Original Research Article

Inverse correlation between the number of CXCR3+ macrophages and the severity of inflammatory lesions in Sjögren's syndrome salivary glands: a pilot study

Keiko Aota1,*, Tomoko Yamanoi1, Koichi Kani 1, Koh-ichi Nakashiro2, Naozumi Ishimaru3 and Masayuki Azuma1

1Department of Oral Medicine, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
2Department of Oral and Maxillofacial Surgery, Ehime University Graduate School of Medicine, Ehime, Japan
3Department of Oral Molecular Pathology, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan

Running title: CXCR3+ macrophage in Sjögren's syndrome

Key words: CXCR3, macrophage, Sjögren's syndrome, salivary gland, LSG

*Corresponding author: Dr. Keiko Aota, Department of Oral Medicine, Tokushima University Graduate School of Biomedical Sciences, 3-18-15 Kuramoto-cho, Tokushima, 770-8504, Japan. Tel: +81-88-633-7352; Fax: +81-88-633-7388. E-mail: aota.keiko@tokushima-u.ac.jp

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ABSTRACT (250 words)

Background: Mechanisms underlying immune cells' recruitment and activation into the inflammatory lesions of lip salivary glands (LSGs) from primary Sjögren's syndrome (pSS) patients are incompletely understood. Chemokines play pivotal roles in these processes, so we investigated the clinical significance of chemokine receptor CXCR3 and its ligands in the autoimmune lesions of pSS.

Methods: We histologically determined the grade of LSG samples from 22 pSS patients and subjected the samples to immunofluorescence analysis to determine the expressions of CXCR3 and its ligands: CXCL9, CXCL10, and CXCL11. To identify the immune cells expressing CXCR3 in the LSGs, we performed double immunofluorescence analysis using antibodies against CD3 (pan-T cells), CD80 (M1 macrophages), CD163 (M2 macrophage), and CD123 (plasmacytoid dendritic cells: pDCs). The relationship between the grade of lymphocytic infiltration and the number of positively stained cells was analyzed by Spearman's rank correlation test.

Results: The expressions of CXCL9 and CXCL10 showed particularly intense staining in the LSG samples' ductal cells. The CXCR3 expression was detected mainly in CD80+ and CD163+ macrophages. The number of CXCR3+CD163+ macrophages inversely correlated with the LSG inflammatory lesions' severity (rs= -0.777, p<0.001).
**Conclusions:** Our results suggest that the enhanced production of CXCL9 and CXCL10 from ductal cells results in the CXCR3$^+$ macrophages' migration. There was an inverse correlation between these two parameters: i.e., the number of CXCR3$^+$CD163$^+$ macrophages decreased as the lymphocytic infiltration grade increased. Although CXCR3 is expressed in all of the innate immune cells, CXCR3$^+$CD163$^+$ M2 macrophages may contribute to the anti-inflammatory functions in pSS lesions.
Introduction

Primary Sjögren's syndrome (pSS), one of the most common autoimmune diseases, is characterized by the eventual total replacement of the acinar structure by marked lymphocytic infiltrates in the salivary and lacrimal glands (1). The pathogenesis of this selective and progressive destruction of the acinar structure in salivary glands is not yet fully understood. However, accumulated evidence indicates a close relationship between cytokine expression in salivary gland tissue and the development and progression of this disease (2). One of the families of effector molecules under the control of the cytokine communication network is the large group of mediators known as chemokines (3).

CXCR3 is a receptor for CXCL9, CXCL10, and CXCL11, all of which are interferon (IFN)-inducible chemokines (4). CXCR3 is well known to contribute to the migration, activation and differentiation of a number of immune cells (5-7). In addition, CXCR3 plays a major role in a wide range of infections, autoimmune diseases, and neoplastic diseases (5-7). CXCR3 is also well known to express on the multiple types of cells of the innate immune system, including dendritic cells, natural killer (NK) cells, NKT cells, neutrophils, and macrophages (8, 9).

Over the years, CXCR3 ligands and their functions in autoimmune diseases such as rheumatoid arthritis (RA) have been extensively reported and reviewed (10). In RA patients, the recruitment of CXCR3-expressing T cells and mast cells to the inflamed
synovium was indeed related to a preferential upregulation of CXCL9 and CXCL10 expression in the synovial fluid and tissue compared to chemokine levels in traumatic arthritis or osteoarthritis (11, 12). Regarding CXCR3 in pSS, Ogawa et al. reported that CXCR3 ligands (i.e., CXCL9, CXCL10, and CXCL11) were involved in the accumulation of CXCR3+ T cell infiltrates in the salivary glands of pSS patients (13, 14). Most of the literature on mammalian systems focuses on the role of CXCR3 in the maturation, priming, activation and migration of T cells, but a few studies demonstrated that CXCR3 also plays an important role in directing macrophages' activities in some animal disease models (15-18).

Although it is evident that CXCR3 and its ligands contribute to the pathogenesis of pSS, an immunopathological analysis to identify the clinical potency of CXCR3 and its ligands in pSS has not been performed. Here we conducted an immunopathological analysis to clarify the clinical significance of CXCR3 and CXCR3 ligands in the autoimmune lesions of pSS.

**Materials and Methods**

**Ethics statement**

This study was approved by the Institutional Review Board (IRB) of Tokushima University Hospital (#2802). Written informed consent was obtained from each
individual prior to participation in the study. All of the study participants provided
signed and informed consent, and this process was documented in an IRB-approved
consent form. The consent procedure was approved by the Ethics Committee of
Tokushima University Hospital. This study was conducted in accordance with the
principles expressed in the Declaration of Helsinki.

Patients

Twenty-two patients with pSS and 3 healthy controls were enrolled in this study, and
they were treated at Tokushima University Hospital between 2011 and 2016. All 22
patients satisfied the revised Japanese Ministry of Health criteria for the diagnosis of SS
(1999) (19). Moreover, all of the pSS patients also satisfied the American College of
Rheumatology (ACR) classification criteria for SS (20).

Table 1 provides a summary of the pSS patients' data. Minor salivary gland
biopsies in the lower lip were performed in all 22 patients. The biopsy specimens were
formalin-fixed, paraffin-embedded, and stained with hematoxylin and eosin. The
lymphocytic focus score (LFS) was recorded as the number of lymphocytic foci
(defined as aggregates of ≥50 mononuclear cells) per 4 mm² of salivary tissue (21).

Based on the biopsy scoring system of Tarpley et al. (22), we categorized the
LSG biopsy samples into four groups according to the grade of infiltration: the Grade 1
group (Tarpley score 1: 1–2 lymphocytic aggregates/lobule), the Grade 2 group (Tarpley score 2: 2–3 lymphocytic aggregates/lobule), the Grade 3 group (Tarpley score 3: diffuse infiltration through acini associated with partial destruction of acinar tissue), and the Grade 4 group (Tarpley score 4: diffuse infiltration associated with complete loss of tissue architecture).

The healthy controls (all females, mean age 60.3 ± 4.2 years) were subjects who had experienced subjective symptoms of oral dryness, but met none of the objective criteria for a diagnosis of pSS.

Double immunofluorescence analysis

To identify the enhanced expression of CXCR3 and its ligands in the LSGs from pSS patients and healthy controls, we examined formalin-fixed paraffin-embedded samples of those LSGs. Sections were deparaffinized in xylene and rehydrated in graded ethanol according to standard procedures. Antigen retrieval was performed by microwave treatment in Antigen Unmasking Solution (Vector, Burlingame, CA). Endogenous biotin was blocked using Blocking One reagent (Nacalai Tesque, Kyoto). The primary antibodies were rabbit polyclonal antibody to human CXCL9, CXCL10, or CXCL11 (1:100 dilution; abcam, Cambridge, UK) and mouse monoclonal antibody to human anti-CXCR3 (1:100 dilution, abcam) at 4°C overnight.
To identify the immune cells expressing CXCR3 and evaluate the number of total macrophages, we incubated sections with the primary antibodies: mouse monoclonal antibody to human anti-CXCR3 (1:100 dilution, abcam), rabbit monoclonal antibody to human anti-CD3 (1:50 dilution, abcam), rabbit polyclonal antibody to human anti-CD80 (1:100 dilution, abcam), rabbit polyclonal antibody to human anti-CD163 (1:100 dilution, abcam), rabbit polyclonal antibody to human anti-CD123 (1:100 dilution, abcam), or rabbit polyclonal antibody to human anti-CD68 (pan-macrophages) (1:50 dilution; abcam) at 4°C overnight. After three washes in phosphate-buffered saline (PBS), the sections were incubated with Alexa Fluor 568 goat anti-mouse IgG (H+L) (1:200 dilution, Invitrogen, Carlsbad, CA) for 30 min. The sections were washed in PBS, then incubated with Alexa Fluor 488 goat anti-rabbit IgG (H+L) (1:100 dilution, Cell Signaling Technology, Beverly, MA) for 2 hr at room temperature. The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI).

The sections were visualized with a laser scanning confocal microscope (Nikon A1, Tokyo) or a light microscope equipped with a digital camera (BZ-X700; Keyence, Tokyo). The number of CXCR3+ cells per 1.5 mm² (objective magnification: ×10) was counted from six different areas. The numbers of CD68+ macrophages and CXCR3+CD163+ macrophages per 0.4 mm² (objective magnification: ×20) were
counted from six different areas. The positively stained populations were counted using BZ-X Analyzer software (Keyence).

**Statistical analysis**

The relationship between the grade of lymphocytic infiltration and the number of CXCR3+ cells, or the number of CXCR3+CD163+ macrophages were analyzed by Spearman's rank correlation test. SPSS Statistics-15.0 J software was used for the analyses. Statistical tests were two-sided, and significance was defined as a p-value <0.05.

**Results**

*Expression of CXCL9, CXCL10, and CXCL11 in the ductal cells of LSGs from pSS patients*

We identified the localization of CXCR3 and its ligands in LSGs by immunofluorescence staining. Formalin-fixed paraffin-embedded sections of LSGs from pSS patients and normal controls were stained with specific antibodies. Figure 1 displays representative LSGs of pSS patients and healthy controls. As shown in Figure 1A, extensive periductal inflammation and parenchymal destruction were apparent in the LSGs of the pSS patients compared to those of the controls (Fig. 1B). The results of
the immunofluorescence study clearly demonstrated the enhancement of CXCL9 (Fig. 1C), CXCL10 (Fig. 1E), and CXCL11 (Fig. 1G) expression in the pSS ductal cells and, to a lesser extent, in the pSS acinar cells. The expressions of CXCL9 and CXCL10 showed particularly intense staining in the ductal cells of pSS, whereas the expression of CXCL9 (Fig. 1D), CXCL10 (Fig. 1F), and CXCL11 (Fig. 1H) was not detected in the normal LSGs. In contrast, as shown in red in Figure 1, CXCR3 was expressed on infiltrating cells, but not on intrinsic salivary structures of pSS. Although we expected that the CXCR3-expressing cells would be lymphocytes infiltrating around the ductal epithelium, most of the infiltrating lymphocytes did not show positive staining for CXCR3 (Fig. 1).


We next performed an immunofluorescence analysis to identify the immune cells expressing CXCR3 in LSGs of the pSS patients. Although it was reported that CXCR3-expressing cells were exclusively activated T cells in SS salivary glands (13), our immunofluorescence analysis demonstrated that most of the CD3$^+$ cells did not show positive staining for CXCR3 (data not shown). To further clarify the CXCR3 expression in LSGs of the pSS patients, we performed double immunofluorescence
staining for CXCR3 and CD80, CD163, or CD123. Interestingly, the expression of CXCR3 was detected in the majority of CD80+ macrophages and CD163+ macrophages (Fig. 2). In contrast, CD3+ T cells were marginally stained for CXCR3 in LSGs of the pSS patients (Fig. 2). The expression of CXCR3+ was also detected in CD123+ plasmacytoid dendritic cells (pDCs) (Fig. 2), but there were few CD123+ pDCs in the pSS LSGs (data not shown). These results indicated that the majority of CXCR3+ cells infiltrating in LSGs from pSS patients are likely to be macrophages.

Association between the number of CXCR3+ cells and the degree of lymphocytic infiltration in LSGs from pSS patients

We then examined the association between the number of CXCR3+ cells and the degree of lymphocytic infiltration. As shown in Figure 3A, a large number of CXCR3+ cells was clearly observed in the Grade 1 pSS patients, in which slight lymphocytic infiltration was detected. However, when we observed the Grade 4 patients, in which diffuse lymphocytic infiltration was associated with a complete loss of tissue architecture, the number of CXCR3+ cells was greatly reduced, and these cells were observed only around salivary glands. The number of CXCR3+ cells was observed to decrease in accord with the infiltration grade. Indeed, the Spearman's rank analysis revealed a strong and significant negative correlation between the existence of CXCR3+
cells and the grade of lymphocytic infiltration ($rs=−0.871; \ p<0.001$) (Fig. 3B). Based on this result, we considered that CXCR3$^+$ cells contributed to the anti-inflammatory functions in pSS lesions. Because the expression of CXCR3 was detected in the majority of macrophages, we hypothesized that CXCR3$^+$ cells might be M2 macrophages.

*Association between the number of CXCR3$^+$CD163$^+$ macrophages and the degree of lymphocytic infiltration in LSGs from the pSS patients*

To investigate this hypothesis, we next evaluated the relationship between CXCR3$^+$CD163$^+$ macrophages and the severity of inflammatory lesions in LSGs of the pSS patients. CD163 is known to be one of the markers of mature macrophages. Figure 4A is a photograph of the double immunofluorescence staining for both CXCR3 and CD163. The number of CD163$^+$ macrophages expressing CXCR3 decreased in accord with the grade of lymphocytic infiltration, and it tended to show a reverse distribution. In fact, a strong negative correlation between the number of CXCR3$^+$CD163$^+$ macrophages and the grade of lymphocytic infiltration was evident ($rs=−0.777; \ p<0.001$) (Fig. 4B). The ratio of CXCR3$^+$CD163$^+$ macrophages/total infiltrating macrophages (CD68$^+$ macrophages) was 90.98% in Grade 1, 84.15% in Grade 2,
91.56% in Grade 3 and 82.46% in Grade 4 (Table 2). Thus, most of the macrophages in the pSS lesions were CXCR3⁺CD163⁺ macrophages.

**Discussion**

The chemokine receptor CXCR3 is well known to contribute to the migration, activation, and differentiation of a number of immune cells, and CXCR3 and its ligands are clearly shown to play important roles in the pathogenesis of infections, autoimmune diseases and neoplastic diseases (5-7). CXCR3 ligands (i.e., CXCL9, CXCL10, and CXCL11) were expressed in the salivary glands of pSS patients (13, 14), and CXCR3-expressing cells were exclusively activated T cells in the salivary glands of pSS patients (13).

It was also reported that the downregulation of CXCL10 reduced the mononuclear cell infiltration in MRL/lpr mice (23). Moreover, in a study of pSS patients, the mRNA expressions of chemokines and chemokine receptors in peripheral blood mononuclear cells (PBMCs) and LSGs from 18 patients with pSS were measured, and the results demonstrated that in the LSGs from the pSS patients, interleukin (IL)-2, IFN-γ, CXCL10, CXCR3, macrophage inflammatory protein (MIP)-1α, RANTES (regulated upon activation normal T cell expressed and secreted), IL-4, IL-10, TARC (thymus- and activation-regulated chemokine), MDC (macrophage-derived chemokine),
CCR4 and IL-17 were higher than those in the control LSGs. In the PBMCs, only CXCL10 and CXCR3 were expressed at higher levels compared to the controls (24). These results suggest the involvement of a Th1 immune response (especially CXCL10 and CXCR3) in the pathogenesis of pSS. However, a comprehensive analysis of CXCR3 in pSS had not been performed; in the present study we thus focused on CXCR3 in the autoimmune lesions of pSS.

Our findings demonstrated that the expressions of CXCL9 and CXCL10 were intense in the ductal cells from the pSS patients' LSGs compared to the controls. These results are consistent with a previous report (13). Another study indicated that minor salivary gland ductal epithelial cells express CXCR3 in both SS patients and healthy controls (25), and that the scavenging function of CXCR3 was impaired in SS so that minor salivary gland epithelial cells were not able to inhibit chemotaxis of CXCR3+ T cells (26). Sfriso et al. detected CXCR3 positivity in SS and normal ductal epithelial cells by immunohistochemistry. In order to confirm their findings, we measured the localization of CXCR3 in LSGs by immunofluorescence staining. The results of this immunofluorescence study demonstrated that CXCR3 was exclusively expressed on infiltrating cells, whereas salivary structures were slightly stained for CXCR3 in both pSS patients and healthy controls.
To identify the immune cells expressing CXCR3 in LSGs from pSS patients, we performed double immunofluorescence staining for CXCR3 and CD3, CD80, CD163, and CD123. Interestingly, CXCR3 was expressed in the majority of CD163+ macrophages and CD80+ macrophages, whereas CD3+ T cells were marginally stained for CXCR3 in LSGs of the pSS patients. Although CXCR3 was also expressed in CD123+ pDC cells, only a few of these cells showed this expression. Our results demonstrated that CXCR3 is expressed in all of the innate immune cells, but the majority of CXCR3+ cells in the pSS patients' LSGs are macrophages. These results are the first finding regarding CXCR3 in LSGs from pSS patients.

CXCR3 is well known as a canonical marker of Th1 cells. Although we also detected a small number of CXCR3+CD3+ T cells in the salivary gland tissue from pSS patients in agreement with the previous studies (13, 14), a much greater increase in the number of CXCR3+ macrophages was detectable. Several more recent studies have raised interest in the expression of CXCR3 by macrophages. These studies have revealed the following: the CXCR3+ macrophage-mediated remodeling of blood vessels (15), CXCR3 signaling in diverse processes such as the recruitment of macrophages to allografts (16), the promotion of tumor progression by the polarization of CXCR3+ macrophages toward an M2 phenotype (17), and the involvement of CXCR3+ macrophage-associated inflammation in the CXCL10-mediated sterile inflammatory
response in murine nonalcoholic steatohepatitis (NASH) (18). Thus, many reports have demonstrated that CXCR3-CXCR3 ligands mediate the recruitment of macrophages in various inflammatory lesions. Our present analyses revealed a strong negative correlation between the number of CXCR3+ macrophages and the grade of lymphocytic infiltration in LSGs of pSS patients, and this is also the first finding of an association between CXCR3+ cells and the severity of lesions in LSGs of pSS patients. This result suggests that CXCR3 ligands would not directly participate in the regulation of T-cell responses, and that the CXCR3 ligands predominantly regulate the recruitment of CXCR3+ macrophages in LSGs of patients with pSS.

Based on this inverse correlation between CXCR3+ macrophages and the severity of LSG inflammatory lesions, we considered that CXCR3+ macrophages may contribute to the anti-inflammatory functions in pSS lesions. Macrophages are classified as M1 (classically activated) and M2 (alternatively activated) macrophages based on the expression of macrophage gene product, including receptors, cytokines, and effector molecules (27). Macrophage polarization was induced by classical macrophage-activating stimuli such as Th1-derived IFN or the Th2-derived anti-inflammatory cytokines IL-4 and IL-13 (27). M1 macrophages produce pro-inflammatory cytokines, such as inducible nitric oxide synthase (iNOS) and tumor necrosis factor (TNF) (28). In contrast, M2 macrophages have been shown to contribute
to angiogenesis, tissue remodeling, and tumor progression by inducing the expression of mannose receptors, scavenging receptors, and angiogenic factors such as vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP)-9 (28, 29). Here we performed double immunofluorescence staining for a CXCR3 and CD163 M2 macrophage marker in the LSGs of pSS patients.

An earlier study showed that the macrophage incidence was positively correlated with the infiltration grade and biopsy focus score (30). Although we could not examine the number of CXCR3+CD80+ macrophages, it was evident that the existence of CXCR3+CD163+ macrophages was significantly decreased in accordance with the grade of lymphocytic infiltration in the pSS patients' LSGs. It is thought that CD163+ macrophages are associated with M2 macrophage-like function. CXCR3+CD163+ macrophages might contribute to the anti-inflammatory functions and the tissue remodeling functions in pSS lesions. There would thus be an inverse correlation between the number of CXCR3+ macrophages and inflammatory grading in the lesions.

Our study had several limitations. First, this was a pilot study with a small number of pSS patients. Second, the correlation with clinicopathologic parameters could not be determined due to the small sample size. And third, because we could not evaluate the number of CD80+CXCR3+ M1 macrophages, the M1/M2 macrophage...
polarization in the LSGs of pSS could not be examined. Nonetheless, our analysis contributed the novel finding that the number of CXCR3⁺CD163⁺ M2 macrophages decreased as the lymphocytic infiltration grade increased.

In summary, we detected high levels of CXCL9 and CXCL10 expression in the ductal cells of LSGs from pSS patients. The majority of CXCR3⁺ cells in the pSS patients' LSGs were macrophages. The level of CXCR3⁺CD163⁺ macrophages was inversely correlated with the severity of LSG inflammatory lesions in the pSS patients. Our results suggest that the enhanced production of CXCL10 from ductal cells results in the migration of CXCR3⁺ macrophages. CXCR3⁺CD163⁺ M2 macrophages may contribute to the anti-inflammatory functions in pSS lesions.

Acknowledgments

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Conflict of Interest Statement

The authors declare that there are no competing interests linked to this study.
References


Table Captions and Figure Legends

**Table 1.** Profile of the 22 female pSS patients

**Table 1 footnote:** ANA: antinuclear antibody, Anti-SSA/SSB: anti-Sjögren's syndrome A and/or B antibodies, CNS: central nervous system, CRP: C-reactive protein, ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index, LFS: lymphocytic focus score, pSS: primary Sjögren's syndrome, RF: rheumatoid factor, WBC: white blood cell.

**Table 2.** Incidence of CXCR3<sup>+</sup>CD163<sup>+</sup> macrophages in total infiltrating macrophages in LSGs from pSS patients with variable grades

**Table 2 footnote:** LSG: lip salivary gland, pSS: primary Sjögren’s syndrome, MΦ: macrophage.

**Fig. 1.** Histological analysis in LSGs from pSS patients and healthy controls. A,E: Hematoxylin and eosin (H&E) staining at 4× magnification. Formalin-fixed paraffin sections of LSGs from pSS patients (B–D) and healthy controls (F–H) were analyzed for the protein expression patterns of CXCL9, CXCL10, CXCL11, and CXCR3. Immunofluorescence staining performed with CXCL9 (green), CXCL10 (green), CXCL11 (green), CXCR3 (red), and DAPI for staining nuclei (blue). The expressions of CXCL9 and CXCL10 showed particularly intense staining in the pSS ductal cells.
Most of the infiltrating lymphocytes around the pSS ductal epithelium seemed to not express CXCR3. Representative photos of 3–5 samples are shown. Scale bar = 100 µm.

Fig. 2. **Double immunofluorescence analysis for CXCR3/CD3, CXCR3/CD80, CXCR3/CD163, and CXCR3/CD123 in LSGs from pSS patients.** Double immunofluorescence staining performed with CXCR3 (red), CD3 (green), CD68 (green), CD163 (green), CD123 (green), and DAPI for staining nuclei (blue). The images for CXCR3 and CD3, CD68, CD163, or CD123 were merged (yellow). CXCR3 was expressed in the majority of CD68+ and CD163+ macrophages. Although CXCR3 was expressed in all the innate immune cells, CXCR3+CD3+ cells and CXCR3+CD123+ cells were few in number. Representative photos of 3–5 samples are shown. Scale bar = 50 µm.

Fig. 3. **Association of CXCR3+ cells with the degree of lymphocytic infiltration in LSGs from pSS patients.** A: Immunofluorescence staining performed with CXCR3 (red) and DAPI for staining nuclei (blue). The existence of CXCR3+ cells decreased with the infiltration grade. Representative photos of 3–5 samples are shown. Scale bar = 100 µm. B: Correlation between the number of CXCR3+ cells and the grade of infiltration. The number of CXCR3+ cells per 1.5 mm² (objective magnification: ×10)
was counted from six different areas. The plot displaying the significant negative correlation ($r_s = -0.872; p<0.001$) was determined by Spearman's rank correlation test.

**Fig. 4. CD163+ macrophages expressing CXCR3 in LSGs from pSS patients. A:** Double immunofluorescence staining performed with CXCR3 (green), CD163 (red), and DAPI for staining nuclei (blue) at LSGs from the four pSS subgroups as classified by the grade of the inflammatory lesion. The number of CXCR3⁺CD163⁺ macrophages decreased in accord with the inflammatory grade. Representative photos of 3–5 samples are shown. Scale bar = 100 µm. **B:** Correlation between the number of CXCR3⁺CD163⁺ macrophages and the grade of infiltration. The number of CXCR3⁺CD163⁺ macrophages per 0.4 mm² (objective magnification: ×20) was counted from six different areas. The plot displaying the significant negative correlation ($r_s = -0.777; p<0.001$) was determined by Spearman's rank correlation test.
Table 1  Profile of the 22 female pSS patients.

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Table 2  Incidence of CXCR3⁺CD163⁺ macrophages in total infiltrating macrophages in LSGs from pSS patients with variable grade.

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<td>CXCR3⁺CD163⁺-MΦ</td>
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<td>/total MΦ (%)</td>
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Figure 2

CXCR3 CD123 Merge
CXCR3 CD3 Merge
CXCR3 CD163 Merge
CXCR3 CD80 Merge
Figure 3

A

Grade 1

Grade 2

Grade 3

Grade 4

rs = -0.871; p < 0.001

B

CXCR3+ cells/1.5 mm²

Grade 1  Grade 2  Grade 3  Grade 4
Figure 4

A

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B

rs = -0.777; p < 0.001