Expression of TLR10 in B cells Correlates With Primary Sjögren's Syndrome Progression

Nannan Lai

The Seventh Affiliated Hospital Sun Yat-sen University

YiChao Qian

The Seventh Affiliated Hospital Sun Yat-sen University

Yilin Wu

The Seventh Affiliated Hospital Sun Yat-sen University

Xi Jiang

The Seventh Affiliated Hospital Sun Yat-sen University

Honghua Sun

The Seventh Affiliated Hospital Sun Yat-sen University

Zhaofan Luo

The Seventh Affiliated Hospital Sun Yat-sen University

Yanli Zhao

Longgang Central Hospital of Shenzhen

Changchun Zeng

Longgang Central Hospital of Shenzhen

Yang Kang

The Seventh Affiliated Hospital Sun Yat-sen University

Xiao-Yong Zhan

The Seventh Affiliated Hospital Sun Yat-sen University

Qingwen Wang

Peking University Shenzhen Hospital

Chun Tang

The Seventh Affiliated Hospital Sun Yat-sen University

Bihui Huang (✉ huangbh7@mail.sysu.edu.cn)

The Seventh Affiliated Hospital Sun Yat-sen University <https://orcid.org/0000-0003-3084-7096>

Research Article

Keywords: Toll-like receptor 10 (TLR10), B cells, Primary Sjögren's Syndrome (pSS), class switch recombination (CSR), autoantibodies

Posted Date: August 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-795510/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Primary Sjögren's Syndrome (pSS) is considered a B cell-mediated disease, yet the precise role of B cells in the pathogenesis is not fully understood. Toll-like receptor 10 (TLR10) is highly expressed in human B cells, indicating that TLR10 probably plays a vital role in regulating B cell function as well as B cell-related diseases. However, the biology of TLR10 in pSS was rarely researched. Here, we examined the TLR10 expression in peripheral B cell subsets isolated from both pSS patients and healthy controls (HCs) and further analyzed the correlations between TLR10 expression and disease activity. We observed that TLR10 expression in peripheral total CD19⁺ B cells, naïve B cells (CD19⁺CD27⁻IgD⁺) and switched memory B cells (CD19⁺CD27⁺IgD⁻) was significantly increased in low-activity pSS patients as compared with HCs and high-activity pSS patients. TLR10 expression in total and switched memory B cells in pSS patients was significantly negatively correlated with serum levels of anti-SSA antibody and B cell activating factor of TNF family (BAFF). As compared with the TLR10 low-, the TLR10 high-expressed pSS patients presented with reduced switched memory B cells. Moreover, a much lower proportion of high-activity pSS patients was observed in TLR10 high- as compared to low-expressed patients. Our study concluded that TLR10 expression in peripheral total and switched memory B is negatively correlated with pSS disease activity, suggesting that TLR10 might suppress pSS progression via inhibiting the B cell class switch recombination. These results should contribute to the diagnosis and treatment of pSS.

Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disorder affecting exocrine glands of the body, preferentially lacrimal and salivary glands. The global prevalence of pSS is approximately 0.4-1%, in which around 30–40% of pSS patients will progress to at least one additional systemic autoimmune complications [1] and nearly 5% of pSS patients may develop to B cell malignancies, most commonly salivary gland mucosa-associated lymphoid tissue lymphomas [2]. Clinical treatments for pSS easily fail due to the heterogeneity of clinical phenotype in pSS patients. Moreover, plenty studies in pSS demonstrated that numerous factors seem to contribute to the progression of the pSS [3, 4]. However, the mechanism by which pSS develops, although being widely researched, remains unclear.

Currently, pSS is considered a B cell-mediated disease characterized by autoantibodies and hypergammaglobulinaemia in patients [4]. Besides the production of autoantibodies, B cell can activate T cell by presenting autoantigen and secrete multiple inflammatory cytokines upon Toll Like Receptor (TLR) activation, thereby contributing to the development of pSS [5]. However, the precise role of B cells in the pathogenesis of pSS is still poorly understood. According to clinical efficacy for rituximab treatment in different trials, it was suggested that B cells at different developmental stages contribute to the broad clinical phenotypes in pSS patients, suggesting that understanding the abnormality of B cell development and differentiation is essential to uncover the pathogenesis of pSS [6–8].

TLRs are pattern recognition receptors that have crucial roles in the initiation of innate immunity and the activation of adaptive immunity. Many studies have proved that TLR signaling is required for human B

cell activation and plays an important role in autoimmune diseases [9, 10]. Among ten human TLRs (TLR1-10), TLR10 remains the least understood one because it presents in human beings, but not in the mice, and its ligand has not been identified yet [11]. TLR10 is expressed at the highest level in B cells, followed by plasmacytoid dendritic cells but not expressed in monocytes, natural killer cells, and T cells [12, 13], indicating that TLR10 might play an important role in regulating B cell function as well as B cell-related diseases. Recently, a few studies have reported that human TLR10 polymorphisms are associated with several diseases, including bacterial infections, cancers and autoimmune diseases [14–16]. Torices et al. showed that TLR10 variant rs11466657 is closely related to rheumatoid arthritis (RA) [17]. Moreover, Zhang et al. revealed that upregulated TLR10 in B cell subsets is positively correlated with disease activity in RA patients [18]. However, the biology of TLR10 in pSS is less researched.

Considering the unique high expression of TLR10 on human B cells and the crucial role of B cells in pSS, we investigated the difference of TLR10 expression in peripheral B cell subsets, including transitional B cells (Tran B), naïve B cells, unswitched memory B cells, switched memory B cells and plasmablasts (PB), between pSS patients and healthy controls (HCs) and further analyzed the correlations of TLR10 expression with disease activity.

Methods And Materials

Patients

This study was approved by the Ethics Committee of the Seventh Affiliated Hospital of Sun Yat-sen University. Each subject provided written informed consent for enrollment in this study. From October 2020 to May 2021, 34 patients diagnosed with pSS based on the 2016 European League Against Rheumatism (EULAR) SS Disease Activity Index (ESSDAI) at the Seventh Affiliated Hospital of Sun Yat-sen University were consecutively recruited [19]. Patients with other autoimmune or inflammatory diseases, severe renal or liver disease, or cancer were excluded. The disease activity of pSS patients was determined according to the ESSDAI. All the pSS subjects were divided into two groups: a high-activity group (ESSDAI $\geq$ 5), a low-activity group (ESSDAI < 5) [20]. In addition, 25 age- and sex-matched HCs were chosen for comparison. More information about age, gender and treatments are shown in Table 1.

Table 1
Clinical characteristics of the study subjects.

	Healthy control group (n = 25)	pSS group (n = 34)	Low-activity pSS group (n = 17)	High-activity pSS group (n = 17)
Female (%)	24 (96)	29 (93.5)	14 (83.3)	17 (100)
Age (years) ^a	40.56 ± 7.22	42.94 ± 12.75	45.75 ± 9.55	41.25 ± 14.29
Disease duration (years)	-	2.88 ± 2.82	2.29 ± 2.48	3.23 ± 3.01
WBC (×10 ⁹)	-	5.96 ± 2.28	6.95 ± 2.85	5.37 ± 1.67
RBC (×10 ¹²)	-	4.46 ± 1.06	4.61 ± 1.67	4.37 ± 0.44
PLT (×10 ⁹)	-	202.34 ± 129.07	177.25 ± 166.62	217.40 ± 102.33
Anti SSA-positive (%) ^b	-	25 (80.6)	7 (58.3)	18 (94.7)
Anti SSB-positive (%) ^b	-	19 (61.3)	3 (25)	16 (84.2)
Anti Ro52-positive (%) ^b	-	20 (64.5)	2 (16.7)	18 (94.7)
C3 (g/L)	-	1.01 ± 0.20	0.99 ± 0.19	1.02 ± 0.21
C4 (g/L)	-	0.22 ± 0.07	0.22 ± 0.04	0.23 ± 0.09
Medicine use	-	25 (80.6)	9 (75)	16 (84.2)
Methotrexate	-	4 (12.9)	3 (25)	1 (5.3)
Prednisolone	-	20 (64.5)	7 (58.3)	13 (68.4)
Hydroxychloroquine	-	18 (58.1)	6 (50)	12 (63.2)
Chinese medicine	-	4 (12.9)	3 (25)	1 (5.2)
No treatment	-	5 (16.1)	3 (25)	2 (10.5)
Unknown ^c	-	1(3.2)	0 (0)	1 (5.3)

The data are expressed as n (%), mean ± standard deviation (SD). ^aThe age of each group is proved to be normal distribution (sample K-S test, $P > 0.05$). And T-test about age between each group ensured that there was no significant difference between them ($P > 0.05$). ^bAnti-SSA antibody, anti-SSB antibody, anti-Ro52 antibody data were lacking in a few subjects. ^cThe patient defined as “unknown” was someone who was on her first visit to our hospital, could not tell which medication to use.

Specimen Collection and Laboratory Testing

For analysis of the peripheral blood, 2 mL venous blood was collected from each participant into ethylene diamine tetra acetic acid (EDTA)-containing collection tubes (Becton Dickinson). Samples were centrifuged to collect the upper serum layer, and then frozen and stored at -80°C until use. After that, the remaining sample was processed to isolate peripheral blood mononuclear cells (PBMCs) by Ficoll density-gradient centrifugation. PBMCs were used for flow cytometry analyses. The levels of clinical laboratory indicators (e.g., WBC, RBC, PLT, and C3, C4) were determined using standard clinical laboratory protocols in the hospital.

Antinuclear antibody (ANA) profile immunoblotting test (IBT)

Serum ANA profile (including Anti-nRNP, Sm, SSA, SSB, Ro52, etc.) titers were determined using commercially available EUROLINE ANA profile (IgG) kits (cat: DL 1590-6401-3/8 G, EUROIMMUN, Ltd. Beijing, China) according to the manufacturers' instructions.

Antibodies and Flow Cytometry Analysis

The immunophenotyping of B cells was performed in the peripheral blood samples using the following fluorochrome-labelled anti-human antibodies: CD19-AF700 (clone SJ25C1), TLR10-PE (clone 3C10C5), CD27-BV421 (clone O323), CD38-APC, CD24-PE-CF594 (clone ML5), IgM-PerCP-Cy5.5 (clone MHM-88), and human Fc receptor blocking solution (cat: 422302) were purchased from Biolegend; IgD-FITC (clone IA6-2) was purchased from BD Biosciences, and 7-amino-actinomycin D (7AAD) (cat:00-6993-50) was purchased from Invitrogen. Fresh isolated PBMCs were first blocked with human Fc blocking reagents and stained with diluted antibodies at 4°C for 15 minutes in the dark. Then, PBMCs were washed twice with cold FACS buffer (1×PBS containing 2% FBS), resuspended in 0.3 ml of FACS buffer and analyzed by flow cytometry. Approximately 300,000 ~ 500,000 events were collected per sample. The data were collected with a FACS Calibur (Beckman CytoFLEX, USA) and analyzed using the FlowJo software version 10.0.

Enzyme-linked immunosorbent assay (ELISA)

Serum anti-SSA, anti-SSB, and BAFF concentrations were determined using commercially available ELISA kits (cat: GOY5611, GOY5501 and GOY5288, Gu Yan Biotech Co., Ltd. Shanghai, China) according to the manufacturers' instructions.

Statistical analysis

The results are expressed as the means $\pm$ standard deviation (SD) and medians (interquartile range). Statistical comparisons were performed by Student's *t*-tests. Differences among the three groups were determined by the Kruskal–Wallis H nonparametric test. Correlation analyses between two parameters were performed by Spearman's correlation method. All statistical analyses were performed using the SPSS software version 20 (SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism (v.8.0, CA). A *P* value < 0.05 was considered statistically significant.

Results

TLR10 expression in B cells in pSS patients

The Human Protein Atlas showed that *TLR10* mRNA mainly enriched in B cells, less in dendritic cells and monocytes, and undetectable in other peripheral blood immune cells (Sup Fig. 1). Therefore, to investigate the difference in TLR10 expression on the B cell surface between pSS patients and HCs, we isolated PBMC from the above two groups and detected by flow cytometry (Fig. 1a). Compared with the HCs, the pSS patients expressed relatively high levels of TLR10 on total CD19⁺ B cells' surface when determined by the mean fluorescence intensity (MFI) (Fig. 1b), but there was no statistically significant difference between the two groups.

Correlation between TLR10 expression in B cells and pSS related autoantibodies

Anti-SSA, anti-SSB and anti-Ro52 are important clinical diagnostic indicators for pSS patients, and their concentrations are usually positively correlated with pSS progression [21–23]. We analyzed the TLR10 expression in B cells in pSS patients according to the extractable nuclear antigen profile results. Interestingly, the expression of TLR10 in total CD19⁺ B cells from anti-SSA⁺⁺⁺ and anti-Ro52⁺⁺⁺ pSS patients is significantly reduced as compared with the anti-SSA^{-/+} and anti-Ro52^{+/-} pSS patients respectively (Sup Fig. 2a-b), and anti-SSB⁺⁺⁺ pSS patients show a moderate reduction in TLR10 expression as compared with anti-SSB^{-/+} pSS patients (Sup Fig. 2c), indicating that the expression of TLR10 in B cells might be related with the production of autoantibodies in pSS patients. Further ELISA results showed that the expression of TLR10 in total CD19⁺ B cells is negatively correlated with serum level of anti-SSA ($r = -0.4599$, $P = 0.0138$), anti-SSB ($r = -0.4028$, $P = 0.0336$) and ANA ($r = -0.7855$, $P = 0.0011$) in pSS patients (Fig. 2a-c). Moreover, the expression of TLR10 in CD19⁺ B cells was negatively correlated with BAFF ($r = -0.4092$, $P = 0.0306$) (Fig. 2d), which is important for survival and activation of B cells and presents excessive level in pSS patients [24]. These results suggested that the expression of TLR10 in CD19⁺ B cells might be correlated with pSS formation and/or progression.

TLR10 expression is mainly upregulated in switched memory B in pSS

Numerous studies have been reported that memory B cells, PB and plasma cells are the key subsets of B cells involved in the pathogenesis of pSS [4]. We further analyzed the expression of TLR10 in peripheral B cell subsets, including CD19⁺CD24⁺⁺CD38⁺⁺ transitional B cells, CD19⁺IgD⁺CD27⁻ naïve B cells, CD19⁺IgD⁺CD27⁺ unswitched memory B cells, CD19⁺IgD⁻CD27⁺ switched memory B cells and CD19⁺CD24⁻CD38⁺⁺ PB (Fig. 3a), obtained from both the pSS patients and HCs. The results showed that TLR10 expression was similar between the pSS patients and HCs in transitional B cells, naïve B cells, unswitched memory B cells and PB (Fig. 3b). Interestingly, the expression of TLR10 in switched memory B cells was significantly increased in pSS patients compared with the HCs (Fig. 3b). Moreover, the expression of TLR10 in switched memory B cells also correlated negatively with serum level of anti-SSA ($r = -0.3953$, $P = 0.0373$) and BAFF ($r = -0.3760$, $P = 0.0486$) in pSS patients (Fig. 3c-d). There was no

significant correlation between TLR10 expression in switched memory B cells and serum level of anti-SSB ($r = -0.2376$, $P = 0.2234$) in pSS patients (Sup Fig. 3a). In addition, the expression of TLR10 in transitional B cells, naïve B cells, unswitched memory B cells and PB showed no obvious correlation with serum level of anti-SSA, anti-SSB and BAFF in pSS patients, respectively (Sup Fig. 3). These results further confirmed that the expression of TLR10 was increased in switched memory B cells, which might play an important role in pSS progression.

TLR10 expression in B cells is negatively correlated with pSS progression

To further clarify whether TLR10 expression in B cells correlated with pSS progression, we analyzed the changes of TLR10 expression in peripheral B subsets in the HCs, low-activity pSS patients and high-activity pSS patients evaluated by ESSDAI according to their clinical features [20]. Interestingly, the expression of TLR10 in total CD19⁺ B cells in low-activity pSS patients significantly increased compared with the HCs, while decreased in high-activity pSS patients compared with low-activity pSS patients (Fig. 4a). Consistent with above results, the expression of TLR10 in transitional B cells and PB was relatively comparable among these groups (Fig. 4b and f). Notably, the expression of TLR10 in naïve B cells and switched memory B cells was obviously increased in low-activity pSS patients compared with the HCs (Fig. 4. c and e). With the progression of pSS, the expression of TLR10 in naïve B cells, unswitched and switched memory B cells was significantly decreased in high-activity pSS patients compared with low-activity pSS patients (Fig. 4c-e), indicating that TLR10 might affect pSS progression by taking part in the process of B cell activation and differentiation, especially in the phase of B cell class-switch recombination (CSR).

TLR10 suppress pSS progression might via inhibiting CSR

There were some evidences suggested that TLR10 might be able to suppress antibody responses of B cell with either T-dependent or independent antigens [25]. The upregulated expression of TLR10 in switched memory B cells indicate that TLR10 could take part in the CSR of B cells in pSS patients. Then we divided the pSS patients and HCs into TLR10 high- and low-expressed groups based on the average value of the TLR10 MFI of total CD19⁺ B cells, respectively, and calculated the percentage change of switched memory B cells. As shown in Fig. 5a, the percentage of switched memory B cells showed no significant difference between the TLR10 low- and the high-expressed group in HCs, but significantly decreased in the TLR10 high- as compared with the low-expressed group in pSS patients (Fig. 5a). Moreover, the pSS progression was closely related with TLR10 expression in total CD19⁺ B cells ($P < 0.001$) (Table 2). In addition, the proportion of high-activity patients in TLR10 low-expressed pSS patients was significantly higher than the TLR10 high-expressed pSS patients (76.19% vs 7.69%) (Fig. 5b). Considering the TLR10 high-expressed pSS patients presented with reduced percentage of switched memory B cells and correspondingly lower proportion of high-activity pSS patients, it suggested that TLR10 could suppress pSS progression via inhibiting the B cell CSR in pSS patients.

Table 2
Relationship between TLR10 expression and pSS progression

	Low-activity (n)	High-activity (n)	<i>P</i> -value*
TLR10-low expressed	5	16	< 0.001
TLR10-high expressed	12	1	
*Chi-Square Test and Fisher's exact Test were used.			

Discussion

pSS is a systemic rheumatic autoimmune disease characterized by abnormal B cell biological function [5]. TLRs, as one kind of pattern recognition receptors, are well known for their significant roles in inflammation and innate immunity. Previous experiments have proved that expression of TLR7 and TLR9 in different kinds of B cells may play an important role in dysregulation of B cells in pSS [26, 27]. TLR10, as the latest identified functional TLR in human, is mainly expressed in B cells [28]. However, few studies on TLR10 expression in pSS have been reported so far. The gene polymorphism analysis showed that TLR10 is involved in autoimmune thyroid disease [14] and rheumatoid arthritis [17]. Recently, Zhang et al reported that the expression of TLR10 in B cell subsets was increased and correlated with disease activity in RA patients [18]. These reports inspired us to detect the TLR10 expression in B cell subsets in the peripheral blood of patients with pSS. Here, we found that TLR10 was highly expressed in total CD19⁺ B cells, naïve B cells and switched memory B cells in low-activity pSS patients as compared with the HCs and high-activity pSS patients. TLR10 expression in CD19⁺ total B cells and switched memory B cells in pSS patients was significantly negatively correlated with anti-SSA autoantibody and BAFF production. Moreover, the TLR10 high-expressed pSS patients usually had relatively lower proportion of high-activity patients accompanied by reduced switched memory B cells as compared with the TLR10 low-expressed pSS patients.

Today, most investigators agree that there are two main CD27⁺ memory B cell compartments in blood, IgM⁺IgD⁺ and IgM⁻IgD⁻ B cells, the former is unswitched memory B cells exhibiting characteristics of marginal zone B cells, whereas the latter most likely represents class switched B cells [29, 30]. Importantly, we found that TLR10 was particularly highly expressed in CD27⁺IgD⁻ switched memory B cells in pSS patients compared with the HCs, while TLR10 expression in CD27⁺IgD⁺ unswitched memory B cells in pSS patients were slightly lower than the HCs. Our results indicate that TLR10 might play an important role in B cell germinal center reaction in patients with pSS. Further studies are necessary to uncover the role and detailed mechanism of TLR10 on B cell activation and class switch response.

pSS disease activity is assessed by physician according to ESSDAI from patient's clinical manifestations [31]. Among all criterion scores, anti-SSA received the highest average weights [32]. Moreover, it has been reported that the presence and increased titers of anti-SSA, anti-SSB and rheumatoid factor serum autoantibodies are correlated with the severity in pSS patients [33]. Intriguingly, we observed that TLR10

expression in CD19⁺ total B cells in pSS patients was negatively correlated with anti-SSA, anti-SSB and ANA autoantibodies (Fig. 2a-c). Moreover, TLR10 expression in switched memory B cells in pSS patients was also negatively correlated with anti-SSA but not anti-SSB autoantibodies. When using anti-SSB autoantibody as the biomarker, different correlation results between TLR10 expression in CD19⁺ total B and switched memory B cells were achieved, but understandable because the anti-SSB shows a weaker correlation with pSS progression as compared with anti-SSA according to the ESSDAI [32]. These results suggested the expression of TLR10, especially in switched memory B cells, could be used as a diagnostic biomarker for pSS progression. Importantly, the expression of TLR10 in CD19⁺ total B cells and switched memory B cells in low-activity pSS patients was significantly increased compared with high-activity pSS patients (Fig. 5). Thus, we postulated that TLR10 could inhibit pSS progression via negatively regulating B cell function.

Although the ligand and downstream signaling pathways of TLR10 remain unclear, it has been identified as an immunomodulatory receptor with inhibitory properties [25, 34, 35]. The high expression of TLR10 in human B cells suggest that TLR10 may regulate B cell function. Hess et al. reported that TLR10 is able to suppress responses mediated by a variety of B cell co-stimulatory signals and attenuate both T cell independent and T cell dependent antibodies production in a TLR10 knock-in mouse model [25]. Therefore, we speculated that TLR10 might inhibit pSS progression via negatively regulating B cell function. In addition, the dysregulation of CSR can cause self-reactive BCRs and B cell lymphomas [36]. Considering the high expression of TLR10 in switched memory B cells in pSS patients, we speculated that TLR10 expression might affect B cell CSR in pSS patients (Fig. 3b). Notably, we further observed that the TLR10 high-expressed pSS patients presented with decreased percentage of switched memory B cells as compared with TLR10 low expression pSS patients (Fig. 5a), suggesting TLR10 suppressed the B cell CSR in pSS patients.

BAFF, an important cytokine for B cell maturation, proliferation and survival, is upregulated in salivary gland tissue and blood from pSS patients, and plays a vital role in the pathogenesis of pSS [37, 38]. We observed that TLR10 expression in B cells and switched memory B cells in pSS patients also was inversely correlated with BAFF level in pSS patients' serum (Fig. 2d and Fig. 3d). All these results further supported our hypothesis that TLR10 inhibited pSS progression by suppressing B cell function.

In summary, for the first time, we found that the protein level of TLR10 expression in peripheral switched memory B cells was increased in pSS patients and was significantly negatively correlated with both anti-SSA and BAFF production. Moreover, TLR10 could inhibit pSS progression via negatively regulating B cell function. These findings suggest that TLR10 could be used as a diagnostic biomarker of pSS progression and be a potential therapeutic target for pSS.

Declarations

FUNDING

We are grateful to the patients and healthy control for their participation and cooperation in this study. This research was supported by National Natural Science Foundation of China (81971470) and Shenzhen municipal Science and Technology Innovation Program (JCY20190809143803732) to Bihui Huang, the Postdoctoral Science Foundation of China (2020M683062) and Guangdong Basic and Applied Basic Research Foundation (2020A1515110012) to Nannan Lai.

ACKNOWLEDGEMENT

We thank Drs. Qing Lv from department of rheumatology, Fuqun Wu from clinical laboratory, the seventh affiliated hospital, Sun Yat-sen University, and Yiping Hu from Shenzhen Hospital, Peking University for their generous help in collecting patient's samples.

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

CODE AVAILABILITY

Not applicable.

AUTHOR CONTRIBUTIONS

B. H. and C. T. designed and supervised the study; N. L. and Y. Q. performed the experiments; X. J., H. S., Z. L., Y. Z., and C. Z. collected patients' samples and carried out lab test; Y. W., C. T., and Q.W. provided clinical data; X. Z. and Y. K. analyzed the data. N. L. and Y. Q. wrote the paper. B. H. corrected the paper. All the authors read and approved the final manuscript.

Conflict of interest

The authors have declared that no competing interests exist.

Ethics Approval and Consent of Participate

This study was performed according to the recommendations of the Declaration of Helsinki and approved by the Ethics Committee of the Seventh Affiliated Hospital of Sun Yat-sen University. All participants carefully read and signed the written informed consent.

Consent of Publication

The manuscript is approved by all authors for publication.

References

1. Mariette, X. and Criswell, L.A. **2018**. Primary Sjögren's Syndrome. *N Engl J Med* 379(1): 97. <https://doi.org/10.1056/NEJMc1804598>.

2. Alunno, A., Leone, M.C., Bartoloni, E., Gerli, R. **2020**. Novel insights on lymphoma and lymphomagenesis in primary Sjögren's Syndrome. *Panminerva Med.* <https://doi.org/10.23736/S0031-0808.20.04079-3>.
3. Nocturne, G. and Mariette, X. **2013**. Advances in understanding the pathogenesis of primary Sjögren's syndrome. *Nat Rev Rheumatol* 9(9): 544-556. <https://doi.org/10.1038/nrrheum.2013.110>.
4. Nocturne, G. and Mariette, X. **2018**. B cells in the pathogenesis of primary Sjögren syndrome. *Nat Rev Rheumatol* 14(3): 133-145. <https://doi.org/10.1038/nrrheum.2018.1>.
5. Reed, J.H., Verstappen, G.M., Rischmueller, M., and Bryant, V.L. **2020**. When B cells break bad: development of pathogenic B cells in Sjögren's syndrome. *Clin Exp Rheumatol* 38 Suppl 126(4): 271-282.
6. Verstappen, G.M., van Nimwegen, J.F., Vissink, A., Kroese, F.G.M. **2017**. The value of rituximab treatment in primary Sjögren's syndrome. *Clin Immunol* 182: 62-71. <https://doi.org/10.1016/j.clim.2017.05.002>.
7. Grigoriadou, S., Chowdhury, F., Pontarini, E., Tappuni, A. **2019**. B cell depletion with rituximab in the treatment of primary Sjögren's syndrome: what have we learnt? *Clin Exp Rheumatol* 37 Suppl 118(3): 217-224.
8. Hoyer, B.F., Moser, K., Hauser, A.E., Peddinghaus, A. **2004**. Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. *J Exp Med* 199(11): 1577-1584.
9. Ruprecht, C.R. and Lanzavecchia, A. **2006**. Toll-like receptor stimulation as a third signal required for activation of human naive B cells. *Eur J Immunol* 36(4): 810-816.
10. Marshak-Rothstein, A. **2006**. Toll-like receptors in systemic autoimmune disease. *Nat Rev Immunol* 6(11): 823-835.
11. Su, S.-B., Tao, L., Deng, Z.-P., Chen, W. **2021**. TLR10: Insights, controversies and potential utility as a therapeutic target. *Scand J Immunol* 93(4): e12988. <https://doi.org/10.1111/sji.12988>.
12. Hasan, U., Chaffois, C., Gaillard, C., Saulnier, V. **2005**. Human TLR10 is a functional receptor, expressed by B cells and plasmacytoid dendritic cells, which activates gene transcription through MyD88. *J Immunol* 174(5): 2942-2950.
13. Hornung, V., Rothenfusser, S., Britsch, S., Krug, A. **2002**. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol* 168(9): 4531-4537.
14. Cho, W.K., Jang, J.-P., Choi, E.-J., Jeon, Y.J. **2015**. Association of Toll-like receptor 10 polymorphisms with autoimmune thyroid disease in Korean children. *Thyroid* 25(2): 250-255.

<https://doi.org/10.1089/thy.2014.0135>.

15. Stappers, M.H.T., Oosting, M., Ioana, M., Reimnitz, P. **2015**. Genetic Variation in TLR10, an Inhibitory Toll-Like Receptor, Influences Susceptibility to Complicated Skin and Skin Structure Infections. *J Infect Dis* 212(9): 1491-1499. <https://doi.org/10.1093/infdis/jiv229>.
16. Purdue, M.P., Lan, Q., Wang, S.S., Kricker, A. **2009**. A pooled investigation of Toll-like receptor gene variants and risk of non-Hodgkin lymphoma. *Carcinogenesis* 30(2): 275-281. <https://doi.org/10.1093/carcin/bgn262>.
17. Torices, S., Julia, A., Muñoz, P., Varela, I. **2016**. A functional variant of TLR10 modifies the activity of NFkB and may help predict a worse prognosis in patients with rheumatoid arthritis. *Arthritis Res Ther* 18(1): 221.
18. Zhang, Y., Cao, R., Ying, H., Du, J. **2018**. Increased Expression of TLR10 in B Cell Subsets Correlates with Disease Activity in Rheumatoid Arthritis. *Mediators Inflamm* 2018: 9372436. <https://doi.org/10.1155/2018/9372436>.
19. Shiboski, C.H., Shiboski, S.C., Seror, R., Criswell, L.A. **2017**. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* 76(1). <https://doi.org/10.1136/annrheumdis-2016-210571>.
20. Seror, R., Bowman, S.J., Brito-Zeron, P., Theander, E. **2015**. EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. *RMD Open* 1(1): e000022. <https://doi.org/10.1136/rmdopen-2014-000022>.
21. Martín-Nares, E. and Hernández-Molina, G. **2019**. Novel autoantibodies in Sjögren's syndrome: A comprehensive review. *Autoimmun Rev* 18(2): 192-198. <https://doi.org/10.1016/j.autrev.2018.09.003>.
22. Li, M.-t., Zhao, Y., Zheng, W.-j., Su, J.-m. **2010**. [The diagnostic value of anti-SSA antibody in primary Sjögren's syndrome]. *Zhonghua Nei Ke Za Zhi* 49(5): 410-413.
23. Zhang, W., Li, X.M., Xu, D., Liu, D.Z. **2020**. [Recommendations of diagnosis and treatment of primary Sjögren's syndrome in China]. *Zhonghua Nei Ke Za Zhi* 59(4): 269-276. <https://doi.org/10.3760/cma.j.cn112138-20200113-00021>.
24. Navarro-Mendoza, E.P., Aguirre-Valencia, D., Posso-Osorio, I., Correa-Forero, S.V. **2018**. Cytokine markers of B lymphocytes in minor salivary gland infiltrates in Sjögren's syndrome. *Autoimmun Rev* 17(7): 709-714. <https://doi.org/10.1016/j.autrev.2018.02.003>.
25. Hess, N.J., Jiang, S., Li, X., Guan, Y. **2017**. TLR10 Is a B Cell Intrinsic Suppressor of Adaptive Immune Responses. *J Immunol* 198(2): 699-707. <https://doi.org/10.4049/jimmunol.1601335>.

26. Ibrahem, H.M. **2019**. B cell dysregulation in primary Sjögren's syndrome: A review. *Jpn Dent Sci Rev* 55(1): 139-144. <https://doi.org/10.1016/j.jdsr.2019.09.006>.
27. Bernasconi, N.L., Onai, N., and Lanzavecchia, A. **2003**. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. *Blood* 101(11): 4500-4504.
28. Bourke, E., Bosisio, D., Golay, J., Polentarutti, N. **2003**. The toll-like receptor repertoire of human B lymphocytes: inducible and selective expression of TLR9 and TLR10 in normal and transformed cells. *Blood* 102(3): 956-963.
29. Weill, J.-C., Weller, S., and Reynaud, C.-A. **2009**. Human marginal zone B cells. *Annu Rev Immunol* 27: 267-285. <https://doi.org/10.1146/annurev.immunol.021908.132607>.
30. Weller, S., Braun, M.C., Tan, B.K., Rosenwald, A. **2004**. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. *Blood* 104(12): 3647-3654.
31. Seror, R., Mariette, X., Bowman, S., Baron, G. **2010**. Accurate detection of changes in disease activity in primary Sjögren's syndrome by the European League Against Rheumatism Sjögren's Syndrome Disease Activity Index. *Arthritis Care Res (Hoboken)* 62(4): 551-558. <https://doi.org/10.1002/acr.20173>.
32. Shiboski, C.H., Shiboski, S.C., Seror, R., Criswell, L.A. **2017**. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis Rheumatol* 69(1): 35-45. <https://doi.org/10.1002/art.39859>.
33. Retamozo, S., Acar-Denizli, N., Rasmussen, A., Horváth, I.F. **2019**. Systemic manifestations of primary Sjögren's syndrome out of the ESSDAI classification: prevalence and clinical relevance in a large international, multi-ethnic cohort of patients. *Clin Exp Rheumatol* 37 Suppl 118(3).
34. Jiang, S., Li, X., Hess, N.J., Guan, Y. **2016**. TLR10 Is a Negative Regulator of Both MyD88-Dependent and -Independent TLR Signaling. *J Immunol* 196(9): 3834-3841. <https://doi.org/10.4049/jimmunol.1502599>.
35. Hess, N.J., Felicelli, C., Grage, J., and Tapping, R.I. **2017**. TLR10 suppresses the activation and differentiation of monocytes with effects on DC-mediated adaptive immune responses. *J Leukoc Biol* 101(5): 1245-1252. <https://doi.org/10.1189/jlb.3A1116-492R>.
36. Roco, J.A., Mesin, L., Binder, S.C., Nefzger, C. **2019**. Class-Switch Recombination Occurs Infrequently in Germinal Centers. *Immunity* 51(2). <https://doi.org/10.1016/j.immuni.2019.07.001>.
37. Mariette, X., Roux, S., Zhang, J., Bengoufa, D. **2003**. The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjögren's syndrome. *Ann Rheum Dis* 62(2): 168-171.

38. Lavie, F., Miceli-Richard, C., Quillard, J., Roux, S. **2004**. Expression of BAFF (BLyS) in T cells infiltrating labial salivary glands from patients with Sjögren's syndrome. *J Pathol* 202(4): 496-502.

Figures

Figure 1

(a) Representative flow cytometry profiles for TLR10 expression in CD19+ B cells in freshly isolated PBMCs. (b) The mean fluorescence intensity (MFI) of TLR10 in CD19+ B cells in the pSS patients (n=34) and HCs (n=25).

Figure 2

TLR10 expression in B cells was negatively correlated to serum autoantibodies in pSS patients. The correlation between the TLR10 MFI in CD19⁺ B cells and the anti-SSA (n=28) (a), anti-SSB (n=28) (b), ANA (n=14) (c) and BAFF (n=28) (d). Statistical comparisons were performed by Student's t-tests. Mean $\pm$ SD are shown. Correlation analyses were performed by Spearman's correlation method.

Figure 3

TLR10 expression was upregulated in switched memory B cells in pSS patients. (a) Representative flow cytometry profiles for TLR10 expression in transitional B cells, naïve B cells, unswitched memory B, switched memory B cells and PB in freshly isolated PBMCs. (b) The MFI of TLR10 in transitional B cells, naïve B cells, unswitched memory B, switched memory B cells and PB in the pSS patients (n=34) and HCs (n=25). The correlation between the TLR10 MFI in switched memory B cells and the anti-SSA (n=28) (c)

and BAFF (n=28) (d) concentration in pSS patients' serum. Statistical comparisons were performed by Student's t-tests. Mean $\pm$ SD are shown. *p<0.05. Correlation analyses were performed by Spearman's correlation method.

Figure 4

TLR10 expression in B cell subsets in the HCs, low-activity and high-activity pSS patients. (a-f) The MFI of TLR10 in CD19+ total B, transitional B cells, naïve B cells, unswitched memory B cells, switched memory B cells and PB in the low-activity pSS patients (n=17), high-activity pSS patients (n=17) and HCs (n=25). Statistical comparisons were performed by the Kruskal–Wallis H nonparametric test. Median $\pm$ 95% CI are shown. *p< 0.05, ***p< 0.001, ****p< 0.0001.

Figure 5

TLR10 inhibits B cell CSR and pSS progression. The pSS patients and HCs were divided into TLR10 low- and high-expressed group according to the average of TLR10 MFI. (a) The proportion of switched

memory B cells between TLR10 low- and high-expressed group in pSS patients (n=34) and HCs (n=25), respectively. (b) Pie-map analysis of the percentage of high- and low-activity pSS patients between TLR10 low- (n=21) and high-expressed (n=13) pSS patients, respectively. Statistical comparisons were performed by Student's t-tests. Mean $\pm$ SD are shown. *p< 0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupFig.13.pdf](#)