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Sjögren Syndrome: What and where we are looking for?

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Abstract

Purpose of review—Sjögren Syndrome (SS) affects exocrine glands leading to dry mouth and dry eye. Dry eye manifestations can precede the diagnosis of SS in by many years. Innumerable spontaneous and inducible SS models have been used to study pathogenesis of SS. This review focuses on recent human data, ocular and extra-glandular manifestations of animal models, what is known, what is still unknown and how we need to look, and their correlation correspondence to the human disease.

Recent findings—Hallmarks of dry eye in SS include increased corneal staining, goblet cell loss and low tear volume. Confocal microscopy and impression cytology is being able to clarify new markers of the ocular disease. Extra-glandular manifestations should alert for more severe complications in the eye. Some models have strong gender and exocrine gland predilection while aging generally worsens disease phenotype. While most models do not display a significant increase in corneal staining or tear secretion impairment, conjunctival infiltration and decrease in goblet cells are frequent seen.

Summary—We have seen great advances in the role of inflammation in ocular, oral and extra-glandular manifestations of SS. Several of the understanding of mechanisms and mediators of SS were elucidated in animal model studies.

Keywords

Sjögren's syndrome; corneal barrier function; goblet cells; dry eye; animal models; dacryoadenitis; sialadenitis

Introduction

Sjögren syndrome (SS) is characterized by symptoms of dry mouth and dry eye, secretory dysfunction of lacrimal and salivary glands, ocular surface staining, autoantibodies and focal lymphocyte infiltrate in salivary glands, and it is about 10 times more frequent in women. The autoimmune mechanism is the mostly accepted, and the primary cause is unknown [1]. The dry eye manifestations, SS or not SS related, are the second most prevalent reason to

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visit an ophthalmologist. The hallmark of dry eye is corneal barrier disruption, clinically measured as increased uptake of fluorescent dyes such as fluorescein. For decades animal models are being used to better understand the physiopathology of SS. Countless rodent strains with or without interventions have been described in an attempt to identify tissues, cells and molecules involved in the pathogenesis and to figure out their interactions, possible causes and potential targets for therapy. However, very frequently, more attention is being given to the novelty of a rodent model, than to identify which strain better fits on the cardinal parameters that define this syndrome [2].

The three major questions regarding SS would benefit from research on an animal model or comparative analysis among those models that mimic better the disease. They are: 1) the genetic and environmental causes and risk factors (etiology), 2) the time-course of the disease (prognosis) and 3) the efficient therapeutic approach (cure). The aim of the present review is to critically evaluate what is known about the relationship among the traditional and emerging animal models of SS and the characteristics that define SS, with special attention to the ocular manifestations. Table 1 shows a summary of the models to be discussed.

Advances understanding Sjögren's syndrome manifestations and approach

A recent meta-analysis that filtered 21 papers from 1800, from the period between 1995 and 2013 showed an incidence of primary Sjögren's syndrome of 7 cases per 100,000 persons/year and the average age of the patients as 56 years old [19]. Clinical studies with large samples of patients with SS revealed that patients with extra-glandular manifestations, including vasculitis and neuropathy have more severe ocular complications and higher risk of mortality [20,21]. In addition, SS secondary to rheumatoid arthritis (RA) is a disease with distinct and more severe systemic characteristics when compared to primary SS [22].

We have seen great advances in understanding the role of diagnostic tools to monitor and correlate inflammation in ocular, oral and extra-glandular manifestations of SS [23,24].

In the last decades, animal models clarified the role of inflammatory cells and mediators, their interactions with the target tissue and autonomic nervous system, effects of the environment, oxidative stress and aging [18,25-27]. They also have being elucidative to the potential of novel treatments with biological, stem cells and gene therapy [28-30].

In vivo confocal microscopy has been a new tool among the clinical instruments that is capable to identify inflammatory cells, goblet cells and nerve fiber density *in vivo* [31]. Evaluation of conjunctival cells obtained by impression cytology has been used to evaluate expression of HLA-DR by flow cytometry, investigation of cytokines by real-time PCR and oxidative stress in SS patients [31-34]. A study showed that aqueous tear deficient patients (irrespective of SS or non-SS status) had the greatest IL-17, IFN- γ and lower MUC5AC mRNA transcripts compared to normal subjects [35]. Another recent study clearly showed that 0.1% dexamethasone eye drops blunted the acute adverse effects of an experimental peri-ocular low humidity challenge [36].

History

In his initial publications regarding keratoconjunctivitis sicca, Dr. Henrik Sjögren described 19 women with dry eye, in part of them other aspects including salivary gland and other organ dryness and inflammatory infiltrates [37,38]. It took three decades to the disease be relabeled as SS and more than 20 years to an autoimmune mechanisms be attributed to SS [39].

Those concepts paved the diagnostic criteria ways and open the opportunities for studies in animal models [40].

The first attempts to induce SS in animal models were with chronic use of hydralazine, isoniazid, and procainamide among other drugs. Both hydralazine and isoniazid, given for 6 months, independently, induced the expression of anti-nuclear factor (AFN), more frequently in C57BL/6 than in Balb/c mice; more frequently in females than in males and in aged mice. These observations were not reverted in most of them after drug discontinuation [41]. Although no other parameters regarding SS were reported for this or other drugs in animal models, there are clinical reports of SS-induced by chronic hydralazine and other drugs in humans that reverted after the drug discontinuation [42,43]. The mechanism that lead those drugs trigger autoimmunity and SS is suggested to include DNA methylation and histone modification influencing gene regulation [44].

In the seventies, the use of spontaneous animal models of autoimmunity took the place of the drug-induced SS in the bench and these models were deeply investigated. In the ninety-decade of XX century the mice strains submitted to gene knockout replaced the spontaneous models. In recent years, despite all of them are available, the animal models more frequently used to study SS are those that combine environmental, drug and/or genetic intervention [45,46].

Spontaneous Animal Models of SS

Among the rodent models to study SS, along the decades of 1970 and 1990, two were more frequently used NZB/w 1 and MRL/lpr. Other emerging models are NOD, C57BL/6.NOD-Aec1Aec2, CD25KO, TSP-1KO mice. Frequent endpoints in animal models are salivary flow, presence of autoantibodies in serum, systemic cytokine expression and within the glands, and, histopathology and focus score. Specific ocular manifestations that have been evaluated and are relevant for the human disease include tear volume, tear protein concentrations, increased uptake of fluorescent dyes (to measure corneal barrier function), number of mucin-filled goblet cells and CD4⁺ T cell infiltration in conjunctiva.

1) NZB/W F1—The first generation of inbred New Zealand Black mouse (NZB) and with New Zealand White mouse (NZW) developed autoimmunity characterized by lymphocytes B hyper reactivity and autoantibodies production. NZB/W F1 also shows lymphocytic infiltration of the lacrimal and salivary glands, initially with foci pattern, that progress with destruction of the acinar structures and *sicca syndrome*. The disease starts by the age of 6 months, it is more aggressive in females. It can be worsened with aging and by inflammatory challenge with Freund's incomplete adjuvant [47-50].

No corneal or other ocular surface changes were observed along of the disease progression [16]. Female mice die in general by the age of 9 month due to autoimmune disease and males live around one year [51].

2 MRL/lpr—MRL/lpr mice develop autoimmune disease comparable to SS, due to a spontaneous deletion in the gene *lpr*, responsible for coding the pro-apoptotic protein Fas, a member of the TNF- α receptor family. The absence of Fas induces proliferation of lymphocytes, in target organs, including lacrimal and salivary glands; markedly positive CD4⁺ T helper (Th) cells, among other cells. There is also infiltration of choroidal and scleral tissues. The infiltrates have a focal distribution and usually surround small arterioles, similar to necrotizing vasculitis. The disease progresses from the first to the fifth month of age. It achieves a rate of 67% by 6 months old, is more frequent in females and commonly the death occurs by 6 months of age with glomerulonephritis, arthritis and vasculitis [51]. These MRL/lpr mice also develop circulating autoantibodies, such as anti-Ro and anti-Sm [52,53]; however, it does not affect 100% of the mice, suggesting the role of an environmental trigger [54].

Despite of its massive lacrimal gland lymphocytic infiltration, these animals do not develop secretory dysfunction or corneal damage measured by fluorescein staining [55].

The genetic background of the *lpr* mutation seems to determine the extension and pattern of inflammation. Balb/c mice are Th-2 prone while C57BL/6 (B6) mice are Th-1 skewed. MRL/lpr mice in Balb/c background have greater lymphocytic infiltration than C57BL/6 mice carrying the same mutation (B6.Lpr) in lacrimal gland [49]. Compared to wild-type mice, MRL/lpr mice have increased numbers of filled goblet cells, [56] probably due to a high protective IL-13 environment [57]. B6.Lpr mouse have increased lymphocytic infiltration in the lacrimal gland at 8 and 12 weeks of age compared to wild-type mice (Fig. 1), increased CD4 infiltration in the conjunctiva and lower goblet cell density (Fig. 1) while no change in corneal barrier function (data not shown). While the both strains developed dacryoadenitis, albeit not at the same level of severity, the opposing findings regarding goblet cell density highlights the importance of genetic background in some of these spontaneous models of SS.

3) NOD—The non-obese diabetic strain (NOD) is a spontaneous mice model of autoimmune disease, used to study diabetes mellitus type 1 (DM) and SS. It was induced by hyperglycemic brother and sister mice inbreeding for more than 20 generations [58].

The disease onset occurs by the 4th week of life with lymphocytic infiltrate of the target tissues, and it is present in about 95 % of the mice by 30 weeks of age [59]. The manifestations have a gender-related incidence and are regulated by sex hormones [60]. About 80% of female and less than 20 % of male become diabetic, the sialoadenitis follow the same pattern; however, the dacryoadenitis is biased towards male mice [49,61,62]. In addition, the disease may be influenced by housing conditions and intestine microbiota [45].

The salivary flow rate and the relative protein concentration in saliva are reduced in comparison to control mice [63]. Tear secretion, conjunctival goblet cells and lymphocytic

infiltration are changed in comparison to C57BL/6 strain [64]. Nevertheless, the ocular surface and cornea integrity seems not to be affected by the disease [17].

4) C57BL/6.NOD-Aec1Aec2—The C57BL/6.NOD-Aec1Aec2 (Aec) mouse model was created by transferring genes located within the 2 chromosomal intervals Idd3 and Idd5 from parenteral NOD into non-susceptible C57BL/6 mice [4]. The manifestations are age-dependent and both submandibular and lacrimal gland are involved. These mice have anti-nuclear and anti-muscarinic 3 receptor antibodies [4]. The disease in Aec mice has three distinct phases with definite lymphocytic infiltration and loss of salivary flow at 20 weeks of age [65].

We have recently characterized the ocular manifestations of female Aec mice and observed decreased number of filled conjunctival goblet cells (GC) as soon as 12 weeks of age, which was accompanied by an increase in infiltrating CD4⁺ T cells in conjunctiva and increased expression of IFN- γ and IL-17 in cornea and conjunctiva [14]. There was mild lymphocytic infiltration in the lacrimal gland (the infiltration was a mix of Th-1 and Th-17 cells, CD8 and B cells) and a paradox increase in tear volume. The ratio of IgA (normally produced by LG) to IgG and IgM (plasma immunoglobulins) increased with aging from 4-20 weeks in wild-type mice while it did not change in Aec mice, indicating that ocular changes occurred independently of tear volume changes.

5) CD25KO—IL-2 signals through a heterodimeric receptor composed of three chains. The alpha chain (also known as CD25) is the binding portion of the receptor. CD25KO mice have no regulatory T cells and the activated T cells escape control of activation-induced T cell death, becoming autoreactive [66]. These mice develop lymphocytic infiltration of several organs, notably salivary and lacrimal glands (LG) and colon [67]. The dacryoadenitis is very severe, develops rapidly and it is age-dependent [68]. Mice start to have LG infiltration around 4 weeks of age [15] and complete LG disorganization and atrophy is observed around 12-16 weeks [7]. There is no gender predilection in this model [68] but anti-muscarinic receptor antibodies can be found in the sera [7]. There is a marked inflammation at the conjunctiva of CD25KO mice and corneal barrier disruption at 12 weeks of age, which paralleled the increase of IL-17A mRNA transcripts in cornea epithelia [18], T cells infiltration and loss of goblet cells (Fig 2). Deletion of interferon- γ delayed dacryoadenitis temporally and improved severity of lacrimal gland infiltration [7] while rescued the number of filled goblet cells (Fig. 2), implicating a pathogenic role for Th-1⁺ cells.

6) TSP-1KO—Thrombospondin-1 (TSP-1) is a physiological activator of TGF- β and it is expressed in both dendritic cells and ocular surface epithelia [69]. TSP-1KO mice develop SS-like disease with aging, inclusive of anti-SSa and anti-SSb antibodies, progressive dacryoadenitis, conjunctival inflammation and loss of filled goblet cells [8,13]. They also develop increased corneal fluorescein staining [8]. Disease phenotype is first observed around 12 weeks of age and worsening of dacryoadenitis and inflammatory cytokines are seen around 48 weeks of age. Similar to another transgenic mouse where TGF- β signaling is disrupted in CD4⁺ T cells [69], TSP-1KO mice show resistance to desiccation-induced dry eye [70]. These results indicate a strong environment component in disease induction.

Inducible SS animal models

There are many inducible SS animal models. A short and non-comprehensive list includes preganglionic parasympathetic denervation [71], administration of benzalkonium eye drops [72,73], injection of adenoviral vectors into salivary gland [74], injection of IL-1 [75] or activated CD4⁺ T cells into lacrimal glands or into immunodeficient mice [76-79], injection of botulinum toxin A into lacrimal glands [80] and pharmacological blockade of lacrimal gland secretion combined with environmental stress [81-86]. Interestingly, these inducible models tend to represent acute changes and many of them have marked corneal barrier disruption, with significantly increased permeability to fluorescent dyes, the hallmark of dry eye disease. Also of note, maintenance of stimulus and prolonged dryness leads to corneal metaplasia and increased expression of skin-like small proline-rich proteins, which are impermeable to dyes [83,84,87]. Therefore, it is plausible to speculate that lack of corneal staining in some of the spontaneous rodent models may not be an indication of lack corneal dysfunction, but rather an indication of corneal metaplasia that warrants further investigation.

Conclusions

A number of SS dry eye models in a variety of species have been developed to improve the understanding of the disease. Here we described the correlations between mice models and clinical and laboratorial aspects of SS. The weakness of those models to the broad understanding of the disease is the lack of one or more features of the disease. The strength is the fact that the starting point, time-course of the signs and the dose-response of the interventions are well controlled. Pathologic and therapeutic aspects of SS have been benefited from those experimental models.

Take-home messages are: a) in the clinic, most of the patients arrive with incomplete features of SS, mostly with symptoms of dry eye; therefore it is mandatory to investigate SS, prior to the irreversible complications appear; b) an animal model that carries association of glandular and extra-glandular manifestations helps to understand the mechanisms of SS, and the initial manifestations of the disease and response to therapy.

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* of special interest

** of outstanding interest

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Key points

- Clinical assessment for early diagnosis of SS in dry eye patients may help to prevent irreversible complications.
- Advances in SS dry eye diagnosis involve confocal microscopy and conjunctiva impression cytology markers.
- Ocular manifestations are frequent findings in spontaneous autoimmune models of SS.
- Different autoimmune models with various genetic backgrounds represent different aspects of glandular and extraglandular manifestations of SS.

Fig. 1.

Lacrimal gland and ocular surface parameters in B6.Lpr mice.

A Representative images of haematoxylin and eosin-stained lacrimal gland in wild-type C57BL/6 and B6.Lpr mice at 8 and 12 weeks of age. Normal LG morphology was noted in the C57BL/6 WT at all time points, in contrast to Aec mice who showed lymphocytic infiltration (circumscribed by black dotted lines and highly magnified in blue dotted lines).

B Representative images of conjunctival frozen sections of immunohistochemistry for CD4-positive T cells. Positive cells are stained red and are indicated by black arrowhead.

C CD4⁺ T-cell density in the conjunctival epithelium (mean ± SEM).

D Number of filled goblet cells in conjunctiva in wild-type C57BL/6 and B6.Lpr mice at 8 and 12 weeks of age (mean ± SEM).

Fig. 2. CD4 infiltration and goblet cell density and in CD25 and CD25/IFN- γ double knock-out strains

A CD4⁺ T-cell density in the conjunctival epithelium (mean $\pm$ SEM). **B** Number of filled goblet cells in conjunctiva in wild-type C57BL/6, CD25KO, CD25/IFN- γ double knock-out (CD25KO/IFN- γ DKO) and IFN- γ KO at 8, 12, and 16 weeks of age (mean $\pm$ SEM).

**P<0.01 within strain comparison to 8 weeks of age.

Table 1
Genetic, immune and clinic characteristics of rodent animal models of Sjögren's syndrome

Model	NZB/NZW F1	MRL.lpr	NOD	Aec	CD25KO	TSP1-KO
Primary mutation	<i>C-beta-1, D-beta-2, and J-beta-2</i> [3]	<i>Lpr</i>	<i>Idd3</i> and <i>Idd5</i> [4]	<i>Idd3</i> and <i>Idd5</i> [4]	<i>IL-2Ra</i>	<i>TSP-1</i>
Background	NZB	Balb/c	NOD	C57BL/6	C57BL/6	C57BL/6
Dacryoadenitis	(+)	(+)	(+)	(+)	(+)	(+)
Sialadenitis	(+)	(+)	(+)	(+)	(+)	?
Colitis	Ø	(+)	(+)	?	(+)	?
Gender predilection	♀	♀	♀ sialadenitis ♂ dacryoadenitis	?	♀= ♂	?
Autoantibodies	Anti-nucleic acid, antithymocyte, antierythrocyte, anti-Ro 60 (SSa)	Anti Ro 52, anti-Sm	Anti Ro [5] Anti-M3R[6]	Anti-nucleic acid, anti-M3R [4]	Anti-M3R [7]	Anti-SSa and anti-SSb[8]
T helper (Th) phenotype	Th2	Th2 [9]	Th1, Th17	Th1, Th17[10-12]	Th1, Th17	Th1[8], Th2[13]; Th17[8]
Youngest age observed	6 months	4 weeks	4 weeks	12 weeks[4,14]	4 weeks[15]	12 weeks
Ocular manifestations	Ø[16]	Sclera Choroid	Ø[17]	(+)[14]	(+)[18]	(+)[8]
Corneal barrier dysfunction	Ø	Ø	Ø	Ø	(+)[18]	(+)
Goblet cell density	?	↑	↓	↓[14]	↓	↓
Conjunctival infiltration	Ø	CD4	CD4	mild	Moderate to severe	CD4

♀=female gender; ♂= male gender, (+) = present, Ø=absent; =non-reported, ↑= increased, ↓ decreased