Association of plasmacytoid dendritic cells with B cell infiltration in minor salivary glands in patients with Sjögren’s syndrome

Jidong Zhao¹, Satoshi Kubo¹, Shingo Nakayamada¹, Shohei Shimajiri², Xiangmei Zhang¹, Kunihiro Yamaoka³, and Yoshiya Tanaka¹

¹The First Department of Internal Medicine and ²Department of Pathology and Cell Biology, University of Occupational and Environmental Health, Fukuoka, Japan, and ³Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan

Abstract

Objectives: Sjogren’s syndrome (SS) is an autoimmune disease with features of both over-production of specific autoantibodies and organ-specific disorders, mainly sialadenitis and dacryoadenitis. However, little is known about the factors that contribute to lymphocytic infiltration of SS.

Methods: Minor salivary gland (MSG) tissue was obtained from 83 patients with primary SS (pSS) and 95 patients with secondary SS and examined pathologically, and correlation between infiltrated immune cells and histological features was evaluated.

Results: Plasmacytoid dendritic cells (pDCs) were increased in MSG of SS compared to Sicca syndrome. The density of pDCs was characteristically correlated with the accumulation of CXCL13+CD68+ macrophages and CXCR5+CD19+ B in the MSG of pSS. In vitro analysis indicated that Type I interferon (IFN) enhanced CXCL13 production by macrophages. Type I IFN was mainly expressed in pDCs and its expression was correlated with the accumulation of CXCL13+ macrophages in the MSG of pSS.

Conclusions: Our histological findings suggest the possible mechanism of type I IFN-CXCL13 axis during the pathological processes of acute/chronic salivary inflammation in SS; local production of type I IFN by pDCs, induction of CXCL13 production in macrophages by type I IFN, induction of accumulation of CXCR5+CD19+ B cells by CXCL13 in the MSG.

Keywords: B cells, CXCL13, Macrophages, Plasmacytoid dendritic cells, Sjogren's syndrome

Introduction

Sjogren’s syndrome (SS) is an autoimmune disease characterized by both over-production of specific autoantibodies and organ-specific disorders, mainly sialadenitis and dacryoadenitis. Lymphocyte activation and infiltration to the salivary glands are known to play an important role in the pathogenesis of SS [1,2]. Although, T cells initiate the pathogenetic process of SS, marked B cell infiltration is found in the lip tissue, and in advanced stages, there is formation of ectopic germinal centers by B cells [3–6]. The infiltrated B cells bring about irreversible destruction of the acini and fibrosis in the lacrimal and salivary glands, causing dryness [7]. However, little is known about the factors that contribute to the lymphocytic infiltration. Understanding exact mechanisms underlying lymphocytic infiltration may help to develop new therapeutic approaches.

Treatments using classical DMARDs, B cell depletion, and CTLA4-Ig have been tested for SS [8–14]; however, there is insufficient evidence of their efficacy. This might be because therapeutic intervention is performed without considering the extent of local lymphocytic infiltration at the sites of inflammation in each SS patient, and there is still insufficient evidence regarding the underlying mechanisms. Thus, a disease-specific treatment approach does not exist in this disease.

Minor salivary gland (MSG) tissue is of high enough diagnostic significance to be used as a classification criterion by the American College of Rheumatology in SS [15]. Along with having diagnostic significance, the MSG tissue also reflects the local pathology at the sites of inflammation in SS and is extremely important in understanding the pathogenesis. We have previously reported that the effects of immunosuppressants, such as mizoribine, are required during the period of marked lymphocytic infiltration and that knowing the histological stage of SS is important to determine the indication of immunosuppressive treatment in SS [16]. Further, because the MSG tissue is exactly the primary site of inflammation, its examination could be useful in seeking for new therapeutic targets.

Plasmacytoid dendritic cells (pDCs) are innate immune cells and the main source of type I IFN in response to foreign nucleic acids. Type I IFNs might be involved in the pathogenesis of SS [2,17,18]. In addition, macrophages are also recruited [19] and produce cytokines and chemokines which contribute to lymphocyte infiltration in SS [20–22]. However, the interaction among pDCs, macrophages, and lymphocytes remains unclear.
Association of plasmacytoid dendritic cells with B cell infiltration

Materials and methods

Specimen and patients

The study subjects were 83 patients with pSS, 95 patients with secondary SS (sSS), and seven patients with Sicca syndrome served as controls. All patients fulfilled the 1999 Ministry of Health and Welfare’s Diagnostic Criteria for SS. After obtaining informed consent, labial MSG biopsies were performed on all patients for diagnostic evaluation of SS. In the sSS group, no patient received bio-DMARDs therapy prior to the labial biopsies. This study was conducted with approval of the ethics committee of University of Occupational and Environmental Health.

Histological evaluation of minor salivary glands

Biopsy specimens of MSG from patients in this study were fixed, embedded, sectioned (5 μm), deparaffinized, rehydrated through alcohol, and stained with H&E [16]. The histological features of MSG were classified as follows. (i) Scores of lymphocytic infiltration per lobule [4]: Score 1 (corresponding to Greenspan’s Grades 1); Score 2 (corresponding to Greenspan’s Grades 2); Score 3 (corresponding to Greenspan’s Grades 3); Score 4 (corresponding to Greenspan’s Grades 4); and Score 5 (infiltration severe enough to form lymph follicles). (ii) Scores of acinar atrophy were rated on a three-grade scale according to the extent of atrophy: Score 1 (less than one-third within a lobule); Score 2 (over one-third and less than two-thirds); and Score 3 (over two-thirds). (iii) Scores of intralobular fibrosis were rated on a three-grade scale according to the extent of fibrosis: Score 1 (less than one-third within a lobule); Score 2 (over one-third and less than two-thirds); and Score 3 (over two-thirds). Histological features were evaluated in a blind manner by two rheumatologists and one pathologist.

Immunohistochemical staining

Paraffin-embedded specimens from lip biopsy specimens were studied by immunohistochemistry (IHC) to define the presence of positive cells. Anti-BDCA-2 (ATLAS Antibodies, Stockholm, Sweden), anti-CD4 (Abcam, Cambridge, UK), anti-CD19 (Abcam), and anti-CD68 Ab (Abcam) were used to define pDCs, T cells, B cells, and macrophages. In addition, CXCR5 (Abcam), IFN-α Ab (Pbl Assay Sci, Piscataway, NJ), CXCL13 Ab (R&D Systems), CCL19 Ab (Abcam), CXCL4 Ab (Abcam), and CXCL 8Ab (R&D Systems, Minneapolis, MN) were used to characterize mononuclear cells.

Expression of both CXCL13 and IFN-α with immune cells was detected by double-staining. CXCL13 was stained with DAB (in the first sequence), and CD68, BDCA-2, CD4 and CD19 was stained (in the second sequence) and binding secondary AP polymer-conjugated antibody with VECTOR Blue (Vector Lab, Burlingame, CA). Likewise, BDCA-2, CD68 and CD4 was stained with DAB (in the first sequence), and IFN-α was stained (in the second sequence) and binding secondary AP polymer-conjugated antibody with VECTOR Blue. Expression of both CXCR5 and CD19 was performed by double-staining. Development of CD19 Ab with DAB followed by those of CXCR5 Ab with VECTOR Blue was performed.

Briefly, paraffin-embedded sections were treated in xylene and rehydrated by gradient of ethanol. Blocking with 5% goat serum (except goat anti-human primary Ab) was performed. Endogenous peroxidase activity was blocked with 3% H2O2 for 30 min. And nuclei were counterstained with hematoxylin (Dako, Glostrup, Denmark). Mouse IgG, rabbit IgG, and goat IgG were used as isotype controls.

NanoZoomer digital pathology (Hamamatsu Photonics K.K., Shizuoka, Japan), a system that converts a histology slide into a high-resolution digital slide (190 million pixels), was utilized for analysis. The density of positive cells (area/mm2) in whole specimen was counted with Nano-Zoomer Digital Pathology apparatus (NDP; Hamamatsu)-viewing. Data are expressed as mean count per 20 × high-power field (Supplementary Figure).

In vitro human monocyte-derived macrophages (HMDMs) generation and cultures

Peripheral blood mononuclear cells were isolated with lymphocyte separation medium (ICN/Cappel Pharmaceuticals, Aurora, OH). Monocytes were obtained by positive magnetic selection using anti-CD14 microbeads (Miltenyi Biotec, San Diego, CA). To generate immature human monocyte-derived macrophages HMDMs [24], monocytes were cultured at 1 × 105 cells/ml in the presence of macrophage colony-stimulating factor (M-CSF) (50 ng/ml; Peprotech, Rocky Hill, NJ) for 6 days. The medium with M-CSF was replaced on day 3.

Immature HMDMs were washed and re-plated in fresh medium at 2.5 × 105/ml and stimulated with IFN-γ (100, 1000, 10,000 unit/ml, Abcam), IFN-γ (50 ng/ml, 100 ng/ml, Miltenyi Biotec), interleukin (IL) -6 (100 ng/ml, Miltenyi Biotec), or TNF-α (100 ng/ml, R&D systems) for 24 and 48 h.

Real-time polymerase chain reaction (PCR) and ELISA

Total RNA was prepared by using the RNasy Mini Kit (Qiagen, Chatsworth, CA). First-strand cDNA was synthesized, and quantitative real-time PCR was performed in the Step One Plus instrument (Applied Biosystems, Foster City, CA). TaqMan target mixes for CXCL13 (Eurofins Genomics, Wolverhampton, UK) was purchased from Applied Biosystems. Expression levels were expressed relative to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The relative quantity was calculated by using the quantification-comparative cycle threshold formula–referenced sample of HMDMs. Supernatants of HMDMs were stored at −80°C until used. Two times diluted supernatant was measured for CXCL13 by a DuoSet ELISA Development Kit (R&D Systems) according to the manufacturer’s instructions. The detection limit of the assay was determined to be 15.6 pg/ml.

Statistical analysis

Statistical analysis was performed by using the SPSS.17 and GraphPad Prism 5 software. Data were expressed as mean ± SD. Differences within multiple (≥3) groups were evaluated by using a one-way ANOVA. Statistically significant differences between groups were subsequently analyzed by using the post hoc test. Correlations analyses were performed using Pearson correlation test. As indicated in the software, whereas p values of less than 0.05 were considered significant for the other tests.
### Results

The accumulation of pDCs was associated with B cell infiltration in MSG from patients with pSS

The characteristics of the 83 patients with pSS, 95 patients with sSS, and seven patients with sicca syndrome are shown in Table 1. Also, the characteristics of sSS subgroups are shown in Supplementary Table. In pathological scoring, accumulation of lymphocytes, intralobular fibrosis, and acinar atrophy was found characteristically in pSS and sSS. On evaluation of the accumulation of human pDCs (Figure 1A), both the pSS (12.75 ± 9.34/mm²) and sSS (12.49 ± 11.06/mm²) groups showed an increased pDCs count in the lip tissue compared with the sicca syndrome group (3.43 ± 3.76/mm²) (Figure 1B).

When pDCs accumulation was divided by pathological scoring, the pDCs count was increased in pSS cases with a high level of lymphocytic infiltration compared with that in cases with a low level of infiltration (Figure 1C). In the tissue with intralobular fibrosis and acinar atrophy, no difference was found in relation to pDCs infiltration. On the other hand, in sSS, no correlation was found in lymphocytic infiltration, intralobular fibrosis, or acinar atrophy (Figure 1D). The immunohistochemical staining revealed that a huge number of CD19⁺ B cells are infiltrated in minor salivary gland of SS (Figure 1E). The double immunohistochemical staining showed that the CD19⁺ B cells express CXCR5⁺ CD19⁺ B cells express CXCR5⁺ (Figure 1F). We found the correlation between CXCR5⁺ CD19⁺ B cells in total MNCs (G) and percentage of CXCR5⁺ CD19⁺ B cells in CD19⁺ B cells in CD19⁺ B cells (H) were analyzed with different lymphocytic infiltration scores in the pSS group. Original magnification ×20 high-power in (A), (E), and (F). Values are the mean ± SD. *p < 0.05. The figure appears in color in online version.

### Table 1. Baseline characteristics of all patients in this study.

| Sicca | pSS | sSS |
|-------|-----|-----|
| Demographic characteristics | | |
| Number | n = 7 | n = 83 | n = 95 |
| Female:Male | 6:1 | 80:3 | 90:5 |
| Age, year (mean ± SD) | 53.7 ± 7.5 | 62.4 ± 14.7 | 60.6 ± 15.9 |
| Histopathological score n (%) | | |
| Lymphocytic infiltration score | | |
| 1 | 2 (28.6) | 2 (2.4) | 1 (1.1) |
| 2 | 5 (71.4) | 19 (22.9) | 19 (20.0) |
| 3 | 0 (0) | 29 (34.9) | 23 (24.2) |
| 4 | 0 (0) | 22 (26.5) | 35 (36.8) |
| 5 | 0 (0) | 11 (13.3) | 17 (17.9) |
| Intralobular fibrosis score | | |
| 1 | 5 (71.4) | 12 (14.5) | 14 (14.7) |
| 2 | 2 (28.6) | 56 (67.5) | 48 (50.5) |
| 3 | 0 (0) | 15 (18.1) | 33 (34.7) |
| Acinar atrophy score | | |
| 1 | 5 (71.4) | 6 (7.2) | 7 (7.4) |
| 2 | 2 (28.6) | 24 (28.9) | 39 (41.1) |
| 3 | 0 (0) | 53 (63.9) | 49 (51.6) |

Sicca, Sicca syndrome; pSS, primary Sjögren’s syndrome; sSS, secondary Sjögren’s syndrome.

The expression of CXCL13 was correlated with the infiltration of CXCR5⁺CD19⁺ B cells in MSG from patients with pSS

Chemokine receptors and their ligands play an important role in the lymphocyte migration. Next, we focused on the expression of various chemokines in the MSG in pSS. Initially, in the salivary glands of pSS patients, among multiple chemokines, the CXCL13-positive mononuclear cell count differed depending on the lymphocytic infiltration score; a higher lymphocytic infiltration score was associated with a higher CXCL13-positive mononuclear cell count (Figure 2A and B). On the other hand, no correlation...
Figure 2. CXCL13 was expressed in mononuclear cells and correlated with lymphocytic infiltration in MSG in pSS. (A) The expression of CXCL13, CXCL8, CCL19, and CXCL4 was detected in MSG of patients with pSS by IHC, isotype control was performed with anti-goat IgG Ab or anti-rabbit IgG Ab. (B–E) The densities of CXCL13+ (B), CXCL8+ (C), CCL19+ (D), and CXCL4+ (E) mononuclear cells with different lymphocytic infiltration scores in pSS were analyzed. (F–I) The correlation between densities of CXCL13+ (F), CXCL8+ (G), CCL19+ (H), CXCL4+ (I) mononuclear cells and pDCs in MSG of pSS was analyzed. Original magnification ×20 high-power field in A. Values are the mean ± SD. * = p < 0.05.
was found between the lymphocytic infiltration score and CXCL8-positive mononuclear cell count (Figure 2A and C), CCL19-positive mononuclear cell count (Figure 2A and D), or CXCL4-positive mononuclear cell count (Figure 2A and E).

Further, examination of the correlation between these chemokines and accumulation of pDC showed a statistical correlation between the CXCL13-positive mononuclear cell count and pDC count (Figure 2F). In contrast, there was no correlation between the accumulation of pDCs and CXCL8 (Figure 2G), CCL19 (Figure 2H), or CXCL4 (Figure 2I). CXCL13 is a chemokine that recruit CXCR5 expressing B cells and T follicular helper (Tfh) cells and contributes to EGC formation. These results suggested that CXCL13 is involved in the infiltration of CXCR5⁺ B cells and that accumulation of pDC is highly correlated with characteristic B cell infiltration and chemokine expression at the sites of inflammation in SS.

The majority of CXCL13⁺ cells were CD68⁺ macrophages which were associated with B cell infiltration in MSG from patients with pSS

Expression of CXCL13 under static conditions is known to occur in the liver, spleen, and intestine [26] and pDCs are also known to produce CXCL13 [27]. We next assessed characteristics of CXCL13-positive mononuclear cells using double staining.

By the double staining for CXCL13 and one of CD68 (macrophages), BDCA-2 (pDCs), CD4 (helper T cells), and CD19 (B cells) were performed (Figure 3A), the most CXCL13-positive mononuclear cells (47.8 ± 24.9/mm²) were macrophages (31.2 ± 17.4/mm²), although pDCs and helper T cells scarcely expressed CXCL13 (Figure 3B). While a correlation of these CXCL13-positive macrophages was found with the lymphocytic infiltration score, no correlation was found between lymphocytic infiltration and CXCL13-negative macrophages (Figure 3C), suggesting that CXCL13-positive macrophages play an important role in B cell infiltration. These results suggested that the majority of CXCL13-producing cells at the sites of inflammation in pSS are macrophages.

Type I IFN enhanced CXCL13 production by macrophages in vitro

Previous studies showed that proinflammatory cytokines including IFN-α, IL-6, IFN-γ, and TNF-α were overexpressed in salivary gland of pSS. We next examined CXCL13 production in macrophages by various cytokines in vitro. Human monocytes-derived macrophages from healthy donors did not contain detectable mRNA for CXCL13 without stimulation. After culture with IFN-α, CXCL13 mRNA was induced in the macrophages and up-regulated in a dose-dependent manner of IFN-α. In contrast to IFN-α, CXCL13 mRNA was not induced in these cells treated with TNF-α, IFN-γ, or IL-6 (Figure 4A). We also evaluated the protein levels of CXCL13 in the supernatant of culture macrophages. The stimulation with IFN-α, but not TNF-α, IL-6, or IFN-γ, induced production of CXCL13 in macrophages in a concentration-dependent manner (Figure 4B).

Figure 3. Macrophages are the main source of CXCL13 and correlated with B cell infiltration. (A) Double IHC staining for both CXCL13 (brown) and CD68 (blue), BDCA-2 (blue), CD4 (blue), or CD19 (blue) was performed in MSG of patients with pSS, isotype control was performed with anti-goat IgG Ab/anti-rabbit IgG Ab. (B) The numbers of different double positive cells were analyzed. (C) The densities of both CXCL13⁺ macrophages and CXCL13⁺ macrophages with lymphocytic infiltration scores in MSG of pSS were analyzed. Original magnification ×20 high-power field in A. Values are the mean ± SD. * = p < 0.05; ** = p < 0.001. The figure appears in color in online version.
Type I IFN was mainly expressed in pDCs and correlated with CXCL13* macrophages in MSG from patients with pSS

Finally, we examined IFN-α Ab and BDCA-2 Ab by double staining of IHC to investigate the link between pDCs and IFN-α expression in MSG. We found that most of the IFN-α were expressed in pDCs, but were not stained with T cells and B cells in MSG from pSS (Figure 5A). On staining for IFN-α producing cells in the lip tissue, pDCs and IFN-α showed an extremely high correlation (Figure 5B).

Furthermore, the positive correlation was found between IFN-α and CXCL13-positive macrophages (Figure 5C), but not with CXCL13-negative macrophages (Figure 5D). Similarly, a correlation was found between pDCs and CXCL13-positive macrophages (Figure 5E), but not with CXCL13-negative macrophages (Figure 5F).

These results suggested that local production of IFN-α from pDCs through innate immune stimuli, associate with the following sequence of events; induction of CXCL13 production by macrophages, accumulation of CXCR5+ B cells responding to CXCL13, acute/chronic salivary inflammation during the pathological processes of sialadenitis in pSS.

Discussion

It was previously reported that pDCs play a pivotal role in SS [2,28–30]; however, the exact mechanism for local action at the actual site of inflammation remains unclear. In the present study, we performed pathological examination from the largest number of patients with pSS and sSS and showed that B cell infiltration, which is important for local pathogenesis at the sites of inflammation in pSS, was closely linked with the presence of pDCs and type I IFN and CXCL13-producing macrophages. Our findings suggest that type I IFN mainly produced by pDCs is closely correlated with B cell infiltration through CXCL13 production by macrophages. Therefore, the innate immune system mediated by pDCs and type I IFN as well as the acquired
immune system mediated by CXCL13 and B cells might be involved in pathological development in SS.

It is well known that pro-inflammatory cytokines, such as TNF-α and IL-6, play a major role in rheumatoid arthritis; however, the role of relevant cytokines in SS remains unclear. In SS, DNA microarray analysis has shown high-level expression of type I IFN signature [18,31], and type I IFN activation has been reported [17,32]. pDCs are the main source of type I IFN [33–35]. Our results suggest a pivotal role of pDCs and type I IFN at the sites of inflammation.

It is well known that T cells infiltration is found in some cases of only mild lymphocytic infiltration; with medium-to-severe infiltration, most infiltrating lymphocytes are B cells in patients with SS [2,25,36]. However, relevant factors related to this accumulation of B cells remain unknown. We demonstrated herein that CXCL13 was important in infiltration CXCR5+ B cells at MSG in pSS. In contrast, no correlation was observed with CCL19 and others, which are important in T cells accumulation. Our data, therefore, suggested that infiltration of B cells was induced by CXCL13 produced by macrophages.

In general, follicular dendritic cells (FDCs) play an important role of germinal center formation [21], and recently study showed that both FDCs and Th cells contribute to the ectopic germinal center formation in autoimmune diseases [37]. In this study, our data showed that macrophages were the main source of CXCL13 in MSG tissue from patients with pSS, and these macrophages were closely associated with B cell infiltration. However, the counts of CXCL13 positive macrophages and pDCs were not different between the ectopic germinal center negative group (score 4) and the positive group (score 5) (data is partly shown). It is possible that pDCs and CXCL13 positive macrophage induce B cell infiltration, whereas not only pDCs and macrophages but also other factors contribute to germinal center formation in MSG.

Figure 5. Type I IFN correlated with pDCs densities and CXCL13+ macrophages in MSG in pSS. (A) Double IHC staining for both IFN-α (blue) and CD68 (brown), BDCA-2 (brown), CD4 (brown), or CD19 (brown) was performed in MSG of patients with pSS, isotype control was performed with anti-rabbit IgG Ab/anti-mouse IgG Ab. (B) The correlation between pDCs population and IFN-α+ cell in MSG of pSS was analyzed. (C–F) In MSG of patients with pSS, positive correlation between CXCL13+ macrophages with IFN-α+ cells (C) and pDCs (E) was observed, but no correlation between CXCL13- macrophages with IFN-α+ cells (D) and pDCs (F). Original magnification ×20 high-power field in A.
In previous in vitro studies, it has been reported that macrophages produce CXCL13 by TLR4 signaling [21]; however, the mechanisms by which CXCL13 is produced at the sites of inflammation in autoimmune diseases are not clearly understood. In this study, we showed that IFN-γ induced CXCL13 production by macrophages in vitro, indicating that CXCL13 production by macrophages might be induced, at least in part, by type I IFN which is produced by pDCs, then induce B cell infiltration at MSG in pSS.

Although, we had observed that high number of pDCs was accumulated in the MSG from both pSS and sSS, the correlation between pDCs and B cell infiltration was found only in pSS and not in sSS. In this study, multiple autoimmune diseases, such as RA, SLE, and SSC, were included in sSS. In contrast to pSS, these patients with sSS had endured different autoimmune environment with pro-inflammatory cytokines [38], such as TNF-α, IL-17, IL-6, and IFN-γ. Moreover, until diagnosing formation of SS, these patients in the sSS group received immunosuppression therapy. All these complicate factors might disturb the correlation between pDCs and histological lesions.

We have previously shown that immunosuppressants are effective for pSS during the period of strong lymphocytic infiltration but ineffective during periods of advanced fibrosis and atrophy [16]. Our findings suggest the validity of the view that lymphocytic infiltration not only plays an important role in the pathogenesis of SS but that it also is a valid target for SS treatment. Yet, in order to control lymphocytic infiltration, our data show that more specific treatments through anti-IFNAR antibodies and CXCL13 suppression could be effective. Another finding was that no correlation was observed between lab data, such as IgG and ESR, and pDCs accumulation. Although, there is no biomarker that reflects SS activity definitely at present, histological findings of minor salivary glands could be useful not only for the diagnosis but also for the decision of treatment.

Taken together, our findings highlight the importance of type I IFN produced by pDCs as an important factor of B cell infiltration at sites of inflammation in pSS. This gives hope for future therapeutic application of a molecular target medication in SS, as well as for the decision of treatment.

Acknowledgements
We thank Ms. Kahoru Sugitani for her working of immunohistochemical staining in this study.

Conflict of interest
Y. Tanaka has received consulting fees, speaking fees, and/or honoraria from Abbvie, Daiichi-Sankyo, Chugai, Takeda, Mitsubishi-Tanabe, Bristol-Myers, Astellas, Eisai, Janssen, Pfizer, Asahi-kasei, Eli Lilly, GlaxoSmithKline, UCB, Teijin, MSD, and Santen; and has received research grants from Mitsubishi-Tanabe, Takeda, Chugai, Astellas, Eisai, Taisho-Toyama, Kyowa-Kirin, Abbvie, and Bristol-Myers. All other authors have declared no conflicts of interest.

This work was supported in part by a Grant-In-Aid for Scientific Research from the Ministry of Health, Labor and Welfare of Japan, the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the University of Occupational and Environmental Health, Japan, through UOEH Grant for Advanced Research.

References
1. Fox RI. Sjogren’s syndrome. Lancet. 2005;366(9482):321–31.
2. Nordmark G, Alm GV, Ronnhblom L. Mechanisms of disease: primary Sjogren’s syndrome and the type I interferon system. Nat Clin Pract Rheumatol. 2006;2(5):262–9.
3. Bombardieri M, Pitzalis C. Ectopic lymphoid neogenesis and lymphoid chemokines in Sjogren’s syndrome: at the interplay between chronic inflammation, autoimmunity and lymphomagenesis. Curr Pharm Biotechnol. 2012;13(10):1089–96.
4. Greenspan JS, Daniels TE, Talal N, Sylvester RA. The histopathology of Sjogren’s syndrome in labial salivary gland biopsies. Oral Surg Oral Med Oral Pathol. 1974;37(2):217–29.
5. Hikichi T, Yoshida A, Tsubota K. Lymphocytic infiltration of the conjunctiva and the salivary gland in Sjogren’s syndrome. Arch Ophthalmol. 1993;111(1):21–2.
6. Yoshimoto K, Tanaka M, Kojima M, Setoymaya Y, Kameda H, Suzuki K, et al. Regulatory mechanisms for the production of BAFF and IL-6 are impaired in monocytes of patients of primary Sjogren’s syndrome. Arthritis Res Ther. 2011;13(5):R170.
7. Szodoray P, Papp G, Horvath IF, Barath S, Sipka S, Nakken B, et al. Cells with regulatory function of the innate and adaptive immune system in primary Sjogren’s syndrome. Clin Exp Immunol. 2009;157(3):343–9.
8. Ramos-Casals M, Brito-Zeron P, Siso-Almirall A, Bosch X, Tzioufas AG. Topical and systemic medications for the treatment of primary Sjogren’s syndrome. Nat Rev Rheumatol. 2012;8(7):399–411.
9. Ramos-Casals M, Tzioufas AG, Font J. Primary Sjogren’s syndrome: new clinical and therapeutic concepts. Ann Rheum Dis. 2005;64(3):347–54.
10. Ramos-Casals M, Brito-Zeron P, Siso-Almirall A, Bosch X. Primary Sjogren syndrome. BMJ. 2012;344:e3821.
11. Carubbi F, Cipriani P, Marrelli A, Benedetto P, Ruscitti P, Berarducci O, et al. Efficacy and safety of rituximab treatment in early primary Sjogren’s syndrome: a prospective, multi-center, follow-up study. Arthritis Res Ther. 2013;15(5):R172.
12. Meiners PM, Vissink A, Kroese FG, Spijkervet FK, Smitt-Kamminga NS, Abdulahad WH, et al. Abatacept treatment reduces disease activity in early primary Sjogren’s syndrome (open-label proof of concept ASAP study). Ann Rheum Dis. 2014;73(7):1391–6.
13. Sada PR, Isenberg D, Curtin C. Biologic treatment in SS. Rheumatology (Oxford). 2015;54(2):219–30.
14. Sugai S, Masaki Y. Current and prospective treatment options for Sjogren’s syndrome. Expert Rev Clin Immunol. 2008;4(4):469–79.
15. Daniels TE, Cox D, Shibsoki CH, Schrodt M, Wu A, Lanfranchi H, et al. Associations between salivary gland histopathologic diagnoses and phenotypic features of Sjogren’s syndrome among 1,726 registry participants. Arthritis Rheum. 2011;63(7):2021–30.
16. Nakayama S, Fujimoto T, Nonomura A, Saito K, Nakamura S, Tanaka Y. Usefulness of initial histological features for stratifying Sjogren’s syndrome responders to mizoribine therapy. Rheumatology (Oxford). 2009;48(10):1279–82.
17. Bave U, Nordmark G, Lovgren T, Ronnelid J, Cajander S, Eloranta P, et al. Activation of the type I interferon system in primary Sjogren’s syndrome: a possible etiopathogenic mechanism. Arthritis Rheum. 2005;52(4):1185–95.
18. Nakamae T, Nakamura Y, Matsumoto I, Goto D, Bo S, Tsutsui A, et al. DNA microarray analysis of labial salivary glands of patients with Sjogren’s syndrome. Ann Rheum Dis. 2007;66(7):844–5.
19. Voulgarelis M, Tzioufas AG. Pathogenic mechanisms in the initiation and perpetuation of Sjogren’s syndrome. Nat Rev Rheumatol. 2010;6(9):529–37.
20. Hernandez-Molina G, Michel-Peregrina M, Hernandez-Ramirez DF, Sanchez-Guerrero J, Llorente L. Chemokine saliva levels in patients with primary Sjogren’s syndrome, associated Sjogren’s syndrome, pre-clinical Sjogren’s syndrome and systemic autoimmune diseases. Rheumatology (Oxford). 2011;50(7):1288–92.
21. Carlson HS, Bækkevold ES, Morton HC, Haraldsen G, Brandtzæg P. Monocyte-like and mature macrophages produce CXCL13 (B cell-attracting chemokine 1) in inflammatory lesions with lymphoid neogenesis. Blood. 2004;104(10):3021–7.
22. Iwasa A, Arakaki R, Honma N, Ushio A, Yamada A, Kondo T, et al. Aromatase controls Sjogren-like disease-like through monocyte chemotactic protein-1 in target organ and adipose tissue-associated macrophages. Am J Pathol. 2015;185(1):151–61.
23. Lindahl G, Hedfors E. Labial salivary gland lymphocytic infiltration in Sjogren’s syndrome. Arthritis Rheum. 1991;34(8):1070–1.
24. Mebouta-Nkamgueu E, Adnet JJ, Itelet D, Laurent-Maquin D, Bouthors S, Potron G, et al. Human monocye-derived macrophages
and dendritic cells as targets for biomaterial cytocompatibility studies using an improved in vitro culture system. J Mater Sci Mater Med. 2001;12(4):351–7.

25. Hansen A, Lipsky PE, Dorner T. B cells in Sjögren’s syndrome: indications for disturbed selection and differentiation in ectopic lymphoid tissue. Arthritis Res Ther. 2007;9(4):218.

26. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggioni M, Moser B. B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. J Exp Med. 1998;187(4):655–60.

27. Perrier P, Martiner FO, Locati M, Bianchi G, Nebuloni M, Vago G, et al. Distinct transcriptional programs activated by interleukin-10 with or without lipopolysaccharide in dendritic cells: induction of the B cell-activating chemokine, CXC chemokine ligand 13. J Immunol. 2004;172(11):7031–42.

28. Nordmark G, Eloranta ML, Ronnblom L. Primary Sjögren’s syndrome and the type I interferon system. Curr Pharm Biotechnol. 2012;13(10):2054–62.

29. Wildenberg ME, van Helden-Meeuwsen CG, van de Merwe JP, Drexhage HA, Versnel MA. Systemic increase in type I interferon activity in Sjögren’s syndrome: a putative role for plasmacytoid dendritic cells. Eur J Immunol. 2008;38(7):2024–33.

30. Gottenberg JE, Cagnard N, Lucchesi C, Letourneur F, Mistou S, Lazure T, et al. Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjögren’s syndrome. Proc Natl Acad Sci USA. 2006;103(8):2770–5.

31. Kimoto O, Sawada J, Shimoyama K, Suzuki D, Nakamura S, Hayashi H, et al. Activation of the interferon pathway in peripheral blood of patients with Sjögren’s syndrome. J Rheumatol. 2011;38(2):310–16.

32. Tateda K, Okazaki S, Nagoya S, Katada R, Mizuo K, Watanabe S, et al. The suppression of TRIM21 and the accumulation of IFN-alpha play crucial roles in the pathogenesis of osteonecrosis of the femoral head. Lab Invest. 2012;92(9):1318–29.

33. McKenna K, Beignon AS, Bhardwaj N. Plasmacytoid dendritic cells: linking innate and adaptive immunity. J Virol. 2005;79(1):17–27.

34. Zhang Z, Wang FS. Plasmacytoid dendritic cells act as the most competent cell type in linking antiviral innate and adaptive immune responses. Cell Mol Immunol. 2005;2(6):411–17.

35. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. Nat Immunol. 2004;5(12):1219–26.

36. Matsumoto I, Okada S, Kuroda K, Iwamoto I, Saito Y, Tokuhisa T, et al. Single cell analysis of T cells infiltrating labial salivary glands from patients with Sjögren’s syndrome. Int J Mol Med. 1999;4(5):519–27.

37. Maehara T, Moriyama M, Nakashima H, Miyake K, Hayashida JN, Tanaka A, et al. Interleukin-21 contributes to germinal centre formation and immunoglobulin G4 production in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz’s disease. Ann Rheum Dis. 2012;71(12):2011–19.

38. Mavragani CP, Moutsopoulos HM. The geoepidemiology of Sjögren’s syndrome. Autoimmun Rev. 2010;9(5):A305–10.

Supplementary material available online