Review

Experimental models of Sjögren's syndrome: differences and similarities with human disease

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ABSTRACT

Mouse models have been employed extensively to provide pathogenetic insights into many complex human disorders including systemic autoimmune diseases. The explosion of biotechnology and molecular biology have simplified the procedures to design and generate mouse models with the phenotype of interest. In this line, more than 30 mouse models have been proposed or developed to resemble Sjögren's syndrome (SS) in humans, in an attempt to better understand the pathophysiology of the disease and design more effective treatments. So far, none of these models has been proven an ideal recapitulation of the human disease, although each model mimics particular aspects of the human SS counterpart. This review summarises the main characteristics of the mouse models of SS that have been developed hitherto, comparing them with the human SS in terms of clinical features, sex predilection, histopathology, autoantibodies production, and propensity for lymphoma. The interpretation of these experimental models with cautiousness and the realisation of the differences between human and mouse physiology and disease pathophysiology, may render mice a useful tool to study in depth SS and reveal new therapeutic perspectives.

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune inflammatory syndrome that occurs either as a primary (not associated with other diseases) or as a secondary disorder that overlaps or complicates rheumatoid arthritis (RA) or another systemic autoimmune disease (1). However, this classification has started to be questioned during the past years, since the majority of SS phenotypes seems to be much more complex and this classification does not take into consideration other autoimmune diseases that may coexist with SS (2, 3). SS is characterised mainly by diminished lacrimal and salivary gland function, but also by a plethora of both exocrine glandular and extra-glandular manifestations that may affect a wide variety of tissues and organs (4). In both primary and secondary SS, impaired exocrine gland function leads to a combination of dry eyes or keratoconjunctivitis sicca (xerophthalmia, from the Greek words xeros = dry and opthalmos = eye) and dry mouth (xerostomia, from the Greek words xeros = dry and stoma = mouth), as well as manifestations from other organs including vagina, skin, larynx and trachea (5-7). On the other hand, extra-glandular manifestations such as Raynaud's syndrome, vasculitis, purpura, inflammatory arthritis, nephritis and interstitial lung disease, are also observed among SS patients (8).

Despite long and extensive research on SS immunopathogenetic mechanisms, the pathophysiology of the disease remains elusive, at least in its entirety. Clinical observations as well as studies in both humans (*in vitro*, epidemiological, immunohistochemical, genetic studies, etc.) (9-12) and animal models, mainly mice, (13, 14), have shed light in many different aspects of disease pathogenesis. Current knowledge gathered hitherto, suggests that the initiation and perpetuation of the aberrant immune responses that drive SS pathogenesis, are caused by a complex interplay between environmental (infections, smoking, etc.), genetic, neuropsychological and hormonal factors (15). The complexity, chronicity and systemic nature of SS have forced the scientific community to recruit or generate different types of mouse models in an attempt to mimic the human SS

disease and study in depth the implicated pathogenetic mechanisms. In this review, the current landscape on mouse models related to SS is being presented along with a critical analysis compared to the main aspects of the human SS disease.

Overview of SS pathogenesis

Glandular dysfunction is mainly the result of a local autoimmune-induced inflammation leading to destruction of the exocrine machinery. The effect of an initial trigger (*e.g.* a viral infection) leads to the activation of innate immunity and elicits a type I interferon response in salivary glands, monocytes and plasmacytoid dendritic cells (16). Subsequently, adaptive immunity is activated, followed by infiltration of T- and B-cells in glandular and extraglandular tissues and local production of (auto)antibodies such as anti-SSA(Ro) and anti-SSB(La) and cytokines such as IL-1, IL-6, TNF-α, IL-17 and BAFF. Additionally, $CD4^+$ T-cells differentiate into T_{FH} cells further supporting the B-cell survival through the formation of ectopic germinal centres (17). Interestingly, salivary duct epithelial cells have been shown to play an active role in SS pathogenesis through: a) expression of MHCII molecules (18), b) release of cytokines such as BAFF, IL-1, IL-6,IL-21 and TNF-α (19, 20), c) release of intracellular antigens that act as self-antigens for the generation of autoantibodies (21) and d) promote lymphocytic and dendritic cell infiltration (22). Therefore, SS has also been termed as "autoimmune epithelitis" (23). Finally, other possible mechanisms have also been suggested as contributors in SS glandular dysfunction. For example, antibodies against the muscarinic receptor could impair the innervation of the gland even in the absence of inflammation (24-26).

The presumed pathogenetic mechanisms underlying the extra-glandular manifestations of SS can be categorised as follows: 1) autoimmune exocrinopathy (*e.g.* interstitial nephritis, biliary cholangitis, etc.), similar to that observed within the inflamed salivary glands, 2) immune-complex mediated tissue injury (*e.g.* cryoglobulinaemic vasculitis), 3) cell- or tissue-specific autoimmunity of unclear aetiology (*e.g.* thrombocytopenia, neuromyelitis optica, etc.) and 4) lymphoproliferation (*e.g.* non-Hodgkin's lymphomas [NHL] of B cell origin either mucosa associated lymphoid tissue (MALT) or Diffuse Large B cell) (4, 27).

The chronic antigenic stimulation of B-cells in SS, driven by known and yet unknown mechanisms, may lead to malignant transformation and generation of NHLs (28-30). The most frequent type of lymphoma in these patients is the extra-nodal marginal zone non-Hodgkin lymphoma MALT type. Less frequently, higher-grade diffuse B-cell lymphomas and T-cell lymphomas have also been described. Moreover, the development of such malignancies in patients with SS, affects diversely their prognosis and survival (31).

Although the clinical, histopathological and laboratory characteristics of human patients with SS have been well documented, the pathophysiological events that take place before, and result in the clinical expression of the disease, are difficult to study. Unfortunately, detecting patients early during disease course and before producing clinical manifestations is still elusive. For this reason, the use of mouse models is more than necessary (32), although the translation to humans should always be interpreted cautiously (33). Many different mouse models have been developed for the study of SS, but none of them can recapitulate the human disease in its entirety (13).

Mouse models of SS

The ideal mouse model of SS must gather some specific features (32):

1. Specific genetic background and female predominance (abnormal MHC-I and MHC-II expression, especially HLA-DQ and HLA-DR).
2. Dominant glandular clinical manifestations (dry mouth and dry eyes, salivary swelling).
3. Specific immunologic characteristics (B-cell activation and autoantibody production, activation of IFN signalling).
4. Typical histopathologic findings (lymphocytic infiltration of the lacrimal and salivary glands, ectopic germinal centres).
5. Common serological biomarkers [hypergammaglobulinaemia, anti-SSA(Ro) antibodies, anti-SSB(La) antibodies, anti-M3R antibodies, RFs, cryoglobulins, hypocomplementaemia, anti-120 kD a-fodrin antibodies].
6. Extra-glandular insult and a predisposition for lymphoma development (multiorgan involvement including vessels, lugs, skin, liver etc.).

As shown in Figure 1, many different techniques (knock-in, knock-out, CRISPR-Cas9, transgenic etc.) have been developed in order to generate mice with specific genetic characteristics (the so called "mouse models" of the disease) (34, 35). Knock-in mice are generated by the genetic manipulation of embryonic stem cells so that a specific gene is substituted with another, or a new sequence of information is inserted into a specific gene. On the contrary, in knock-out mice, the expression of a gene is blocked either by replacing the gene or by disrupting its sequence. Additionally, transgenic mice, are generated by the random insertion of a completely new (exogenous) gene into the nucleus of a fertilised mouse egg. In order to silence or insert the target gene in a more tissue-specific way, in the conditional knock-in or knock-out or transgenic mice, the gene of interest is silenced or expressed only in specific tissues with the use of specific promoters that are expressed in these tissues (34). Finally, the CRISPR-Cas9 technique exploits a bacterial genome editing system in order to inflict very small (even on a single-base level) alterations in one or more specific genes (35).

For the study of SS, 6 different types of mouse models have been used and will be described in the following sections of this review: 1) Genetic or spontaneous mouse models, consisting of mice the spontaneously after consecutive breeding for many generations or after accidental mutagenesis developed a phenotype that resembles, more or less, SS. 2) Knock-out mouse models. 3) Transgenic mouse models. 4) Immunisation-induced mouse models, where the injection of a specific

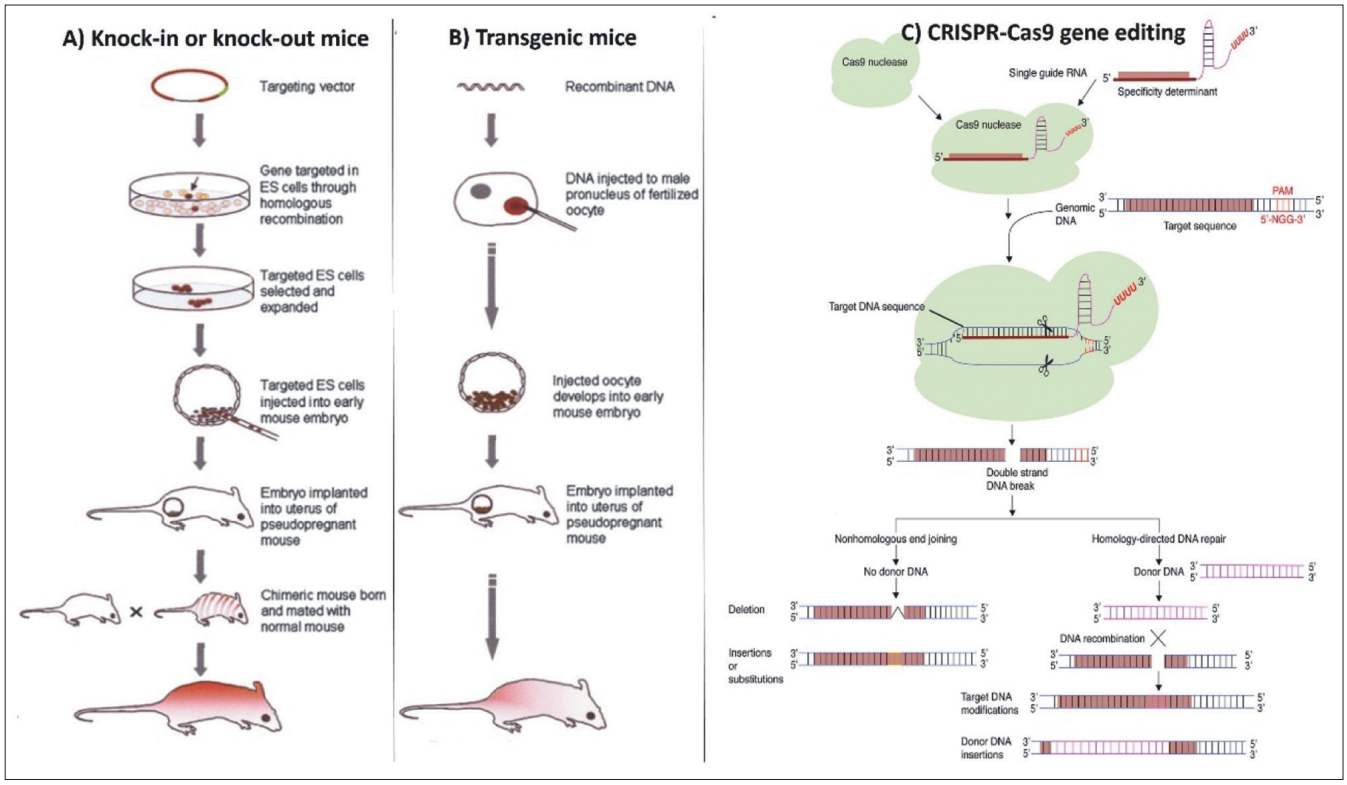

Fig. 1. Generation of mouse models using the knock-in, knock-out, transgenic and CRISPR-Cas9 techniques.
A: Generation of knock-in or knock-out mice. The genetic sequence of interest is inserted into a targeting vector that shows homology to the genomic region where it is planned to be inserted. The vector containing the sequence of interest is inserted via electroporation into mouse embryonic stem cells (ES). The ES cells that were successfully targeted are then selected and isolated in order to be expanded. After that, they are injected into an early mouse embryo that is then implanted into the uterus of a pseudopregnant female mouse. The chimeric mice that are born from this embryo, are subsequently mated with wild-type mice in order to finally get mice with stem exclusively from the genetically modified ES cells. With the use of specific promoters, the expression (in the knock-in mice) or silencing (in the knock-out mice) of the target gene can be limited only in specific tissues of interest.
B: Generation of transgenic mice. The recombinant DNA sequence of interest (transgene) is injected in a fertilised mouse oocyte into the male pronucleus. The resulting embryo is implanted into the uterus of a female pseudopregnant mouse and the mouse that is born carries the transgene in a random location and therefore its expression is uncontrolled. With the use of a specific promoter though, the expression of the transgene can be limited to specific tissues of interest.
C: Gene editing using the CRISPR-Cas9 technique. The Cas9 protein together with one or more guide RNAs that are complementary to the DNA that needs to be edited, constitute the CRISPR-Cas9 system. Initially, the guide RNA-Cas9 binary complex cleaves specifically the DNA and creates a double-strand DNA break. This break initiates cellular DNA repair mechanisms like homology-directed repair (HDR) and non-homologous end joining (NHEJ). During this repairing process and according to the guide RNAs used, short deletions or insertions, nucleotide substitutions or even gene insertion can be achieved. [Figure adopted and modified by Chen and Roop (36) and El-Mounadi *et al*. (37)].

peptide from the salivary glands or of salivary gland homogenate, leads to the development of (auto)antibodies that eventually destroy structures of the normal salivary glands of these mice. 5) Infection-induced mouse models, where the infection of the mice with a specific virus leads to the development of an inflammatory reaction and autoantibody production against the salivary glands of these mice. Finally, 6) a humanised mouse model of SS has been developed with the injection of peripheral blood mononuclear cells from SS patients into immunodeficient NOD-scid IL2rγ mice. Notably, SCID mice exhibit severe immunodeficiency due to lack of functional B- and T-cells, and therefore, they have been used in the formation of xenografts with human tissues (38).

For the study of these mice many different techniques and approaches have been applied: a) Tissue biopsies and histopathological studies -even with the use of electron microscopy- of salivary and lacrimal glands as well as from other tissues/organs, b) serological studies and (auto)antibody measurement (mainly using ELISA), c) FACS analysis to study cellular populations, d) Western blot analysis to study different proteins, e) measurement of salivary gland spleen weight, f) measurement of salivary production upon intraperitoneal injection of pilocarpine plus isoproterenol (to stimulate salivary secretion) and g) measurement of lacrimal gland secretion upon intraperitoneal injection of pilocarpine plus isoproterenol (to stimulate lacrimal gland secretion) using the Schirmer's test. For the study of neuropsychiatric features, different tests were applied to assess anxiety (elevated-plus maze, open field etc.), depression/sickness-like behaviour (sucrose preference test, forced swim test, open field locomotor activity, etc.), learning and memory (novel object recognition, Morris water maze, fear-conditioning paradigm, etc.) and motor function (beam-walking, climbing, etc.).

As it will be discussed in the following sections of this review, current mouse models present with a variety of manifestations. Most of them exhibit oral and eye dryness with simultaneous

lymphocytic infiltration of the salivary and lacrimal epithelium, while in some of them extra-glandular manifestations and serologic markers may occur. On the contrary no mouse model exhibits the formation of ectopic germinal centres in the inflammatory lesions of the SGs and/or LGs as seen in humans apart from maybe one virally induced model that shows only locally the formation of ectopic lymphoid tissue. Moreover, only two mouse models displayed a propensity for lymphoma development, which however showed some similarities to the presentation of such lymphomas in SS patients. Finally, only one mouse model exhibits cryoglobulinaemia and another one exhibits hypocomplementaemia, features that are essential in human SS. Table I depicts the main mouse models of SS and the characteristics they share with human disease.

Genetic ("spontaneous") mouse models

Inbred and mutated mice

NZB/NZW F_1 mice

The first mouse model of spontaneous systemic SS was the NZB/NZW F_1 mouse (New Zealand Black x New Zealand White F_1 generation) (39), that was initially utilised as a model of systemic lupus erythematosus with nephritis (40). NZB/NZW F_1 mice develop inflammatory infiltration in both SGs and LGs (largely in the latter) mainly by Th1 $CD4^+$ T-cells, which is more severe in females and in older mice (39, 41). Additionally, NZB/NZW F_1 mice show decreased tear production and conjunctivitis (42). Finally, hypocomplementaemia has been described in these mice, which, interestingly, correlated with the appearance of anti-DNA autoantibodies and immunocomplexes in the renal glomeruli of the mice (43). However, in comparison to human disease, this mouse model has some weaknesses. On the one hand, the clinical significance (the exhibition of dry mouth) of the histopathological findings (lymphocytic infiltration) in the SGs of these mice has not been evaluated. On the other hand, no SS-specific autoantibodies have been detected (32, 39, 44). Regarding other tissues, these mice show an SLE-like phenotype with increased expression of antinuclear autoantibodies, hemolytic anaemia, hepatitis and immune complex glomerulonephritis with proteinuria, but no propensity to develop lymphoma (40).

MRL/lpr (MRL/Mp-lpr) mice

The MRL/lpr mouse is a congenic mouse strain of the MRL mouse that has been utilised as spontaneous model for SLE, rheumatoid arthritis and SS (45, 46). They exhibit inflammatory infiltrations in their SGs and LGs consisting mainly of $CD4^+$ T-cells, which lead to decreased salivary production and dry eyes with a female predilection (42, 47, 48). Additionally, some of these mice express SG-specific autoantibodies as well as anti-Ro/SSA and anti-La/SSB autoantibodies, and they show ectopic MHCII expression in their SGs even before the development of inflammation (49, 50). Interestingly, MRL/lpr mice may show other systemic manifestations such as peripheral neuropathy, arthritis, pneumonitis and lymphoproliferation, which are also common in human SS and SLE patients (51, 52).

The MRL/lpr mouse model could very well serve as model of systemic and/or secondary SS (53). However, the susceptibility of these mice to develop autoimmune phenomena in general, restricts them from being a SS-specific mouse model. Moreover, no SS-specific autoantibodies have been detected in these mice, and they do not show any propensity to develop lymphoma (32, 44).

Interestingly, the administration of a monoclonal IgG3 antibody -isolated from unimmunised MRL/lpr mice- that shows both Rheumatoid Factor (RF) activity (anti-IgG2a) and cryoglobulin activity (Fc-Fc interaction), showed the importance of both these activities in the development of vasculitis. On the contrary, genetic manipulation of this antibody, showed that its cryoglobulin activity can still induce glomerulonephritis even without its RF activity, but it cannot induce vasculitis (54).

Non-obese diabetic (NOD) mice and NOD-derived strains

The nonobese diabetic (NOD) inbred mouse strain was initially used as a model of type 1 diabetes (55). Further studies revealed a SS-like phenotype which consists of inflammatory cell infiltration of the salivary (SG) and lacrimal (LG) glands with subsequent apoptosis of glandular epithelial cells, impaired salivation and lacrimation, as well as detection of serum autoantibodies [anti-SSA(Ro), anti-SSB(La), anti-muscarinic type 3 acetylcholine receptors (M3Rs) and anti-120 kD a-fodrin]. Due to the simultaneous presence of type 1 diabetes and SS-like symptoms, NOD mice can be used as a model of secondary SS (56-59). Replacement of the MHC I-A^{g7} molecule of NOD mice with MHC I-A^b from the B10 strain, resulted in the NOD.B10.H^b mouse that exhibits the above described SS-like phenotype without autoimmune diabetes, which renders this mouse as a perfect candidate model for primary SS. Interestingly, these mice also showed inflammation in their kidneys and lungs (60-62). Finally, NOD mice on a C57BL/6 background carrying two autoimmune exocrinopathy loci – the C57BL/6.NOD-Aec1Aec2 mice- may constitute a model of primary SS, since they exhibit impaired salivation and lacrimation, increased protein content in their saliva, focal lymphocytic infiltrates in the exocrine glands, autoantibodies and enhanced proteolytic enzyme activity (63).

NOD and NOD-derived mouse models are the most extensively used and studied models for SS. Genetically engineered NOD mice have also been used in order to study specific aspects of SS. For example, NOD.*IFN-*$\gamma^{-/-}$, NOD.*IFN-*γ $R^{-/-}$ (64), NOD.*IL4*$^{-/-}$ (65) and NOD Ig$\mu^{-/-}$ mice (26), have been used to study the role of IFN-γ, IL-4 and B-cells respectively in SS pathogenesis.

Interestingly, NOD and NOD-derived mice develop disease symptoms spontaneously as a polygenic trait with a female predilection which resembles the development in humans. However, they show some shortcomings regarding their resemblance to human SS. Firstly, none of them shows ectopic expression of MHCII. Secondly, apart from the NOD.B10.H^b mice, the other 2 strains do not show inflammatory infiltration

Table I. The main mouse models used in the study of SS and their characteristics in comparison to human disease.

Mouse model		Local disease		Inflammatory infiltration		Systemic disease	Autoantibodies		Lymphoma	Sex	Ref.
		Dry mouth	Dry eyes	SG	LG		anti-SSA/Ro	anti-SSB/La			
Genetic or "spontaneous" mouse models	NZB/NZW F_1	N	Y	Y	Y	Y	N	N	N	F>M	(39, 41-43)
	MRL/lpr (MRL/Mp-lpr)	Y	Y	Y	Y	Y	Y	Y	N	F>M	(42, 48-50)
	NOD	Y	Y	Y	Y	Y	Y	Y	N	F>M	(56-59)
	NFS/sld	Y	N	Y	Y	Y	N	N	N	F>M	(67-69)
	McH-*lpr*/*lpr*-RA1	N	N	Y	N	N	Y	Y	N	F>M	(73)
	IQI/Jic	N	N	Y	Y	Y	N	N	N	F>M	(74-76)
	Aly/aly	N	N	Y	Y	Y	N	N	N	F=M	(79)
Knock-out mouse models	Ar-KO	N	N	Y	Y	Y	Y	Y	N	F=M	(82, 83)
	Id3-KO	Y	Y	Y	Y	Y/N	Y	Y	N	F=M	(85, 88)
	T-cell specific PI3K-KO	N	Y	N	Y	Y	Y	N	N	F=M	(93)
	Act1-KO	Y	Y	Y	Y	Y	Y	Y	N	F=M	(99)
	Aire-KO	N	Y	Y	Y	N	N	N	N	F=M	(103, 105)
	ERdj5-ko	Y	N	Y	N	N	Y	Y	N	F>M	(107)
	CD25-KO	N	Y	N	Y	Y	N	N	N	F=M	(108-111)
	$Nfkbiz^{-/-}$	N	Y	N	Y	Y	N	N	N	F=M	(113)
	$I\varkappa B\alpha^{M/M}$	N	N	Y	Y	Y	Y	Y	N	N/A	(116)
Transgenic mouse models	RBAp48 Tg	Y	Y	Y	Y	N	Y	Y	N	F>M	(117, 118)
	BAFF Tg	Y	N	Y	Y	Y	N	N	Y	F=M	(119-122)
	IL-12 Tg	Y	N	Y	Y	N	N	Y	N	F=M	(125)
	IL-14a Tg	N	N	Y	N	Y	Y*	Y*	Y	F=M	(126)
	IL-10 Tg	Y	Y	Y	Y	N	N	N	N	F=M	(127)
	Opn Tg	Y	N	Y	Y	N	Y	N	N	F>M	(128)
Immunization – induced mouse models	Salivary gland protein	Y	N	Y	N	N	N	N	N	F=M	(133)
	M3R	Y	N	Y	N	N	N	N	N	F=M	(141)
	Ro60 peptide	Y**	Y**	Y**	Y**	N	Y	Y	N	F=M	(143-146)
Infection – induced mouse models	Adenovirus 5	Y	N	Y	N	N	N	N	N	F=M	(147, 148)
	Murine CMV#	Y	N	Y	Y	N	Y	Y	N	F=M	(149, 150)
Humanised mouse model		Y	N	Y	Y	N	N	N	N	N/A	(151)

Y: the discussed characteristic is present in this mouse model. N: the discussed characteristic is either absent or it has not been studied in this mouse model. N/A: sex predilection has not been studied in this mouse model.
*Only rarely. **Depending on the Ro peptide used, mice develop either SG inflammation and dry mouth or LG inflammation and dry eyes. #The phenotype depends on the genetic background of the mice used and is more pronounced in autoimmunity-prone mice.

in other organs (with the exception of pancreas in the NOD mice)(13). Moreover, female C57BL/6.NOD-Aec1Aec2 mice, do not show dry eyes, while the disease onset is delayed in comparison to their male counterparts, which is the opposite of that observed in humans (63). Finally, no propensity to develop lymphoma or cryoglobulins have been described in these mice (13, 63).

NFS/sld mice

The NFS/sld mice bear an autosomal recessive mutation in the gene sld (sublingual gland differentiation arrest), which inhibits the differentiation of sublingual gland acinar cells into mucus-secreting cells (66). Thymectomy at 3 days after birth, drives these mice to develop a primary SS-like disease with lymphocytic infiltrates in both SGs and LGs (mainly $CD4^+$ but also $CD8^+$ and B-cells) and autoantibodies against 120kDa alpha-fodrin with a female predilection (67, 68). Similar results, with additional inflammatory infiltrations in other organs, were obtained using 2,3,7,8-tetrachlorodibenzo-p dioxin (TCDD) at 3 days after birth of NFS/sld mice without thymectomy, thus supporting the role of environmental fac-

tors in SS pathogenesis (69). Finally, ovariectomised NFS/sld mice have also been used to study the role of oestrogens in SS pathogenesis (70).

In comparison to human disease, this mouse model shows some weaknesses. Firstly, apart from anti-fodrin antibodies, no other -typical for human SS- antibodies have been detected (67, 68). It is of interest that the anti-fodrin antibodies may play a less important role in human SS pathogenesis as initially thought (71). Finally, inflammatory infiltration in other organs except from SGs and LGs in thymectomised mice, as well as inflammatory infiltration in SGs and LGs in non-thymectomised mice, has not been reported (67).

McH-lpr/lpr-RA1 mice

The McH-*lpr/lpr*-RA1 mice were initially used as model of autoimmune arthritis and ankylosis (72). Interestingly, it was found recently that these mice also develop autoimmune sialadenitis with local destruction of the salivary glands as well as vasculitis in their submandibular glands with a female predilection. Immunohistochemical analysis has revealed the expression of SSA and SSB autoantigens in the inflamed salivary tissues (73).

Although this could be an interesting model to study autoimmune sialadenitis in parallel with arthritis, it is far from being used as a good model for human SS. On the one hand, the clinical phenotype of the observed sialadenitis (meaning dry mouth) has not been evaluated in these mice. On the other hand, the lacrimal glands as well as other tissues have not been studied to see if they are also infiltrated, as usually is the case in humans (73).

IQI/Jic mice

The inbred IQI/Jic mice have been used as model for systemic SS since they show spontaneous inflammatory infiltrates in both SGs and LGs as well as in other organs starting at 6 months of age and accompanied by ectopic MHCII expression on glandular epithelial cells (74, 75). Interestingly, autoantibodies against tissue kallikrein have been shown to play a key role in disease pathogenesis of this model (76). Moreover, thymectomy at 3 days after birth accelerates and enhances inflammatory infiltration in the LGs but not in SGs of IQI/Jic mice (77).

However, this mouse model has some serious weaknesses compared to human disease. Firstly, while small inflammatory infiltrates are mainly consisting of $CD4^+$ T-cells (as seen in humans), large infiltrates consist mainly of B-cells (74). Secondly, the clinical significance of the histopathologically detected sialadenitis and dacryocystitis has not been examined adequately (44). Thirdly, no classical "human" autoantibodies (anti-SSA, anti-SSB etc.) have been detected in these mice (44, 74). Finally, the fact that IQI/Jic mice develop autoinflammation at a late age, renders this model difficult to use for long-term studies (13, 74).

Aly/aly mice

The aly/aly mice show immunodeficiency regarding both cellular and humoral responses due to a homozygous recessive mutation (the so-called alymphoplasia mutation) within the NF-kappa B-inducing kinase (NIK) gene(78). Simultaneously, they exhibit inflammatory infiltrations consisting mainly by $CD4^+$ T-cells in many different exocrine and non-exocrine tissues and organs including the SGs, the LGs, the pancreas, and the lungs (79).

Aly/aly mice develop systemic disease with inflammatory infiltrations in many different tissues and therefore could be used as a model of secondary SS (80); however, they display serious disadvantages. Firstly, the observed histopathological findings are not accompanied by relevant clinical manifestations while no autoantibodies and no difference in the frequencies between males and females have been documented (78, 79). Additionally, the serious immunological abnormalities observed in these mice, make the translation of the findings quite tricky (44).

Knock-out mice

Aromatase KO mice (Ar KO mice)

The Ar KO mice were generated to study the role of oestrogens in SS, since they lack aromatase cytochrome P450, the enzyme that catalyses the conversion of C19 steroids to oestrogens (81). Interestingly, these mice exhibit splenomegaly and inflammatory infiltration in the exocrine glands including SGs and LGs, as well as in the kidneys followed by proteinuria (82, 83). Additionally, Ar KO mice showed increased inflammatory cytokines such as INF-γ, IL-1, IL-6 and TNF-α, as well as $B220^+$ B-cell hyperactivation with subsequent production of anti-SSA, anti-SSB, anti-ssDNA and anti-a-fodrin autoantibodies (82, 83).

However, Ar KO mice do not show a sex-predominance regarding the clinically observed phenotype (83). Additionally, it has not been evaluated weather the inflammatory infiltration in the SGs of these mice leads to dry mouth, as opposed to LG inflammation which seems to be clinically silent, not producing eye dryness (13, 84). Finally, the fact that the described phenotype is observed mainly in old mice (around 12 months old) is also another limitation of this mouse model (82).

Id3-KO mice

Id-3 KO mice (85) lack Id-3, a member of the transcription family called basic helix-loop-helix, which is essential for the development of both T and B lymphocytes (86, 87). At 2-4 months of age, these mice develop a SS-like phenotype with dry mouth and dry eyes. Additionally, they exhibit inflammatory infiltration of both SGs and LGs (mainly by $CD4^+$ T-cells followed by $CD8^+$ T-cells and B-cells) and occasionally of lungs and kidneys in older mice (>1 year of age). Finally, they also produce anti-SSA and anti-SSB autoantibodies in their sera (85, 88).

Although these mice recapitulate important aspects of human SS such as the role of both T-cells (89) and B-cells (90) in the disease pathogenesis, they also show some weaknesses. Firstly, males and females seem to be affected in the same frequency, while secondly, the phenotype seems to be local and not systemic (85). Finally, no genetic variant in the Id3 locus has been associated with human SS (91).

T-cell specific PI3K KO mice

PI3K is a kinase that plays an important role in lymphocyte function (92). Mice

lacking PI3K specifically in their T-cells, develop some features of SS such as dry eyes, inflammatory infiltration of LGs (mainly by $CD4^+$ T-cells followed by $CD8^+$ T-cells and B-cells) and anti-SSA as well as ANA autoantibodies. Additionally, some of them also develop inflammatory infiltrations (on a histopathological level) in their lung, liver or intestine, but not in their kidneys (93).
Unfortunately, these mice could better serve as a model only for dry eyes since they do not seem to exhibit any other dryness symptoms such as dry mouth. Additionally, in comparison to human SS, they do not show a female sex-predilection (93).

TGF-β1- KO mice and TSP-1 KO mice
TGF-β1 has a suppressive effect on T-cells, B-cells and macrophages in order to protect or recover from autoimmune conditions (94). TGF-β1 KO mice die at 3 weeks of age due to organ failure accompanied by inflammatory infiltration in many different organs and tissues including liver, stomach, kidney, heart, muscle and SGs (95). Additionally, these mice show anti-SSA, anti-dsDNA, anti-ssDNA and anti-RNP autoantibodies (96). However the fact that these mice die so early and they do not show SS-related symptoms such as dry mouth and dry eyes, renders them inappropriate candidate for the study of human SS (95, 96).
To overcome the extremely high lethality of the TGF-β1 mice, Turpie *et al*. generated the TSP-1 KO that lack thrombospondin-1, one of the main activators of TGF-β1 (97). TSP-1 KO mice exhibit a less severe phenotype than the TGF-β1 KO mice, and they show inflammatory infiltration of their LG, accompanied by dry eyes and anti-SSA and anti-SSB autoantibodies. Additionally, CD4+ T-cells of these mice produce increased levels of IL-17, a marker of chronic inflammation (97). However, these mice could serve only as a model of dry eyes and not systemic SS.

Act1 KO mice
Act1 acts as a negative regulator of both T and B-cells (98) and mice lacking this protein (Act1 KO mice) develop a systemic autoimmune disease with features from both SS and lupus-like nephritis (99). By the age of 6 months, all Act1 KO mice have developed inflammatory infiltrations in both SGs and LGs with subsequent development of dry mouth and dry eyes, as well as anti-SSA and anti-SSB autoantibodies in their serum (99).
However, the above-described phenotype does not show a female sex predilection as in human SS. Additionally, they do not show inflammatory infiltration in other tissues apart from kidneys, which do not consist a common target in human SS (99).

Aire KO mice
Autoimmune regulator (Aire) is a transcription factor that plays a crucial role in self-tolerance and therefore, Aire deficient mice develop an autoimmune exocrinopathy that resembles SS and is affected also by the genetic background of the mice (100, 101). These mice develop lymphocytic infiltrations in their SGs and LGs with subsequent severe dry eyes and anti-a-fodrin autoantibodies (102, 103). Interestingly, in these mice, another lacrimal gland autoantigen, the odorant binding protein 1 (OBP1a) has been discovered and has been associated with disease development (104). Moreover, studies in the initial stages of the disease in Aire KO mice, have shown enhanced inflammatory marker production (IFN-γ, IL1β, IL6-STAT3 etc.), increased vascularisation, tissue remodeling and decreased innervation of the lacrimal glands even before the development of the clinical syndrome (102). Therefore, it has been suggested that this mouse model can be used to study the corneal and lacrimal gland neuropathy observed in human SS, which may be driven by a new pathway, the semaphoring-plexin pathway (102, 105).
However, Aire KO mice do not show a female sex predilection and they do not show any systemic symptoms or dry mouth, in contrast to human SS (103).

ERdj5 KO mice
ERdj5 is a chaperone protein with various functions in the Endoplasmic Reticulum, and it has been shown to play an important role in protein quality control in the SGs (106). Interestingly, this protein has been found to be highly expressed in the acinar and ductal epithelial cells and the infiltrating mononuclear cells in the minor SGs of SS patients, while its levels correlate positively with anti-SSA positivity and the severity of inflammation (107). ERdj5 KO mice exhibit lymphocytic infiltration in their SGs (mainly by B-cells and less by T-cells) with subsequent dry mouth, anti-SSA (Ro52 and Ro60), anti-SSB (in older age in comparison to the anti-SSA) autoantibody production and increased proinflammatory cytokine milieu (IL17, IL18, IL23), all of which show a female sex predilection (107).
However, ERdj5 KO mice do not exhibit dry eyes or systemic symptoms, and they do not show any inflammatory infiltration in their LGs or other organs (apart from rare infiltrations in the renal pelvis) in contrast to human SS (107).

CD25 KO (IL-2Rα$^{-/-}$) mice
Signalling through IL-2Ra (CD25) is important in Th17 differentiation and inflammation, and as a result, CD25 KO mice show inflammatory infiltrations in many different organs and tissues such as colon, exocrine glands, pancreas and bone marrow (108). Especially in their LGs, CD25 KO mice show dacryocystitis along with inflammatory infiltrations characterised by early Th17 and late Th1 cytokine predominance, damage in the cornea and conjunctiva and reduced innervation (109, 110). The severity of dacryocystitis correlates with anti-M3R autoantibodies in the LGs of these mice (111) and the whole inflammatory process eventually leads to fibrosis, atrophy of the LGs and dry eyes (112). Interestingly, CD25 KO phenotype is considered to be at least partially, IFN-γ-dependent (109, 111).
Unfortunately, CD25 KO mice do not show a sex predilection and they do not show dry mouth and anti-SSA/anti-SSB autoantibodies as seen in human SS (112).

Nfkbiz$^{-/-}$ mice
Nfkbiz$^{-/-}$ mice (or IkB-ζ KO mice) which lack IkB-ζ, develop dacryocys-

titis, conjunctivitis, facial dermatitis and often interstitial pneumonia, accompanied by splenomegaly, lymphadenitis and anti-SSA and anti-SSB autoantibodies. Inflammatory infiltration consists mainly of T and B-cells, and is followed by increased Th1 proinflammatory cytokines such as IFN-γ. Interestingly, epithelial cell death preceded lymphocytic infiltration in the LGs of these mice (113). Moreover, the deletion -specifically on epithelial cells- of either IkB-ζ (K5-Cre;Nfkbiz$^{flox/flox}$ mice) or its upstream molecule STAT3 (K5-Cre;Stat3$^{flox/flox}$ mice), leads to a similar phenotype, thus supporting an essential role of epithelial cells (113).
However, all these three mouse models lack a sex predilection and do not develop dry mouth or other systemic symptoms similar to human SS.

IκBα$^{M/M}$ mice

IκBα is a negative regulator of NFkB which is induced by NFkB activation, acting as a negative feedback loop (114). Interestingly, genetic polymorphisms in the promoter region of IκBα have been associated with SS susceptibility (115). IκBα$^{M/M}$ mice bear a knock-in mutation in the enhancer of the promoter of kB that blocks the NFkB-induced upregulation of IkBα, therefore showing enhanced NFkB activity and signalling. These mice show increased proinflammatory markers (IL1α, IL17, TNF-α), lymphocytic infiltration in SG, LG, liver, pancreas and lungs (mainly by CD4$^+$ T-cells and also by B220$^+$ B-cells), as well as anti-DNA, anti-SSA/Ro and anti-SSB/La autoantibodies that resemble human SS. Additionally, they show decreased numbers of naive T-cells with simultaneously increased numbers of activated T-cells. The uncontrolled NFkB activation seems to break tolerance (both central and peripheral) and drive autoimmunity either by affecting thymic selection or by enhancing T-cell Receptor responsiveness and decreasing CD4$^+$ T-cell apoptosis, leading to the development of autoimmunity (116).
However, the clinical significance of the histopathological findings as well as any possible sex predilection have not been evaluated to assess whether this mouse model resembles human SS.

Transgenic mice

RBAp48 transgenic mice

Retinoblastoma-associated protein 48 (RBAp48) – transgenic C57BL/6 mice, overexpress RBAp48 in their exocrine glands, similar to the overexpression observed in ovariectomised mice (117). These mice exhibit lymphocytic infiltration in their SGs and LGs (mainly by CD4$^+$ cells and less by CD8$^+$ and B220$^+$ cells), increased exocrine gland apoptosis (p53 mediated), decreased saliva and tear production, ectopic MHCII expression on glandular epithelial cells, and anti-SSA, anti-SSB and anti-a-fodrin autoantibodies, all of which show a female predilection (117, 118).
Despite the big resemblance of the above phenotype to human SS and its significance in studying the importance of oestrogens to autoimmune exocrinopathies, the exocrine gland-specific overexpression of RBAp48 is artificial and does not mimic the development of the disease in humans. Moreover, RBAp48 transgenic mice do not show lymphocytic infiltration or symptoms from other organs/tissues as observed in human SS (118).

BAFF transgenic mice

BAFF transgenic C57BL/6 mice (BAFF-tg) overexpress B-cell Activating Factor which results in B-cell hyperproliferation and systemic autoimmunity with lymphocytic infiltration in their SGs and LGs, decreased saliva production, SLE-like nephritis and increased production of rheumatoid factor and anti-DNA autoantibodies. Therefore, these mice could serve as a mouse model of SS secondary to SLE (119). Finally, BAFF-tg mice exhibit neuroinflammation and anxiety-like behaviour, which could probably mimic some of the neuropsychiatric symptoms seen in human SLE-SS (120, 121).
However, these mice do not produce anti-SSA, anti-SSB or other human SS-specific autoantibodies, and do not develop dry eyes or symptoms from other organs/tissues (apart from kidneys). Moreover, they do not show a sex predilection and they exhibit dry mouth only at older ages (>1 year)(119).
Interestingly, the TNF$^{-/-}$BAFF-tg mice, apart from the above-described phenotype, show a surprisingly high prevalence (>35%) of B-cell lymphomas mainly in their cervical, inguinal or mesenteric lymph nodes, but also in their small intestine (Mucosa Associated Lymphoid Tissue-like lymphoma), which is something unique in a mouse model of human SS (despite its weaknesses) (122).

HTLV-1 Tax transgenic mice

In HTLV-1 Tax transgenic mice, the expression of the HTLV-1 *tax* gene is controlled by the long terminal repeats (LTRs) of the virus and its expression correlates initially with the development of exocrine gland cellular proliferation, followed by inflammatory infiltrations in the SGs and LGs of the transgenic mice (123).
Although these mice have been proposed as a model to study the possible connection between SS and viral infections (124), they are far from recapitulating human SS phenotype. Firstly, no clinical features (dry mouth, dry eyes, systemic symptoms) or autoantibodies have been described in these mice. Secondly, they do not show any sex predilection. Finally, they often develop neuroblastomas, something that is not observed in human SS (123).

IL-12 transgenic mice and IL-14a transgenic mice

SJL IL-12 transgenic mice that overexpress the IL-12 heterodimer (p70) under the control of the thyroglobulin promoter show lymphocytic infiltration in their SGs and LGs consisting mainly by B220$^+$ cells and less by CD4$^+$ cells, followed by decreased saliva production. Additionally, they exhibit increased ANA and anti-SSB/La autoantibodies, in an age-dependent manner (125). However, these mice do not show dry eyes or symptoms from other organs/tissues as it happens with human SS (125).
IL-14a transgenic mice overexpress IL-14a mainly in the B-cell compartment, and they exhibit features of sialadenitis, hypergammaglobulinaemia, SLE-like nephritis and rarely positive autoantibodies (anti-SSA, anti-SSB, anti-dsDNA, anti-RNP etc.), thus making them a possible model for SS sec-

ondary to SLE (126). However, these mice do not show any clinical relevance to human SS phenotype (dry mouth, dry eyes, other systemic symptoms etc.), and are not characterised by a female sex predilection. Additionally, the presence of typical SS-specific autoantibodies is rare (126). It is of great interest however, that the majority of these mice develop large B-cell lymphomas ($CD5^+CD19^+CD21^-$) in their liver, gastrointestinal tract or lung, in a pattern that very much resembles the one seen in human SS-associated lymphomas (126).

IL-10 transgenic mice and Opn transgenic mice

IL-10 transgenic mice that overexpress IL-10 under the control of salivary gland amylase promoter exhibit lymphocytic infiltration in both SGs and LGs, consisting mainly by $CD4^+$ T-cells ($FasL^+$), while the salivary glands ectopically express MHCII. Subsequently, these mice exhibit decreased saliva and tear production (127). However, IL-10 transgenic mice do not show any systemic symptom or autoantibody, and lack sex predilection as observed in human SS (127).

Mice overexpressing osteopontin in bone tissue (using the immunoglobulin enhancer/SV40 promoter) show significantly reduced saliva production, lymphocytic infiltrations in almost half of their SGs and LGs, anti-SSA autoantibodies in their sera and increased levels of IL-4, IL-6, IL-2 and TNF-α, with a clear female predilection (128). Interestingly, Opn levels have been found to be increased both in salivary glands and sera of patients with SS (129, 130). However, in comparison to human SS, these mice do not show dry eyes, any systemic symptoms or infiltration of other organ/tissue (128).

Immunisation-induced mouse models

Salivary gland protein-induced model

Immunisation of SL/Ni or C57BL/6 mice with syngeneic submandibular gland homogenate leads to lymphocytic infiltration within the SGs of these mice (131, 132). Additionally, the C57BL/6 mice show decreased saliva secretion, production of anti-M3R autoantibodies and increased expression of proinflammatory cytokines including TNF-α, IL1 and IL-6 (both locally in the SGs and systemically), in an Th17-IL17 – dependent manner (133, 134).

However, these mice do not show dry eyes or inflammatory infiltration in their LGs, nor do they show systemic symptoms or inflammatory infiltration in other organs/tissues. Additionally, they do not produce anti-SSA or anti-SSB autoantibodies and they do not display a sex predilection. Finally, the submandibular gland homogenate is a complex mixture of variable possible antigens that could trigger the observed phenotype, so the causative antigen remains unknown (134).

Carbonic anhydrase II-induced model

Patients with SS or SLE have been found to express autoantibodies against CAII (135). Intradermal immunisation of PL/J ($H\text{-}2^u$) mice with human CAII leads to the development of sialadenitis with lymphocytic infiltration in the SGs of these mice. Interestingly, some of these mice develop similar infiltrations in their kidneys and pancreas (136). Additionally, these mice show a urine acidification defect, that could mimic renal tubular acidosis described in SS patients (137, 138).

However, these mice do not show infiltrations in their LGs, nor do they present with dry mouth, dry eyes or any other systemic symptom, while SS specific autoantibodies are not detected (136). Finally, CAII seems unlikely to be a major target antigen in the pathogenesis of human SS, and therefore this mouse model cannot be used to study/explain main aspects of the disease (139).

M3R (M3 muscarinic acetylcholine receptor) peptide immunisation

M3R plays a key role in the parasympathetic innervation of SGs and LGs. Many SS patients show either anti-M3R autoantibodies or M3R reactive T-cells in their blood (140). To establish an M3R mouse model, $M3R^{-/-}$ mice were injected with fragments of M3R and then splenocytes from these mice were transferred to $Rag1^{-/-}$ mice. These mice show very high levels of anti-M3R autoantibodies, lymphocytic infiltration in their SGs (mainly by CD4 T-cells and fewer B-cells, IFN-γ and IL-17 producing T-cells) and decreased saliva production. Interestingly, transfer of only $CD3^+$ T-cells from $M3R^{-/-}$ immunised mice into $Rag1^{-/-}$ mice (that completely lack mature B- and T-cells), led to the same phenotype of autoimmune sialadenitis, suggesting a key pathogenic role for $CD3^+$ T-cells (141).

However, the above-described model could only serve as a model of autoimmune sialadenitis since no other systemic infiltration or symptom has been described. Additionally, no sex predilection was found in the phenotype of these mice and no other autoantibodies such as anti-SSA or anti-SSB were detected in their sera, in line with human SS (141).

On the contrary, although the immunisation of BALB/c with modified peptides of the 2^{nd} extracellular loop of M3R (a region suspected to carry a disease-promoting epitope), induced the production of autoantibodies against M3R that could bind on the SGs, it did not result in any histological or clinical manifestation as described above. Therefore, anti-M3R autoantibodies, at least in these mouse models, might not play a pathogenic role in the observed phenotype (142).

Ro60 peptide immunisation

Different Ro60 peptides have been used for mice immunisation. The peptides used by Scofield *et al*. for the immunisation of BALB/c mice, led to anti-SSA/Ro and anti-SSB/La autoantibody production, lymphocytic infiltration in their SGs (mainly by $CD4^+$ T-cells, and also by $CD8^+$ T-cells and B-cells) and decreased saliva production. (143). However, the above-described phenotype is strain dependent, since other mouse strains immunised with the same protocol did not exhibit the same phenotype (144).

On the other hand, Zheng *et al*. used another Ro peptide that contains a predominant T-cell epitope for the immunisation of mice. This immunisation resulted in anti-Ro/SSA and anti-La/SSB autoantibody production, lymphocytic infiltration in the LGs of the mice and decreased tear production, in a B-cell

dependent manner. Again, the observed phenotype was strain dependent (145). Interestingly, these mice showed ectopic MHCII expression in their LGs even before disease presentation (146). The generation of anti-La autoantibodies in both models, suggests intermolecular epitope spreading.

These models could possibly shed light to some aspects of human SS pathogenesis (for example the pathogenic role of a T-cell epitope within the human Ro antigen). Additionally, the variability in their phenotype could reflect the variability seen in human genetic background and the difference in SS development and manifestation. However, both models show only local infiltration (either in the SGs or in the LGs of the mice) of lymphocytes and subsequent local clinical manifestations, but no systemic infiltrations or symptoms. Additionally, they do not show any sex predilection similar to the human SS (143, 145).

Infection-induced mouse models

Adenovirus 5-induced model

The infection of C57BL/6 submandibular salivary glands by replication deficient adenovirus-5 (Adv5) using retrograde excretory duct cannulation, leads to local infiltration by T and B-cells, formation of ectopic lymphoid structures, production of ANA (suggesting breach of immune tolerance related to the antiviral immune response) and decreased saliva production only by the infected SG (147). In addition, more detailed analysis of this model has shed light in the step-by-step procedure of immune cell infiltration of the SG and the formation of ectopic lymphoid structures. Interestingly, it seems that the initial recruitment and activation of innate immune cells (monocytes/macrophages, dendritic cells etc.) plays a crucial role in T- and B-cell recruitment and activation and the subsequent ectopic lymphoid structure formation (148).

Although this model could serve as a useful tool to study the mechanisms underlying viral infections and autoimmunity, as well as formation of ectopic lymphoid structures in autoimmune diseases, it does not recapitulate the entirety of human SS. The histological and clinical findings are contained only in the infected gland. Additionally, no pathology is described from the LGs or other organs/tissues. Finally, no sex predilection has been described in this model (147). On the other hand, since the Adv5 does not have the ability to replicate, local inflammation resolves spontaneously after 4-5 weeks and therefore, this mouse model cannot be used to study chronic SG inflammation (as is the case in SS) (148).

Murine CMV-induced model

Intraperitoneal injection of 4 different mouse strains (C57BL/6, Fas-deficient C57BL/6-lpr/lpr, C57BL/6-tnfr1$^{-/-}$, and C57BL/6-tnfr1$^{-/-}$-lpr/lpr) with the murine CMV (that replicates into the acinar cells of exocrine glands), results in extensive inflammatory cell infiltration of the SGs of these mice at 28 days after the infection. However, only the lpr/lpr mice show severe chronic inflammation at 100 days after the infection, as well as anti-SSA and anti-SSB autoantibodies(149).

Simultaneously, infection of NZM2328 mice with murine CMV leads to inflammatory infiltration in both SGs and LGs (mainly by CD4$^+$ T-cells and B-cells), as well as decreased saliva production and autoantibodies against exocrine gland antigens, mainly in female mice. The development of autoimmune features from the kidneys of these mice, was not affected by the infection (150).

The above-described model could be used to study the connection between viral infections and SS. However, these mice do not develop the whole phenotype observed in human patients since their "disease" is mostly local in the SGs ± LGs. Additionally, they do not produce "classical" human SS autoantibodies such as anti-SSA and anti-SSB. Finally, the fact that the infection with murine CMV leads to SS-like features only in autoimmunity-prone mouse strains such as lpr/lpr or NZM2328, limits its utility as a model for human SS, although in some cases, SS can be triggered by an environmental factor such as a viral illness in a person with an autoimmunity-prone genetic background (149, 150).

A humanised model of SS

To better mimic the human disease, immunodeficient NOD-scid IL2rγ mice (NSG) received peripheral blood mononuclear cells (PBMCs) from patients with SS. These chimeric mice produce increased levels of cytokines such as IFN-γ, TNF-α and IL-10 and exhibit inflammatory infiltration in their SGs and LGs consisting mainly by CD4$^+$ T-cells and less often by CD8$^+$ T-cells and B-cells, with subsequently decreased salivary flow (151).

However, in comparison to human disease, decreased lacrimal flow has not been established, while inflammatory infiltration has not been detected in other organs. Additionally, since SS in humans is a quite heterogenous disease, each chimeric mouse of the above model will represent only the patient's PBMCs used for the generation of the mouse. Finally, since NSG mice commonly show graft-versus-host inflammation at least 30 days after the transfer, the above model cannot be safely used for long-term studies (151, 152).

Discussion

It is known that humans and mice show a significant synteny, with less than 1% of mouse genes lacking any homology to their human counterparts (153, 154). Additionally, their small size, the easiness of their breeding, practicality, short lifespan, easiness in genetic manipulation, high fertility and a relatively low maintenance cost compared to bigger animal models (such as primates), have made mice perfect candidates for modelling human disease (155, 156). More than 30 mouse models have been developed in order to study SS pathogenesis, which has led to the identification of many important aspects of this disease in humans (13).

However, there are many established differences between mice and humans, that make the translation of research findings using mouse models quite intriguing. First, as described previously in our review, many of the mouse models utilised in SS research are used mainly because of the histological findings that resemble human SS, which do not necessarily translate into a phenotypical similarity. Secondly, the hu-

man immune system differs profoundly from that of mice, not only due to species-specific differences, but also due to genetic irregularities in mouse immune system that result from inbreeding, lifestyle differences, evolutionary divergence and smaller infection "burden". These differences concern not only the effector arm of the immune system *per se*, but also its regulatory components (154, 157). Additionally, mouse studies usually utilise inbred strains that exhibit a high genetic homogeneity which contrasts strongly with the heterogeneity found in human patients. This means that a mouse model can mimic only a specific subset of human patients (33). Moreover, the initiation of the disease, especially in the induced mouse models, is highly artificial, and the timing of events (*e.g.* disease triggering or perpetuation) differs from what happens in humans (156). Furthermore, especially regarding SS, researchers should consider other basic factors such as strain and age of the mice used, due to differences in the time-course (or other aspects) of the disease. The huge differences between the lifespan of humans and mice, cannot go unnoticed when interpreting the results from mouse studies. In addition, the exocrine glands show functional and morphological differences both between species (humans vs mice) and among humans. Finally, it has also been suggested that anaesthesia used in mouse experiments might affect measurements such as saliva secretion (158).

For all the above reasons, no ideal mouse model exists for SS. Each different model mimics a specific aspect of the human counterpart SS disease. Therefore, all the existing models shed light in different aspects of the disease and all these information must be complemented and translated cautiously to get a more spherical view of the disease pathogenesis. Additionally, it is noteworthy that, in the current literature, the mouse models of SS do not correlate with some important biologic features of human SS such as cryoglobulinaemia, cryoglobulinaemia-related manifestations, RFs connoting type II cryoglobulins, hypocomplementaemia and the formation of germinal centres. There is the possibility however, that some of the above-described mouse models might show such features, but researchers have not described them yet. Another very important deficit in the current armamentarium used in SS research, is the lack of a mouse model that develops the variable phenotype of SS including lymphoma. One such model could help us understand the lymphomagenesis process of this malignant transformation in SS patients in order to prevent it. Moreover, it could shed light in the development of lymphomas and other haematological malignancies in non-SS patients. As described previously, the IL-14a transgenic mice and the $tnf^{-/-}$BAFF transgenic mice, are mice that develop SS-like phenotype and lymphomas that quite often present in a similar pattern to that observed in humans. However, the phenotype of these mice is far from what is seen in human SS. Additionally, both of them overexpress a protein that plays a key role in B-cell physiology and therefore, the development of a B-cell lymphoma, even without the detection of sialadenitis or other histological findings that resemble SS, is not a surprise.

Despite the above-described weaknesses of the mouse models used in the field of SS research, we also need to think how we could better exploit them. First of all, each mouse model can be used to study specific aspects of the disease (*e.g.* study the role of oestrogens or the role of genetic factors etc.). Secondly, some of the models can be used to study the initial stages of disease development (such as infection-induced models). Thirdly, the use of conditional knock-out or transgenic mice, can better compartmentalise and localise diseases aspects into specific cells or tissues. Finally, different models can be used for the study of different cell- or tissue- or organ-specific or even biological treatments. It is well known that mouse models are perfect models for mice. However, their interpretation with cautiousness and the realisation of the differences between human and mouse (patho)physiology can make them a useful tool in our hand in order get better insights into human disease pathogenesis.

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