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Animal Models in Autoimmune Diseases: Lessons Learned from Mouse Models for Sjögren's Syndrome

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Abstract

The mouse model is the one of the most frequently used and well-established animal models, and is currently used in many research areas. To date, various mouse models have been utilized to elucidate underlying causes of multi-factorial autoimmune conditions, including pathological immune components and specific signaling pathways. This review summarizes the more recent mouse models for Sjögren's syndrome, a systemic autoimmune disease characterized by lymphocytic infiltration in the exocrine glands, such as the salivary and lacrimal glands, and loss of secretory function, resulting in dry mouth and dry eyes in patients. Although, every Sjögren's syndrome mouse model resembles the major symptoms or phenotypes of Sjögren's syndrome conditions in humans, the characteristics of each model are variable. Moreover, to date there is no single mouse model that can completely replicate the human conditions. However, unique features of each mouse model provide insights into the roles of potential etiological and immunological factors in the development and progression of Sjögren's syndrome. Here, we will overview the Sjögren's syndrome mouse models. Lessons from these mouse models will aid us to understand underlying immune dysregulation in autoimmune diseases in general, and will guide us to direct future research towards appropriate diagnostic and therapeutic strategies to predict autoimmune disease including Sjögren's syndrome.

Keywords

Sjögren's Syndrome; mouse models; T/B lymphocytes; autoantibodies; autoimmunity

1. Introduction

Autoimmune diseases occur when our immune system attacks and destroys our own cells and tissues due to breakdown of tolerance. They are usually characterized by the presence of autoreactive T/B lymphocytes and autoantibodies. There are more than 80 clinically distinct autoimmune conditions, which include insulin dependent type 1 diabetes mellitus (T1D), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), and Sjögren's syndrome (SjS). Animal models provide insights into underlying pathologies, etiologies, and specific signaling pathways of human diseases because of their physiological similarities to humans and their shorter life span that allows the entire disease onset and

development to be studied within a relatively short amount of time. Although there are potential drawbacks in using animal models, such as incomplete disease manifestations or limited genetic similarities due to the heterogeneity of genetic background in humans, they have been an indispensable tool to understanding disease pathogenesis in autoimmune conditions. Among all of the animal models for human diseases, mouse models and other rodents such as rats and hamsters consist of more than 90% of the total number of animals used in research [1]. Spontaneous mouse models for autoimmune diseases have been generated through the crossing of mouse strains that have genetic susceptibility genes or loci followed by careful monitoring of these mice for the development of disease phenotype. For example, nonobese diabetic (NOD) mouse model and NZB/W F1 mouse model spontaneously develop T1D and SLE-like conditions, respectively. Since spontaneous mouse models are not always available for all human autoimmune conditions, extrinsic factors have been introduced to generate mice that resemble human conditions. The experimental autoimmune encephalomyelitis mouse generated by autoantigen injection for the study of multiple sclerosis is an example of an inducible model [2]. In addition, with recent developments in biotechnology and molecular biology, genetic manipulations, such as knock-outs, knock-ins, and transgenics, are commonly utilized to generate mouse models of interest.

To discuss the roles and importance of mouse models in understanding human autoimmune diseases, in this review, we will focus on SjS mouse models that exhibit a wide array of underlying disease pathogenesis for SjS-like disease manifestations. SjS is a chronic autoimmune condition, which primarily affects the salivary and lacrimal glands resulting in severe dry mouth and dry eye. Although, SjS has been studied for decades since it was first described by Henrik Sjögren in 1933, its pathogenic mechanisms and etiologies still remain obscure. Because every SjS patient exhibits different aspects of clinical symptoms and/or disease phenotypes, utilizing animal models that can resemble human SjS can be useful to understand the heterogeneity of SjS in humans. It has been reported that there are more than a dozen SjS-like mouse models that resemble human SjS. We will describe more recent SjS mouse models in this review with an emphasis on the salivary gland pathophysiology to explain how the mouse models have contributed to better understanding of SjS etiologies and underlying disease pathogenesis.

2. Mouse Models for Sjögren's Syndrome

One of the main characteristics of autoimmune diseases is their genetic inheritance, which is generally associated with MHC alleles or chromosomal locations [3, 4]. Although SjS is known to have a relatively weak familial tendency compared to other autoimmune conditions, susceptibility genes in certain mouse models are essential in SjS onset and disease progression.

2.1 Genetic predisposition

2.1.1 Nonobese Diabetic Mice (NOD)—The NOD mouse model is the most extensively studied animal model in research for human T1D and SjS. It is known that more than 20 chromosomal regions, defined as genetic intervals influencing susceptibility to diabetes (*Idd*), contribute to the development of T1D in NOD mice. In addition to diabetes, NOD mice spontaneously develop SjS-like disease phenotype, such as lymphocytic infiltrates in the salivary and the lacrimal glands and secretory dysfunction at approximately 12-16 weeks old [5, 6]. Delaleu et al. reported that critical factors in serum and saliva of NOD mice are associated with either salivary flow or glandular inflammation. These factors include: IL-10, interferon- γ (IFN- γ), IL-7, granulocyte macrophage-colony stimulating factor (GM-CSF), IL-17, IL-11, IL-5, and IL-18 [7]. Furthermore, blocking of intracellular adhesion molecule-1 (ICAM-1), which recruits leukocytes into inflammatory sites and is known to be

dysregulated in NOD mice, showed prevention or delayed SjS when the intervention was carried out prior to lymphocytic infiltration in the NOD mice [8]. A number of modified and/or intervened NOD mice, such as NOD-*Ifn* $\gamma^{-/-}$, NOD-*Ifn* $\gamma^{\text{tr}^{-/-}}$, and NOD-*IL4^{-/-}* mice, have also been utilized to investigate SjS pathogenesis or delineate roles of individual immunological factors in disease processes [9, 10].

2.1.2 C57BL/6.NOD-Aec1Aec2 Mice—Our previous studies have shown that of all the *Idds* screened, both *Idd3* and *Idd5* were involved in salivary and lacrimal gland dysfunction and required for the development of the SjS-like disease in the NOD mouse [11]. As a reverse approach, we introduced these two *Idd* loci into C57BL/6 mice by crossing C57BL/6.NODc3 mice carrying *Idd3* (*Aec1*, autoimmune exocrinopathy 1) locus with C57BL/6.NODc1t mice carrying *Idd5* (*Aec2*) locus. The newly generated strain was designated as the C57BL/6.NOD-*Aec1Aec2* mouse [12]. This strain shows SjS-like disease phenotype without developing T1D and exhibits altered underlying glandular homeostasis prior to disease onset and abnormal immunological responses with age [13]. Briefly, phase 1 (approximately 0-8 weeks old) is characterized by aberrant genetic and physiological activities, resulting in retarded gland development and increased acinar cell apoptosis. The mice start developing exocrine gland pathology with the appearance of leukocytic infiltrates in phase 2 (8-16 weeks old). Mice in phase 3 (older than 16 weeks old) exhibit clinical hallmark of SjS, loss of secretory function. Further analyses confirmed that *Idd3* and *Idd5* loci in C57BL/6.NOD-*Aec1Aec2* are responsible for the immunological aspects of the disease (expression of inflammatory cytokines, activated B- and T- lymphocytes, and production of autoantibodies) and pre-clinical aspects of the disease (increased apoptosis and altered protein secretions in exocrine glands), respectively [12, 14-19].

2.2 Environmental triggers and pathological injury

SjS is a multifactorial disease of which etiologies are not clear to date. However, the genetic and environmental factors, such as bacterial/viral infection, hormones, and pathological injury, among other factors, are known to play a role in triggering SjS development [20]. SjS mouse models induced by extrinsic factors have been intensively studied to identify and address critical roles of these factors in the etiology of SjS (Figure 1).

2.2.1 Murine cytomegalovirus (MCMV)—Viruses infecting the exocrine glands have been speculated as a trigger for SjS. It is thought that an environmental insult to the glands causes cell death in the context of producing danger signals, which initiates an autoimmune response to the glands in genetically predisposed individuals [21, 22]. Human cytomegalovirus (HCMV) infects the salivary glands and is preferentially replicated in the ductal epithelium of the glands, whereas murine cytomegalovirus (MCMV) replicates in the acinar cells of the glands long after it is cleared from other organs. MCMV infection of the salivary glands is not cytolytic, but induces a prolonged destructive immune response resulting in epithelial cell death [23, 24]. Thus, CMV dissemination in mice and humans involves viral replication in the salivary gland epithelium and consequent immune cell destruction of the glands. Depending on the individual's immune properties, cytomegalovirus can persist in the glands causing sub-threshold level of chronic infection or can remain latent with periodic reactivation.

In a new murine model of SjS, MCMV induced a chronic sialadenitis despite the absence of detectable infectious MCMV in Fas-mutant B6-*lpr/lpr* mice [25]. This chronic inflammation has been mainly mediated by T cells [26]. More interestingly, the levels of anti-La/SSB and anti-Ro/SSA autoantibodies in the sera of these mice were significantly increased with MCMV infection in comparison with the uninfected control or the C57BL/6 mice after MCMV infection [25]. In the process of chronic sialadenitis, infiltrating mononuclear cells

and acinar/ductal cells in the salivary glands showed an altered apoptosis pathway. Increased gene expression of anti-apoptotic Bcl-2 in infiltrating lymphocytes associated with resistance to Fas-induced apoptosis has been described in patients with SjS [27]. Therefore, a viral infection could trigger chronic inflammation in the target tissue, but the presence of infectious virus may not be required to maintain the inflammation. Local expression of FasL would be a useful experimental approach for SjS-like chronic sialadenitis mediated by altered apoptotic pathway [28].

2.2.2 Aromatase-deficiency Mice—Autoimmune conditions tend to be more prevalent in females than males in general, suggesting an important role of estrogen in autoimmune diseases [29]. Previous studies show that estrogen suppresses SjS-like disease in normal mice, whereas ovariectomy leads to a condition mimicking SjS [21]. In addition, estrogen can ameliorate T cell-mediated sialoadenitis and prevent cell death in the lacrimal gland [21]. As for estrogen receptors (ERs), ER- α -mRNA has been broadly detected in the murine salivary gland tissue and cultured human non-neoplastic salivary gland epithelial cells, but ER- β is the predominant ER subtype in human salivary glands [30-32]. However, no evidence of SjS has been observed in either ER- $\alpha^{-/-}$ or ER- $\beta^{-/-}$ mice. Interestingly, mice that are estrogen-free because of inactivation of aromatase gene (ArKO mice) develop SjS-like autoimmune exocrinopathy and occasional renal failure due to B cell dominated infiltration of the kidneys [33]. These results suggest that the roles of estrogen may have clinical value in the prevention or treatment of SjS [33].

In addition, a study reported that prominent retinoblastoma-associated protein (RbAp48) expression with increased apoptosis was observed in the exocrine glands of C57BL/6 ovariectomized (OVX) mice but not in OVX estrogen receptor(ER) $^{-/-}$, p53 $^{-/-}$, and E2F-1 $^{-/-}$ mice [34]. RbAp48, initially identified as a retinoblastoma-binding protein (Rb), is a multifunctional protein that binds to transcription factors and kinases to regulate both cell growth and apoptosis [34]. This indicates that prominent expression of RbAp48 induces tissue-specific apoptosis in the exocrine glands when estrogen is deficient [34]. Indeed, transgenic expression of the RbAp48 gene induced apoptosis in the exocrine glands but not in other organs in the RbAp48 Tg mice [35]. These findings indicate that estrogen deficiency initiates p53- mediated apoptosis in the exocrine gland cells through RbAp48 overexpression and exerts a possible gender-based risk of autoimmune exocrinopathy in women [36].

2.3 Autoreactive B cells

The clinical manifestations of SjS, such as hyposalivation and autoantibody production, are suggested to be mediated through disruptions in B cell differentiation, activation, and altered B cell tolerance. The following section describes how the various mouse models of SjS have elucidated the potential roles for B cells in the pathogenesis of SjS.

2.3.1 BAFF Transgenic Mice—The BAFF transgenic C57BL/6 (BAFF Tg) mouse has been utilized to show the critical regulation of B cell survival and maturation. The excess production of BAFF has been shown to lead to autoreactive B cell formation [37]. BAFF is a B cell survival factor required for B cell maturation and known to regulate B lymphocyte proliferation and survival. BAFF Tg initially develops an autoimmune condition similar to SLE where as they age they will develop secondary SjS that manifests as sialadenitis and destruction of salivary glands [37]. The likely cause for the autoimmune diseases in BAFF Tg mice is excess survival signals allowing autoreactive B cells to survive and mature. Especially the marginal zone B cell subset, which are the most similar in phenotype to the B cells infiltrating the salivary glands of BAFF Tg mice and may be directly involved in the tissue damage of SjS [38, 39]. The loss of marginal zone B cells, due to a deficiency of

lymphotoxin- β , in the BAFF Tg mice has been shown to prevent the development of sialadenitis as they age. However, these mice still develop nephritis associated with the presence of B1 B cells in the kidneys [39]. Overall, these experiments suggest that marginal zone B cells potentially participate in the tissue damage in salivary glands and coordinate the development of SjS. Interestingly, no detectable anti-Ro or -La autoantibodies were present in BAFF Tg mice [37].

2.3.2 Act1-Deficient Mice—The Act1-deficient BALB/c mouse model has been developed to elucidate its effects on B cell survival [40]. Act1 was found to be a crucial negative regulator of BAFF and CD40 [40]. Knockout of this molecule leads to a significant increase in the number of peripheral B cells and exhibits lymphoid system abnormalities such as lymphadenopathy, splenomegaly, hypergammaglobulinemia and autoantibody production [40]. In addition, these mice deficient in Act1 developed systemic autoimmune disease consistent with features of SjS and SLE nephritis [41]. For example, deficiency in Act1 caused production of SjS-associated anti-SSA/Ro and anti-SSB/La autoantibodies in addition to anti-DNA autoantibodies unlike in the BAFF Tg mice [41]. The utilization of double knockout mouse models Act1^{-/-}CD40^{-/-} and Act1^{-/-}BAFF^{-/-} revealed Act1 regulation of different stages of disease development through its distinct negative regulatory role on both CD40 and BAFF [41]. The regulatory influence of Act1 on the survival of autoreactive B cells appears to be primarily through its negative regulation of BAFF. Act1^{-/-}BAFF^{-/-} mice still had detectable anti-SSA/Ro and anti-SSB/La autoantibodies suggesting autoantibody production was not affected, but there was no increase in the B cell sub-populations that were observed in the Act1-deficient mice [41]. In contrast, the Act1 influence on the production of autoantibodies is likely mediated through its modulation of the CD40-mediated T cell dependent antibody response since Act1^{-/-}CD40^{-/-} mice prevented production of SjS-associated anti-SSA/Ro and anti-SSB/La autoantibodies. These mouse models are a strong indication of the distinct regulatory steps of autoimmunity and regulation of self tolerance in relation to SjS [40, 41].

2.3.3 IL-14 Transgenic Mice—IL-14 is a cytokine that was previously identified as a B cell growth factor [42]. In the area of altered B cell growth and development of autoimmunity, studies in the IL-14 α transgenic C57BL/6 mouse model (IL-14 Tg) have indicated hypergammaglobulinemia, autoantibody production, sialadenitis (as in SjS), immune complex mediated nephritis (as in SLE nephritis), as well as, a higher incidence of CD5⁺ B cell lymphomas (consistent with later stages of SjS) [43, 44]. This model reproduces several critical features of human SjS, supporting the influence of IL-14 in the development of autoimmunity in general, as well as, providing a link between SLE and SjS. Further analyses of this mouse model have indicated a strong dependence of SjS on the local expression of lymphotoxin-alpha (LTA), where the loss of LTA retains normal saliva secretion [45]. The IL-14 Tg mice were shown to express significantly elevated levels of LTA mRNA and soluble protein in their salivary glands compared to C57BL/6 controls. Crossing of the IL-14 Tg with LTA^{-/-} retained normal salivary gland secretions, and did not develop either lymphocytic infiltration of salivary glands or secondary lymphomas [45]. The IL-14 Tg LTA^{-/-} mice had essentially no change in B cell function or deposition of IgM autoantibodies in their salivary glands. In addition, the autoantibody deposition alone was not sufficient to produce salivary gland dysfunction. This suggests that local production of LTA in the salivary glands of IL-14 Tg mice is necessary for the initiation and progressive development of overt SjS [45].

2.3.4 NOD-modified Mice—The NOD mouse model, as described previously, is a standard mouse model of autoimmune T1D and SjS. Previous genomic microarray analyses of submandibular glands from the NOD derived double congenic C57BL/6.NOD-*Aec1Aec2*

mouse model of SjS have supported the association of B cell activation during full-blown disease onset with hyposalivation and the decrease of lacrimation [46]. This evidence supports previous microarray analyses of human minor salivary gland biopsies showing a general increase in B cell recruitment and activation in primary SjS patients [47]. Another NOD-related mouse model, the NOD-*Igμ*^{-/-}, was utilized to directly study the pathogenicity of B cells to the development of SjS [48]. The NOD-*Igμ*^{-/-} mice lack functional B lymphocytes and exhibits the focal infiltration of T lymphocytes in the submandibular and lachrymal glands as seen in the NOD mice, however, these mice fail to lose their stimulated secretory function [48]. Since the loss of functional B cells prevented disease progression to secretory dysfunction, the authors subsequently tested whether B cell autoantibodies produced in the SjS condition were the mediators of the secretory dysfunction [48]. Infusion of either parental NOD or human primary SjS patient serum IgG or F(ab')₂ fragments into the NOD-*Igμ*^{-/-} mice led to decreased stimulated saliva production in comparison to healthy control serum IgG [48]. Conclusions from these experiments suggest that autoantibodies present in SjS bind to cellular receptors, subsequently inhibiting the binding of agonists to the receptors and preventing the stimulation of secretion.

In addition, knockout studies of IL-4 (NOD.*IL4*^{-/-} and NOD.B10-*H2^b.IL4*^{-/-}) and STAT6 (NOD.B10-*H2^b.C-Stat6*^{-/-}) in NOD mice showed normal salivation in the absence of anti-muscarinic acetylcholine type-3 receptor (M3R) IgG1 while still exhibiting leukocytic infiltration of exocrine glands and production of anti-nuclear autoantibodies [49]. Both IL-4 and STAT6 knockout mice were unable to synthesize IgG1 Ab, which correlates with an inability to develop to the end-stage of SjS. Furthermore, the IgG fraction of NOD.B10-*H2^b.C-Stat6*^{-/-} sera was unable to induce glandular dysfunction when introduced into naive recipient C57BL/6 mice. This suggests a role for these genes in the differentiation to T_H2, the expression of cell surface markers and the class switching of immunoglobulins. In addition, it implies a requirement for IL-4 -STAT6 pathway for the onset of the clinical phase of SjS at least in the NOD mouse model [49].

2.4 Production of autoantibodies

SjS, like other autoimmune diseases, is typically accompanied by the production of autoantibodies. In humans with SjS, some of these autoantibodies observed include: anti-SSA/Ro 52 kDa, anti-SSA/Ro 60 kDa, anti-SSB/La, rheumatoid factor, anti-α-fodrin, anti-muscarinic type 3 (M3R) receptor, and anti-M1R antibodies [50]. The presence of these autoantibodies has been suggested to have an active participation in the SjS disease process through their interactions with critical processes. Of the mentioned autoantibodies and their autoantigens, Ro and M3R autoantigens have shown strong associations to the loss of secretory capacity in experiments involving peptide immunization and in knockout mouse models [51-53]. The association of Ro and M3R as autoantigens for the loss of salivation and salivary gland injury in SjS will be discussed in the following section.

2.4.1 Ro Immunization—Immunization experiments in mice have yielded important information as to the potential relationship between autoantibodies and the autoimmune SjS condition. BALB/c mice immunized with short peptides from 60-kDa Ro antigen resulted in an immune response against the entire Ro/La ribonucleoprotein, which is a similar in reactivity to what is found in humans with SLE or SjS [51]. In addition, functional studies showed a decrease in salivary flow and lymphocytic infiltration in the salivary glands of immunized animals when compared to controls [51]. Further studies into this experimental SjS phenomenon showed that oral feeding of Ro60 peptide or Ro60 autoantigen to BALB/c mice that were then later immunized with Ro60 274-289 peptide could prevent the susceptibility to experimental-induced SjS autoimmune disease and epitope spreading, presumably, via induction of immune tolerance [54]. The increased presentation of self

peptides to the immune system as a result of apoptosis has been tested in vitro, where Ro autoantigens are redistributed during apoptosis leading to surface exposure [55]. In a different approach to understanding the Ro autoantigen, the loss of Ro52 in Ro52^{-/-} mice has been shown to lead to enhanced production of proinflammatory cytokines [56]. This research indicates that defects in Ro52-mediated pathway may potentially contribute to tissue inflammation and systemic autoimmunity [56].

2.4.2 Muscarinic Type 3 Receptor (M3R) Peptide—There is considerable evidence supporting the presence of anti-muscarinic receptor autoantibodies that are potentially interacting with muscarinic acetylcholine receptors on the surface of acinar cells in SjS (Figure 1) [57]. These autoantibodies are of importance to the examination of SjS since they are believed to alter glandular secretion and contribute to hyposalivation [57]. The M3R^{-/-} mouse model was used to initially characterize the role of M3R in salivary glands, where induced saliva production was reduced to about 20% of normal production while no significant reduction was observed in M1R^{-/-} [53]. Interestingly M1R^{-/-} M3R^{-/-} double knockouts showed almost complete loss of secretion, indicating that M3R has a predominant role in secretion from the salivary glands [53]. The role of M3R as an autoantigen in the pathogenesis of SjS has been evaluated through the immunization of C57BL/6.M3R^{-/-} mice with M3R peptides, where subsequent transfer of these splenocytes into the C57BL/6.Rag^{-/-} mice were able to confer development of inflammation and glandular secretory hypofunction [52]. In addition, transfers of only CD3⁺ splenocytes into the C57BL/6.Rag^{-/-} mice resulted in cell infiltration and destruction of epithelial cells in the salivary glands, suggesting that M3R-reactive CD3⁺ T cells play a pathogenic role in the development of autoimmune sialoadenitis [52]. Overall, M3R as an autoantigen could play a role in the generation of the lymphocytic infiltration as well as glandular hypofunction via M3R reactive T cells, as well as, autoantibody production against M3R.

2.5 Autoreactive T cells

Abnormal activation and expansion of various T cell subsets that secrete pro-inflammatory cytokines is one of the key characteristics of SjS (Figure 1). Most prominent T cells in terms of SjS disease progression are known to be CD4⁺ T cells rather than CD8⁺ T cells [58]. For decades, imbalance between T_H1 cells mainly releasing IFN- γ and T_H2 cells mainly secreting IL-4 was believed to be the main pathogenesis of autoimmune diseases including SjS. Recently, the importance of IL-17A producing T_H17 cells has been brought to attention by numerous studies on SjS [59-61].

2.5.1 *Id3*-deficient mice—Inhibitor of differentiation 3 (*Id3*) is one of a transcriptional regulator that inhibits basic helix-loop-helix transcription factors and involved in both negative and positive regulations of cell growth and differentiation [62]. *Id3* is also known to play important roles in mediating T cell receptor signals during T cell lineage differentiation [63]. Interestingly, *Id3* germline knockout (C57BL/6-*Id3*^{-/-}) mice exhibited an autoimmune disease similar to SjS with alterations in the humoral immune reaction [64]. In addition, C57BL/6-*Id3*^{-/-} mice showed reduced tear and saliva secretion around two months of age prior to lymphocytic infiltration in the exocrine organs at around 6 months of age and anti-Ro and anti-La autoantibodies production at 12 months of age. In this model, secretory dysfunction appears to be independent of infiltrates in the target tissues or autoantibodies against Ro and La. Adoptive transfer of lymphocytes confirmed that autoreactive T cells were the main pathogenesis in this C57BL/6-*Id3*^{-/-} mice [65]. Furthermore, genetic ablation of T cells or neonatal 3 day thymectomy in *Id3*-deficient mice resulted in restoration of secretory function, which further confirmed that autoreactive T cells are of thymic origin [65]. T cell lineage specific *Id3* conditional knock mouse (and *Id3*^{fl/fl}; LckCre) showed similar phenotypic features to what was observed in *Id3* germline

knockout mouse model with the exception of anti-Ro or anti-La antibodies [66]. It has also been reported that depletion of B cells with CD20 antibody significantly improved histopathology and recovered saliva secretory function with reduction in IgG3 in the *Id3*-deficient mouse [67]. Findings from the studies above may support the idea that different subsets of immune cells play roles in different stages of SjS-like disease development (i.e., autoreactive T cells initiating the diseases at a young age as opposed to autoreactive B cells exacerbating the disease symptoms at an older age). Surprisingly, a clinical study with 212 Caucasian primary SS patients showed that no *Id3* were impaired in salivary glandular epithelial cells, peripheral T cells and in labial salivary glands. Moreover, in the same study, any *Id3*-relevant SNPs did not give evidence for genetic predisposition in primary SjS patients [68], underscoring the difference between disease in animal models and in humans.

2.5.2 PI3K Knockout Mice—Another molecule investigated in SjS is phosphoinositide 3-kinase (PI3K). PI3K is an enzyme lipid kinase that has been shown to regulate diverse aspects of lymphocyte behavior [69]. PI3K acts as an effective cell signaling molecule and generate 3-phosphorylated phosphoinositides that act as second messengers downstream of numerous cellular receptors. Moreover, PI3K is also activated downstream of many receptors, such as T cell receptor and CD28 that mediate proliferation and survival of T cells [70]. A mouse model with T cells lacking PI3K 1A exhibited a relatively inclusive organ specific SjS phenotype in the lacrimal glands with antinuclear antibodies and anti-SSA/Ro autoantibodies in the serum [71]. It has been reported that in B cells, class IA PI3K is the dominant subgroup whereas both class IA and IB PI3K contribute to development and immune function of T cells [72]. PI3K also regulates both chemokine responsiveness and antigen-driven changes in lymphocyte trafficking. In addition, PI3K modulates the function of not only of effector T cells, but also regulatory T cells [72]. This complexity of PI3K functions and its regulations can contribute to onset of unexpected autoimmune phenotypes in mice with PI3K-deficient T cells [72].

2.5.3 NOD-modified mice—It was believed in the past that SjS is a T_H1 -mediated disease because patients with SjS have high levels of IFN- γ in serum and IFN- γ mRNA expression in the salivary glands [73, 74]. Studies with NOD.IFN- $\gamma^{-/-}$ or NOD.IFN- $\gamma R^{-/-}$ revealed that IFN- γ is the critical cytokine for SjS-like disease development [10]. NOD.IFN- $\gamma^{-/-}$ or NOD.IFN- $\gamma R^{-/-}$ mice failed to develop any subsequent autoimmune responses, including acinar cell apoptosis and leukocyte infiltration against the salivary glands at clinical disease stage [10]. Although, NOD.*IL4*^{-/-} and NOD.B10-*H2b.IL4*^{-/-}, and NOD.B10-*H2b.C-Stat6*^{-/-} mice showed pathophysiological abnormalities and leukocyte infiltrations in the exocrine glands along with production of anti-nuclear autoantibodies, they prevented secretory dysfunction and failed to produce anti-M3R antibody as mentioned elsewhere in this review [9, 49, 75]. Adoptive transfer of T lymphocytes from NOD.B10-*H2b.Gfp* mice to NOD.B10-*H2b.IL4*^{-/-} mice confirmed that IL-4 plays an essential role during the clinical phase (manifested as loss of secretion), while having no or less effect on the preclinical disease state [75]. Overall, these studies indicate that IFN- γ plays a critical role in early disease stage while IL-4 is essential in the generation of secretory dysfunction in NOD mice.

T_H17 cell population is a subset of CD4⁺ effector T cells. Although, current research focuses on T_H17 cells for their pathogenic role in autoimmune diseases, knockout or transgenic mouse models are not available to date to study SjS pathogenesis. Nguyen *et al.* identified IL-17 secreting T_H17 cells within the lymphocytic foci in the salivary and lacrimal glands of C57BL/6.NOD-*Aec1Aec2* mice and human SjS patients [60]. Moreover, blocking IL-17 binding in salivary glands by injection of adenovirus serotype 5 (Ad5) vector expressing IL17R:Fc blocking vector (Ad5-IL17R:Fc) in early and late stage of C57BL/6.NOD-*Aec1Aec2* mice showed decreased lymphocytic infiltration, antinuclear antibodies

and increased saliva secretion [76]. In the reverse approach, Ad5-IL17 injection within salivary glands of SjS-non-susceptible C57BL/6 mice showed appearance of lymphocytic infiltration, increased pro-inflammatory cytokine levels, changes in antinuclear autoantibodies profile, and temporal loss of saliva secretion [77]. One possible mechanism is that increased IL-17 level at the clinical disease stage may effect the formation of lymphocytic infiltration in the salivary glands and the promotion of the autoreactive B cells. According to Hsu's study in 2008, T_H17 cells can drive autoimmune responses by promoting the formation of germinal centers in autoimmune BXD2 mice [78]. These studies demonstrate the importance of IL-17 secreting T_H17 cells as a major pathogenic player and potential therapeutic target for SjS.

3. Conclusion

SjS is a multifactorial and heterogeneous condition, which usually takes years for patients to be diagnosed. Moreover, pathogenesis and etiologies of SjS are also intricate in the sense that they encompass extrinsic and intrinsic factors, such as genetic, environmental, and abnormal immune regulation. Animal models, especially mouse models, have provided insights into the roles of these extrinsic and intrinsic factors and various underlying immune pathologies including innate and adaptive immune responses in SjS (Figure 1). In addition, mouse models with a single etiology for developing SjS-like disease manifestations can serve as a platform to invent diagnostic tools and novel therapies targeting that particular etiological factor. Therefore, these mouse models have been invaluable tools in SjS research. Because of the complexity of SjS in humans, it needs to be taken into consideration that one specific mouse model would neither reflect the complete disease profile of SjS in patients nor sufficiently address disease pathogenesis and etiology in humans. Some can explain genetic susceptibility, as in spontaneous mouse models, while others can pinpoint the pathological roles of molecules of interest in SjS onset and development. Nevertheless, lessons that we learned from the mouse models for SjS have been a corner stone for advancement in our understanding of disease pathogenesis and for applications in clinical trials.

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Figure 1.

Schematic illustration of etiopathogenic factors involved in Sjögren's syndrome. Mouse models are listed under the categories of etiological factors in this figure. A mouse model used to study one etiological factor does not necessarily exclude a possibility of involving other etiological factors. Factors of secretory dysfunction, such as immune cell infiltrates and anti-M3R autoantibodies, are included in this figure. Anti-M3R autoantibodies are known to block the binding site of receptor for acetylcholine, resulting in inhibition of fluid secretion from the acinar cells. ACh, acetylcholine; M3R, muscarinic type 3 receptor; Abs, autoantibodies; SMX, salivary glands

Table 1

Sjögren's Syndrome Mouse Models

Mouse Strain	Sjögren's syndrome (SjS)-like disease characteristics	Classification	Ref.
Nonobese Diabetic (NOD)	Type 1 diabetes (T1D) mouse model. Infiltration in the salivary and lacrimal glands. Loss of secretory function around at 12-16 weeks of age.	Secondary w/ T1D	[5, 6]
NOD- <i>Igμ</i> ^{-/-}	No functional B lymphocytes. Focal T cell infiltration in the submandibular and lacrimal glands. Normal secretory function.	-	[48]
NOD- <i>IL4</i> ^{-/-}	Unable to synthesize IgG1. Pathophysiological abnormalities in the exocrine glands with normal salivation.	-	[9, 49]
NOD- <i>IFNγ</i> ^{-/-} & NOD- <i>IFNγR</i> ^{-/-}	Lack of any subsequent autoimmune responses in the absence of acinar cell apoptosis in the salivary glands.	-	[10]
NOD.B10- <i>H2b</i>	Diabetogenic MHC replaced with H2b of C57BL/10. SjS-like condition without T1D.	Primary	
NOD.B10- <i>H2b.IL4</i> ^{-/-} ,	Pathophysiological abnormalities with anti-nuclear autoantibody production. Normal secretion in the absence of anti-M3R antibodies.	-	[49, 75]
NOD.B10- <i>H2b.C-Stat6</i> ^{-/-}	Unable to synthesize IgG1 antibody. Normal secretion.	-	[49]
C57BL/6- <i>Id3</i> ^{-/-}	<i>Id3</i> germline knockout mice exhibiting SjS-like phenotype. Reduced secretion between 6 and 18 weeks of age prior to lymphocytic infiltration in the exocrine glands at 6-12 months of age. Anti-Ro and anti-La antibody positive.	Primary	[64]
<i>Id3</i> ^{fl} ; LckCre	T cell lineage specific <i>Id3</i> conditional knock mouse. Similar to <i>Id3</i> germline knockout mice. Anti-Ro or anti-La antibody negative.	Primary	[66]
C57BL/6.NOD- <i>Aec1Aec2</i>	C57BL/6 with two NOD loci on chromosomes 1 and 3. SjS-like disease phenotype without T1D. Altered underlying glandular homeostasis prior to disease onset similar to NOD. Full-blown disease at 16 weeks of age.	Primary	[12, 13]
Murine cytomegalovirus (MCMV)	Virus-induced chronic sialadenitis. Infiltrating mononuclear cells and acinar/ductal cells in the glands exhibiting an altered apoptosis pathway. Clinical disease onset approximately at 4 weeks of age.	Secondary w/ SLE	[23, 24, 27]
Aromatase-deficient	Lack of estrogen due to inactivation of aromatase gene (ArKO). SjS-like autoimmune exocrinopathy and occasional renal failure due to B cell dominated infiltration of the kidneys.	Primary	[33]
<i>RbAp48</i> transgenic	Prominent expression of RbAp48 showing exocrine tissue-specific apoptosis in the absence of estrogen.	Primary	[34, 35]
<i>BAFF</i> transgenic	B cell activating factor transgenic mice. SLE phenotype followed by SjS-like exocrinopathy. Marginal zone B cells infiltrating in the salivary glands. Anti-Ro and anti-La negative.	Secondary w/ SLE	[37, 38, 39]
<i>Act1</i> -deficient	Act1, negative regulator of BAFF and CD40, knockout mice. Similar to BAFF transgenic mice except for anti-Ro and anti-La autoantibody production.	Secondary w/ SLE	[40, 41]
<i>IL-14α</i> transgenic	Hypergammaglobulinemia, exocrine gland inflammation, immune complex mediated nephritis, and a high incidence of CD5 ⁺ B cell lymphoma.	Secondary w/ SLE	[43, 44]
<i>Ro</i> immunization	Intraperitoneal injection of Ro peptides into Balb/c mice. Tolerance developed for Ro with oral feeding of Ro or Ro peptides. Decreased salivary flow and lymphocytic infiltration in the salivary glands.	Primary	[51, 56]
<i>M3R</i> immunization	M3R peptides immunization to C57BL/6-M3R ^{-/-} mice. Infiltration in the glands and hyposalivation with adoptive	Primary	[52, 53]

Mouse Strain	Sjögren's syndrome (SjS)-like disease characteristics	Classification	Ref.
	transfer of splenocytes or CD3+ T cells from the immunized mice into immunodeficient C57BL/6-Rag1 ^{-/-} mice.		
<i>PI3K class IA</i> knockout	T cell-specific loss of PI3K class IA leading to relatively inclusive organ specific SjS phenotype in the lacrimal glands with antinuclear antibodies and anti-SSA/Ro autoantibody production.	Primary	[71]