function, anti-Ro and La	Exocrine gland dysfunction precedes SG and LG infl.	[125]
Anti-CD20	↓ SG and LG infl., ↑ SG function	Depletion of B cells	[127]
C57BL/6- <i>Baff</i> Tg	SG and LG infl., ↓ SG function	MZ B-cell dominated infl., SLE-like disease	[106]
<i>Ltβ^{-/-}</i>	↓ SG infl., ↑ SG function	MZ B-cell dependence of the SS-like disease	[107]
<i>Tnfa^{-/-}</i>	Unchanged	Increased incidence of B-cell lymphoma	[110]
BALB/c- <i>Act1^{-/-}</i>	SG and LG infl., anti-Ro and anti-La	MZ B-cell dominated infl., SLE-like disease	[115]
<i>CD40^{-/-}</i>	SG and LG infl. unchanged	Absence of anti-Ro and anti-La	[115]

Selection of genetic modifications and specific intervention, which gave insight into the mechanisms underlying either the etiology or the pathogenesis of Sjögren's syndrome (SS)-like disease in the original strain. ↑, increased; ↓, decreased; AIRE, autoimmune regulator; ANA, antinuclear antibodies; DC, dendritic cells; E2F1, E2F transcription factor 1; HSP, heat shock protein; IFA, Freund's incomplete adjuvant; infl., inflammation; LFA, leukocyte function-associated antigen; LG, lacrimal gland; LT, lymphotoxin; M3R, muscarinic acetylcholine type-3 receptor; MZ, marginal zone; NOD, nonobese diabetic mice; OB1a, odorant binding protein 1a; PNAd, peripheral node addressin; poly(I:C), polyinosinic:polycytidylic acid; *scid*, severe combined immunodeficiency; SG, salivary gland; SLE, systemic lupus erythematosus; STAT, signal transducer and activator of transcription; T1D, type 1 diabetes; Tg, transgenic; TLR, Toll-like receptor; T_{reg}, regulatory T cell; VCAM, vascular cell adhesion molecule. ^aFor all modified strains and interventions, listings refer to relative changes compared with the original, not indented, strain listed above.

complex (MHC) haplotype H2^{s7} [44]. Whereas NOD.B10-*H2^b* mice are resistant to the onset of overt T1D, they still exhibit the main disease manifestations of SS [42]. The exact extent and cellular composition of the glandular inflammation in NOD.B10-*H2^b* mice, however, remains to be defined.

NOD mice in which the original MHC H2^{s7} haplotype was replaced with an H2^g or H2^p haplotype were also investigated. In summary, while the difference in H2 haplotype did not seem to affect the frequency of sialoadenitis, the disease severity varied among these strains [43]. Interestingly, introduction of the H2^g haplotype directed the autoimmune response towards the production of SLE-associated autoantibodies and a higher incidence of kidney pathology [43].

Autoimmune manifestations in NOD mice represent a complex disease involving genetics, sensitivity to exogenous factors and defects in central and peripheral tolerance [44]. These factors have also been reported to contribute to the susceptibility of the strain to develop autoimmune thyroiditis [45], SLE [46], myasthenia gravis [47] and autoimmune encephalomyelitis [44] subsequent to specific intervention.

In NOD mice, focal inflammation in the submandibular salivary glands and the lacrimal glands develops from approximately 8 weeks of age onwards. The foci appear comparable in structure and cellular composition with infiltrates found in human salivary glands (Figure 1) [48,49], and gender-related differences in the degree of exocrine gland inflammation have also been reported in this strain [50]. As in patients with SS, in NOD mice the relationship between histopathological changes and hyposalivation is not always obvious – which indicates a certain autonomy of the autoimmune manifestations of SS (Figure 2) [51]. Exocrine gland inflammation in NOD mice appears to precede the onset of hyposalivation by a considerable amount of time [52]. Interestingly, transition to an overt disease does not necessarily need to be associated with a significantly higher degree of glandular inflammation [52], but hyposalivation and reduction in lacrimation were rather correlated with the occurrence of B-cell response-related gene transcripts in the exocrine glands [53,54].

Supporting the notion of a certain independence between degree of inflammation and glandular hypofunction, introduction of an NZW-derived interval of chromosome 7 (annotated *Ssial3*) into NOD mice moderated sialoadenitis without ameliorating salivary gland function [55]. Analyses of dozens of inflammatory mediators in serum and saliva obtained from NOD mice, furthermore, only revealed a minimal number of biomarkers correlating with several SS-related disease manifestations in an association network [56]. In addition, successful prevention of hyposalivation – through

administration of 60 kDa heat-shock protein and of 60 kDa heat-shock protein-derived peptide amino acids 437 to 460 – did not coincide with a corresponding decrease in salivary gland inflammation [57]. In contrast, biomarker signatures generated from saliva, indicating qualitative changes in salivary gland inflammation, predicted treatment outcome and salivary gland function with high accuracy [57]. Several lines of evidence indicate that as T1D progresses from early insulinitis to overt diabetes there is a loss of immune cell subsets, such as regulatory T cells (T_{regs}) and invariant natural killer T cells within the islets [17]. Unfortunately, little is still known about the role of these cell subsets in the progression of SS. Nevertheless, NOD mice deficient for E2F transcription factor 1 – a regulator of T-cell proliferation, differentiation, and apoptosis – have a pronounced decrease in CD4⁺CD25⁺ T_{regs} and seem to be highly predisposed not only to T1D but also to SS [58]. In order to investigate the effects of E2F transcription factor 1 deficiency prior to the involvement of the adaptive immune system, the SS disease profile was later assessed in NOD-*E2f1*^{-/-} mice, which, in addition, carried the severe combined immunodeficiency (*scid*) mutation. Interestingly, this strain's saliva secretion capacity was found impaired [59] irrespective of the severe deficiencies in adaptive immunity and the absence of exocrine gland inflammation reminiscent of SS mediated by the *scid* mutation [60].

Another possible connection between SS and T1D in NOD mice might involve common autoantigens. Disruption of the islet-cell autoantigen 69 kDa gene in NOD mice, a self-antigen associated with diabetes that is expressed not only in the pancreas but also in the exocrine glands, reduced SS-related histopathology and glandular hypofunction [61]. A study investigating a large cohort of patients with SS could not, however, confirm a role or true frequency of islet-cell autoantigen 69 kDa autoimmunity in patients with SS [62]. Studying the role of autoimmune regulator deficiency and central tolerance in the context of SS in NOD and Balb/c mice identified odorant binding protein 1a as a potential autoantigen involved in the etiology of autoimmune-mediated lacrimal gland pathogenesis [63].

To determine whether B cells contribute to the SS-like disease, experiments were carried out in NOD-*Igμ*^{null} mice, which lack mature B cells [64]. The results indicate that in SS, in contrast to T1D, B cells do not significantly participate in the initiation phase of the disease [44,64]. However, B-cell activity appears to be critical in the transition to an overt disease stage in these mice, since, despite the presence of T cells in the salivary glands, NOD-*Igμ*^{null} mice fail to develop hyposalivation [64]. Subsequent studies also documented the concomitant lack of hyposalivation and anti-muscarinic acetylcholine

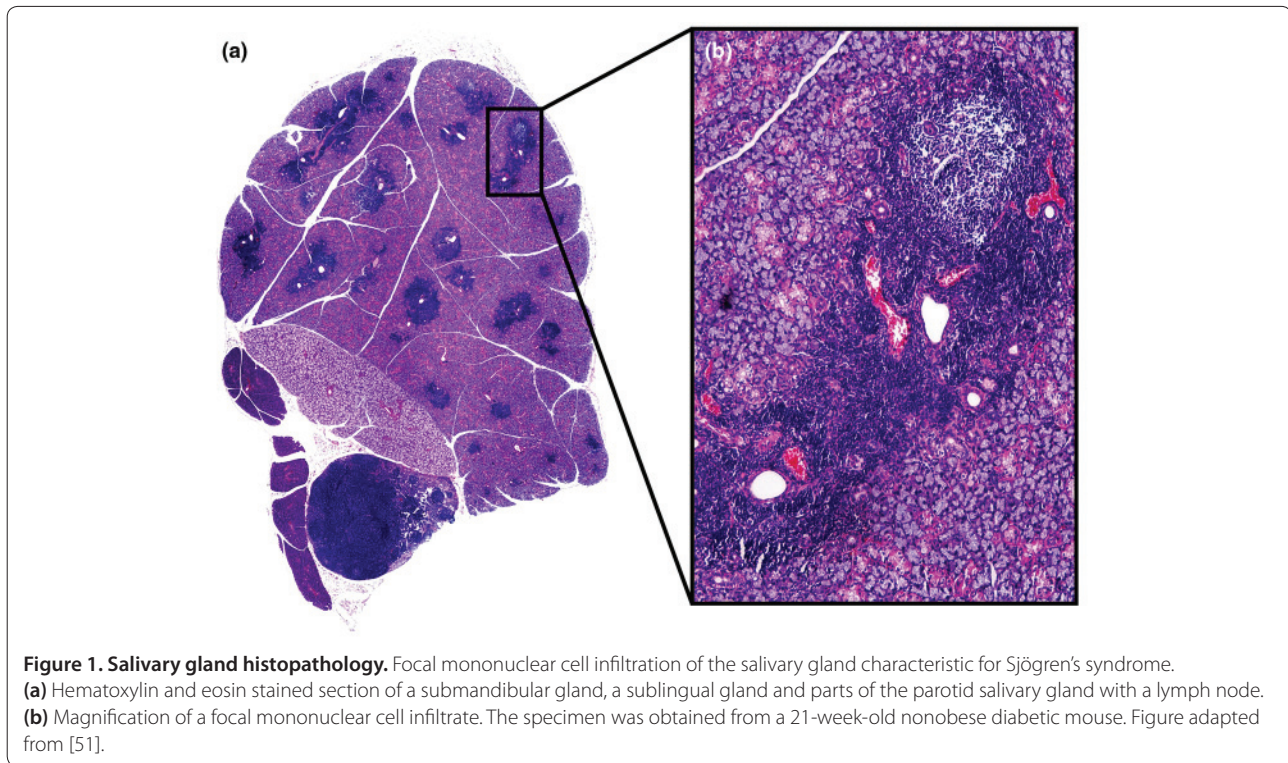


Figure 1. Salivary gland histopathology. Focal mononuclear cell infiltration of the salivary gland characteristic for Sjögren's syndrome. (a) Hematoxylin and eosin stained section of a submandibular gland, a sublingual gland and parts of the parotid salivary gland with a lymph node. (b) Magnification of a focal mononuclear cell infiltrate. The specimen was obtained from a 21-week-old nonobese diabetic mouse. Figure adapted from [51].



Figure 2. Assessment of salivary gland secretion capacity. Saliva collection and measurement of the salivary flow rate in an anesthetized nonobese diabetic mouse subsequent to the intraperitoneal injection of pilocarpine. Figure reproduced with kind permission from Springer Science & Business Media [13], photograph by Nicolas Delaleu.

type-3 receptor (M3R) autoantibodies of the IgG₁ isotype in IL-4-deficient and signal transducer and activator of transcription 6-deficient NOD strains [65,66]. In connection with possible non-inflammatory mechanisms underlying the onset of hyposalivation, an altered aquaporin 5 distribution – similar to the patterns observed in human specimens – has also been described in exocrine glands obtained from NOD mice [67,68].

Protection from T1D in NOD mice has been associated with a shift from a Th1 to a Th2 cytokine expression profile in autoreactive T cells [17]. Results obtained in subsequent studies, however, indicated that compartmentalization into disease-promoting Th1 and protective Th2 cytokines cannot be applied to the overall pathogenesis manifested in NOD mice [69]. The emergence of novel immune-cell subsets such as T_{regs} and Th17 cells further questions the validity of such models [70,71].

Cytokine expression in the exocrine glands obtained from NOD mice has been analyzed [72,73]. In a later study, taking advantage of recent technological developments, more comprehensive sets of inflammatory mediators were analyzed in serum and saliva obtained from NOD mice [56]. Furthermore, blocking of either lymphotoxin β R or TNFR1 signaling has given insight into the implication of these two TNF family members in the development of the SS-like disease in NOD mice [48,74]. Whereas lymphotoxin β R signaling appears to affect the degree and cellular composition of salivary gland inflammation [48], inhibition of TNFR1 engagement has been suggested to exacerbate the manifestation of hyposalivation [74]. In an earlier study, however, transgenic overexpression of TNFR1 inhibited exocrine gland inflammation [75]. Investigation of antibody-mediated inhibition of lymphocyte migration as a potential treatment strategy demonstrated that $\alpha_4\beta_1$ -integrin, leukocyte selectin and leukocyte function-associated

antigen 1 expression on lymphocytes and that vascular cell adhesion molecule 1 expression and peripheral node addressin on endothelial cells are required for lymphocyte homing to the lacrimal gland of NOD mice [76].

The functional roles of Th1 and Th2 cytokines in the pathogenesis of SS have been assessed in some detail by comparing a set of gene knockout mice: NOD-*Il4*^{-/-} [77], NOD.B10-*H2^b-Il4*^{-/-} [65], NOD.B10-*H2^b-C.Stat6*^{-/-} [66], NOD-*Ifny*^{-/-} and NOD-*Ifny^r*^{-/-} [78] mice. *Il4*^{-/-} NOD mice and *Stat6*^{-/-} NOD mice retain salivary secretion rates similar to Balb/c mice despite the fact that they continued to present with exocrine gland inflammation [65,66]. NOD-*Ifny*^{-/-} mice and NOD-*Ifny^r*^{-/-} mice were found to develop neither sialoadenitis or hyposalivation nor to present the signs of delayed salivary gland organogenesis present in the salivary gland of the parental NOD strain [60,78]. Of note, the mononuclear cell infiltrates within the lacrimal glands persisted in these two latter strains [78]. Results regarding the more recently described Th17-cell subset suggest that the Th17/IL-23 system is activated in a NOD-derived strain during the overt state of the disease [79]. Interestingly, local IL-17A expression as a result of adenovirus vector-associated *Il17a* delivery to the salivary gland of SS nonsusceptible C57BL/6 mice recapitulated to a large extent the SS-like disease phenotype described in the NOD strain [80]. Subsequent investigation of IL-17 as a therapeutic target at different disease stages showed that gene therapy-induced inhibition of IL-17, through expression of its receptor in the salivary gland, had the capacity to significantly reduce several important features of the SS-like disease, including salivary gland inflammation and severity of hyposalivation [81].

To investigate the importance of specific gene regions with regard to SS-like disease manifestations, NOD-specific genetic loci were introduced into either a C57BL/6 background [41] or a C57BL/10 background [82]. For both strains, gene expression of the salivary gland tissues was compared with their respective parental strain [54,82,83]. Unfortunately, the C57BL/10-based model termed B10.Q-*Nss1/Idd5* has not been assessed for salivary gland hypofunction [82].

The principal aim for the development of the C57BL/6-based model named C57BL/6.NOD-*Aec1Aec2* was primarily to circumnavigate three problems associated with its parental NOD strain: the known impact of overt T1D on the physiological process of saliva and tear secretion as well as the possible interference of T1D, overt or asymptomatic, with biological readouts obtained from the NOD strain; the fact that there is no appropriate comparative nondiseased control strain for NOD mice; and the presence of a multitude of immune system-associated defects in the NOD strain [44].

The genes within the genetic regions designated *Aec1* (*Idd5* on chromosome 1) and *Aec2* (*Idd3* on chromosome 3) appear sufficient for the manifestation of a SS-like disease phenotype comparable with the one manifested in NOD mice [41]. First steps towards fine-mapping of *Aec2* were undertaken with the purpose of identifying candidate genes potentially regulating SS-associated autoimmunity [84]. Nevertheless, although considered nonsusceptible to the development of a SS-like disease, the genomes of C57BL/6) or C57BL/10 might still contribute to the congenic strain's disease phenotype by enhancing the primary effects introduced by the congenic regions [85]. Such phenomena render it more difficult to discriminate between disease-causing and disease-promoting gene segments. In addition, the two recipient strains may develop spontaneous sialoadenitis as they age [82,86]. The improved applicability of the C57BL/6.NOD-*Aec1Aec2* strain compared with the original NOD mice, however, facilitated the study of proteases in the initiation phase of the disease [87], a more distinct delineation of the salivary and lacrimal gland transcriptome prior to and during the onset of the SS-like disease [53,54], as well as assessment of a potential role of complement 3 in SS [88].

Gene knockout and transgenic models

Genetic modifications have been shown to trigger different aspects of the SS-like disease in murine models. The fact that silencing or overexpression of a single gene can result in a disease profile reminiscent of SS points to pathways downstream of this particular gene. These pathways are commonly associated with either regulating the immune response, governing developmental processes or contributing to exocrine gland homeostasis. A discussion of gene knockout and transgenic models of SS follows (Tables 1 to 3).

IL-2-deficient, IL-2R α -deficient and forkhead box P3-deficient mice

The first indication that IL-2 activities are diminished in NOD mice was first reported in 1993 [89] and was later found to be associated with *Idd3* [90]. Today, IL-2 is recognized as a critical factor in promoting differentiation and activation of T_{regs}. Concordantly, inhibition of circulating IL-2 led to aggravation of diverse autoimmune manifestations in NOD mice [45], and both IL-2-deficient and IL-2R α -deficient C57BL/6 mice present with exocrine gland inflammation and hyposalivation [91,92]. The SS-like disease in the two strains develops, however, secondary to a generalized lymphoproliferative disease characterized by autoimmune hemolytic anemia and inflammatory bowel disease [93]. Nevertheless, data collected on IL-2 in a SS-related context indicate that in conditions with decreased regulatory cell populations the salivary glands are prone to exhibit autoimmune

Table 3. Extrinsic factor-induced models of Sjögren's syndrome

Strain	Extrinsic factor	SS-like disease manifestations	Remarks	Reference
BALB/c	Ro amino acids 480 to 494	SG infl., ↓ SG function, anti-Ro and anti-La	Low disease penetrance rate	[136]
	Ro amino acids 274 to 290	SG infl., ↓ SG function, anti-Ro and anti-La	Low disease penetrance rate	[136]
C57BL/6- <i>Rag1</i> ^{-/-}	Six peptides from M3R	SG and LG infl., ↓ SG function	Splenocytes from vaccinated <i>M3R</i> ^{-/-} mice	[140]
PL/J	CAII	SG infl.	Inflammatory lesions in multiple organs	[142]
C57BL/6- <i>gld/gld</i>	MCMV	SG infl., anti-Ro and anti-La	Incomplete clearance of the MCMV	[144]

Selection of murine strains in which Sjögren's syndrome (SS)-like disease manifestations develop after the administration of specific extrinsic factors. ↑, increased; ↓, decreased; CAII, carbonic anhydrase II; infl., inflammation; LG, lacrimal gland; M3R, muscarinic acetylcholine type-3 receptor; MCMV, murine cytomegalovirus; SG, salivary gland.

manifestations. Inconsistently, however, mice that carry the forkhead box P3 (*Foxp3*)^{sf} mutant gene and are therefore deficient for Foxp3⁺-positive T_{regs} remain free of glandular inflammation as long as they are not exposed to lipopolysaccharide [94]. Nonetheless, cells isolated from their lymph nodes had the capacity to induce sialoadenitis in immunocompromised, recombination activating gene-1-deficient recipient mice [94].

IL-10 transgenic mice

Overexpression of IL-10 in C57BL/6J mice provokes progressive histopathology and hyposalivation suggestive of SS [95]. *Il10* transfer into NOD mice, however, partially suppressed the appearance of SS-like features [96] – indicating a dual role of IL-10 in SS, most probably dependent on temporal or site-specific expression patterns of IL-10.

IL-12 transgenic mice

The influence of IL-12 is considerably clearer. Both CBA [97] and SJL [98] mice transgenic for *Il12* exhibit focal inflammation within their exocrine glands, with the latter strain showing an additional array of SS-related manifestations, including hyposalivation and modest increases in autoantibody levels upon aging [98]. In this context, it should be noted that SJL mice are generally susceptible to pathogenic autoimmunity and are also highly prone to develop B-cell lymphoma [99]. In an earlier study, concurrent intraperitoneal injection of IL-12 and IL-18 triggered severe atrophy of the salivary and lacrimal glands of various strains [100]. These degenerative changes in glandular tissues, however, occurred without lymphocytes concomitantly infiltrating the affected glands [100].

IL-14α transgenic mice

By promoting expansion and activation of specific B-cell subsets, *Il14α* transgenic C57BL/6 mice present with hypergammaglobulinemia by 3 months of age, with exocrine gland inflammation by 6 months of age [101,102]. In addition, this strain develops immune-complex-mediated nephritis, as well as exhibiting a high incidence of CD5⁺ B-cell lymphoma [101]. Strengthening the relevance of this model, a recent study demonstrated a

strong dependence of the SS-like disease manifestations on local expression of lymphotoxin α, a molecule crucial for the maintenance of organized lymphoid micro-environments in target tissues of autoimmune diseases [103]. *Il14α* transgenic mice deficient for lymphotoxin α retained normal saliva secretion and presented no signs of salivary gland inflammation or secondary lymphoma development [104]. This strain also no longer presented the disproportionately large CD5⁺ B-cell compartment, characteristic for mice overexpressing *Il14α* [104].

B-cell-activating factor transgenic mice

B-cell-activating factor (BAFF), also known as B-lymphocyte stimulator, has emerged as a critical regulator of B-cell survival and maturation, demonstrating the need for an obligate survival signal for both maturing and fully differentiated B cells [105]. Excess BAFF-mediated survival signals are thought to aid autoreactive B cells to escape apoptosis, to expand and, subsequently, to exert their potentially pathogenic activities [105].

BAFF received considerable attention following development of *Baff* transgenic strains, of which one was shown to develop features reminiscent of SS, including lymphoid infiltrates in the salivary and lacrimal glands and hyposalivation [106]. The manifestation of the SS-like disease thereby critically depends on B cells with a marginal zone B-cell like phenotype, which are the dominant lymphocyte population infiltrating the salivary glands from this strain [107]. Despite the high numbers of B cells, anti-Ro antibodies or anti-La antibodies were not detected. Strains overexpressing BAFF also develop severe autoimmune manifestations traditionally associated with SLE, including circulating immune complexes, anti-DNA antibodies and immunoglobulin deposition in the kidneys [108,109]. Disruption of *Tnfa* in *Baff* transgenic mice furthermore revealed a critical role of the anti-tumor activity of TNFα in this strain [110].

Transforming growth factor beta 1 transgenic and deficient mice

Transforming growth factor (TGF) beta1 is a multifunctional molecule that has effects on many developmental, physiological and immunological processes. Animals

carrying a mutated *Tgfb1* allele present a syndrome marked by mixed inflammatory cell responses and tissue necrosis, in many cases leading to organ failure and death [111]. In surviving mice, the syndrome includes inflammation of the exocrine glands in a large share of the animals that can, however, be prevented by systemic injections of synthetic fibronectin peptides [112]. Mice overexpressing TGF β 1 in the secretory cells of both the mammary and salivary glands exhibit impaired salivary gland architecture concomitant with salivary gland hypofunction [113]. It is important to note that altered TGF β 1 expression in mice results in poor viability and surviving mice suffer from hyposalivation in association with inflammation, acinar cell atrophy and fibrosis in the salivary glands [111-113].

Adaptor molecule Act-1-deficient mice

As a negative regulator of BAFF and CD40, the adaptor molecule Act 1 (Act1) crucially modulates the survival of all B cells [114]. In Act1-deficient mice, similar to mice transgenic for BAFF, marginal-zone-like B cells dominate the inflammation in the exocrine gland that develops around 6 months of age [115]. At 8 months of age, levels of salivary secretion appear to be slightly diminished, while indications for dry eyes – such as scratching and skin lesions around the eyes – were observed during the breeding process as early as 3 weeks postpartum [115]. Another shared trait between the Act1-deficient strain and *Baff* transgenic mice is the production of SLE-associated anti-DNA autoantibodies and the manifestation of glomerulonephritis [115]. In contrast with *Baff* transgenic mice [106], however, deficiency in Act1 causes the production of autoantibodies specific for Ro and La [115]. Further investigation of this phenomenon revealed that, compared with the other autoantibody specificities found in these mice, the production of anti-Ro autoantibodies and anti-La autoantibodies critically depends on functional CD40. As Act1 was more recently also identified as a critical signaling component of the IL-17 signaling pathway, this aspect needs to be addressed in further studies [114].

Thrombospondin-1-deficient mice

A recent study showed that silencing the thrombospondin 1 gene (*Thbs1*) in C57BL/6 mice causes the development of a severe and remarkably complete SS-like disease with respect to the involvement of the eye [116]. Increased apoptosis in the lacrimal glands accompanies their progressive deterioration and, in addition, anti-Ro autoantibodies and anti-La autoantibodies were detected in this strain [116]. Some THBS1-deficient mice also displayed external signs of dry eyes, although the tear volume secreted upon stimulation did not differ between the genetically modified mice and the wild-type strain

[116]. Although a few parameters of lacrimal gland function significantly decreased before the significant influx of inflammatory cells into the glands, the authors argue for a critical involvement of the immune system, in particular the Th17 system, in the observed pathology [116].

THBS1 is capable of activating latent TGF β and, as alluded to previously, dysregulation of the TGF β system affects the immune system as well as multiple developmental processes. Although less devastating compared with direct deletion of *Tgfb1*, THBS1-deficient mice exhibit a similar inflammatory condition and display histological abnormalities described for TGF β -deficient mice [117]. Being a multidomain matrix glycoprotein capable of interacting with multiple cell adhesion molecules and proteases involved in angiogenesis, it is not surprising that THBS1 plays important roles in the development of diverse tissues [118]. Taking into account these properties of THBS1, investigating immune system-unrelated alterations in the exocrine tissues of *Thbs1*-deficient mice would further clarify the etiology of the disease they manifest.

Aromatase-deficient mice

The high female predominance and the late onset of SS in humans suggest a possible role of estrogen in the etiology of SS. Whereas neither estrogen receptor-alpha-deficient nor estrogen receptor-beta-deficient strains exhibit SS-like disease manifestations, another model for estrogen deficiency – the aromatase knockout mouse – develops a lymphoproliferative condition that in some aspects resembles the histopathological manifestation of SS in the salivary glands [119]. In parallel with sialoadenitis, B-cell-dominated inflammation of the kidneys and enlargement of the spleen were also reported for this strain [119].

Retinoblastoma-associated protein 48 transgenic mice

Estrogen deficiency caused by ovariectomy was shown to increase the number of epithelial cells undergoing apoptosis in the salivary glands as well as to have an effect on the cleavage of structural proteins [120]. The observation that retinoblastoma-associated protein 48 (RbAp48) contributes to the observed estrogen-dependent modulation of apoptosis exclusively in the salivary glands gave reason to investigate its role in a strain overexpressing RbAp48 under the control of a salivary gland specific promoter [121,122]. From 20 weeks of age onwards, *RbAp48* transgenic mice exhibit inflammatory lesions in the salivary and lacrimal glands, which comprise mostly T cells and fewer B cells. At 30 weeks of age, salivary gland function was shown to be impaired in the transgenic mice compared with the wild-type strain. Increased levels of anti-Ro autoantibodies and anti-La autoantibodies complemented the SS-like phenotype observed in *RbAp48* transgenic mice [122]. The investigators also

collected considerable data that suggest a dominant role of resident cells in the initiation and perpetuation of the disease, especially by contributing to MHC-II-dependent antigen presentation and modulation of the cytokine milieu [122].

Id3-deficient mice

Inhibitors of DNA binding (ID) proteins are inhibitors of basic helix–loop–helix transcription factors and act as regulators of proliferation and differentiation of immune and non-immune cells [123]. The immune system of C57BL/6-*Id3*^{-/-} mice is characterized by alteration in humoral immune reactions, marginal zone B-cell development, B-cell precursor survival and both MHC-I-restricted and MHC-II-restricted positive and negative selection [124]. In these mice, T-cell-dominated focal inflammation develops between 6 and 12 months of age, coinciding with anti-Ro autoantibody and anti-La autoantibody production. The same strain, however, shows severe exocrine gland dysfunction as early as between 6 and 18 weeks of age – long before the appearance of focal lymphocytic foci in their exocrine glands [125]. Nevertheless, the notion that disruption of certain genes triggers distinct pathological changes, almost exclusively limited to the salivary and lacrimal glands, may encourage further investigation of possible interrelationships between organ and lymphocyte development and the etiology of autoimmune diseases. In this context, investigation of a T-cell-targeted conditional *Id3* knockout strain revealed the strong dependence of the original C57BL/6-*Id3*^{-/-} strain's disease profile on ID3-deficient T cells in particular [126]. Interestingly, depletion of B cells ameliorated the SS-like disease in this strain [127]. The role of ID3 in SS is now under investigation in humans, but to date there are no SNPs in *Id3* associated with SS in humans [128].

Phosphoinositide 3 kinase class-IA-deficient mice

There is an abundance of information that implicates phosphoinositide 3 kinase class IA in the development and onset of autoimmune diseases [129]. Mice with a T-cell-specific loss of phosphoinositide 3 kinase class IA (r1ΔT/r2n) develop an inflammatory condition reminiscent of SS in the lacrimal glands that parallels the occurrence of inflammatory lesions in the lungs, liver and intestines in these mice [130]. While exocrine gland function was not assessed in detail, the authors report a decrease in T_{regs} in the periphery and increased anti-Ro antibodies and anti-La antibodies as a result of this specific genetic modification.

Knockin mice with mutated κB enhancers in the *IkBa* promoter

Aberrant regulation of nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) has been

associated with inflammatory and autoimmune diseases since its crucial role in both innate and adaptive immunity was reported [131]. Among other autoimmune conditions, certain polymorphisms in the promoter of NF-κB inhibitor nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (*IkBa*) might contribute to an individual's susceptibility to develop SS [132]. Removal of the feedback regulation of NF-κB by introducing κB enhancers in the *IkBa* (*IkBa*^{M/M}) promoter of C57BL/6 mice altered the expression of NF-κB-associated genes such as *Il17* and genes involved in T-cell development [133]. Subsequently, inflammation in the exocrine glands, concomitant with production of anti-Ro autoantibodies, anti-La autoantibodies and anti-DNA autoantibodies, were observed in these mice without, however, assessing exocrine gland function [133]. The overall phenotype of this strain, in addition, is characterized by involvement of various other organs, shortened lifespan and hypersensitivity to septic shock [133].

Extrinsic factor-induced models

For the strains described earlier in this manuscript, the etiology of SS-like disease manifestations is assumed to be, to a large extent, associated with the mouse's specific genetic background. The experimental models presented below are strains in which the development of a SS-like pathology requires administration of extrinsic factors, such as proteins and peptides or viruses (Table 3). Such protocols are based on the concept that injecting specific components emulsified in an adjuvant can break immunological tolerance to certain organ-specific or organ-unspecific structures. This event might subsequently be followed by an immune-system-initiated pathogenesis. The components injected in such studies are mostly selected on the basis of their suspected role in the disease of interest. As alluded to previously, unfortunately, the current knowledge about disease-relevant autoantigens in SS is limited – which might be one reason why induced models are not of equal importance in SS as, for example, in studying multiple sclerosis [134] or rheumatoid arthritis [135].

Ro peptides

Repeated intraperitoneal injection of the Ro peptides – Ro amino acids 480 to 494 or Ro amino acids 274 to 290 – emulsified in complete Freund's adjuvant and later in Freund's incomplete adjuvant has been shown to recapitulate some manifestations of SS in Balb/c mice [136]. These mice present with hyposalivation, SS-like histopathology and production of anti-Ro antibodies and anti-La antibodies by 38 weeks of age [136]. Unfortunately, the actual penetrance rate of the SS-like disease proved to be low, thereby limiting the potential value of the model [136]. Oral feeding of Ro or Ro peptides

abolished the susceptibility of Balb/c mice to SS-like disease induction through the experimental procedure described above [137]. While these studies were designed to determine whether Ro, as an autoantigen, is important in the etiology of SS, there is still a question as to how Ro might actually be presented to the immune system [138]. In light of a recent study indicating that Ro52 is a negative regulator of proinflammatory cytokine production [139], if and how these newly described properties of Ro52 contribute to SS remain to be investigated.

Muscarinic acetylcholine type-3 receptor peptides

As alluded to above, antibodies targeting the M3R may directly mediate the inhibition of exocrine gland secretion by inhibiting neuronal innervation of acinar cells. A recent study assessed the question further by vaccinating C57BL/6-*M3R*^{-/-} mice with a six-valent mixture of free-form extracellular peptides of M3R [140]. Indeed, the inoculation of splenocytes or CD3⁺ T cells into immunodeficient C57BL/6-*Rag1*^{-/-} mice triggered the development of marked mononuclear cell inflammation in the exocrine glands accompanied by salivary gland hypofunction [140]. This study further supports the notion of a direct pathogenic role of anti-M3R immunity in SS [10].

Carbonic anhydrase

A subset of patients with autoimmune diseases, including patients with SS, produces autoantibodies against carbonic anhydrase II [141]. Studies carried out in mice revealed that experimental sialoadenitis can be induced through carbonic anhydrase II immunization of PL/J mice [142] as well as congenic strains of PL/J mice carrying the a H2^d or a H2^u haplotype [142]. Additional studies are required, however, to be able to estimate in more detail the resemblance of the disease manifested in this model with SS in humans.

Murine cytomegalovirus

Intraperitoneal injection of murine cytomegalovirus has been documented to lead to sialoadenitis and production of anti-Ro autoantibodies and anti-La autoantibodies in genetically modified C57BL/6 mice [143]. The modifications, affecting either FAS-mediated or TNFR1-mediated apoptosis, resulted in an incomplete clearance of murine cytomegalovirus, suggesting that any defect in this response may evoke chronic inflammation that resembles the histopathological changes characteristic for SS [143]. In a subsequent study, C57BL/6-*gld/gld* mice, which are *Fas* ligand deficient, were treated with an adenoviral viral vector inducing the overexpression of *Fas* ligand [144]. In light of high levels of *Fas* ligand expression following injection of the vector, fewer than 5% of ductal and acinar cells proved to be apoptotic. Nevertheless, the intervention caused significant reductions in the number

of inflammatory foci and the degree of tissue destruction in the salivary glands [144].

Conclusions

SS is a complex autoimmune exocrinopathy that over time often progresses to a systemic disease. Interpatient heterogeneity is a major component of this rheumatic disease, as evidenced by the array of symptoms exhibited by patients at clinic visits. Although numerous mouse strains are being proposed as models of SS, it is not surprising that no single model can perfectly match the full spectrum of SS observed in a human population. In the present review, we describe how the genetic background of these models and intervention protocols modulated the disease profile they project. Individual genetic alterations and their contribution to different disease stages and specific manifestations of SS might one day be assembled to depict a more complete and integrated picture of SS.

Today researchers are presented with several alternatives regarding spontaneous and genetically modified models of SS: yet, because of the complexity of SS, additional models will undoubtedly be required. Unfortunately, the limited knowledge about SS disease-relevant autoantigens and SS-related genetic risk factors continues to impede the development of extrinsic factor-induced models of SS.

In recent years there has been a positive trend towards testing hypotheses through genetic modification or intervention protocols in established models of SS. Results from these studies have often yielded insight into mechanisms potentially associated with the pathology of SS. The identification of B-cell-dependent mechanisms of pathogenesis in murine models, coupled with investigation of treatment strategies such as anti-CD20 antibodies targeting B cells in patients with SS, exemplify such translational advances. Nevertheless, it has become ever more challenging to keep pace with the developments in immunology and to be able to, at least partly, assess the importance of newly discovered components such as novel immune-cell subsets or regulatory pathways in a SS-related context. An assortment of well-characterized murine strains is needed in order to investigate possible roles of these components at the different stages of SS. In the past few years, there has also been an increase in the number of studies assessing the role of possibly relevant and immune system-unrelated processes in the etiology of SS. Again depending greatly on animal models, such research initiatives are expected to yield an increasing number of relevant biomarkers, which may specify an individual's risk of developing SS or may indicate an early stage of disease.

It also has become clear that murine strains, in some cases, represent a stereotypic or incomplete picture of

their human disease counterpart. To counteract this issue, in-depth characterization of individual models as well as reliance on results obtained in multiple models is, however, anticipated to increase the success rate of translational studies.

In conclusion, many advances in the field of SS have their basis founded in discoveries initially made in animal models. Improved collaboration among scientists that develop animal models, researchers that apply animal models to investigate SS-related aims and clinicians that have access to well-defined SS patient cohorts should accelerate the discovery of novel disease mechanisms that lead to development of effective treatment regimens.

Autoimmune Basis of Rheumatic Diseases

This article is part of a series on *Sjögren's syndrome*, edited by Thomas Dörner, which can be found online at <http://arthritis-research.com/series/Sjögrens>

This series forms part of a special collection of reviews covering major autoimmune rheumatic diseases, available at: <http://arthritis-research.com/series/abrd>

Abbreviations

Act1, adaptor molecule Act 1; BAFF, B-cell-activating factor; dsRNA, double-strand RNA; E2F1, E2F transcription factor 1; ID, inhibitors of DNA binding; IFN, interferon; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; IL, interleukin; M3R, muscarinic acetylcholine type-3 receptor; MHC, major histocompatibility complex; NOD, nonobese diabetic mice; NF, nuclear factor; RbAp48, retinoblastoma-associated protein 48; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; SS, Sjögren's syndrome; STAT, signal transducer and activator of transcription; T1D, type 1 diabetes; TGF, transforming growth factor; Th, T-helper type; THBS1, thrombospondin 1; TNF, tumor necrosis factor; T_{reg}, regulatory T cell.

Competing interests

The authors declare that they have no competing interests.

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