

# Increased expression of interferon- $\lambda$ in minor salivary glands of patients with primary Sjögren's syndrome and its synergic effect with interferon- $\alpha$ on salivary gland epithelial cells

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## ABSTRACT

**Objective.** To investigate the expressions of interferon (IFN)- $\lambda$ s 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- $\lambda$ s 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- $\lambda$ s 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- $\lambda$ s 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- $\lambda$  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- $\alpha$  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- $\lambda$  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 periepithelial 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

**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 (n=5)	pSS patients (n=15)	<i>p</i> -value*	Healthy controls (n=22)	pSS patients (n=44)	<i>p</i> -value*
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]	
Dry eye symptoms	3 (60.0%)	14 (93.3%)	0.140		36 (81.8%)	
Dry mouth symptoms	5 (100%)	14 (83.3%)	1.000		36 (81.8%)	
Lacrimal dysfunction <sup>a</sup>	3 (60.0%)	10 (66.7%)	1.000		34 (77.3%)	
Salivary dysfunction <sup>b</sup>	4 (80.0%)	15 (100%)	0.250		43 (97.7%)	
Focus score $\geq 1$	0 (0.0%)	10 (66.7%)	0.033		21/24 (87.5%)	
Antinuclear antibody	1 (25.0%)	8 (53.3%)	0.319		28/41 (68.3%)	
Rheumatoid factor	1 (20.0%)	5/14 (35.7%)	1.000		17/31 (54.8%)	
Anti-Ro	0 (0.0%)	13 (86.7%)	0.001		43 (97.7%)	
Anti-La	0 (0.0%)	7 (46.7%)	0.114		26 (59.1%)	
White blood cell, / $\mu$ L	5270 [4760 ~ 6860]	4850 [4050 ~ 5750]	0.455	5255 [4298 ~ 6115]	4730 [3480 ~ 5633]	0.136
Haemoglobin, g/dL	13.3 [13.1 ~ 14.0]	12.5 [11.8 ~ 13.1]	0.035	13.1 [12.8 ~ 14.3]	12.8 [11.8 ~ 13.4]	0.010
Platelet, $\times 10^3$ / $\mu$ L	257 [212 ~ 284]	220.0 [186 ~ 270]	0.296	244 [208 ~ 291]	217 [181 ~ 263]	0.021
ESR, mm/hr	3 [3 ~ 28]	18 [5 ~ 31]	0.456	5 [2 ~ 9]	18 [9 ~ 29]	<0.001
C3, mg/dL		105 [83 ~ 117]			99 [39 ~ 109]	
C4, mg/dL		21 [14 ~ 27]			19 [14 ~ 23]	
IgG, mg/dL		2000 [1670 ~ 2240]			2075 [1613 ~ 2713]	
ESSDAI		2.0 [1.0 ~ 5.0]			2.5 [2.0 ~ 4.8]	
ESSPRI		NA			4.39 [3.28 ~ 5.75]	
$\beta 2$ -microglobulin		2.10 [1.90 ~ 2.85]			2.15 [1.85 ~ 2.77]	
Extra-glandular manifestations						
Arthralgia/arthritis		10 (66.7%)			19 (43.2%)	
Raynaud's phenomenon		4 (26.7%)			11 (25.0%)	
Interstitial lung disease		1 (6.7%)			0 (0%)	

Values are expressed as the median [IQR 25-75%] or *n* (%). <sup>a</sup>Schirmer test  $\leq 5$  mm/5 min, or Rose Bengal staining score  $>3$ ; <sup>b</sup>Unstimulated salivary flow rate  $\leq 0.1$  ml/min or abnormal salivary scintigraphy; \**p*-value by Mann-Whitney U-test or Fisher's exact test.

pSS: Sjögren's syndrome; ESR: erythrocyte sedimentation rate; IgG: immunoglobulin G; ESSDAI: EULAR Sjögren's syndrome disease activity index; ESSPRI: EULAR Sjögren's syndrome patient-reported index; NA: not available.

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- $\lambda$ s), have recently been discovered as the members of the IFN superfamily. There are four known type III IFNs: IFN- $\lambda 1$  (IL [interleukin]-29), IFN- $\lambda 2$  (IL-28A), IFN- $\lambda 3$  (IL-28B), and IFN- $\lambda 4$ . These cytokines signal through a heterodimeric receptor that consists of a ligand binding chain, IFN- $\lambda R1$  (IL28RA), and an accessory chain, IL-10R2 (11, 12). Although several aspects of IFN- $\lambda$  biology differ from that of type I IFN, IFN- $\lambda$ s

and type I IFNs share similar signaling pathways and biological activities (12). Initially, studies have focused on IFN- $\lambda$ s 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- $\lambda$ s are inducible by viruses and type I IFNs and IL28RA are expressed preferentially on the epithelial cells (11), we hypothesised that IFN- $\lambda$ s are also involved in the pathogenesis of pSS.

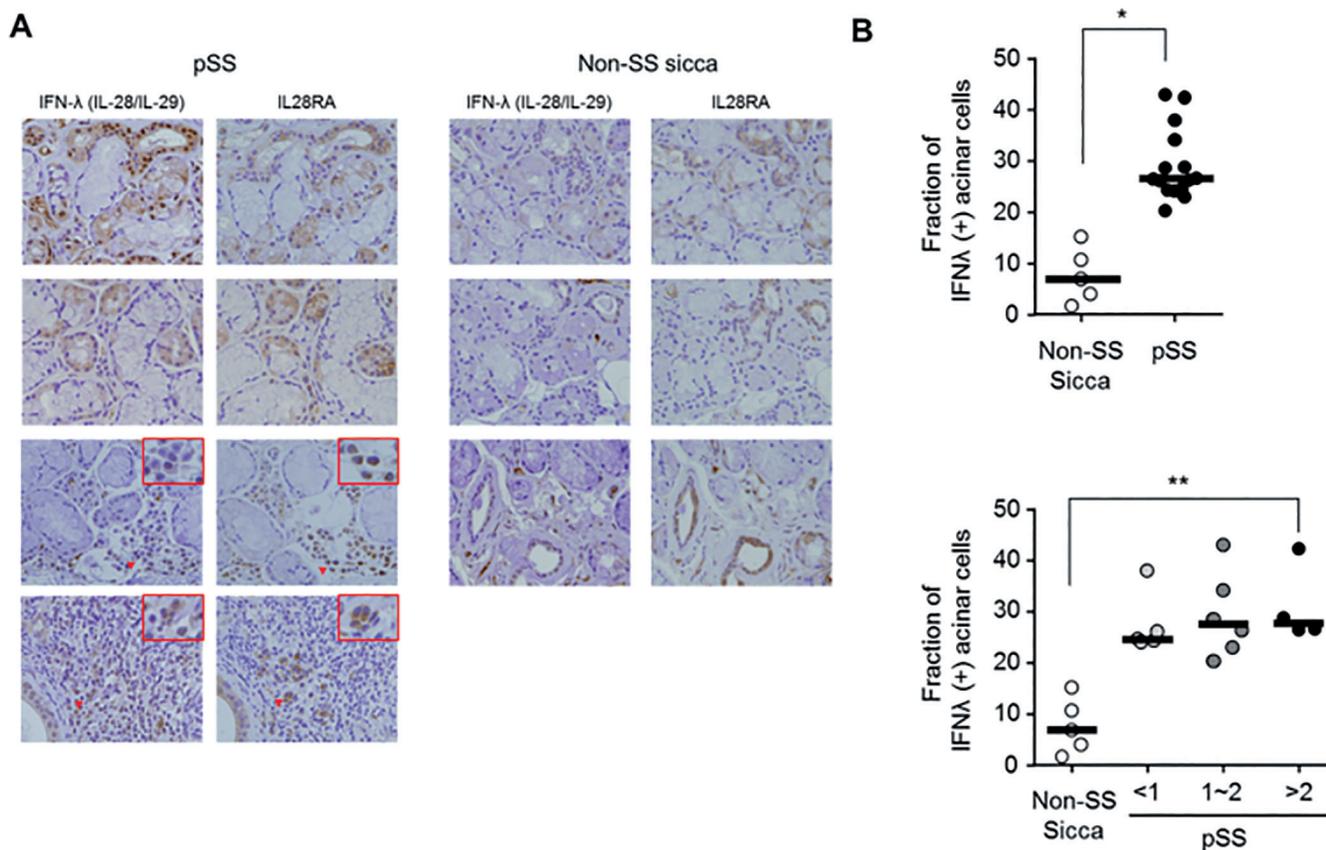
Accordingly, we investigated the expressions of IFN- $\lambda$ s and their receptor (IL28RA) in minor salivary gland (MSG) tissues from pSS patients. We also examined the TLR3-mediated IFN- $\lambda$  expression, the effect of IFN- $\lambda$  on the expression of B-cell activating factor (BAFF) and CXCL10, and the synergistic effect between IFN- $\alpha$  or IFN- $\gamma$  and IFN- $\lambda$  in the salivary epi-

thelial cell lines. We also measured the serum IL-29 levels in pSS patients and healthy controls.

## Materials and methods

### Patients and controls

To evaluate the expression of IFN- $\lambda$  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  $\leq 1$  and  $>1$ , respectively) were from pSS patients who fulfilled the American-European Consensus Group criteria (16) and 5 samples from gender- and age-matched patients, 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



**Fig. 1.** Immunohistochemistry staining for IFN- $\lambda$  (IL-28/IL-29) and its receptor IL28RA in the MSG tissues.

**A:** Representative photos showed IFN- $\lambda$  expression in acinar and ductal epithelial cells in pSS patients and in non-pSS sicca controls (original magnification  $\times 400$ ). The immunostaining of IFN- $\lambda$  was more pronounced in the ductal epithelial cells than in acinar cells and was increased in pSS patients when compared to that in non-pSS sicca controls. IL28RA was detected in both acinar and ductal epithelial cells. Within mononuclear cell infiltrates, some IFN- $\lambda$  positive or IL28RA positive cells were observed (The red box at the right upper corner is a double-magnified photograph of the arrowhead area).

**B:** Fractions of IFN- $\lambda$  immunoreactive acinar epithelial cells were significantly higher in pSS patients than in controls (upper panel). When the pSS patients were stratified into 3 subgroups according to focus score (low, focus score  $<1$ ,  $n=5$ ; intermediate,  $1 \leq$  focus score  $\leq 2$ ,  $n=6$ ; high, focus score  $>2$ ,  $n=4$ ), there was no significance in the fractions among 3 pSS subgroups (lower panel).  $p$ -values calculated by the Mann-Whitney U-test or by the Kruskal-Wallis test. \* $p=1.29 \times 10^{-4}$ ; \*\* $p=0.008$ .

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  $\mu$ g/mL streptomycin. All cells were grown at 37°C in a humidified 5% CO<sub>2</sub> atmosphere.

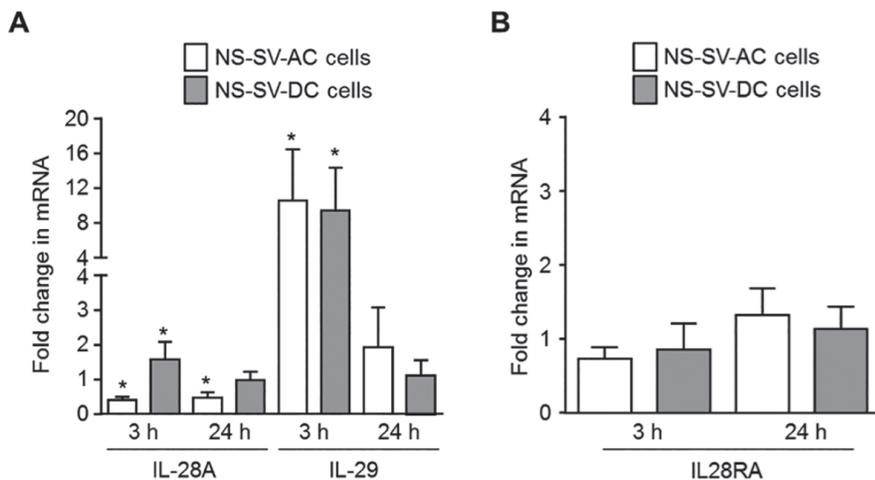
#### Immunostaining for IFN- $\lambda$ and IL28RA

IFN- $\lambda$  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 Biotechnology, 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 was 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  $\geq 1$ ) was counted in  $\geq 1000$  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- $\lambda$  (IL-28A or IL-29; PeproTech, Rocky Hill, NJ, USA). The expressions of IL-28A, IL-29, IL28RA, TLR3, BAFF, CXCL10 and GAPDH transcripts were determined using an RT-PCR assay with specific primer pairs (Supplementary Table S1). Quantitative real-time PCR was per-



**Fig. 2.** TLR3-mediated expression of IFN- $\lambda$ s and IL28RA in salivary gland epithelial cells. **A:** When NS-SV-AC and NS-SV-DC cells were stimulated, under serum-free conditions, with poly (I:C) (30  $\mu$ g/mL) for 3 h and 24 h, IL-29 mRNA was significantly upregulated at 3 h in both cell lines, and IL-28A mRNA was increased at 3 h in NS-SV-DC cells. **B:** IL28RA expression did not show a remarkable change after TLR3 stimulation. Error bars indicate the SEM (n=3). \*  $p_c < 0.001$  vs. no stimulation.

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  $1 \times 10^4$  cells/uL. Then, the cells were stained with PE anti-human IL-28RA (BioLegend) or PE mouse IgG2a,  $\kappa$  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  $\mu$ g) 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 phosphor-STAT1, using the ImageJ software (NIH, Bethesda, MD, USA).

#### Determination of IL-29 levels in serum

Serum IL-29 concentrations were measured using the commercial human IL-29 enzyme-linked immunosorbent assay kits (eBioscience, San Diego, CA, USA) in accordance with the manufacturer's recommendations. The assays were performed in duplicate.

#### Statistical analysis

The results are expressed as the median (interquartile range) or mean  $\pm$  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  $p_c$ -value of  $< 0.05$  was considered statistically significant.

## Results

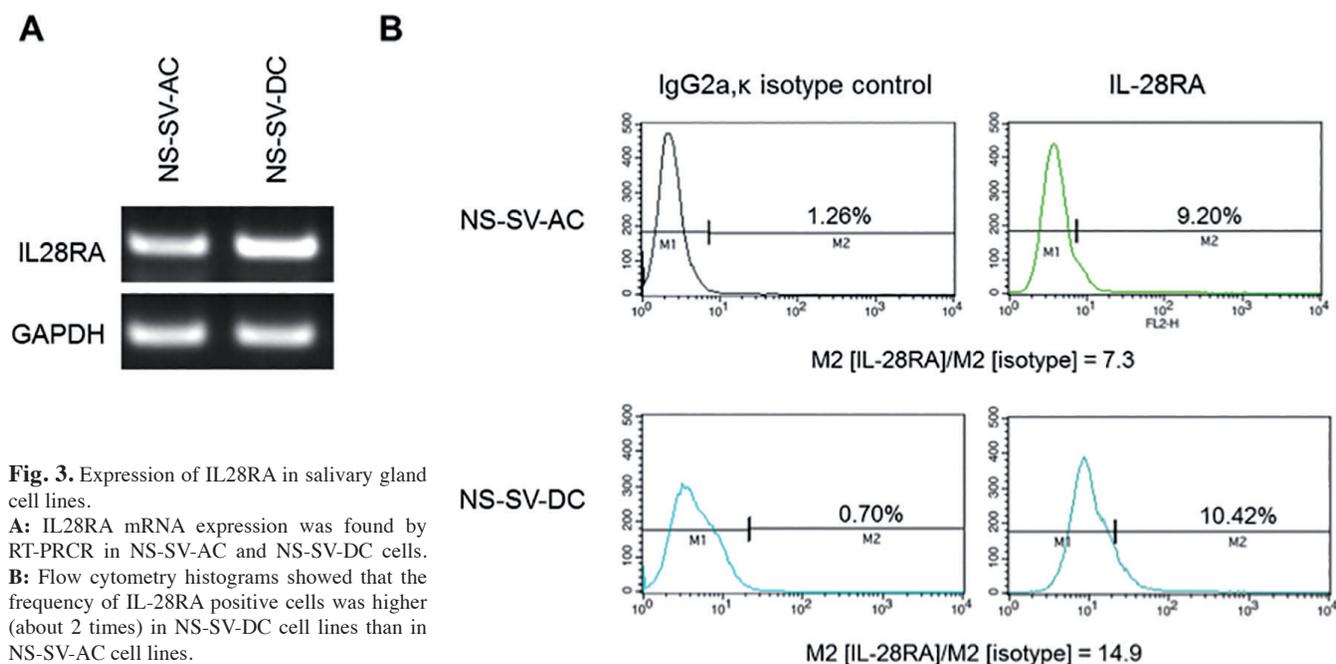
### Overexpression of IFN- $\lambda$ detected in the MSG of pSS patients

We examined the expressions and distributions of IFN- $\lambda$  (IL-28/IL-29) and IL28RA from the MSG biopsy tissues using immunohistochemical staining. As shown in Fig. 1A, IFN- $\lambda$  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- $\lambda$  in MSG, we calculated the percentage of IFN- $\lambda$ -positive acinar epithelial cells. The fractions of IFN- $\lambda$ -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- $\lambda$ -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- $\lambda$ -positive cell frequency. The IL28RA expression was comparable between the pSS and non-SS sicca groups.

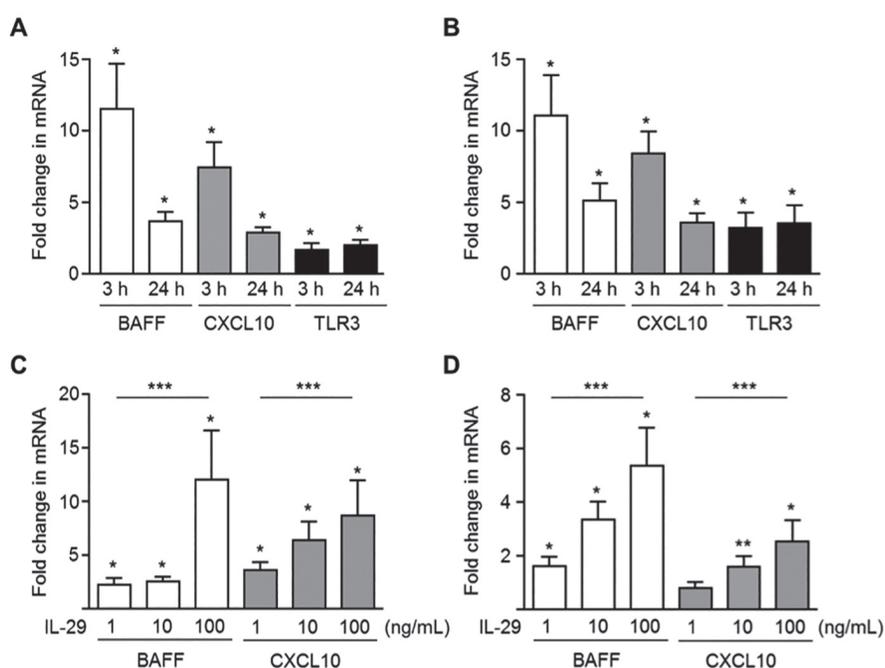
### TLR3-mediated IFN- $\lambda$ expression in salivary gland cell lines

Because of the higher IFN- $\lambda$  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. In the NS-SV-DC cells, poly(I:C) stimulation significantly increased the IL-28A and IL-29 mRNA levels at 3 h compared



**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- $\lambda$  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 ( $n=3$ ). \* $p_c < 0.001$ ; \*\* $p_c = 0.016$  vs. no stimulation.

with those in the unstimulated cells (Fig. 2A). 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 at 3 h. However, IL28RA mRNA was minimally or not changed (Fig. 2B).

#### IL28RA expression in salivary gland cell lines

Because the biological functions of IFN- $\lambda$ s depend on the IL28RA receptors, we investigated the expression of IL28RA in the salivary gland cell lines. The IL28RA mRNA was detected in

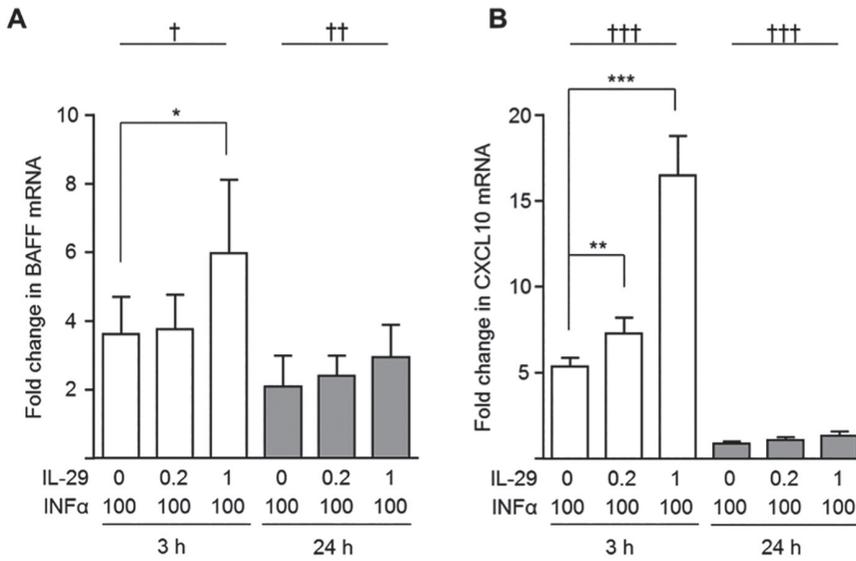
the NS-SV-AC and NS-SV-DC cells in an unstimulated condition (Fig. 3A). The FACS analysis revealed that the frequency of IL-28RA expressing cells was higher in NS-SV-DC cells than in NS-SV-AC cells (Fig. 3B). Therefore, the NS-SV-DC cells were used in subsequent experiments.

#### The effects of IFN- $\lambda$ in salivary gland epithelial cells

To study the effects of IFN- $\lambda$  on the salivary 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 upregulated, 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).

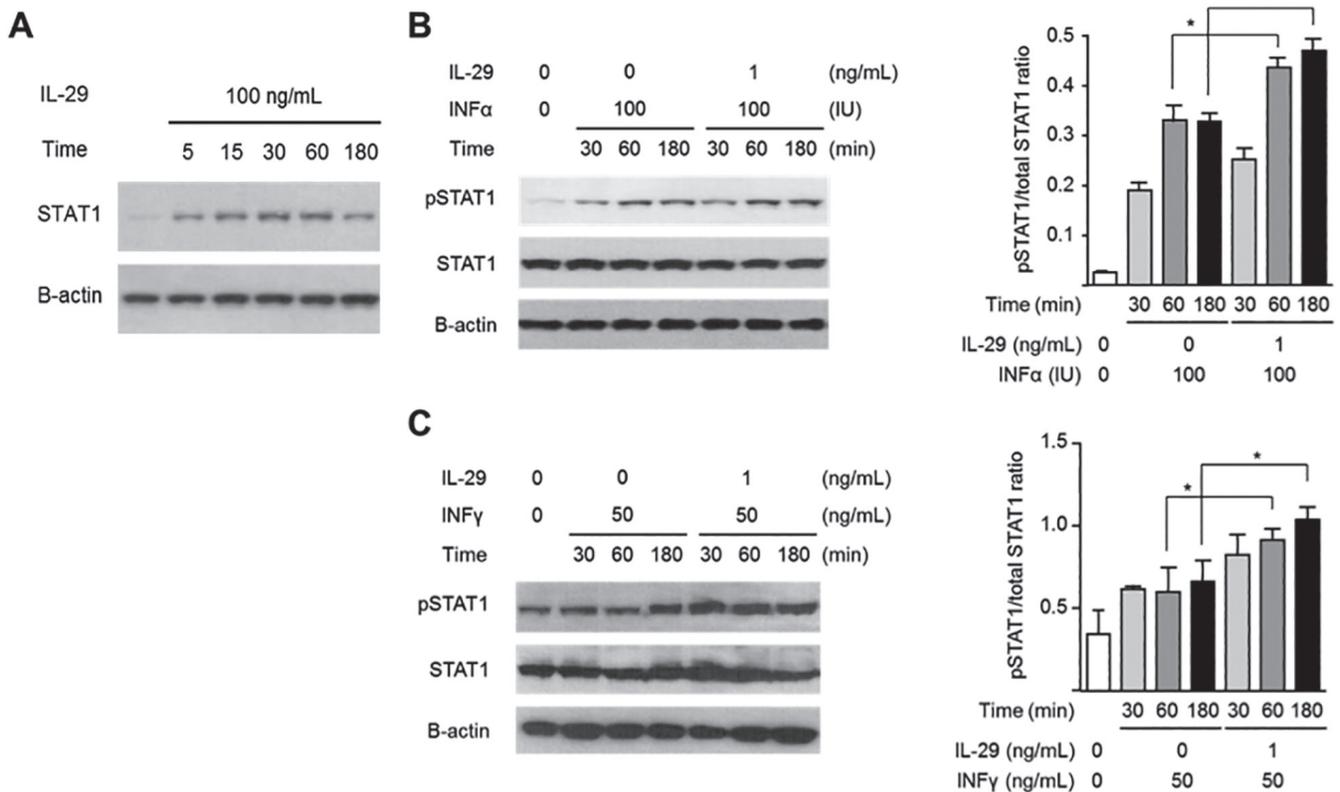
#### Interaction of IFN- $\alpha$ and IL-29 in the induction of BAFF and CXCL10

Type I IFN is known to be upregulated in MSG of pSS patients, and BAFF and CXCL10 are under the control of IFN- $\alpha$  (8). Because there have been conflicting results with respect to the interaction between IFN- $\alpha$  and IFN- $\lambda$

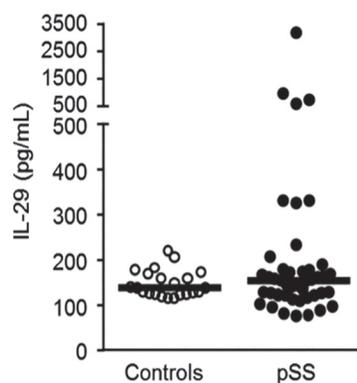


**Fig. 5.** Synergistic upregulation of BAFF and CXCL10 expression by IFN-α and IL-29. **A-B:** After stimulation of NS-SV-DC cells with 100 IU/mL IFN-α under serum-free conditions, gene expression levels of BAFF (**A**) and CXCL10 (**B**) significantly increased (all  $p < 0.001$ ); except CXCL10 at 24 h. Co-stimulation with IFN-α and different concentrations of IL-29 (0.2~1 ng/mL) resulted in significant enhancement of IFN-α-induced upregulation of BAFF and CXCL10 transcripts at 3 h. At both time points, dose-dependent synergistic effects of IL-29 on BAFF and CXCL10 gene expression were evident (all trend  $p < 0.05$ ). The bars show the means  $\pm$  SEM (n=3). The  $p_c$  value was calculated by the Kruskal-Wallis test with Bonferroni correction, and the  $p$ -value was determined by Jonckheere's trend test. \* $p_c = 0.033$ ; \*\* $p_c = 0.001$ ; \*\*\* $p_c < 0.001$ ; †  $p = 0.005$ ; ††  $p = 0.028$ ; †††  $p < 0.001$ .

(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,  $p_c = 0.033$ ). 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 ( $p_c = 0.001$ , Fig. 5B). The synergistic effect of IL-29 on the expression of IFN-α-induced CXCL10 was significantly dose-dependent ( $p < 0.001$  at 3 h and 24 h). Since both IFN-α and IFN-λ activate the JAK1/STAT1 pathway and



**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  $\pm$  SEM (n=3). \* $p < 0.05$ ; \*\* $p < 0.01$ .



**Fig. 7.** Comparison of serum IL-29 levels between pSS patients and controls. Serum IL-29 levels showed no differences between two groups.

pSTAT1 induces BAFF and CXCL10 expressions (5), we investigated the effects of IL-29 on the IFN- $\alpha$ - and IFN- $\gamma$ -induced phosphorylation of STAT1. After stimulation with 100 ng/mL of IL-29, STAT1 phosphorylation peaked at 30 to 60 min (Fig. 6A). IFN- $\alpha$ - or IFN- $\gamma$ -induced STAT1 phosphorylation was significantly enhanced by co-stimulation with 1 ng/mL of IL-29 at 60 min and 180 min (Fig. 6B-C).

#### *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 ( $p=0.025$ , Spearman's correlation coefficient  $\rho=0.349$ ). 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- $\alpha$  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- $\gamma$  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- $\alpha$  and IFN- $\gamma$  (22, 27, 28).

In this study, we found that IFN- $\lambda$  (IL-28/IL-29) was significantly increased in MSG tissues of pSS patients compared with those of non-SS sicca controls, although the IFN- $\lambda$  expression levels in MSG tissues were not correlated with the focus scores. Immunostaining for IFN- $\lambda$  was more prominent in the glandular epithelial cells than in other cells including infiltrating mononuclear cells, with the highest intensity in the ductal cells, followed by the acinar cells. While this manuscript was under preparation, a recent paper reported that all members of IFN- $\lambda$  and IL-28RA were detected in MSG tissue and IFN- $\lambda$ s 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- $\lambda$  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 ( $\geq 3$  focal infiltrates per lobule). In the present study, we used the antibody bound to all subtypes and the expression of IFN- $\lambda$ s was significantly increased in total pSS patients when IFN- $\lambda$ s 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- $\lambda$  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 immunohis-

tochemical results. We also observed some IFN- $\lambda$  or IL28RA positive cells within the area of lymphocytic infiltration, as shown in Fig. 1. It is unclear which immune cells expressed IFN- $\lambda$ , but IL-28 transcripts were detectable in the peripheral blood mononuclear cells from patients with SLE (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, though 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 reported to be elevated in patients with pSS (29). But, they pointed out that the data contained several outliers with extremely 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- $\lambda$  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- $\lambda$  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- $\lambda$ s, as well as type I IFN expression, in the salivary glands. IFN- $\lambda$  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- $\lambda$ s in the salivary gland epithelial cells, suggesting that TLR3 and IFN- $\lambda$  pathways reciprocally stimulate their expression.

Because CD4<sup>+</sup> T helper cells migrate and accumulate within the exocrine glands and Th1 cytokines, including IFN- $\gamma$ , 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<sup>+</sup> 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- $\lambda$  increases the expression of CXCL10 in the peripheral mononuclear cells and hepatocytes (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- $\lambda$ -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- $\lambda$ . Although the

IL-29-producing Th17 cells have not been examined in pSS patients, Th17 subsets (IL-17 producing CD4<sup>+</sup> 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. Since 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- $\alpha$  induces BAFF production and IFN- $\alpha$  and IFN- $\lambda$  share a common signalling pathway, it is not surprising that IFN- $\lambda$  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 naïve and memory B cells were reported to express IL28RA, IFN- $\lambda$  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- $\lambda$  levels in the salivary gland could create a niche for the persistently activated B cells, both directly and indirectly.

Although IFN- $\alpha$  and IFN- $\lambda$  activate different receptors, they are induced by similar stimuli and activate similar IFN-stimulated genes (47). Therefore, we investigated where IFN- $\lambda$  and IFN- $\alpha$  or IFN- $\gamma$  can act synergistically or antagonistically. Several previous studies have reported synergistic effects between IFN- $\alpha$  and IFN- $\lambda$ , while others have revealed antagonistic effects between two (18-21). In the current study, we clearly showed synergic effects between IFN- $\alpha$  and IL-29 on the dose-dependent induction of BAFF and CXCL10. To further elucidate the mechanisms underlying the synergism between IFN- $\alpha/\gamma$  and

IL-29, we evaluated the phosphorylation level of STAT1 – a transcriptional activator that is central to IFN- $\alpha$  signalling – after the stimulation of IFN- $\alpha/\gamma$  with or without IL-29. IL-29 prolonged IFN- $\alpha$ - or IFN- $\gamma$ -induced STAT1 phosphorylation in the salivary gland epithelial cells. These novel findings suggest that increased IFN- $\lambda$  in the salivary glands can potentiate the pathogenic effects of IFN- $\alpha$  and IFN- $\gamma$  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- $\lambda$  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- $\lambda$  in the pathogenesis of pSS.

In conclusion, the elevated expression of IFN- $\lambda$  was detected in MSGs of pSS patients, and the expression of TLR3-induced IFN- $\lambda$  was observed in the salivary epithelial cells. BAFF and CXCL10 expressions were increased by IFN- $\lambda$  in the salivary epithelial cells, and their induction was synergistically enhanced by IL-29 and IFN- $\alpha$ . These results suggest the possibility that IFN- $\lambda$  – both directly and indirectly – drives chronic local inflammation and induces the response of salivary gland cells in pSS.

## References

1. JONSSON R, BOLSTAD AI, BROKSTAD KA, BRUN JG: Sjögren's syndrome--a plethora of clinical and immunological phenotypes with a complex genetic background. *Ann N Y Acad Sci* 2007; 1108: 433-47.
2. BARONE F, COLAFRANCESCO S: Sjögren's syndrome: from pathogenesis to novel therapeutic targets. *Clin Exp Rheumatol* 2016; 34: 58-62.

3. NEZOS A, MAVRAGANI CP: Contribution of genetic factors to Sjögren's syndrome and Sjögren's syndrome related lymphomagenesis. *J Immunol Res* 2015; 2015: 754825.
4. FERRO F, VAGELLI R, BRUNI C *et al.*: One year in review 2016: Sjögren's syndrome. *Clin Exp Rheumatol* 2016; 34: 161-71.
5. MEYER O: Interferons and autoimmune disorders. *Joint Bone Spine* 2009; 76: 464-73.
6. NEZOS A, GRAVANI F, TASSIDOU A *et al.*: Type I and II interferon signatures in Sjögren's syndrome pathogenesis: contributions in distinct clinical phenotypes and Sjögren's related lymphomagenesis. *J Autoimmun* 2015; 63: 47-58.
7. YAO Y, LIU Z, JALLAL B, SHEN N, RONNBLUM L: Type I interferons in Sjögren's syndrome. *Autoimmun Rev* 2013; 12: 558-66.
8. MAVRAGANI CP, CROW MK: Activation of the type I interferon pathway in primary Sjögren's syndrome. *J Autoimmun* 2010; 35: 225-31.
9. SZCZERBA BM, RYBAKOWSKA PD, DEY P *et al.*: Type I interferon receptor deficiency prevents murine Sjögren's syndrome. *J Dent Res* 2013; 92: 444-9.
10. KAPSOGEORGOU E, MANOUSSAKIS MN: The central role of epithelial cells in Sjögren's syndrome or autoimmune epithelitis. *Autoimmun Rev* 2004; 3 Suppl. 1: S61-3.
11. DONNELLY RP, KOTENKO SV: Interferon-lambda: a new addition to an old family. *J Interferon Cytokine Res* 2010; 30: 555-64.
12. LAZEAR HM, NICE TJ, DIAMOND MS: Interferon-lambda: Immune Functions at Barrier Surfaces and Beyond. *Immunity* 2015; 43: 15-28.
13. WU Q, YANG Q, LOURENCO E, SUN H, ZHANG Y: Interferon-lambda1 induces peripheral blood mononuclear cell-derived chemokines secretion in patients with systemic lupus erythematosus: its correlation with disease activity. *Arthritis Res Ther* 2011; 13: R88.
14. WU Q, YANG Q, SUN H, LI M, ZHANG Y, LA CAVA A: Serum IFN-lambda1 is abnormally elevated in rheumatoid arthritis patients. *Autoimmunity* 2013; 46: 40-3.
15. DANTAS AT, GONCALVES SM, PEREIRA MC *et al.*: Interferons and systemic sclerosis: correlation between interferon gamma and interferon-lambda 1 (IL-29). *Autoimmunity* 2015; 48: 429-33.
16. VITALI C, BOMBARDIERI S, JONSSON R *et al.*: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; 61: 554-8.
17. AZUMA M, TAMATANI T, KASAI Y, SATO M: Immortalization of normal human salivary gland cells with duct-, myoepithelial-, acinar-, or squamous phenotype by transfection with SV40 ori- mutant deoxyribonucleic acid. *Lab Invest* 1993; 69: 24-42.
18. VOIGT EA, YIN J: Kinetic differences and synergistic antiviral effects between type I and type III interferon signaling indicate pathway independence. *J Interferon Cytokine Res* 2015; 35: 734-47.
19. BORDI L, LALLE E, CAGLIOTI C *et al.*: Antagonistic antiviral activity between IFN-lambda and IFN-alpha against lethal Crimean-Congo hemorrhagic fever virus *in vitro*. *PLoS One* 2015; 10: e0116816.
20. SHINDO H, MAEKAWA S, KOMASE K *et al.*: IL-28B (IFN-lambda3) and IFN-alpha synergistically inhibit HCV replication. *J Viral Hepat* 2013; 20: 281-9.
21. FUJIE H, TANAKA T, TAGAWA M *et al.*: Antitumor activity of type III interferon alone or in combination with type I interferon against human non-small cell lung cancer. *Cancer Sci* 2011; 102: 1977-90.
22. ZHENG L, ZHANG Z, YU C, TU L, ZHONG L, YANG C: Association between IFN-alpha and primary Sjögren's syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 107: e12-8.
23. HJELMERVIK TO, PETERSEN K, JONASSEN I, JONSSON R, BOLSTAD AI: Gene expression profiling of minor salivary glands clearly distinguishes primary Sjögren's syndrome patients from healthy control subjects. *Arthritis Rheum* 2005; 52: 1534-44.
24. KIMOTO O, SAWADA J, SHIMOYAMA K *et al.*: Activation of the interferon pathway in peripheral blood of patients with Sjögren's syndrome. *J Rheumatol* 2011; 38: 310-6.
25. 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: 2024-33.
26. BAVE U, NORDMARK G, LOVGREN T *et al.*: Activation of the type I interferon system in primary Sjögren's syndrome: a possible etiopathogenic mechanism. *Arthritis Rheum* 2005; 52: 1185-95.
27. SHIOZAWA S, SHIOZAWA K, SHIMIZU S *et al.*: Immunoreactive circulating alpha-interferon is low in Sjögren's syndrome. *Br J Rheumatol* 1990; 29: 50-2.
28. KANG EH, LEE YJ, HYON JY, YUN PY, SONG YW: Salivary cytokine profiles in primary Sjögren's syndrome differ from those in non-Sjögren sicca in terms of TNF-alpha levels and Th-1/Th-2 ratios. *Clin Exp Rheumatol* 2011; 29: 970-6.
29. APOSTOLOU E, KAPSOGEORGOU EK, KONSTA OD *et al.*: Expression of type III interferons (IFN-lambdas) and their receptor in Sjögren's syndrome. *Clin Exp Immunol* 2016; 186: 304-12.
30. LIN SC, KUO CC, TSAO JT, LIN LJ: Profiling the expression of interleukin (IL)-28 and IL-28 receptor alpha in systemic lupus erythematosus patients. *Eur J Clin Invest* 2012; 42: 61-9.
31. XU D, YAN S, WANG H *et al.*: IL-29 enhances LPS/TLR4-mediated inflammation in rheumatoid arthritis. *Cell Physiol Biochem* 2015; 37: 27-34.
32. KILLEDAR SJ, ECKENRODE SE, MCINDOE RA *et al.*: Early pathogenic events associated with Sjögren's syndrome (SjS)-like disease of the NOD mouse using microarray analysis. *Lab Invest* 2006; 86: 1243-60.
33. TISSARI J, SIREN J, MERI S, JULKUNEN I, MATIKAINEN S: IFN-alpha enhances TLR3-mediated antiviral cytokine expression in human endothelial and epithelial cells by up-regulating TLR3 expression. *J Immunol* 2005; 174: 4289-94.
34. LI J, YE L, WANG X, HU S, HO W: Induction of interferon-gamma contributes to Toll-like receptor 3-mediated herpes simplex virus type 1 inhibition in astrocytes. *J Neurosci Res* 2012; 90: 399-406.
35. WOLK K, WITTE K, WITTE E *et al.*: Maturing dendritic cells are an important source of IL-29 and IL-20 that may cooperatively increase the innate immunity of keratinocytes. *J Leukoc Biol* 2008; 83: 1181-93.
36. OSTERLUND P, VECKMAN V, SIREN J *et al.*: Gene expression and antiviral activity of alpha/beta interferons and interleukin-29 in virus-infected human myeloid dendritic cells. *J Virol* 2005; 79: 9608-17.
37. NOCTURNE G, MARIETTE X: Advances in understanding the pathogenesis of primary Sjögren's syndrome. *Nat Rev Rheumatol* 2013; 9: 544-56.
38. LEE YJ, SCOFIELD RH, HYON JY *et al.*: Salivary chemokine levels in patients with primary Sjögren's syndrome. *Rheumatology (Oxford)* 2010; 49: 1747-52.
39. YOON KC, PARK CS, YOU IC *et al.*: Expression of CXCL9, -10, -11, and CXCR3 in the tear film and ocular surface of patients with dry eye syndrome. *Invest Ophthalmol Vis Sci* 2010; 51: 643-50.
40. HERNANDEZ-MOLINA G, MICHEL-PEREGRINA M, HERNANDEZ-RAMIREZ DF, SANCHEZ-GUERRERO J, LLORENTE L: Chemokine saliva levels in patients with primary Sjögren's syndrome, associated Sjögren's syndrome, pre-clinical Sjögren's syndrome and systemic autoimmune diseases. *Rheumatology (Oxford)* 2011; 50: 1288-92.
41. FREEMAN J, BAGLINO S, FRIBORG J *et al.*: Pegylated interferons Lambda-1a and alfa-2a display different gene induction and cytokine and chemokine release profiles in whole blood, human hepatocytes and peripheral blood mononuclear cells. *J Viral Hepat* 2014; 21: e1-9.
42. WOLK K, WITTE K, WITTE E *et al.*: IL-29 is produced by T(H)17 cells and mediates the cutaneous antiviral competence in psoriasis. *Sci Transl Med* 2013; 5: 204ra129.
43. ALUNNO A, BISTONI O, BARTOLONI E *et al.*: IL-17-producing CD4-CD8- T cells are expanded in the peripheral blood, infiltrate salivary glands and are resistant to corticosteroids in patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2013; 72: 286-92.
44. LIN X, RUI K, DENG J *et al.*: Th17 cells play a critical role in the development of experimental Sjögren's syndrome. *Ann Rheum Dis* 2015; 74: 1302-10.
45. GROOM J, KALLED SL, CUTLER AH *et al.*: Association of BAFF/BlyS overexpression and altered B cell differentiation with Sjögren's syndrome. *J Clin Invest* 2002; 109: 59-68.
46. COCCIA EM, SEVERA M, GIACOMINI E *et al.*: Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells.

- Eur J Immunol* 2004; 34: 796-805.
47. DOYLE SE, SCHRECKHISE H, KHUU-DUONG K *et al.*: Interleukin-29 uses a type I interferon-like program to promote antiviral responses in human hepatocytes. *Hepatology* 2006; 44: 896-906.
48. NANDULA SR, DEY P, CORBIN KL, NUNEMAKER CS, BAGAVANT H, DESHMUKH US: Salivary gland hypofunction induced by activation of innate immunity is dependent on type I interferon signaling. *J Oral Pathol Med* 2013; 42: 66-72.
49. LI Y, CHENG H, ZUO XB *et al.*: Association analyses identifying two common susceptibility loci shared by psoriasis and systemic lupus erythematosus in the Chinese Han population. *J Med Genet* 2013; 50: 812-8.
50. ANK N, IVERSEN MB, BARTHOLDY C *et al.*: An important role for type III interferon (IFN- $\lambda$ /IL-28) in TLR-induced antiviral activity. *J Immunol* 2008; 180: 2474-85.