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Systemic manifestations of primary Sjögren's syndrome in the NOD.B10Sn-H2^b/J mouse model

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Abstract

Animal models that recapitulate human disease are crucial for the study of Sjögren's Syndrome (SS). While several SS mouse models exist, there are few primary SS (pSS) models that mimic systemic disease manifestations seen in humans. Similar to pSS patients, NOD.B10Sn-H2b/J (NOD.B10) mice develop exocrine gland disease and anti-nuclear autoantibodies. However, the disease kinetics and spectrum of extra-glandular disease remain poorly characterized in this model. Our objective was to characterize local and systemic SS manifestations in depth in NOD.B10 female mice at early and late disease time points. To this end, sera, exocrine tissue, lung, and kidney were analyzed. NOD.B10 mice have robust lymphocytic infiltration of salivary and lacrimal tissue. In addition, they exhibit significant renal and pulmonary inflammation. We identified numerous autoantibodies, including those directed against salivary proteins. In conclusion, the NOD.B10 model recapitulates both local and systemic pSS disease and represents an excellent model for translational studies.

Keywords

Sjögren's syndrome; autoantibodies; extra-glandular disease; NOD.B10

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1. Introduction

Sjögren's syndrome (SS) is a debilitating disease that is primarily seen in middle-aged women. There are two forms of the disease: termed primary and secondary. Primary SS (pSS) affects the exocrine glands and may also cause systemic disease manifestations including pulmonary, renal and hematopoietic abnormalities [1]. In secondary SS (sSS) patients also have an additional autoimmune connective tissue disease. While studies in human subjects are critical to evaluate disease mechanisms and validate therapeutic targets, work in mouse models is equally valuable, as it allows for initial pathway discovery and interventional studies that are not currently possible in pSS patients [2].

Current work in numerous autoimmune diseases is focused on the identification of early disease markers in order to deliver treatment in a timely fashion and ultimately prevent disease development or reduce its severity in afflicted individuals [3–5]. Good mouse models are critical to discover biomarkers that carry diagnostic and prognostic relevance for human disease. To this end, we performed extensive work in a commercially available pSS mouse model to characterize both exocrine-specific and systemic disease manifestations, thereby enabling clinical translational studies that are highly relevant to human disease.

This work focuses on the pSS mouse model NOD.B10Sn-*H2^b*/J (NOD.B10). NOD.B10 mice are derived from the well-characterized NOD/ShiLtJ strain that develops SS in addition to type I diabetes (T1D). NOD.B10 mice were generated by replacing the NOD/ShiLtJ major histocompatibility locus with that of the healthy C57BL/10 strain [6]. The resultant congenic animals still develop SS but are protected from T1D [6]. SS pathogenesis in NOD.B10 mice shares many similarities with the human disease. Specifically, NOD.B10 mice exhibit a female disease predilection, have anti-nuclear autoantibodies (ANA), and develop the disease spontaneously [7–9]. Moreover, exocrine tissue from female NOD.B10 mice shows histopathologic features of human disease and mice lose salivary flow with disease progression, similar to pSS patients [7–9]. NOD.B10 animals are reported to develop pre-clinical disease at 3 months and clinical disease at 6 months of age [7, 9]. However, careful studies detailing SS-related pathology over time are lacking and extra-glandular manifestations (EGM) of disease in this model are poorly characterized. Therefore, we conducted studies in NOD.B10 female mice at pre-clinical, clinical and advanced stage disease time points to characterize the incidence and severity of exocrine and systemic disease.

2. Materials and Methods

2.1. Mice

NOD.B10 and C57BL10/SnJ (BL/10) mice were purchased from Jackson Laboratories. Animals were bred and maintained at the University at Buffalo. Females were used in all experiments. All animals were cared for and handled in accordance with NIH and IACUC guidelines.

2.2. Sera and saliva collection

To assess disease kinetics, sera and saliva were harvested from female NOD.B10 and age and gender-matched BL/10 controls. We classified the mice as pre-disease (1.5 months of age), pre-clinical disease (3 months of age), clinical disease (6–7 months of age) or as advanced disease (10 – 12 months of age) in accordance with the literature [7, 9]. Sera were harvested by retroorbital bleed or cardiac puncture immediately following euthanasia and stored at –20° C until use. Saliva was collected for 10 minutes following intraperitoneal injection with Pilocarpine HCl (0.3 mg/100 µL). Saliva was centrifuged briefly, measured by pipette, and stored at –80° C as previously described [10].

2.3. Histological Processing and Analyses

Submandibular gland (SMG), sublingual, parotid, and lacrimal gland (LG) tissue was collected. In addition, lung and kidney were harvested. All tissue was formalin fixed, paraffin embedded, and stained with hematoxylin and eosin (H&E). Pulmonary and renal pathology was confirmed by a veterinary pathologist at the Cornell University College of Veterinary Medicine. Slides were scanned using Aperio software (Leica Biosystems) and ImageJ was used to quantify the inflammation present in the tissue [11]. The area of lymphocytic infiltration was assessed and divided by the total tissue area examined, as previously published [10].

2.4. ELISAs

Total serum IgG and IgM ELISAs were performed in accordance with manufacturer instructions (Bethyl Laboratories). Murine anti-ANA ELISAs (Alpha Diagnostics) were also carried out in accordance with manufacturer instructions with the following modifications: horseradish peroxidase conjugated to anti-IgG or IgM respectively was used to detect isotype-specific ANA (Southern Biotech), as previously described [10]. Murine anti-carbonic anhydrase 6 (CA6), anti-parotid secretory protein (PSP), and anti-salivary protein-1 (SP1) ELISAs were developed and performed by Immco Diagnostics, as previously described [12].

2.5. Autoantigen Array

Sera were harvested from NOD.B10 and BL/10 females with clinical disease as described above. IgG and IgM autoantigen arrays were performed at the Microarray Core Facility of the University of Texas Southwestern Medical Center as previously described [13]. The background-subtracted median signal intensity of each antigen was normalized by the Microarray Core Facility using standard practices. Individual autoantigen array assay values are shown in the supplemental data. Statistical analysis was performed by Dr. Gaile using R (<https://www.r-project.org/>). A detailed description of this analysis is provided in the supplemental data. The autoantigen array accession number is GSE97905. The data can be accessed at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE97905>.

2.6. Statistical Analyses

Mann-Whitney tests were done using Prism (GraphPad Software Inc.).

3. Results

3.1. NOD.B10 females develop salivary and lacrimal disease

Tissue was harvested from NOD.B10 mice with clinical (age 6 – 7 months) and advanced disease (age 10 – 12 months) and age and gender-matched controls. We first assessed salivary and lacrimal inflammation. The majority of mice exhibited focal lymphocytic infiltration of SMG and lacrimal tissue at the clinical disease stage, and this was increased at the advanced time point. As expected, SMG and lacrimal inflammation was higher than that seen in BL/10 controls at the clinical stage ($p = 0.01$ and $p = 0.02$, respectively). Moreover, NOD.B10 females exhibited significant lymphocytic infiltration of SMG tissue at the advanced disease time point as compared to BL/10 controls ($p = 0.04$) (Figure 1A – D). Inflammation was minimal or absent in all sublingual and parotid tissue examined at both disease stages (data not shown).

We then assessed salivary flow in NOD.B10 females at different disease stages and compared this to age and gender-matched BL/10 controls. We found that saliva production was decreased in the NOD.B10 mice as compared to BL/10 animals at the pre-clinical ($p = 0.001$), clinical ($p = 0.003$), and advanced stage diseases ($p < 0.0001$). Importantly, compared with the pre-clinical disease stage animals, NOD.B10 females showed decreased saliva by 6 – 7 months of age ($p = 0.05$), and this diminished production was maintained in the 10 – 12 month old animals ($p = 0.022$). In contrast, BL/10 saliva levels remained constant over time ($p = 0.44$) (Figure 1E).

3.2. NOD.B10 mice demonstrate systemic histopathology consistent with a subset of pSS patients

To assess systemic disease manifestations, lung and kidney were harvested from NOD.B10 females with clinical and advanced disease and compared with those from age and gender-matched controls. Examination of the kidneys of NOD.B10 animals with clinical and advanced disease revealed perivascular nodules of lymphocytes and macrophages at the corticomedullary junction, with a few dilated tubules with protein in the medulla. These findings are consistent with moderate perivascular lymphocytic interstitial nephritis and mild tubular proteinosis. Pathologic changes in the lung were also observed, as we found mild to moderate bronchus-associated lymphoid tissue (BALT) hyperplasia and multifocal perivascular lymphocytic aggregates. Mild multifocal alveolar hemorrhage was also seen. We quantified the lymphocytic infiltrates in lung and renal tissue using ImageJ. We found inflammatory infiltrates in NOD.B10 females were significantly increased as compared to age and gender-matched controls in animals with clinical stage disease ($p = 0.02$ and $p = 0.002$, respectively). These findings were consistent in NOD.B10 mice with advanced stage disease ($p = 0.02$ and $p = 0.003$, respectively) (Figure 2). Table 1 summarizes histopathologic results of all animals examined in Figures 1 and 2.

3.3. Serum IgM and IgG is elevated and increases with disease progression in NOD.B10 females

Sera were harvested from NOD.B10 females with pre-clinical disease, clinical disease, and advanced disease and from equivalent numbers of age and gender-matched BL/10 controls.

We found BL/10 and NOD.B10 females have similar levels of IgM and IgG at the pre-clinical time point ($p = 0.7$ and $p = 0.6$, respectively). However, NOD.B10 mice had elevated IgM and IgG at the clinical disease stage ($p = 0.01$ and $p < 0.0001$, respectively). Although both IgM and IgG titers tended to be higher in NOD.B10 animals at the advanced disease time points than in controls, these differences were not significant ($p = 0.2$ and $p = 0.2$, respectively) (Figure 3A and B).

3.4. NOD.B10 mice express IgM and IgG autoantibodies that increase with disease progression

We then performed an autoantigen array to identify ANA-specific IgG autoantibodies. We assayed sera from NOD.B10 mice with clinical disease ($n = 5$) and age and gender-matched BL/10 controls ($n = 5$). We found anti-nuclear antibodies were enriched in NOD.B10 females, namely double-stranded DNA (dsDNA) ($p = 0.03$), single-stranded DNA (ssDNA) ($p = 0.008$), and U1-snRNP68 ($p = 0.008$) (Figure 4A). Tables 1 and 2 in the supplementary data provide a summary of the most significant results from the autoantigen array study.

To validate the array data, we performed ANA-specific IgM and IgG ELISAs on NOD.B10 and BL/10 sera at both the pre-clinical and clinical disease stages. We found ANA-specific IgM was similar at the pre-clinical stage between BL/10 ($n = 6$) and NOD.B10 mice ($n = 6$) ($p = 0.24$). However, autoreactive IgM was elevated in NOD.B10 females with clinical disease as compared to the NOD.B10 animals with pre-clinical disease ($p = 0.0087$) and age-matched BL/10 females ($n = 5$) ($p = 0.0087$). Of note, there was no difference in ANA-specific IgM in BL/10 animals as they aged ($p = 0.54$). Our findings were similar for the ANA-IgG ELISAs. ANA-specific IgG levels were comparable at the pre-clinical disease stage between BL/10 ($n = 6$) and NOD.B10 mice ($n = 6$) ($p = 0.29$). We found that ANA-IgG titers were increased in NOD.B10 females with clinical disease ($n = 6$) as compared to age-matched BL/10 females ($n = 6$) ($p = 0.026$). Similar to our findings for ANA-specific IgM, there was no difference in ANA-specific IgG in BL/10 animals as they aged ($p > 0.99$). Finally, NOD.B10 animals with clinical disease tended to have higher ANA-specific IgG than the pre-clinical disease mice, although this did not reach statistical significance ($p = 0.063$) (Figure 4B and C, respectively).

3.5. NOD.B10 mice express the novel SS autoantibodies anti-CA6 and anti-SP1

Recent studies identified novel SS autoantibodies in other SS mouse models and in SS patients, termed anti-PSP, anti-CA6, and anti-SP1 [14–16]. This work suggested that these antibodies arose relatively early in disease, prior to anti-Ro and anti-La [16]. We sought to determine whether NOD.B10 females have these autoantibodies and to establish their expression kinetics. We harvested sera from females with pre-disease ($n = 22$), pre-clinical disease ($n = 21$), clinical disease ($n = 7$), and advanced disease ($n = 10$). Sera were also harvested from age and gender-matched BL/10 controls.

We found anti-CA6 autoantibodies increased with disease progression in NOD.B10 females, as anti-CA6 IgM titers were higher in NOD.B10 animals relative to age-matched controls at the clinical and advanced disease time points ($p = 0.0068$ and $p < 0.0001$, respectively) (Figure 5B). We observed similar findings for anti-CA6 IgG ($p = 0.0001$ and $p = 0.021$).

Anti-CA6 IgM levels increased with disease progression, as levels were increased in NOD.B10 animals with clinical disease as compared to sera from both pre-disease ($p = 0.0012$) and pre-clinical disease time points ($p = 0.019$). This observation was consistent in animals with advanced disease ($p < 0.0001$ and $p = 0.0006$, respectively), although there was no difference in anti-CA6 IgM titers between animals with clinical and advanced disease ($p = 0.19$) (Figure 5B).

We then quantified anti-CA6 IgG autoantibodies, and found increased titers of anti-CA6 IgG in NOD.B10 mice with clinical disease as compared to sera from both pre-disease ($p < 0.0001$) and pre-clinical disease ($p < 0.0001$). Our findings were similar in animals with advanced disease ($p < 0.0001$ and $p < 0.0001$, respectively), although there was no difference in anti-CA6 IgG titers between animals with clinical and advanced disease ($p = 0.89$). Of note, we also observed increased anti-CA6 IgG levels in control BL/10 with age, as significant differences were seen between animals 1.5 and 3 months of age ($p = 0.012$), 1.5 and 10–12 months of age ($p = 0.0057$), and from 6–7 to 10–12 months of age ($p = 0.011$). However, the magnitude of these increases were much less than those seen in the NOD.B10 animals (Figure 5C).

We found no differences in anti-PSP IgA, IgM or IgG titers between NOD.B10 females and controls at any of the time points (Figure 5D – F). In addition, BL/10 and NOD.B10 females have similar levels of IgM, IgA, and IgG specific anti-CA6 and anti-SP1 at the pre-disease and pre-clinical disease time points (Figure 5).

Next, we examined anti-SP1 IgA autoantibodies in NOD.B10 and control animals. We found these autoantibodies were increased in NOD.B10 animals with clinical and advanced disease as compared to age-matched controls ($p = 0.0001$ and $p < 0.00001$, respectively). Anti-SP1 IgA autoantibodies increased with disease in the NOD.B10 strain, as animals with both clinical and advanced disease had elevated titers as compared to pre-disease ($p < 0.0001$ and $p < 0.0001$, respectively) and pre-clinical disease time points ($p < 0.0001$ and $p < 0.000$, respectively). Finally, we saw no increase in titers between clinical and advanced disease stage NOD.B10 animals ($p = 0.19$) (Figure 5G).

We then examined anti-SP1 IgM autoantibodies. We found no change in BL/10 animals with age. However, titers were elevated in NOD.B10 animals as compared to controls at both clinical and advanced disease stages ($p = 0.0001$ and $p < 0.0001$, respectively). Anti-SP1 IgM titers increased with disease progression in NOD.B10 mice, as significant differences were noted between sera from pre-disease and pre-clinical disease ($p = 0.0099$), and between pre-clinical and clinical disease females ($p = 0.042$). Titers were also increased in clinical disease animals as compared to those with pre-clinical disease ($p = 0.42$). Anti-SP1 IgM was elevated in animals with advanced disease as compared to those with pre-disease or pre-clinical disease ($p < 0.0001$ and $p = 0.0009$, respectively). Finally, we saw no difference in IgM autoantibodies directed against SP1 between clinical and advanced disease stage in NOD.B10 mice ($p = 0.47$) (Figure 5H).

Lastly, we examined anti-SP1 IgG autoantibodies. We found similar results as those for anti-SP1 IgM. Specifically, anti-SP1 IgG titers were elevated in NOD.B10 animals as compared

to age-matched controls at both clinical and advanced disease stages ($p = 0.0001$ and $p < 0.0001$, respectively). Anti-SP1 IgG levels increased over time in NOD.B10 mice, as titers increased in sera between pre-disease and pre-clinical disease ($p = 0.0045$), and between pre-clinical and clinical disease females ($p = 0.0012$). NOD.B10 mice with clinical disease had elevated SP1 IgG as compared to those with pre-clinical disease ($p < 0.0001$). In addition, anti-SP1 IgG was higher in animals with advanced disease than those with pre-disease or pre-clinical disease ($p < 0.0001$ and $p = 0.0006$, respectively). Finally, we observed similar levels of IgG autoantibodies directed against SP1 in NOD.B10 mice with clinical and advanced stage disease ($p = 0.19$). Of note, although we observed differences between anti-SP1 IgG levels in BL10 controls, the levels remained relatively low across each time point and did not increase consistently with age as was observed in the NOD.B10 animals (Figure 5H).

4. Discussion

This study provides a comprehensive analysis of local and systemic disease manifestations in female NOD.B10 mice. As is the case for many complex pathoses, there is a pressing need for good animal models that recapitulate findings in humans in order to understand disease progression and to design targeted therapeutics. This is particularly true for SS, as early disease markers, staging criteria, and therapeutics are lacking [2]. Our findings indicate that NOD.B10 mice are ideally suited for the study of SS, as they develop robust sialadenitis and dacryoadenitis. In addition to exocrine gland inflammation, we observed lymphocytic infiltration in both lung and kidney of NOD.B10 females with clinical disease and this increased with disease progression in both organs. Moreover, this strain expresses many autoantibodies seen in SS patients, including those recently reported to be early markers of disease. Finally, NOD.B10 females develop both local and systemic disease at comparable rates or even more quickly than other published pSS models, thereby establishing the relevance and feasibility of this model for SS research [17, 18].

Interestingly, NOD.B10 females with clinical stage disease show renal inflammation that worsens with disease progression. It is estimated that approximately 13% of pSS patients are afflicted by renal pathology [19]. Patients tend to develop renal pathoses relatively early in the disease course, with a mean diagnosis age of 41 years [19]. Recent studies suggest tubular interstitial nephritis is the most common renal disease manifestation in SS patients [20, 21], and this is characterized by renal inflammation [19, 22, 23]. Thus, our findings in NOD.B10 mice mimic the renal histopathology seen in SS patients, although further studies are needed to establish the functional consequence of this in our model.

In addition, approximately 9 to 20% of SS patients experience pulmonary dysfunction, including airway abnormalities, follicular bronchiolitis (FB), interstitial lung disease and lymphoproliferative disorders [24]. These typically present late in the disease course and patients with pulmonary dysfunction tend to be older than those who experience kidney pathology [19, 25]. FB is a relatively common pulmonary manifestation of SS. This condition represents a rare type of cellular bronchiolitis characterized by hyperplastic lymphoid follicles with reactive germinal centers distributed along the bronchovascular bundles. The lymphoid infiltrates are restricted to bronchioles and to the immediate

peribronchiolar interstitium [26]. FB is thought to be caused by antigenic stimulation of the BALT, resulting in polyclonal lymphoid hyperplasia [27]. Interestingly, NOD.B10 mice display pulmonary histopathology that is consistent with FB at both clinical and advanced disease stages (Figure 2). Importantly, SS patients with pulmonary involvement have an increased risk of mortality after 10 years of disease [28]. Therefore, studies suggest that pulmonary involvement is a relatively late-stage disease manifestation that may be seen in SS patients with serious clinical consequence. NOD.B10 mice show pulmonary inflammation that increases with disease progression, suggesting that this model represents a valuable tool to study pulmonary pathoses that are observed in SS patients.

It is important to point out that we observed inflammation in the tissues of the BL/10 animals that increased with age, albeit this was significantly reduced as compared to the NOD.B10 strain (Figures 1, 2, and Table 1). Careful studies performed in the congenic C57BL/6 strain demonstrate that 10% of females exhibit both salivary and pulmonary inflammation at 6 months of age and approximately 30% of C57BL/6 females show immune cells in the lungs, kidney and salivary tissue by 12 months of age [29]. These results are consistent with those observed in our BL/10 colony (Figure 1 and 2). Of note, BL/10 animals aged 6 – 7 months or 10 – 12 months maintained salivary flow, suggesting that the lymphocytic infiltrate in salivary tissue is not associated with glandular damage. Therefore, the presence of inflammation in the organs of BL/10 mice is likely a normal response to physiological aging. This is in contrast to the NOD.B10 animals, in which the lymphocytic infiltration is associated with aberrant immune activation.

In addition to histopathologic analyses, we assayed sera from NOD.B10 mice at different disease stages to determine the specificity and kinetics of total and self-reactive antibody production. Of note, anti-Ro, anti-La, ANA, and RF are included in the SS diagnostic criteria [1]. While previous studies reported ANA autoantibodies in NOD.B10 females with clinical disease [7, 8], the specific antinuclear antibodies remained unknown, and data regarding the presence of other types of autoantibodies are lacking. Autoantibodies are a consistent feature of numerous autoimmune diseases and are indicative of B cell hyperactivity. Although the significance of many autoantibodies in SS is unknown, some are known to cause decreased salivation [9, 30, 31]. Moreover, sialadenitis is strongly associated with anti-Ro, -La, and RF antibodies and ocular disease manifestations [32].

We found that IgG antibodies directed against dsDNA, ssDNA, and U1-snRNP68 are highly expressed in the sera of NOD.B10 mice by autoantigen array. While these autoantibodies are characteristic of SLE [33, 34], recent studies suggest these autoantigens may also have relevance to SS. Importantly, animals immunized with a La peptide generated autoantibodies to both La and U1-snRNP [35]. A corroborative study found that animals immunized with both anti-La and dsDNA showed higher anti-La levels than those immunized with La alone. In addition, animals immunized with both anti-La and dsDNA antibodies produced anti-RNP autoantibodies [36]. Both La and RNP are RNA binding proteins that share similar RNA recognition motifs (RRMs). These RRM motifs are major epitopes in SLE [37], and it is hypothesized that RNP autoantibodies that arise from immunization with La alone or in combination with dsDNA could be generated as a result of intermolecular epitope spreading

in SS [35, 36, 38]. Thus, specific ANAs identified in NOD.B10 females with clinical disease likely have relevance to pSS patients.

Finally, we found novel SS autoantibodies (anti-SP1 and anti-CA6) are elevated in the sera of NOD.B10 mice with clinical and advanced disease. SP1 and CA6 are expressed in salivary glands of NOD.B10 mice, suggesting a possible link between salivary pathology and disease onset and/or exacerbation. SP1 (also referred to as Spt1 or mucin-like 2 protein) is classified as a highly acidic salivary protein that is expressed in SMG and lacrimal tissue in mice [39]. While the human homologue of the SP1 protein is unknown, a preliminary study shows a murine anti-SP1 antibody binds to human parotid tissue [14]. While SP1 antibodies are elevated in sera of SS patients as compared to healthy controls, further work is needed to characterize the protein and to determine whether its expression is restricted to exocrine tissue in humans [14].

CA6 is a secreted enzyme produced by serous acini of the parotid and SMG in humans [40, 41], although it has been detected in other tissues including lacrimal and mammary glands, and serous acini and ductal cells of tracheobronchial glands in other mammals [42–45]. Interestingly, CA6 levels are reduced in parotid saliva of pSS patients as compared to healthy controls [46]. Although SP1 and CA6 clearly localize to exocrine tissue, it is unclear at present why mice and humans with SS exhibit loss of tolerance to these proteins. Nonetheless, several studies have detected these autoantibodies in pSS models and SS patients, suggesting that they may represent valuable biomarkers for disease diagnosis and monitoring [16].

Our results are consistent with those in other SS mouse models, as early disease stage pSS IL-14 α transgenic mice exhibit antibodies to CA6 and SP1 [47]. Sera from sSS mice (NOD/ShiLtJ) with clinical disease showed similar findings [16]. Of note, in IL-14 α transgenic mice, anti-SP1 and anti-CA6 antibodies are detected earlier than those directed against Ro and La [16]. Data in the present study support these findings, as we found elevation of anti-SP1-specific IgM and IgG at the pre-clinical disease time point in NOD.B10 mice prior to elevation of ANA (Figure 4, 5H, and I).

Of note, we did not detect any autoantibodies to PSP. While the reasons for this are unknown at present, it is important to point out that the NOD.B10 model expresses an aberrantly expressed and processed PSP [7]. It is possible that this truncated PSP protein lacks an antigenic epitope, and this could account for the absence of anti-PSP autoantibodies observed.

Studies in pSS patients support the clinical-translational relevance of these findings, although further data are needed to determine whether these autoantibodies can serve as biomarkers for SS. Compelling work demonstrates that anti-Ro and anti-La antibodies are present years before patients develop pSS [48, 49], so these autoantibodies clearly have the potential to serve as early disease markers. However, a significant percentage of pSS patients lack these autoantibodies [1]. Therefore, these novel autoantibodies may be useful in establishing the diagnosis and early treatment strategies for pSS patients, particularly those who do not express autoantibodies directed against Ro and La.

5. Conclusion

This study demonstrates numerous local and systemic pSS disease manifestations in NOD.B10 mice that mimic human disease, thereby establishing this model as an excellent tool for clinical translational studies, particularly those designed to evaluate EGM in pSS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ANA	Anti-nuclear autoantibodies
BALT	Bronchus-associated lymphoid tissue
CA6	Carbonic anhydrase 6
dsDNA	Double-stranded DNA
EGM	Extra-glandular manifestations
FB	Follicular bronchiolitis
LG	Lacrimal gland
M3R	Muscarinic 3 acetylcholine receptor
PSP	Parotid secretory protein
pSS	Primary Sjögren's syndrome
RF	Rheumatoid factor
RRM	RNA recognition motif
SMG	Submandibular gland
SP1	Salivary Protein 1
sSS	Secondary Sjögren's syndrome
SS	Sjögren's syndrome
ssDNA	Single-stranded DNA

References

1. Malladi AS, Sack KE, Shiboski SC, Shiboski CH, Baer AN, Banushree R, Dong Y, Helin P, Kirkham BW, Li M, Sugai S, Umehara H, Vivino FB, Vollenweider CF, Zhang W, Zhao Y, Greenspan JS, Daniels TE, Criswell LA. Primary Sjogren's syndrome as a systemic disease: a study of participants enrolled in an international Sjogren's syndrome registry. *Arthritis Care Res (Hoboken)*. 2012; 64:911–918. [PubMed: 22238244]
2. Donate A, Voigt A, Nguyen CQ. The value of animal models to study immunopathology of primary human Sjogren's syndrome symptoms. *Expert Rev Clin Immunol*. 2014; 10:469–481. [PubMed: 24506531]
3. Lu R, Munroe ME, Guthridge JM, Bean KM, Fife DA, Chen H, Slight-Webb SR, Keith MP, Harley JB, James JA. Dysregulation of innate and adaptive serum mediators precedes systemic lupus erythematosus classification and improves prognostic accuracy of autoantibodies. *J Autoimmun*. 2016
4. Levitsky A, Wick MC, Mottonen T, Leirisalo-Repo M, Laasonen L, Korpela M, van Vollenhoven RF, Rantalaiho V. Early treatment intensification induces favourable radiographic outcomes according to predicted versus observed radiographic progression in early rheumatoid arthritis: a subanalysis of the randomised FIN-RACo and NEO-RACo trials. *Clin Exp Rheumatol*. 2016
5. James JA, Kim-Howard XR, Bruner BF, Jonsson MK, McClain MT, Arbuckle MR, Walker C, Dennis GJ, Merrill JT, Harley JB. Hydroxychloroquine sulfate treatment is associated with later onset of systemic lupus erythematosus. *Lupus*. 2007; 16:401–409. [PubMed: 17664230]
6. Wicker LS, Appel MC, Dotta F, Pressey A, Miller BJ, DeLarato NH, Fischer PA, Boltz RC Jr, Peterson LB. Autoimmune syndromes in major histocompatibility complex (MHC) congenic strains of nonobese diabetic (NOD) mice. The NOD MHC is dominant for insulinitis and cyclophosphamide-induced diabetes. *J Exp Med*. 1992; 176:67–77. [PubMed: 1613467]
7. Robinson CP, Yamachika S, Bounous DI, Brayer J, Jonsson R, Holmdahl R, Peck AB, Humphreys-Beher MG. A novel NOD-derived murine model of primary Sjogren's syndrome. *Arthritis Rheum*. 1998; 41:150–156. [PubMed: 9433880]
8. Gao J, Killedar S, Cornelius JG, Nguyen C, Cha S, Peck AB. Sjögren's syndrome in the NOD mouse model is an interleukin-4 time-dependent, antibody isotype-specific autoimmune disease. *J Autoimmun*. 2006; 26:90–103. [PubMed: 16413168]
9. Nguyen CQ, Gao JH, Kim H, Saban DR, Cornelius JG, Peck AB. IL-4-STAT6 signal transduction-dependent induction of the clinical phase of Sjögren's syndrome-like disease of the nonobese diabetic mouse. *J Immunol*. 2007; 179:382–390. [PubMed: 17579059]
10. Sharma A, Kiripolsky J, Klimatcheva E, Howell A, Fereidouni F, Levenson R, Rothstein TL, Kramer JM. Early BAFF receptor blockade mitigates murine Sjogren's syndrome: Concomitant targeting of CXCL13 and the BAFF receptor prevents salivary hypofunction. *Clin Immunol*. 2016; 164:85–94. [PubMed: 26826598]
11. Rasband WS. ImageJ. U.S. National Institutes of Health; Bethesda, Maryland, USA: 1997–2016. <http://imagej.nih.gov/ij/> (Ed.)
12. Shen L, Gao C, Suresh L, Xian Z, Song N, Chaves LD, Yu M, Ambrus JL Jr. Central role for marginal zone B cells in an animal model of Sjogren's syndrome. *Clin Immunol*. 2016; 168:30–36. [PubMed: 27140729]
13. Kramer JM, Holodick NE, Vizconde TC, Raman I, Yan M, Li QZ, Gaile DP, Rothstein TL. Analysis of IgM antibody production and repertoire in a mouse model of Sjogren's syndrome. *Journal of leukocyte biology*. 2015
14. Shen L, Kapsogeorgou EK, Yu M, Suresh L, Malyavantham K, Tzioufas AG, Ambrus JL Jr. Evaluation of salivary gland protein 1 antibodies in patients with primary and secondary Sjogren's syndrome. *Clin Immunol*. 2014; 155:42–46. [PubMed: 25178982]
15. Suresh L, Malyavantham K, Shen L, Ambrus JL Jr. Investigation of novel autoantibodies in Sjogren's syndrome utilizing Sera from the Sjogren's international collaborative clinical alliance cohort. *BMC Ophthalmol*. 2015; 15:38. [PubMed: 25881294]
16. Shen L, Suresh L, Lindemann M, Xuan J, Kowal P, Malyavantham K, Ambrus JL Jr. Novel autoantibodies in Sjogren's syndrome. *Clin Immunol*. 2012; 145:251–255. [PubMed: 23123440]

17. Li H, Dai M, Zhuang Y. A T cell intrinsic role of Id3 in a mouse model for primary Sjogren's syndrome. *Immunity*. 2004; 21:551–560. [PubMed: 15485632]
18. Shen L, Suresh L, Malyavantham K, Kowal P, Xuan J, Lindemann MJ, Ambrus JL. Different stages of primary Sjogren's syndrome involving lymphotoxin and type 1 IFN. *J Immunol*. 2013; 191:608–613. [PubMed: 23772034]
19. Ramos-Casals M, Brito-Zeron P, Seror R, Bootsma H, Bowman SJ, Dorner T, Gottenberg JE, Mariette X, Theander E, Bombardieri S, De Vita S, Mandl T, Ng WF, Kruize A, Tzioufas A, Vitali C, Force ESST. Characterization of systemic disease in primary Sjogren's syndrome: EULAR-SS Task Force recommendations for articular, cutaneous, pulmonary and renal involvements. *Rheumatology (Oxford)*. 2015; 54:2230–2238. [PubMed: 26231345]
20. Ren H, Wang WM, Chen XN, Zhang W, Pan XX, Wang XL, Lin Y, Zhang S, Chen N. Renal involvement and followup of 130 patients with primary Sjogren's syndrome. *J Rheumatol*. 2008; 35:278–284. [PubMed: 18085734]
21. Maripuri S, Grande JP, Osborn TG, Fervenza FC, Matteson EL, Donadio JV, Hogan MC. Renal involvement in primary Sjogren's syndrome: a clinicopathologic study. *Clin J Am Soc Nephrol*. 2009; 4:1423–1431. [PubMed: 19679669]
22. Evans R, Zdebek A, Ciurtin C, Walsh SB. Renal involvement in primary Sjogren's syndrome. *Rheumatology (Oxford)*. 2015; 54:1541–1548. [PubMed: 26070939]
23. Talal N, Zisman E, Schur PH. Renal tubular acidosis, glomerulonephritis and immunologic factors in Sjogren's syndrome. *Arthritis Rheum*. 1968; 11:774–786. [PubMed: 4178221]
24. Flament T, Bigot A, Chaigne B, Henique H, Diot E, Marchand-Adam S. Pulmonary manifestations of Sjogren's syndrome. *Eur Respir Rev*. 2016; 25:110–123. [PubMed: 27246587]
25. Cain HC, Noble PW, Matthay RA. Pulmonary manifestations of Sjogren's syndrome. *Clin Chest Med*. 1998; 19:687–699. viii. [PubMed: 9917960]
26. Stojan G, Baer AN, Danoff SK. Pulmonary manifestations of Sjogren's syndrome. *Curr Allergy Asthma Rep*. 2013; 13:354–360. [PubMed: 23797265]
27. Tashtoush B, Okafor NC, Ramirez JF, Smolley L. Follicular Bronchiolitis: A Literature Review. *Journal of clinical and diagnostic research : JCDR*. 2015; 9:OE01–05.
28. Palm O, Garen T, Berge Enger T, Jensen JL, Lund MB, Aalokken TM, Gran JT. Clinical pulmonary involvement in primary Sjogren's syndrome: prevalence, quality of life and mortality--a retrospective study based on registry data. *Rheumatology (Oxford)*. 2013; 52:173–179. [PubMed: 23192906]
29. Hayashi Y, Utsuyama M, Kurashima C, Hirokawa K. Spontaneous development of organ-specific autoimmune lesions in aged C57BL/6 mice. *Clin Exp Immunol*. 1989; 78:120–126. [PubMed: 2805415]
30. Robinson CP, Brayer J, Yamachika S, Esch TR, Peck AB, Stewart CA, Peen E, Jonsson R, Humphreys-Beher MG. Transfer of human serum IgG to nonobese diabetic Igmu null mice reveals a role for autoantibodies in the loss of secretory function of exocrine tissues in Sjögren's syndrome. *Proc Natl Acad Sci U S A*. 1998; 95:7538–7543. [PubMed: 9636185]
31. Fayyaz A, Kurien BT, Scofield RH. Autoantibodies in Sjogren's Syndrome. *Rheum Dis Clin North Am*. 2016; 42:419–434. [PubMed: 27431345]
32. Daniels TE, Cox D, Shiboski CH, Schiødt M, Wu A, Lanfranchi H, Umehara H, Zhao Y, Challacombe S, Lam MY, De Souza Y, Schiødt J, Holm H, Bisio PA, Gandolfo MS, Sawaki T, Li M, Zhang W, Varghese-Jacob B, Ibsen P, Keszler A, Kurose N, Nojima T, Odell E, Criswell LA, Jordan R, Greenspan JS. Groups SsICCART. Associations between salivary gland histopathologic diagnoses and phenotypic features of Sjögren's syndrome among 1,726 registry participants. *Arthritis Rheum*. 2011; 63:2021–2030. [PubMed: 21480190]
33. Pisetsky DS. Anti-DNA antibodies--quintessential biomarkers of SLE. *Nat Rev Rheumatol*. 2016; 12:102–110. [PubMed: 26581343]
34. Cozzani E, Drosara M, Gasparini G, Parodi A. Serology of Lupus Erythematosus: Correlation between Immunopathological Features and Clinical Aspects. *Autoimmune Dis*. 2014; 2014:321359. [PubMed: 24649358]

35. Routsias JG, Kyriakidis N, Latreille M, Tzioufas AG. RNA recognition motif (RRM) of La/SSB: the bridge for interparticle spreading of autoimmune response to U1-RNP. *Molecular medicine*. 2010; 16:19–26. [PubMed: 19838329]
36. Ding M, Zhang J. Epitope spreading induced by immunization with synthetic SSB peptides. *Exp Ther Med*. 2016; 12:147–150. [PubMed: 27347030]
37. Routsias JG, Vlachoyiannopoulos PG, Tzioufas AG. Autoantibodies to intracellular autoantigens and their B-cell epitopes: molecular probes to study the autoimmune response. *Crit Rev Clin Lab Sci*. 2006; 43:203–248. [PubMed: 16574554]
38. Monneaux F, Muller S. Epitope spreading in systemic lupus erythematosus: identification of triggering peptide sequences. *Arthritis Rheum*. 2002; 46:1430–1438. [PubMed: 12115171]
39. Dickinson DP, Abel K, Near J, Taylor BA, Gross KW. Genetic and tissue-specific variation in the expression of a closely linked murine multigene family on chromosome 15 that encodes salivary and lacrimal proteins. *Biochem Genet*. 1989; 27:613–637. [PubMed: 2482730]
40. Kivela J, Parkkila S, Parkkila AK, Leinonen J, Rajaniemi H. Salivary carbonic anhydrase isoenzyme VI. *J Physiol*. 1999; 520(Pt 2):315–320. [PubMed: 10523402]
41. Parkkila S, Kaunisto K, Rajaniemi L, Kumpulainen T, Jokinen K, Rajaniemi H. Immunohistochemical localization of carbonic anhydrase isoenzymes VI, II, and I in human parotid and submandibular glands. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*. 1990; 38:941–947. [PubMed: 2113069]
42. Ogawa Y, Matsumoto K, Maeda T, Tamai R, Suzuki T, Sasano H, Fernley RT. Characterization of lacrimal gland carbonic anhydrase VI. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*. 2002; 50:821–827. [PubMed: 12019298]
43. Kitade K, Nishita T, Yamato M, Sakamoto K, Hagino A, Katoh K, Obara Y. Expression and localization of carbonic anhydrase in bovine mammary gland and secretion in milk. *Comp Biochem Physiol A Mol Integr Physiol*. 2003; 134:349–354. [PubMed: 12547264]
44. Patrikainen M, Pan P, Kuleshkaya N, Voikar V, Parkkila S. The role of carbonic anhydrase VI in bitter taste perception: evidence from the Car6(-)/(-) mouse model. *J Biomed Sci*. 2014; 21:82. [PubMed: 25134447]
45. Leinonen JS, Saari KA, Seppanen JM, Myllyla HM, Rajaniemi HJ. Immunohistochemical demonstration of carbonic anhydrase isoenzyme VI (CA VI) expression in rat lower airways and lung. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*. 2004; 52:1107–1112. [PubMed: 15258187]
46. Ryu OH, Atkinson JC, Hoehn GT, Illei GG, Hart TC. Identification of parotid salivary biomarkers in Sjogren's syndrome by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and two-dimensional difference gel electrophoresis. *Rheumatology (Oxford)*. 2006; 45:1077–1086. [PubMed: 16522680]
47. Xuan J, Shen L, Malyavantham K, Pankewycz O, Ambrus JL Jr, Suresh L. Temporal histological changes in lacrimal and major salivary glands in mouse models of Sjogren's syndrome. *BMC oral health*. 2013; 13:51. [PubMed: 24093879]
48. Jonsson R, Theander E, Sjöström B, Brokstad K, Henriksson G. Autoantibodies present before symptom onset in primary Sjögren syndrome. *JAMA*. 2013; 310:1854–1855. [PubMed: 24193084]
49. Theander E, Jonsson R, Sjöström B, Brokstad K, Olsson P, Henriksson G. Prediction of Sjögren's Syndrome Years Before Diagnosis and Identification of Patients With Early Onset and Severe Disease Course by Autoantibody Profiling. *Arthritis & rheumatology*. 2015; 67:2427–2436. [PubMed: 26109563]

Figure 1. NOD.B10 females exhibit salivary and lacrimal gland inflammation and lose salivary flow with disease progression

SMG and lacrimal glands were harvested from NOD.B10 females with (A) clinical and (B) advanced stage disease and age and gender-matched BL/10 controls. Tissue was H&E stained and representative photomicrographs are shown. Black arrows represent salivary ducts and white arrows show lymphocytic infiltrates. Original magnification is 200X. Lymphocytic infiltration was quantified in (C) SMG and (D) lacrimal tissue from clinical and advanced stage time points. (E) Saliva production was quantified from NOD.B10 females at the early clinical (n = 8), clinical (n = 7) and advanced disease stages (n = 10). Saliva was also measured from age and gender-matched controls. (N.S. = non-significant, *p 0.05, **p 0.01, ****p 0.0001).

Figure 2. NOD.B10 females display systemic inflammation characteristic of human SS
Lung and kidney were harvested from NOD.B10 females with (A) clinical and (B) advanced stage disease and age and gender-matched BL/10 controls. Tissue was H&E stained and representative photomicrographs are shown. White arrows indicate lymphocytic infiltrates. Original magnification is 200X. Inflammation in (C) kidney and (D) lung tissue from NOD.B10 mice with clinical disease (n = 8) and age and gender-matched controls (n = 5) and advanced disease was quantified (N.S. = non-significant, *p < 0.05).

Figure 3. NOD.B10 mice with clinical disease have elevated serum IgM and IgG

Serum was harvested from NOD.B10 and BL/10 females at 3 (n = 7 and 8, respectively, 6–7 (n = 8 and 10, respectively), 10 – 12 months of age (n = 10 and 8, respectively) and (A) IgM and (B) IgG titers assessed by ELISA. Mean and SEM are shown, (N.S. = non-significant, **p < 0.01, ****p < 0.0001).

Figure 4. Anti-nuclear autoantibodies are elevated in NOD.B10 females

(A) Serum was harvested from NOD.B10 females with clinical disease (n = 5) and age and gender-matched females (n = 5) and IgG autoantigen arrays were performed. Normalized (relative) autoantibody values are shown for specific ANAs. (B) ANA-specific IgM and (C) IgG were quantified by ELISA using sera from NOD.B10 females with pre-clinical or clinical disease (n = 6) and age and gender matched controls (n = 6). Relative ANA-specific IgM values are shown. ANA-specific IgG values are shown as Units/mL. Mean and SEM are shown, (N.S. = non-significant, *p < 0.05, **p < 0.01).

Figure 5. CA6 and SP1 autoantibodies are elevated in NOD.B10 mice with clinical and advanced stage disease

Serum was harvested from NOD.B10 and BL/10 females at 1.5 (n = 22 and 14, respectively), 3 (n = 21 and 7, respectively), 6–7 (n = 7 and 10, respectively), 10 – 12 months of age (n = 10 and 9, respectively) and (A) Anti-CA6 IgA, (B) Anti-IgM CA6, (C) Anti-CA6 IgG, (D) Anti-PSP IgA, (E) Anti-PSP IgM, (F) Anti-PSP IgG, (G) Anti-SP1 IgA, (H) Anti-SP1 IgM, and (I) Anti-SP1 IgG titers assessed by ELISA. Mean and SEM are shown, (*p 0.05, **p 0.01, ***p 0.001, ****p 0.0001).

Table 1

Strain (age)	SMG	LG	Lung	Kidney
BL/10 (6 – 7 mos)	0/9 *	1/6	0/6	0/6
NOD.B10 (6–7 mos)	6/10	6/7	5/7	6/6
BL/10 (10 – 12 mos)	4/8	4/5	2/7	1/7
NOD.B10 (10 – 12 mos)	7/7	7/7	6/7	7/7

* Number of animals with lymphocytic infiltration/total number of animals examined