Increased expression of interferon-λ in minor salivary glands of patients with primary Sjögren’s syndrome and its synergic effect with interferon-α on salivary gland epithelial cells

Y.-J. Ha¹, Y.S. Choi¹, E.H. Kang¹, J.-H. Chung², S. Cha³, Y.W. Song⁴,⁵, Y.J. Lee¹,⁶

¹Division of Rheumatology, Department of Internal Medicine, and ²Department of Pathology, Seoul National University Bundang Hospital, Seongnam, Korea; ³Departments of Oral and Maxillofacial Diagnostic Sciences and Oral Biology, University of Florida College of Dentistry, Gainesville, USA; ⁴Department of Internal Medicine, Seoul National University Hospital, Korea; ⁵WCU Department of Molecular Medicine and Biopharmaceutical Sciences, Medical Research Institute, and ⁶Department of Internal Medicine, Seoul National University College of Medicine, Korea.

You-Jung Ha, MD* Yong Seok Choi, PhD* Eun Ha Kang, MD, PhD Jin-Haeng Chung, MD, PhD Seunghee Cha, DDS, PhD Yeong Wook Song, MD, PhD Yun Jong Lee, MD, PhD

*These authors contributed equally to this study.

Please address correspondence and reprint requests to:
Dr Yun Jong Lee,
Department of Internal Medicine,
Seoul National University
Bundang Hospital,
82 Gumi-ro, 173 Beon-gil,
Bundang-gu, Seongnam-si,
Gyeonggi-do, 463-707 Korea.
E-mail: yn35@snu.ac.kr

Funding: this research was supported by Astellas Pharma Korea, Inc. (grant no. 800-20120306 to Y.J. Lee) and NIH/NIDCR (grant no. DE019644 to S. Cha).

Competing interests: none declared.

ABSTRACT

Objective. To investigate the expressions of interferon (IFN)-λs and their receptor, IL28RA, in minor salivary glands (MSG) of pSS patients and their effects on the salivary gland cells.

Methods. The expressions of IFN-λs and IL28RA were evaluated in MSG by immunohistochemistry in 15 patients with pSS and in 5 patients with non-SS sicca. Poly(I:C)-induced IL-28A and IL-29 expressions were determined in immortalised human salivary gland acinar (NS-SV-AC) and ductal (NS-SV-DC) cell lines. We assessed the effect of IFN-λs on the expressions of typical interferon-inducible genes, B-cell activating factor (BAFF) and CXCL10, and the synergistic effect of IL-29 and type I or II IFN on their expressions. The serum IL-29 levels were measured in 44 patients with pSS and 22 healthy controls.

Results. IFN-λs expression was significantly higher in MSG from pSS than from non-SS sicca controls. Poly(I:C) treatment led to the induction of IL-28A and IL-29 in the salivary gland cell lines. In the NS-SV-DC cells, IFN-λ significantly increased the levels of BAFF and CXCL10 in a time and dose-dependent manner. Moreover, there was a synergistic effect between IL-29 and IFN-α in the induction of BAFF and CXCL10 expressions by prolonged STAT1 phosphorylation. However, the serum IL-29 levels were not significantly higher in pSS patients than in healthy controls.

Conclusion. Our results suggest the possibility for IFN-λ to play a role by participating local inflammation in the salivary glands of pSS through direct and indirect regulations of the expressions of BAFF and CXCL10 in salivary gland epithelium.

Introduction
Primary Sjögren’s syndrome (pSS) is a chronic systemic autoimmune disease that mainly affects the exocrine glands, which may eventually lead to dry mouth or eyes. The typical immuno-pathological features of pSS include focal peripherally lymphocytic infiltration in the exocrine glands and the presence of organ-specific or organ-nonspecific autoantibodies. Although the pathogenic mechanisms of pSS remain unclear, environmental triggers, i.e. viral infection, are believed to stimulate the innate and acquired immune systems in genetically predisposed individuals (1, 2).

To date, it has been established that genes that are involved in the activation of the interferon (IFN) signalling pathway and affect B cell function and autoantibody production are important in pSS (3, 4). IFNs are a family of key antiviral cytokines produced in response to viral infection. However, they can also modulate numerous genes that encode proteins involved in inflammation, apoptosis, and immune responses (5). In pSS, gene expression profiling studies have shown aberrant activation of type I and type II IFNs and IFN-inducible transcripts in minor salivary glands and peripheral blood (6). IFNs can contribute to the pathophysiology of pSS through apoptosis induction, upregulation of autoantigens, such as Ro52, production of B cell survival/proliferation factors, and enhancement of chemokines or co-stimulatory molecules for lymphocytes in the salivary gland epithelial cells (7, 8). Previous animal studies have shown that mice lacking type I IFN signalling had a significantly lower lymphocytic infiltration in the exocrine glands and less salivary gland dysfunction (9). These data indicate the pivotal role of type I IFN in the pathogenesis of pSS.

Many studies have suggested that glandular epithelial cells are important players in the pathogenesis of pSS as well as the main targets of autoimmunity.
in pSS. They actively contribute to the induction and perpetuation of inflammation, serving as a non-professional antigen presenting cells and proinflammatory cytokine-producing cells (10). Although plasmacytoid dendritic cells (pDCs) are the professional producers of type I IFNs, glandular epithelial cells can also produce type I IFNs, especially when their toll-like receptors (TLRs) are activated via extracellular nucleic acids (8).

Type III IFNs, or IFN-lambdas (IFN-λs), have recently been discovered as members of the IFN superfamily. There are four known type III IFNs: IFN-λ1 (IL10RA), IFN-λ2 (IL28A), IFN-λ3 (IL28B), and IFN-λ4. These cytokines signal through a heterodimeric receptor that consists of a ligand binding chain, IFN-λR1 (IL28RA), and an accessory chain, IL10R2 (11, 12). Although several aspects of IFN-λ biology differ from that of type I IFN, IFN-λs and type I IFNs share similar signaling pathways and biological activities (12). Initially, studies have focused on IFN-λs with respect to viral infections and cancers; however, recent data demonstrated their role in several autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic sclerosis (13-15). Since IFN-λs are inducible by Toll-like receptors (TLRs) and their receptor (IL28RA) in minor salivary gland (MSG) tissues, we collected 20 formalin-fixed paraffin-embedded tissue blocks from the pathology archives of our hospital. Fifteen samples (8 and 7 samples from gender- and age-matched controls) and an additional 44 patients with focus scores ≤1 and >1, respectively, were from pSS patients who fulfilled the American-European Consensus Group criteria (16) and 5 samples from gender- and age-matched healthy controls. We also measured the serum IL-29 levels in pSS patients and healthy controls.

Materials and methods

To evaluate the expression of IFN-λ in MSG tissues, we collected 20 formalin-fixed paraffin-embedded tissue blocks from the pathology archives of our hospital. Fifteen samples (8 and 7 samples with focus scores ≤1 and >1, respectively) were from pSS patients who fulfilled the American-European Consensus Group criteria (16) and 5 samples from gender- and age-matched healthy controls, who did not fulfill the criteria, but presented with dry mouth or dry eye. To quantify the serum IL-29 levels, an additional 44 patients with pSS and 22 gender- and age-matched healthy controls were also enrolled. Clinical features of study subjects are presented in Table I. The study was approved by the ethics committee of Interferon-λ in Sjögren’s syndrome / Y.-J. Ha et al.

| Table I. Baseline characteristics of study participants. |
|------------------------|------------------------|------------------------|------------------------|
| Study subjects for immunohistochemical analysis | Study subjects for the measurement of serum IL-29 |
| Non-SS sicca controls | pSS patients | p-value* | Healthy controls | pSS patients | p-value* |
| (n=5) | (n=15) | | (n=22) | (n=44) | |
| Age, years | 47.0 [41.0 – 56.0] | 43.0 [31.0 – 52.0] | 0.223 | 43.0 [37.0 – 51.3] | 44.0 [35.3 – 51.0] | 0.978 |
| Female sex | 5 (100%) | 15 (100%) | 1.000 | 21 (95.5%) | 42 (95.5%) | 1.000 |
| Duration of Sicca symptoms, years | 3.0 [1.0 – 5.5] | 5.0 [3.8 – 10.0] | 0.147 | 4.0 [2.0 – 10.0] | 36 (81.8%) | 0.817 |
| Dry eye symptoms | 3 (60.0%) | 11 (83.3%) | 1.000 | 36 (81.8%) | 34 (77.3%) | 0.436 |
| Lacrimal dysfunction | 3 (60.0%) | 5 (66.7%) | 0.001 | 34 (77.3%) | 43 (97.7%) | 0.001 |
| Salivary dysfunction | 4 (80.0%) | 15 (100%) | 0.250 | 34 (77.3%) | 43 (97.7%) | 0.001 |
| Focus score ≥1 | 0 (0.0%) | 10 (66.7%) | 0.033 | 21/24 (87.5%) | 28/41 (68.3%) | 0.194 |
| Antinuclear antibody | 1 (25.0%) | 8 (53.3%) | 0.319 | 21/24 (87.5%) | 28/41 (68.3%) | 0.194 |
| Rheumatoid factor | 1 (20.0%) | 5/14 (35.7%) | 1.000 | 17/31 (54.8%) | 43 (97.7%) | 0.001 |
| Anti-Ro | 0 (0.0%) | 13 (86.7%) | 0.001 | 43 (97.7%) | 43 (97.7%) | 0.001 |
| Anti-La | 0 (0.0%) | 7 (46.7%) | 0.114 | 26 (59.1%) | 26 (59.1%) | 0.114 |
| Platelet, x10³ /μL | 257 [212 – 284] | 220.0 [186 – 270] | 0.296 | 244 [208 – 291] | 217 [181 – 263] | 0.021 |
| ESSDAI | 2.0 [1.0 – 5.0] | 2.5 [2.0 – 4.8] | 0.021 | 2.5 [2.0 – 4.8] | 2.5 [2.0 – 4.8] | 0.021 |
| ESSPRI | NA | 4.39 [3.28 – 5.75] | 0.001 | 4.39 [3.28 – 5.75] | 4.39 [3.28 – 5.75] | 0.001 |
| β2-microglobulin | 2.10 [1.90 – 2.85] | 2.25 [1.85 – 2.77] | 0.001 | 2.15 [1.85 – 2.77] | 2.15 [1.85 – 2.77] | 0.001 |

Values are expressed as the median [IQR 25-75%] or n (%). *Schirmer test ≤5mm/5 min or Rose Bengal staining score >3; †Unstimulated salivary flow rate ≤0.1 ml/min or abnormal salivary scintigraphy; ‡p-value by Mann-Whitney U-test or Fisher’s exact test.

our institution (B-0506/021-004), and written informed consent was obtained from all participants.

Cell culture
Two SV40-immortalised normal human salivary gland cell lines—NS-SV-AC and NS-SV-DC cells were kindly provided by Professor Masayuki Azuma (Department of Oral Medicine, University of Tokushima Graduate Faculty of Dentistry, Japan) (17). The characteristics of NS-SV-AC and NS-SV-DC cells are similar to those of primary acinar and ductal cells, respectively. These cell lines were grown in keratinocyte serum-free media. Each medium contained 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin. All cells were grown at 37°C in a humidified 5% CO₂ atmosphere.

**Immunostaining for IFN-λ and IL28RA**
IFN-λ and IL28RA immunostainings were performed on MSG tissues from 15 pSS patients and 5 non-pSS sicca controls. After deparaffinisation and rehydration of formalin-fixed paraffin-embedded tissue samples, the sections were immunostained with anti-human IL-28/29 (sc-66933; Santa Cruz Technology, Santa Cruz, CA, USA) and anti-IL28RA (HPA017319; Sigma-Aldrich, St. Louis, MO, USA). The antibody against human IL-28/29 can detect human IL-28A, IL-28B, and IL-29. The binding of the primary antibodies were assessed by an incubation of secondary antibody (Dako REAL™ EnVision™/HRP, Rabbit/Mouse (ENV) K5007; DakoCytomation, Glostrup, Denmark). The slides were counterstained with haematoxylin. Specimens were evaluated by an expert pathologist (J-H. C.). Staining intensity was assigned as follows: 0 (negative), 1 (weak), 2 (moderate), or 3 (strong); the number of stained cells (staining intensity of score ≥1) was counted in ≥1000 cells of each sample.

**RT-PCR**
Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) from NS-SV-AC and NS-SV-DC cells stimulated with high molecular weight poly(I:C) (InvivoGen, San Diego, CA, USA) or IFN-λ (IL-28A or IL-29; PeproTech, Rocky Hill, NJ, USA). The expressions of IL-28A, IL-28B, IL28RA, TLR3, BAFF, CXCL10 and GAPDH transcripts were determined using an RT-PCR assay with specific primer pairs (Supplementary Table SI). Quantitative real-time PCR was per-
formed in triplicate on an ABI-7500 machine (Applied Biosystems, Foster City, CA, USA) with a Power SYBR Green PCR Master Mix kits (Applied Biosystems). The fold difference in the expression of target mRNA was calculated using the comparative Ct method\(2^{-\Delta\Delta Ct}\) normalised to GAPDH.

**Fluorescence-activated cell sorting (FACS)**

The NS-SV-AC and NS-SV-DC cells were suspended in a cell-staining buffer (BioLegend, San Diego, CA, USA) at \(1\times10^4\) cells/ul. Then, the cells were stained with PE anti-human IL-28RA (BioLegend) or PE mouse IgG2a, κ as a control isotype (BioLegend) on ice for 20 min in the dark. After washing with the buffer, the cells were analysed immediately by a FACScalibur flow cytometer (BD Biosciences, San Jose, CA, USA) to evaluate the expression of IL-28RA in the salivary gland cell lines.

**Western blotting**

To prepare the total cell lysates, the cells were washed twice with ice-cold PBS and lysed with RIPA buffer. The whole cell extracts (20 μg) were separated on 10% polyacrylamide gel and transferred to polyvinylidene fluoride membranes. After blocking, the membranes were incubated with anti-STAT1 and anti-phospho-STAT1 (Cell Signaling Technology, Beverly, MA, USA). The membranes were developed with enhanced chemiluminescence (Amersham Biosciences, Little Chalfont, UK). Densitometric analysis was performed to determine the relative intensity ratio of the bands from total STAT1 and phospho-STAT1, using the ImageJ software (NIH, Bethesda, MD, USA). The results are expressed as the median (interquartile range) or mean ± standard error (SE). Mann-Whitney U-test was used to make a comparison of continuous variables between the 2 groups, and Kruskal-Wallis test was performed to compare the quantitative variables among 3 or more groups. To adjust for multiple comparisons, a Bonferroni correction was done. The non-parametric Jonckheere’s trend test was also applied where appropriate. Chi-square or Fisher’s exact test was used to compare categorical variables. To test for bivariate correlation, Spearman’s correlation coefficient was calculated. Statistical analyses were performed using IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA). A \(p\) or \(p^*\)-value of \(<0.05\) was considered statistically significant.

**Results**

**Overexpression of IFN-λ detected in the MSG of pSS patients**

We examined the expressions and distributions of IFN-λ (IL-28/IL-29) and IL28RA from the MSG biopsy tissues using immunohistochemical staining. As shown in Fig. 1A, IFN-λ was detected in the acinar/ductal epithelial cells of non-SS sicca controls and in the acinar/ductal epithelial cells and some infiltrating mononuclear cells of pSS patients. IL28RA was detected mainly in the acinar/ductal epithelial cells in both groups. IL28RA-positive mononuclear cells were also observed in the focal infiltrates of pSS patients.

To compare the expression levels of IFN-λ in MSG, we calculated the percentage of IFN-λ-positive acinar epithelial cells. The fractions of IFN-λ-positive acinar epithelial cells were significantly higher in pSS patients than in non-SS sicca controls (Fig. 1B, upper panel, \(p=1.29 \times 10^{-4}\)). Additionally, when pSS patients were stratified into pSS with low focus scores (<1), intermediate focus scores (1~2) and high focus scores (>2), there were significant differences in the fractions of IFN-λ-staining acinar epithelial cells (\(p=0.008\) by Kruskal-Wallis test). However, in pSS patients, we did not observe an association between the inflammatory cell infiltration grade and IFN-λ-positive cell frequency. The IL28RA expression was comparable between the pSS and non-SS sicca groups.

**TLR3-mediated IFN-λ expression in salivary gland cell lines**

Because of the higher IFN-λ expression levels in MSG tissues of pSS patients, we studied the TLR3 ligand-induced expressions of IL-28A and IL-29 in the salivary gland cell lines. Poly(I:C) stimulation significantly increased the IL-28A and IL-29 mRNA levels in the NS-SV-DC cells compared with those...
The extent of induction was greater for IL-29 than for IL-28A. In the NS-SV-AC cells, the gene expression of IL-29 was significantly induced. However, IL28RA mRNA was minimally or not changed (Fig. 2B).

**The effects of IFN-λ in salivary gland epithelial cells**

To study the effects of IFN-λ on the salivary gland epithelial cells, we determined the expressions of BAFF, CXCL10, and TLR3 after stimulation with IL-28A and IL-29. When stimulated with 100 ng/mL of IL-28A or 100 ng/mL of IL-29, the levels of BAFF, CXCL10, and TLR3 transcripts were significantly increased, with a decrease in the BAFF and CXCL10 levels over time (Fig. 4A-B). After stimulating the NS-SV-DC cells with IL-29, the levels of BAFF and CXCL10 mRNA were increased in a dose-dependent manner at both 3 h and 24 h (Fig. 4C-D).

**Interception of IFN-α and IL-29 in the induction of BAFF and CXCL10**

Type I IFN is known to be upregulated in MSG of pSS patients, and BAFF and CXCL10 are under the control of IFN-α (8). Because there have been

---

**Fig. 3. Expression of IL28RA in salivary gland cell lines.**

A: IL28RA mRNA expression was found by RT-PCR in NS-SV-AC and NS-SV-DC cells. B: Flow cytometry histograms showed that the frequency of IL-28RA positive cells was higher (about 2 times) in NS-SV-DC cell lines than in NS-SV-AC cell lines.

**Fig. 4. Induction of BAFF, CXCL10, and TLR3 expression by IFN-λ in salivary gland epithelial cells.**

A-B: When NS-SV-DC cells were stimulated, under serum-free conditions, with IL-28A (A: 100 ng/mL) or IL-29 (B: 100 ng/mL) for 3 h or 24 h, BAFF, CXCL10, and TLR3 transcripts were significantly increased. C-D: After NS-SV-DC cells were stimulated with different concentrations of IL-29 for 3 h (C) or 24 h (D), BAFF and CXCL10 were dose-dependently induced (**p<0.001 by Jonckheere’s trend test). Error bars indicate the SEM (n=3). **p<0.001; ***p<0.001 vs. no stimulation.
conflicting results with respect to the interaction between IFN-α and IFN-λ, (18-21), we investigated the expression of IFN-α-induced BAFF and CXCL10 with or without IL-29. In the NS-SV-DC cell lines, IFN-α significantly increased the levels of BAFF and CXCL mRNA at 3 h (Fig. 5, all p<0.001), but decreased their levels at 24 h. With 1 ng/mL of IL-29, IFN-α-induced BAFF expression was significantly enhanced when compared with stimulation with IFN-α only at 3 h (Fig. 5A, p=0.033). At both 3 h and 24 h, the ability to induce BAFF by IFN-α significantly increased with the increase in IL-29 concentration (p<0.005 and p=0.028, respectively). IFN-α-induced CXCL10 expression was significantly upregulated even after adding 0.2 ng/mL of IL-29 at 3 h (p=0.001, Fig. 5B). The synergistic effect of IL-29 on the expression of IFN-α-induced CXCL10 was significantly dose-dependent (p<0.0001 at 3 h and 24 h).

Fig. 6. Enhanced IFN-α- and IFN-γ-induced activation of STAT1 by co-stimulation with IL-29.
A: When NS-SV-DC cells were stimulated, under serum-free conditions, by 100 IU/mL of IL-29, the peak pSTAT1 level was reached at 30 or 60 min.
B-C: IFN-α (B) or IFN-γ (C) induced activation of STAT1 was enhanced by co-stimulation with 1 ng/mL of IL-29. Densitometric analyses revealed that IFN-α (B) or IFN-γ (C) induced pSTAT1/total STAT1 ratios were further augmented at 60 min and 180 min by adding IL-29 (n=3). The bars show the means ± SEM (n=3). *p<0.05; **p<0.01.
pSS patients and controls. Serum IL-29 levels showed no differences between two groups.

Serum IL-29 levels in pSS patients and controls
We determined the IL-29 levels in the sera of 44 pSS patients and 22 healthy controls. The serum levels of IL-29 showed no significant difference between pSS patients and controls (151 [120–177] vs. 138 [124–171] pg/mL; p=0.683, Fig. 7). In pSS patients, there was no significant association between the serum IL-29 levels and clinical or laboratory variables, with the exception of a positive bivariate correlation with patient’s global assessment for pSS (r=0.025, Spearman’s correlation coefficient p=0.349). Four patients showed exceptionally high serum IL-29 levels (>500 pg/mL) but a detailed medical records review did not reveal any clinical or laboratory features suggestive of a viral-like illness.

Discussion
In the salivary glands of pSS patients, immunostaining for IFN-α and microarray analyses of IFN-induced genes showed an increase in type I IFN activity (22, 23). The systemic activation of type I IFN has been confirmed in the peripheral blood, circulating monocytes, and pDCs (24-26). Moreover, due to its overexpression in salivary gland tissues, IFN-γ has also been considered as a player in the pathogenesis of pSS (6). However, contradictory results have been published on whether there was an increase in the salivary or serum IFN-α and IFN-γ (22, 27, 28).

In this study, we found that IFN-λ (IL-28/IL-29) was significantly increased in MSG tissues of pSS patients compared with those of non-SS sicca controls, although the IFN-λ expression levels in MSG tissues were not correlated with the focus scores. Immunostaining for IFN-λ was more prominent in the glandular epithelial cells than in other cells including infiltrating mononuclear cells, with the highest intensity in the ducal cells, followed by the acinar cells. While this manuscript was under preparation, a recent paper reported that all members of IFN-λ and IL-28RA were detected in MSG tissue and IFN-λs were induced in salivary gland epithelial cells by TLR-3 stimulation (29).

Our findings are in line with the results of Apostolou et al. They used antibodies specific to IFN-λ subtypes (IL-28A, IL-28B, or IL-29) and showed that IL-28A expression was significantly higher in only pSS patients with intermediate MSG lesions (≥3 focal infiltrates per lobule). In the present study, we used the antibody bound to all subtypes and the expression of IFN-λs was significantly increased in total pSS patients when IFN-λs positive acinar cells were counted.

Our and Apostolou et al. (29) studies showed that the IL28RA expression levels in MSG tissues were comparable between the two groups, and IL28RA positivity was mainly observed in the glandular epithelial cells. These findings are consistent with the previously reported patterns of IL28RA expression. Unlike the shared receptors for type I IFN, which are expressed on most cell types, the receptors for IFN-λ are known to be limited to the cells of epithelial origin (11). We found that the surface expression of IL28RA protein was greater in the NS-SV-DC cells than in the NS-SV-AC cells, and this was consistent with the immunohistochemical results. We also observed some IFN-λ or IL28RA positive cells within the area of lymphocytic infiltration, as shown in Fig. 1. It is unclear which immune cells expressed IFN-λ, but IL-28 transcripts were detectable in the peripheral blood mononuclear cells from patients with pSS (30). Moreover, Apostolou et al. have reported that strong IL28RA-positive mononuclear cells were mainly pDCs by co-staining with immune cell-specific surface markers (29).

In patients with SLE, the serum IL-29 levels were elevated compared with healthy controls and correlated with their disease activity (13). However, although increased IL-29 levels were also observed in sera of RA and systemic sclerosis patients, the IL-29 levels did not show a clinically meaningful association (14, 15, 31). In this study, patients with pSS did not show significantly higher serum IL-29 levels than normal controls. Moreover, the serum IL-29 levels did not show a significant association with the clinical features or disease activity indices in pSS patients. In the study of Apostolou et al., serum IL-29 levels were reproted to be elevated in patients with pSS (29). But, they pointed out that the data contained several outliers with exremly high levels of IL-29 in pSS patients and it could drive the statistical significance. When considering the above findings and our immunohistochemical findings from MSG of pSS patients, IFN-λ could be involved in the disease process of pSS as a local factor, and not as a systemic one. However, because the sample size was small and 75% (33/44) of our pSS patients had low disease activity (ESS-DAI <5), the role of systemic IL-29 in pSS pathogenesis should be further investigated in a study with a larger sample size and one that includes patients with high disease activity.

TLR3 recognises the double-stranded RNA, and TLR3 activation induces type I IFN production. A definitive role for viral infection in the induction of pSS has not been confirmed; but there is some evidence to suggest the role of TLR3 activation in the pathogenesis of...
pSS (32). In our study, a TLR3 ligand significantly increased IL-28A, and especially increased the IL-29 mRNA expression in the NS-SV-DC cells. These findings are consistent with recently published data showing TLR3-stimulated IFN-λ expression in salivary gland epithelial cells from SS patients (29). Also, similar findings were also observed in the non-immune cells (33-35). Thus, TLR3-mediated events may induce IFN-λs, as well as type I IFNs, expression, in the salivary glands. IFN-λ was previously reported to increase the expression of TLR3, similar to type I and II IFNs (36). In our study, TLR3 gene expression was significantly enhanced by the stimulation with IFN-λs in the salivary gland epithelial cells, suggesting that TLR3 and IFN-λ pathways reciprocally stimulate their expression. Because CD4+ T helper cells migrate and accumulate within the exocrine glands and Th1 cytokines, including IFN-γ, are increased in the target tissues, pSS has been regarded as a Th1 cell-mediated disease (37). In fact, the majority of infiltrating cells in the exocrine glands are made up by CD4+ T cells. Therefore, it is possible for Th1-associated chemokines to be relevant in the pathogenesis of pSS. It has previously been reported that CXCL10 in patients with pSS is elevated in the saliva and tears as well as in the salivary glands (38-40). These findings highlight the pathogenic importance of elevated CXCL10 expression in pSS. Both types I and II IFNs are known to induce CXCL10, and IFN-λ increases the expression of CXCL10 in the peripheral mononuclear cells and hepaticocytes (13, 41). Our results that show the expression of IL-28A/IL-29-induced CXCL10 in the salivary ductal epithelial cells are consistent with the previous findings. However, although CXCL10 was found to be expressed predominantly in the ductal epithelium adjacent to lymphoid infiltrates in other studies (25), our study indicates that the distribution of IFN-λ-positive cells was not confined to these sites. Wolk et al. recently reported that the Th17 cells are a novel source of IL-29 in psoriasis (42). We found that some infiltrating mononuclear cells were positive for IFN-λ. Although the IL-29-producing Th17 cells have not been examined in pSS patients, Th17 subsets (IL-17 producing CD4+ or double negative T cells) are suggested to play a role in the pathogenesis of pSS (43, 44). It remains to be investigated whether the IL-29-producing Th17 cells are present in the salivary glands tissues of pSS patients. Recently, B cells have been regarded as the central cell in the pathomechanism of pSS. Because BAFF is critical for B cell survival and differentiation, and because it can rescue self-reactive B cells from deletion, BAFF has been considered as the key cytokine in the B cell hyperactivity of pSS. Patients with pSS have elevated levels of BAFF in the serum and saliva, in addition to having increased expression of BAFF in the mononuclear cells and ductal epithelial cells of MSG tissues (37). BAFF transgenic mice develop a pSS-like pathology and show reduced saliva production (45). Given that IFN-α induces BAFF production and IFN-α and IFN-λ share a common signalling pathway, it is not surprising that IFN-λ increased the expression of BAFF. In our study, IL-28A and IL-29 significantly induced the production of BAFF from the salivary gland epithelial cells. Additionally, since both naive and memory B cells were reported to express IL28RA, IFN-λ could enhance the TLR-mediated activation of B cells that lead to the production of IgG, IgM, and IL-6 (46). Thus, the elevated IFN-λ levels in the salivary gland could create a niche for the persistently activated B cells, both directly and indirectly. Although IFN-α and IFN-λ activate different receptors, they are induced by similar stimuli and activate similar IFN-stimulated genes (47). Therefore, we investigated where IFN-λ, IFN-α, or IFN-γ can act synergistically or antagonistically. Several previous studies have reported synergistic effects between IFN-α and IFN-λ, while others have revealed antagonistic effects between two (18-21). In the current study, we clearly showed synergic effects between IFN-α and IL-29 on the dose-dependent induction of BAFF and CXCL10. To further elucidate the mechanisms underlying the synergism between IFN-α/γ and IL-29, we evaluated the phosphorylation level of STAT1 – a transcriptional activator that is central to IFN-α signalling – as the stimulation of IFN-α/γ with or without IL-29. IL-29 prolonged IFN-α- or IFN-γ-induced STAT1 phosphorylation in the salivary gland epithelial cells. These novel findings suggest that increased IFN-λ in the salivary glands can potentiate the pathogenic effects of IFN-α and IFN-γ in pSS. Ifnar1-deficient mice, which lack type I IFN signalling, have been reported to show significantly lower lymphocytic infiltration in the exocrine glands and less salivary gland dysfunction when compared with the wild-type B6.Aec1Aec2 mice (9, 48). Although the IL28RA locus has been associated with psoriasis and SLE in a genome-wide association study (49), the effect of IL28RA deficiency on autoimmune diseases has not been investigated thus far. Surprisingly, IL28RA-deficient mice did not exhibit altered virus susceptibility, but mice lacking the type I IFN receptors were highly susceptible to viral infection (50). Thus, the contribution of IFN-λ alone may be more limited than that of type I IFN in infectious or inflammatory diseases. However, our results provide new information on the role of IFN-λ in the pathogenesis of pSS. In conclusion, the elevated expression of IFN-λ was detected in MSGs of pSS patients, and the expression of TLR3-induced IFN-λ was observed in the salivary epithelial cells. BAFF and CXCL10 expressions were increased by IFN-λ in the salivary epithelial cells, and their induction was synergistically enhanced by IL-29 and IFN-α. These results suggest the possibility that IFN-λ – both directly and indirectly – drives chronic local inflammation and induces the response of salivary gland cells in pSS.

References


