Animal models of Sjögren’s syndrome: an update

F. Xiao¹, M. Han², X. Wang¹, X. Gong², E. Huang¹, Z. Zhu³, F. Zhao³, Y. Zhao⁴, Q. Jiang², L. Lu¹

¹Department of Pathology and Shenzhen Institute of Research and Innovation, The University of Hong Kong; ²Division of Rheumatology, Guang’anmen Hospital, China Academy of Chinese Medical Sciences, Beijing; ³Department of Rheumatology and Immunology, Shanghai Ninth People’s Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai; ⁴Department of Rheumatology, Peking Union Medical College Hospital, Beijing, China.

Funding: see page 214.
Competing interests: none declared.

ABSTRACT

Sjögren’s syndrome (SS) is a chronic autoimmune disease characterised by lymphocytic infiltration in exocrine glands with secretory dysfunction. Although both environmental triggers and genetic predisposition have been recognised as important factors in the initiation and development of SS, the pathogenesis of SS is complex and still largely unclear. Animal models have served as useful tools for studying SS pathogenesis with several advantages. A number of animal models recapitulating key characteristics of primary SS patients including secretory dysfunction, glandular inflammation and presence of autoantibodies were developed in the past years. The studies based on the animal models of SS have provided significant insight in SS pathogenesis and therapeutic intervention. This review summarises current animal models with primary SS-like symptoms including spontaneous models, genetically modified models, induced models and humanised models, and discusses their contribution to the understanding of SS aetiology and therapies.

Introduction

Sjögren’s syndrome (SS) is a heterogeneous autoimmun disease characterised by lymphocytic infiltration in exocrine glands, predominantly salivary and lachrymal glands (LG), resulting in dry mouth and dry eyes (1). SS is also a systemic disease with the involvement of multiple extraglandular organs including lung, kidney, and skin (1, 2). SS may either occur alone as primary SS or exist in the presence of other autoimmune diseases as secondary SS (1). Glandular infiltration of lymphocytes and the presence of anti-SSA autoantibodies are key evidence for the diagnosis of SS while ocular problems and salivary hypofunction are also important criteria (3, 4).

Although SS has been studied for decades, the pathogenesis is still largely unclear. Both B cells and T cells activation as well as disturbed cytokine network in SS pathogenesis were demonstrated in clinical research and animal models studies (5-7). The predominant infiltration of CD4 T cells and B cells in salivary gland (SG) tissues of both SS patients and animal models with SS-like symptoms strongly suggested the involvement of dysregulated B and T cell responses in SS pathogenesis (6, 8). However, the exact roles of the immune populations in initiation and maintenance of SS are illusive. Increasing evidence suggests that both genetic predisposition and environmental triggers including virus infection are significant mediators in SS pathogenesis (5, 9). Clinical observations have provided important information on disease manifestations but can hardly delineate immunological and pathogenic changes before the occurrence of overt clinical signs (10). The studies based on SS animal models have the advantages of illustrating the whole disease spectrum before and after the appearance of disease symptoms, and bridging basic research and clinical observation. During recent years, a number of animal models with primary SS-like symptoms have been developed and those studies have provided new insight in the understanding of SS aetiologies (11-13).

This review summarises and discusses recent studies on animal models that resemble key features of primary SS patients, including spontaneous models, genetically modified models, induced models and humanised models. Current understanding and future perspectives of SS animal models studies are also discussed.

Spontaneous models

A number of mouse strains spontane-
ously develop SS-like phenotypes. The lupus-prone mice such as MRL/lpr and NZB/NZW F1 mice exhibited lymphocytic infiltration in SG tissues and were considered as secondary SS models (14, 15). Non-obese diabetic (NOD) mice with insulin-dependent diabetes also showed SS-like symptoms (16). Moreover, the NOD derivatives with only SS but not diabetic symptoms were developed. Other spontaneous models include NFS/sld model, IQI/Jic model and Aly/aly model. Although the spontaneous models are useful tools for studying SS pathogenesis, the accompanying phenotypes of other diseases have limited their applications.

**NOD and derivative mice**

The NOD model is widely used to study the immune-pathogenesis and develop potential therapeutics for human SS. The NOD mice spontaneously develop both diabetic and SS-like symptoms, which serves as a typical secondary SS model. Lymphocytic infiltration was observed in exocrine glands including SG and LG, resulting in sialadenitis and dacryoadenitis (17). The autoantibody signature in NOD model resembles human SS patients with the presence of anti-SSA, anti-SSB and anti-muscarinic receptor type III (M3R) autoantibodies but usually with low levels (13, 18-20). Moreover, a number of NOD derivative models including NOD.B10-H2^d and NOD.H-2h4 mice recapitulated key symptoms of SS patients without the occurrence of diabetes, indicating the independent development of glandular dysfunction and diabetic symptoms (21, 22). Multiple insulin dependent diabetes (idd) loci were identified in NOD mice for diabetes development while two of them Idd3 and Idd5 were found to be critical for SS-like phenotypes (23). C57BL/6 mice with congenic loci of Idd3 and Idd5 from NOD mice termed as C57BL/6.NOD-Aec1Aec2 also mirrored human SS characteristics without diabetic symptoms, thus serving as another primary SS model (24). Recent studies on NOD and derivative models have indicated key features of SS pathogenesis and provided strategies for developing novel therapies. Recently, it’s reported that blocking CD40-CD40L interactions markedly reduced lymphocytic infiltration and glandular ectopic lymphoid structures (ELS) in NOD model (25). Even a single treatment of anti-CD40L significantly decreased ELS formation and serum autoantibodies levels in NOD.H-2h4 mice (26). Consistently, clinical studies also indicated the benefits of anti-CD40 monoclonal antibody CFZ533 in SS patients (27). Together, these results demonstrated the involvement of CD40–CD40L pathway in SS pathogenesis and suggested the therapeutic potential of targeting CD40/CD40L for treating SS.

**NFS/sld mice**

NFS/sld mutant mice bear defects of salivary glands differentiation. The mice with thymectomy at 3 days after birth (3d-Tx NFS/sld) exhibited significant inflammatory lesions in SG and LG with secretory dysfunctions (28). Aging-associated CD4 T cells dysregulation and Fas-mediated epithelial cell apoptosis were suggested to be involved in autoimmune development in this model (29). Moreover, α-fodrin appeared to be an important autoantigen. Neonatal immunisation with recombinant 120-kDa α-fodrin decreased inflammatory lesions in 3d-Tx NFS/sld mice (30) while immunisation in 4-week old normal NFS/sld mice induced autoimmune SS symptoms (31). However, anti-α-fodrin antibodies are less specific and sensitive when compared with anti-SSA autoantibodies in SS patients (32), suggesting that anti-α-fodrin antibodies may not be suitable for SS diagnosis.

**Aly/aly mice**

The alymphoplasia mutated (aly/aly) mice possess a spontaneous mutation which causes a systemic lack of Peyer’s patches and lymph nodes (33). Aly/aly mice developed inflammatory infiltration in exocrine glands including SG, LG and pancreas (34). Inflammatory lesions in liver and lung were also observed but autoantibodies were not detected in aly/aly mice. Transfer of T cells from aly/aly mice into RAG2 knockout (KO) mice induced inflammation in exocrine organs, suggesting that autoreactive T cells are critical for disease initiation and progression in this model.

**IQI/Jic mice**

IQI/Jic mice spontaneously develop lymphocytic inflammation in SG and LG with increased incidence and severity in aged mice (35). Female IQI/Jic mice exhibit severer tissue damage when compared with male mice. CD4 T cells were found to be the dominant population in small foci while the frequencies of B cells were elevated in accordance with increased disease severity, which was consistent with B and T cells quantification in minor salivary glands of SS patients (35, 36). However, anti-SSA autoantibodies were not detected (35). Notably, inflammatory lesions within multiple organs including pancreas, lung and kidney were observed in IQI/Jic mice, resembling systemic manifestation in SS patients (37). Increased expression of kallikrein (Klk)-13 in salivary tissues and elevated levels of antibodies against Klk-13 in diseased mice were detected, suggesting that Klk-13 may function as an autoantigen in the disease development (38).

**Genetically modified models**

Genetic factors play critical roles in SS development. Various genetically modified models were established in the past decades. Some genetically modified mice such as BAFF transgenic mice exhibited secondary SS phenotypes (39). Moreover, genetically modified models recapitulating primary SS symptoms were also studied. The gene editing technologies will further elucidate new pathogenic pathways in SS pathogenesis.

**Inhibitor of differentiation 3(Id3) KO mice**

Id3 is a critical transcriptional regulator in cell proliferation and differentiation of various cell types, including B and T cells (40, 41). The Id3 KO mice developed glandular lymphocytic infiltration and secretory dysfunction at early stages while the presence of autoantibodies was detected at late stage (42). Adaptive transfer of Id3 KO T cells induced SS-like secretory dysfunction in recipi-
ent mice, suggesting a T cell–intrinsic role of Id3. Furthermore, mice with Id3 deficiency in T cell lineage also exhibited comparable SS-like phenotypes with Id3 germine KO mice (43), further demonstrating the critical roles of T cells in driving disease development. However, B cells depletion ameliorated glandular inflammation with improved secretory functions (44), suggesting differential roles of B and T cells at different stages of disease progression. The clinical relevance of Id3 in SS patients has not been confirmed as available studies suggested no obvious predisposition of Id3-related SNPs in SS patients (45).

Aromatase KO mice
The higher incidence of SS in females has suggested the involvement of oestrogen in disease pathogenesis (2). Ovariectomy of the normal mice led to SS-like disease symptoms while administration of oestrogen prevented apoptosis of SG epithelial cells in oestrogen-deficient mice, indicating the protective effects of oestrogen in SS (46,47). Aromatase is an enzyme that controls a key step in the biosynthesis of oestrogens. Aromatase KO mice developed severe inflammatory infiltrates in SG with glandular dysfunction, which was possibly related to adipose tissue-associated macrophages (48,49).

Autoimmune regulator (Aire) KO mice
Aire is a transcription factor that regulates self-tolerance by promoting ectopic expression of autoantigens in thymus and controlling peripheral autoreactive B cells (50, 51). Aire is not only expressed in thymic medullary epithelial cell but also in peripheral lymphoid organs (52). Previous clinical observations revealed that Aire deficient patients harboured unique autoantibodies profiles with loss of B cell tolerance (53). Aire deficient mice exhibited spontaneous development of autoimmune profiles and served as an animal model for SS (54). The Aire KO mice developed SS-like lymphocytic infiltration in lacrimal glands, parotid glands and submandibular glands together with severe dry eyes (54–56). It was found that autoimmunity against α-fodrin and odorant binding protein 1a were associated with disease development while both the autoantigens were identified in thymus (54, 57). Moreover, mouse genetic background markedly affected SS progression in Aire KO mice because disease development differed in C57/BL6, BALB/c and NOD background (54, 55). Recently, neuropathic changes were found to be associated with chronic lacrimal inflammation in Aire KO mice (58), suggesting disturbed neuronal regulation in SS pathogenesis. Additionally, gene expression analysis in Aire KO mice revealed the involvement of multiple signalling pathways in early regulation of inflammation, initiation, and cell survival during autoimmune SS development (55).

T cell-specific phosphoinositide 3-kinase (PI3K) KO mice
PI3K functions as a second messenger downstream of multiple receptors and has key roles in regulating immune cell functions (59). Mice with T cell-specific PI3K deficiency showed autoimmune development with SS-like phenotypes. Glandular lymphocytic infiltration, increased antinuclear antibodies (ANA) and anti-SSA antibodies were detected (60). A recent study reported that in vivo blockade of PI3Kδ activity ameliorated disease symptoms in a SS-like sialoadenitis model, suggesting that PI3K pathway might be a novel therapeutic target for SS treatments (61).

IkBαMM mice
NF-κB represents a family of transcription factors with diverse roles. Defective NF-κB signalling substantially contributed to the development of autoimmunity (62). IkBα is induced by NF-κB activation and in turn inhibits NF-κB activity with a feedback loop. Polymorphisms in IkBα promoter region were suggested to be related with SS susceptibility (63). IkBαMM mice with mutated κB enhancers inIkBα promoter developed autoimmune symptoms which were very similar to SS patients (64). The IkBαMM mice exhibited inflammatory infiltrates in SG, LG and lung tissues. Moreover, autoantibodies against SSA and SSB were detected.

Osteopontin (OPN) transgenic mice
OPN is a multifunctional cytokine with diverse sources (65). Previous studies suggested that aberrant expression and function of OPN were associated with a number of autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis and multiple sclerosis (66-68). The association between OPN and SS pathogenesis was further revealed by the SS-like phenotypes in OPN transgenic mice which served as another SS model (69). OPN transgenic mice recapitulated SS characteristics in terms of exocrine histopathology, saliva hypofunction, increased anti-SSA antibodies and female predilection.

Overexpression of retinoblastoma-associated protein 48 (RbAp48) in SG
RbAp48 is a histone binding protein with multiple functions. Previous studies reported that RbAp48 induced apoptosis in the exocrine glands which was dependent on oestrogen deficiency (70). Moreover, transgenic expression of RbAp48 in SG tissues resulted in autoimmune exocrinopathy (71). Inflammatory infiltrates with CD4 T cells in SG and LG tissues and reduced saliva and tear secretion were observed. In addition, increased anti-SSA/SSB autoantibodies levels were detected. SG epithelial cells may serve as antigen-presenting cells and promote disease progression in this model.

Overexpression of TNF-α in SG
TNF-α is an important cytokine in autoimmunity. Increased TNF-α expression was observed in patients with autoimmune diseases, including SS (72). Moreover, TNF-α levels were implicated to be associated with secretory dysfunctions and inflammation in SS (73). A recent study showed that overexpression of TNF-α within salivary glands induced SS-like sialadenitis symptoms, including lymphocytes aggregation in SG tissues and secretory dysfunction (74). However, autoantibodies were not observed in this model, suggesting the autoantibody-independent induction of SG hypofunction in the model.

Induced models
In addition to genetic background,
extrinsic triggers are also important factors involved in SS pathogenesis. Immunisation with specific autoantigens can induce breakdown of the immune-tolerance and result in glandular inflammation and hypofunction, which resembles the symptoms of SS patients. Currently, the antigens used for SS induction include Ro peptides, M3R peptides, carbonic anhydrise 2 (CA2) and SG protein extracts. Moreover, adenovirus infection in SG tissues also induced SS-like symptoms. The induced models serve as powerful tools for translational studies and have the advantage of exploring the whole disease development spectrum from a precise point of disease onset.

Ro immunisation
The presence of anti-SSA/Ro autoantibody is important for the diagnosis and classification of SS patients. Repeated immunisation with short peptide derived from Ro 60 induced profound lymphocytic infiltrates in SG tissues with glandular hypofunction in BALB/c mice (75). Moreover, the immunised mice exhibited anti-SSA/Ro autoantibodies while oral feeding of Ro 60 peptides prevented disease progress through induction of immune tolerance (76). Recent studies reported that immunisation of mRo60_316-335 peptide antigen with a predominant T cell epitope induced several major SS-like symptoms in mice, including glandular dysfunction and tissue inflammation as well as the increase of autoantibodies and inflammatory cytokines (77). An indispensable role of B cells was demonstrated in this model when B cell depletion prevented the development of SS-like disease. Genetic predisposition was also important because immunisation with Ro 60 peptides induced different degrees of preclinical autoimmune with diverse phenotypes in different strains of mice (78). Autoantibodies against SSA/Ro recognise two types of ribonucleoprotein antigens with 52 kDa and 60 kDa, respectively. Not only Ro 60 but also Ro 52 peptides immunisation induced SS-like phenotypes in mice. NZM2758 mice with immunisation of Ro52 exhibited symptoms resembling those of SS patients (79). Moreover, Ro52-induced antibodies were capable of inducing SG hypofunction, which was dependent on activation of innate immunity (79).

M3R peptide immunisation
Anti-M3R autoantibodies detected in SS patients are suggested to be involved in SS pathogenesis (80). M3R is located on the surface of SG acinar cells and plays key roles in saliva secretion. The predominant role of M3R in regulating saliva production was revealed by findings that M3R KO mice showed 20% reduction of saliva secretion when compared with wild type (WT) mice (81). Previous studies suggested that anti-M3R autoantibodies bound to M3R in SG tissues and contributed secretory dysfunction (82). Adoptive transfer of splenocytes from the M3R KO mice which received immunisation with M3R-derived peptides into Rag KO immunodeficient mice resulted in profound hyposalivation accompanied with severe glandular inflammation (83). The immune pathogenesis in this model was dependent on T cells because the transfer of CD3+ T cells but not CD3- cell population conferred development of SS-like phenotypes in recipient mice. M3R-specific effector T cells especially Th17 cells were critically involved in M3R-induced SS development. RORγt antagonist treatment significantly improved SG secretory functions with suppressed IFN-γ and IL-17 production (84), indicating that RORγt antagonism might be a potential therapeutic strategy for SS patients. However, immunisation with second extracellular loop of M3R in BALB/c mice induced neither secondary hypofunction nor histological abnormality though the presence of high levels of anti-M3R autoantibodies (85), suggesting that anti-M3R autoantibodies for the second extracellular loop may not exert any pathogenic function in SS development.

SG protein immunisation
Early studies reported the induction of salivary gland inflammation in SMA mice with repeated immunisation of salivary gland extract with Klebsiella O3 Lipopolysaccharide as adjuvant (86). Moreover, SS-like keratoconjunctivitis sicca symptoms were also observed in Lewis rats with immunisation of a mixture of lacrimal and salivary gland extract (87), suggesting that salivary and lacrimal glands-derived proteins may contain autoantigens for the induction of SS-like symptoms. Our recent work has established an experimental SS (ESS) model induced in C57BL/6 mice, which may serve as a powerful tool for studying SS pathogenesis and screening potential therapeutic candidates (88). The proteins were prepared from homogenised SG tissues in normal mice. Upon immunisation with the SG-derived proteins, the mice displayed decreased saliva production, increased anti-SSA and anti-M3R autoantibodies, profound glandular inflammation with the infiltration of both T and B cells. Pulmonary and renal inflammation were also observed in some mice at chronic disease stage (unpublished data), suggesting that the ESS mice also mimicked systemic manifestation with multiple organs-injury in SS patients. Using this ESS model, we demonstrated a critical role of Th17 cells in SS development (88). IL-17 KO mice were resistant to ESS induction while adoptive transfer of polarised Th17 cells into IL-17 KO recipient mice induced hyposalivation and focal sialadenitis (88). Moreover, the proteasome inhibitor Bortezomib suppressed Th17 but not Th1 responses in ESS development accompanied with ameliorated disease pathology, suggesting that targeting Th17 cells might be a promising therapeutic strategy for treating SS patients (89). In addition, IL-25 pathway was demonstrated to be involved in human primary SS patients and ESS model (90). Furthermore, our recent work revealed the regulatory roles of IL-10-producing B cells and myeloid-derived suppressor cells in SS pathogenesis (91, 92).

CA2 immunisation
CA2 is a metalloenzyme that catalyses the reversible hydration of carbon dioxide. Antibodies against CA2 were detected and associated with renal damage in a subset of SS patients (93). PL/J(H-2u) mice immunised with human CA2 exhibited lymphocytic infiltration.
In SG tissues with the presence of anti-CA2 antibodies (94, 95). Moreover, impaired renal functions were also observed in immunised mice.

**Virus-induced model**

Environmental triggers including virus infections have been suggested to be important for SS initiation and progression. Several viruses such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV) were associated with SS development (96). Previous studies showed that murine CMV (MCMV)-infected autoimmune-prone mice including B6-lpr/lpr and NZM2328 mice exhibited SS-like disease symptoms and served as secondary SS models (97, 98). Notably, SG administration of a replication-defective adenovirus 5 in WT C57BL/6 mice reproduced several phenotypic and functional features of SS patients including lymphocytic infiltration in SG tissues, decreased saliva secretion and functional B cell activation with increased ANA levels (99). Ectopic lymphoid structures with germinal centres were observed in the majority of infected mice. Therefore, the adenovirus-infected mice may also serve as a SS model for studying cellular and molecular mechanisms.

**SG cannulation of Ad5-IL17 vectors**

A number of studies strongly suggested the involvement of IL-17 in SS pathogenesis (100-103). Our previous studies also showed the critical roles of Th17 cells in driving SS progression in a murine model (88). Moreover, overexpression of IL-17 in SG tissues directly induced SS-like disease profiles, serving as another SS model. The mice with retrograde salivary gland cannulation of adenovirus serotype 5 vectors expressing IL-17 (Ad5-IL-17) exhibited increased IL-17 production accompanied with elevated inflammatory cytokines, glandular lymphocytic infiltration, presence of autoantibodies, and reduction of saliva secretion (103). In addition, blocking IL-17 reduced SS pathology in C57BL/6.NOD-Aec1Aec2 model (104, 105), further demonstrating that IL-17 might be a promising therapeutic target for treating SS.

**Humanised model**

Animal models are proved to be useful tools for studying human diseases, but the differences between animals and human biology limit the translation of knowledge from animal models to clinical outputs. In current research, humanised mouse models have been increasingly recognised as important pre-clinical tools to fill the gap between mouse and human. The humanised SS model was developed by adoptive transfer...
Animal models of Sjögren’s syndrome / F. Xiao et al.

of peripheral blood mononuclear cells (PBMCs) from SS patients into immunodeficient NOD-scid IL-2rγ(null) recipient mice (NSG mice) (106). The chimeric mice with PBMCs from SS patients exhibited inflammation in SG and LG tissues together with reduced saliva production. Further histological analysis revealed primarily CD4 T cells infiltration with minimal CD8 T cells and B cells. This chimeric humanised model represents an important platform for future translational studies.

Conclusions and future perspectives
SS is a heterogeneous disease with complex pathogenesis. Both external triggers and intrinsic factors contribute to SS development. Clinical research has provided important evidence for the understanding of SS aetiology. However, the pathological changes before the clinical diagnosis can hardly be studied from clinical observations. Animal models, especially murine models with the advantages of genetic consistency, short life span and sufficient sample size, have provided a myriad of information regarding disease initiation, progression and treatment. During recent decades, a number of animal models recapitulating the key characteristics of SS patients have been developed (Table I). Those animal models of SS serve as excellent tools for studying full disease spectrum. The advancement and applications of genetically modified mice in SS models will identify the direct roles of target genes in disease pathogenesis. In addition, pre-clinical studies based on animal models will benefit the potential development of novel therapies for SS patients.

Although there are still discrepancies between SS animal models and patients, numerous studies on SS models have contributed substantially to the current understanding of SS pathogenesis. Notably, the humanised mouse model has markedly narrowed the gap between animal models and SS patients, allowing the in vivo study of human immune cells under SS disease condition. Moreover, the humanised mouse model is usually patient-specific, which may benefit the advancement of clinical applications of personalised therapies. Further research in animal models is needed for a comprehensive understanding of SS pathogenesis. The future translational studies from animal models to clinical research will certainly provide new insight in understanding SS pathogenesis and further developing effective therapies for patients.

Funding
This work was supported by the National Natural Science Foundation of China (no. 91842304 and 81771761), General Research Fund, Hong Kong Research Grants Council (17114515 and 17149716), Chinese National Key Technology R&D Program, Ministry of Science and Technology (2017YFC0907601, 2017YFC0907605 and 2018YFC1705200), Hong Kong Croucher Foundation (26096016) and HKU Seed Funding for Strategic Interdisciplinary Research Scheme (2018/19).

References
25. WIECZOREK G, BIGAUD M, PFISTER S et al.: Blockade of CD40–CD154 pathway interactions suppresses ectopic lymphoid structures and inhibits pathology in the


Animal models of Sjögren’s syndrome / F. Xiao et al.


75. SLOCIFIELD RH, ASFA S, OBESO D, JOHNSON R, KURIEN BT: Immunization with short peptides from the 60-kDa Ro antigen recapitulates the serological and pathological findings as well as the salivary gland dysfunction of Sjögren’s syndrome. J Immunol 2005; 175: 8409-14.


