Expression of TLR10 in Peripheral B Cell Subsets of Patients with Primary Sjögren’s Syndrome

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Research Article

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Abstract

Primary Sjögren's syndrome (pSS) is considered as 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 is less 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 was highly expressed in switched memory B cells (CD19^+CD27^*IgD^-) in the pSS patients compared with the HCs. TLR10 expression in CD19^+ B cells, memory B cells (CD19^+CD27^+) and switched memory B cells in pSS patients was negatively correlated with serum levels of anti-SSA antibody and B cell-activating factor of TNF family (BAFF), respectively. A much lower proportion of high-activity pSS patients was observed in TLR10 high-expression compared to low-expressed patients. TLR10 expression in CD19^+ B cells, naïve B cells (CD19^+CD27^-IgD^+), memory B cells, and switched memory B cells was significantly increased in low-activity pSS patients as compared with HCs and high-activity pSS patients. Our study concluded that TLR10 expression in CD19^+ B, naïve B, and memory B cells was negatively correlated with pSS disease activity, suggesting that TLR10 might play a critical role in the progression 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 common 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 of studies on 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 as a B cell-mediated disease characterized by autoantibodies and hypergammaglobulinaemia in patients [4]. Besides the production of autoantibodies, B cells can activate T cells by presenting autoantigen and secreting 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 [17]. Moreover, Zhang et al. revealed that upregulated TLR10 in B cell subsets is positively correlated with disease activity in rheumatoid arthritis patients [18]. However, the biology of TLR10 in pSS is rarely 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, naïve B cells, memory 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≥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  | pSS group  | Low-activity pSS group | High-activity pSS group |
|---------------------------|----------|------------|------------------------|------------------------|
|                           | group (n=25) | (n=34)     | (n=17)                 | (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 (\(\times10^9\))     | -        | 5.96±2.28  | 6.95±2.85              | 5.37±1.67              |
| RBC (\(\times10^{12}\))  | -        | 4.46±1.06  | 4.61±1.67              | 4.37±0.44              |
| PLT (\(\times10^9\))     | -        | 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). \(^a\)The 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 (\(P>0.05\)). \(^b\)Anti-SSA antibody, anti-SSB antibody, anti-Ro52 antibody data were lacking in a few subjects. \(^c\)The 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-labeled 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 ± 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

Both previous studies \[12, 13\] and 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. Therefore, to investigate the differences in TLR10 expression on the B cell surface between pSS patients and HCs, we isolated PBMC from the above two groups and detected them by flow cytometry. The proportion of CD19+ B cells in PBMC between pSS patients and HCs was comparable (Sup. Fig. 1). Compared with the HCs, the pSS patients expressed relatively high levels of TLR10 on CD19+ B cells’ surface when determined by the mean fluorescence intensity (MFI) (Fig. 1a-b), but there were no statistical differences between the two groups. In addition, the proportion of TLR10+CD19+ B cells was also similar between the two groups (Fig. 1c-d).

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 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 (Fig. 2a-b), and anti-SSB+++ pSS patients show a moderate reduction in TLR10 expression as compared with anti-SSB−/+ pSS patients (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 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. 2d-f). Moreover, the expression of TLR10 in CD19+ B cells was negatively correlated with BAFF (r = -0.4092, p = 0.0306) (Fig. 2g), 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+CD27+ memory 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, memory 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 memory B cells and switched memory B cells was negatively correlated with serum level of anti-SSA (memory B, $r = -0.4034$, $p = 0.0333$; switched memory B, $r = -0.3953$, $p = 0.0373$) and BAFF (memory B, $r = -0.3966$, $p = 0.0367$; switched memory B, $r = -0.3760$, $p = 0.0486$) in pSS patients (Fig. 3c-f), respectively. The expression of TLR10 in memory B cells and switched memory B cells showed no significant correlation with the serum level of anti-SSB (memory B, $r = -0.2288$, $p = 0.2416$; switched memory B, $r = -0.2376$, $p = 0.2234$) in pSS patients (Sup Fig. 2a-b). 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. 2c-e). 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

Although TLR10 expression in CD19$^+$ B cells showed no significant differences between the HCs and pSS patients, we wondered that whether TLR10 expression in B cells changed with pSS progression. Firstly, the expression of TLR10 in B cells in pSS patients with low- and high-activity evaluated by ESSDAI according to their clinical features[20] was analyzed by flow cytometry. As shown in Sup Fig. 3a, the proportion of CD19$^+$ B cells among the HCs, low- and high-activity pSS patients was comparable. Interestingly, the expression of TLR10 in 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). Then we divided the pSS patients into TLR10 high- and low-expressed groups based on the average value of the TLR10 MFI of CD19$^+$ B cells, and calculated the proportion of low- or high-activity patients between the two groups. As shown in Fig. 4h, the proportion of high-activity patients in TLR10 low-expressed pSS patients was significantly higher than that in TLR10 high-expressed pSS patients (76.19% vs 7.69%). Conversely, the proportion of low-activity patients in TLR10 low-expressed pSS patients was significantly lower than that in TLR10 high-expressed pSS patients (23.81% vs 92.31%). Moreover, correlation analysis showed that the pSS progression was closely related to TLR10 expression in CD19$^+$ B cells ($p < 0.001$) (Table 2). These results suggested that TLR10 expression in CD19$^+$ B cells was negatively correlated with pSS progression.

| Relationship between TLR10 expression and pSS progression |
|----------------------------------------------------------|
| Low-activity (n) | High-activity (n) | $p$-value$^*$ |
|------------------|-------------------|--------------|
| TLR10-low expressed | 5 | 16 | <0.001 |
| TLR10-high expressed | 12 | 1 | |

*Chi-Square Test and Fisher’s exact Test were used.

TLR10 expression in naive and memory B cells is upregulated in low-activity pSS patients
To clarify whether the TLR10 expression in B subsets changed during the pSS progression, we analyzed the expression of TLR10 in peripheral B subsets in the HCs, low- and high-activity pSS patients. Compared with the HCs, the high-activity pSS patients presented with a significantly increased proportion of transitional B cells, naive B cells, and PB proportion, respectively, but decreased proportion of memory B cells, as well as unswitched and switched memory B cells (Sup. Fig. 3b-g). Consistent with the above results, the expression of TLR10 in transitional B cells and PB was relatively comparable among these groups (Fig. 4b and g). Notably, the expression of TLR10 in naïve B cells, memory and switched memory B cells was increased in low-activity pSS patients compared with the HCs (Fig. 4c, d, and f). With the progression of pSS, the expression of TLR10 in naïve and memory (including unswitched and switched) B cells was significantly decreased in high-activity pSS patients compared with low-activity pSS patients (Fig. 4c-f). These results suggested that TLR10 expression might suppress pSS progression by taking part in the process of B cell activation and differentiation.

**Discussion**

pSS is a systemic rheumatic autoimmune disease characterized by abnormal B cell biological function [5]. TLRs, as one kind of pattern recognition receptor, are well known for their significant roles in inflammation and innate immunity. Previous studies have proved that the expression of TLR7 and TLR9 in B cells may play an important role in the dysregulation of B cells in pSS [25, 26]. TLR10, as the latest identified functional TLR in human beings, is mainly expressed in B cells [27]. However, few studies on TLR10 expression in pSS have been reported so far. Here, we found that TLR10 was highly expressed in switched memory B cells in the pSS patients compared with the HCs. TLR10 expression in CD19⁺ B cells, memory and switched memory B cells in pSS patients was significantly negatively correlated with the anti-SSA autoantibody and BAFF production, respectively. The TLR10 high-expressed pSS patients usually had a relatively lower proportion of high-activity condition as compared with the TLR10 low-expressed pSS patients. Moreover, TLR10 was highly expressed in CD19⁺ B cells, naïve B cells, and memory B cells in low-activity pSS patients as compared with the high-activity 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 [28, 29]. 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 was 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 about B cell activation and class switch response.

We also assessed the proportion change of B cell subsets in the peripheral blood of patients with pSS. It was found that the pSS patients presented with a comparable proportion of CD19⁺ B cells, increased proportions of transitional B, naïve B, and PB, but a reduced proportion of memory B, as compared with
the HCs (unpublished data), which were consistent with previous studies [30, 31]. Importantly, the proportion change of B cell subsets mentioned above was even more obvious between HCs and high-activity pSS patients, but showed no statistical difference between HCs and low-activity pSS patients, suggesting that the proportion change of B cell subsets focused on high-activity pSS patients. Notably, the expression of TLR10 was increased only in switched memory B cells in pSS patients compared with the HCs, whereas that was reduced in CD19+ B, naive B, memory B (including unswitched and switched memory B) in high-activity compared with low-activity pSS patients. Considering that the change of TLR10 expression mainly occurs in naïve B and memory B, TLR10 might play an important role in B cell activation and differentiation during the pSS progression. In addition, we observed that the TLR10 expression in B cells had a complex effect on the proportion change of patients’ corresponding B subsets.

pSS disease activity is assessed by the physician according to ESSDAI from the patient’s clinical manifestations [32]. Among all criterion scores, anti-SSA received the highest average weights [33]. 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 [34]. Intriguingly, we observed that TLR10 expression in CD19+ B cells in pSS patients was negatively correlated with anti-SSA, anti-SSB, and ANA autoantibodies. Moreover, TLR10 expression in memory and 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+ 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 [33]. Importantly, the expression of TLR10 in CD19+ B, naïve B, and memory B cells in low-activity pSS patients was significantly increased compared with high-activity pSS patients. 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 [35–37]. The high expression of TLR10 in human B cells suggests that TLR10 may regulate B cell function. Hess et al. reported that TLR10 can 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 [35]. 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 [38, 39]. We observed that TLR10 expression in CD19+ B cells, memory and switched memory B cells in pSS patients also was inversely correlated with BAFF level in pSS patients’ serum (Fig. 2d and Fig. 3d, f), which 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 expression in peripheral CD19+ B, naïve B, and memory B is negatively correlated with pSS disease activity. These findings suggest that TLR10 might participate in
the pathogenesis of pSS by negatively regulating B cell function, and support the further investigation on TLR10 biological function in B cells.

**Declarations**

**FUNDING**

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**AVAILABILITY OF DATA AND MATERIAL**

Not applicable.

**CODE AVAILABILITY**

Not applicable.

**AUTHOR CONTRIBUTIONS**

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 tests; 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.

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Figures
Figure 1

The expression of TLR10 in CD19+ B cells in HCs and pSS patients. (a) Representative flow cytometry profiles showing the expression of TLR10 and (b) the mean fluorescence intensity (MFI) of TLR10 in CD19+ B cells in the HCs (black) and pSS patients (red). (c)Representative flow cytometry profiles of CD19+TLR10+ B cells in freshly isolated PBMCs from HCs and pSS patients. (d)The proportion of CD19+TLR10+B cells in CD19+ B cells in HCs and pSS patients. pSS patients (n=34) and HCs (n=25). Statistical comparisons were performed by Student’s t-tests. Mean ± SD are shown. ns, no significance.
Figure 2

TLR10 expression in B cells was negatively correlated to serum autoantibodies in pSS patients. (a) The mean fluorescence intensity (MFI) of TLR10 in CD19+ B cells in the anti-SSA-/+ (n=5), anti-SSA++ (n=5) and anti-SSA+++ (n=15) pSS patients. (b) The MFI of TLR10 in CD19+ B cells in the anti-Ro52-/+ (n=11) and anti-Ro52+++ (n=7) pSS patients. (c) Anti-SSB+++pSS patients (n=8) show a moderate reduction in TLR10 expression as compared with anti-SSB-/pSS patients (n=16). The correlation between the TLR10 MFI in CD19+ B cells and the anti-SSA (n=28) (d), anti-SSB (n=28) (e), ANA (n=14) (f) and BAFF (n=28) (g) autoantibodies.
(g). Statistical comparisons were performed by Student’s t-tests or the Kruskal–Wallis H nonparametric test. Correlation analyses were performed by Spearman’s correlation method. Mean ± SD are shown. ns, no significance, **p< 0.01, ***p< 0.001, ****p< 0.0001.

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, naïve B, memory B, unswitched memory B,
switched memory B, and PB in freshly isolated PBMCs. (b) The MFI of TLR10 in transitional B, naïve B, memory B, unswitched memory B, switched memory B cells and PB in the pSS patients (n=34) and HCs (n=25). (c-f) The correlation between the TLR10 MFI in memory and switched memory B cells and the anti-SSA (n=28) and BAFF (n=28) concentration in pSS patients’ serum, respectively. Statistical comparisons were performed by Student’s t-tests. Correlation analyses were performed by Spearman’s correlation method. Mean ± SD are shown. ns, no significance, *p<0.05.

Figure 4

TLR10 expression in B cell subsets in the HCs, low-activity and high-activity pSS patients. (a-g) The MFI of TLR10 in CD19+ B, transitional B, naïve B, memory B, unswitched memory B, switched memory B and PB in the low-activity pSS patients (n=17), high-activity pSS patients (n=17), and HCs (n=25). (h) 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 the Kruskal–Wallis H nonparametric test. Mean±SD are shown. ns, no significance, *p<0.05, **p<0.01, ****p<0.0001.
Supplementary Files

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- SupFig.13.pdf