

Original Article



Type I Interferon Increases Inflammasomes Associated Pyroptosis in the Salivary Glands of Patients with Primary Sjögren's Syndrome

Seung-Min Hong ¹, Jaeseon Lee ¹, Se Gwang Jang ¹, Jennifer Lee ^{1,2},
Mi-La Cho ¹, Seung-Ki Kwok ^{1,2}, Sung-Hwan Park ^{1,2,*}

¹Rheumatism Research Center, Catholic Research Institute of Medical Science, College of Medicine, The Catholic University of Korea, Seoul, Korea

²Division of Rheumatology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea



Received: Apr 3, 2020
Revised: Aug 11, 2020
Accepted: Aug 21, 2020

*Correspondence to

Sung-Hwan Park, MD, PhD

Division of Rheumatology, Department of Internal Medicine, College of Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea.
E-mail: rapark@catholic.ac.kr

Copyright © 2020. The Korean Association of Immunologists

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID iDs

Seung-Min Hong
<https://orcid.org/0000-0002-5289-8487>
Jaeseon Lee
<https://orcid.org/0000-0003-2549-0889>
Se Gwang Jang
<https://orcid.org/0000-0003-0518-5975>
Jennifer Lee
<https://orcid.org/0000-0001-6873-6629>
Mi-La Cho
<https://orcid.org/0000-0001-5715-3989>
Seung-Ki Kwok
<https://orcid.org/0000-0002-6142-8364>
Sung-Hwan Park
<https://orcid.org/0000-0003-1711-2060>

<https://immunenetw.org>

ABSTRACT

Sjögren's syndrome (SS) is a chronic and systemic autoimmune disease characterized by lymphocytic infiltration in the exocrine glands. In SS, type I IFN has a pathogenic role, and recently, inflammasome activation has been observed in both immune and non-immune cells. However, the relationship between type I IFN and inflammasome-associated pyroptosis in SS has not been studied. We measured IL-18, caspase-1, and IFN-stimulated gene 15 (ISG15) in saliva and serum, and compared whether the expression levels of inflammasome and pyroptosis components, including absent in melanoma 2 (AIM2), NLR family pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein (ASC), caspase-1, gasdermin D (GSDMD), and gasdermin E (GSDME), in minor salivary gland (MSG) are related to the expression levels of type I IFN signature genes. Expression of type I IFN signature genes was correlated with mRNA levels of caspase-1 and GSDMD in MSG. In confocal analysis, the expression of caspase-1 and GSDMD was higher in salivary gland epithelial cells (SGECs) from SS patients. In the type I IFN-treated human salivary gland epithelial cell line, the expression of caspase-1 and GSDMD was increased, and pyroptosis was accelerated in a caspase-dependent manner upon inflammasome activation. In conclusion, we demonstrate that type I IFN may contribute to inflammasome-associated pyroptosis of the SGECs of SS patients, suggesting another pathogenic role of type I IFN in SS in terms of target tissue -SGECs destruction.

Keywords: Sjogren's Syndrome; Pyroptosis; Inflammasomes; Type I interferon; Salivary gland epithelial cell (SGEC)

INTRODUCTION

Sjögren's syndrome (SS) is a systemic autoimmune disease that causes xerophthalmia and xerostomia, and lymphomagenesis in particular. Salivary gland and lacrimal gland epithelia are attacked by infiltrating leukocytes, including T cells and B cells, which leads to glandular dysfunction and apoptosis (1).

Conflicts of Interests

The authors declare no potential conflicts of interest.

Abbreviations

AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein; BAFF, B-cell activating factor; cDNA, complementary DNA; GSDMD, gasdermin D; GSDME, gasdermin E; HMGB1, high mobility group box 1; HSG, human salivary gland epithelial cell line; ISG15, IFN-stimulated gene 15; MSG, minor salivary gland; NET, neutrophil extracellular trap; NLRP3, NLR family pyrin domain containing 3; PI, propidium iodide; qPCR, quantitative PCR; RIPA, radioimmunoprecipitation assay; SGEC, salivary gland epithelial cell; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome.

Author Contributions

Conceptualization: Hong SM, Park SH; Investigation: Hong SM, Jang SG; Methodology: Hong SM, Lee J¹; Project administration: Hong SM, Lee J¹, Jang SG, Lee J², Cho ML, Kwok SK, Park SH; Supervision: Lee J¹, Lee J², Cho ML, Kwok SK, Park SH; Visualization: Hong SM; Writing - original draft: Hong SM; Writing - review & editing: Lee J¹, Lee J², Cho ML, Kwok SK, Park SH.

¹Lee J, Jaeseon Lee; ²Lee J, Jennifer Lee.

There have been many studies of the association between the level of type I IFN and SS pathogenesis. For example, type I IFN signature gene expressions were correlated with the levels of anti-Ro/SSA and anti-La/SSB autoantibodies (1). Furthermore, type I IFN stimulation induced B-cell activating factor (BAFF) expression on monocytes and salivary gland epithelial cells (1). Type I IFNs have a pathogenic role in other autoimmune diseases including SLE (2), whereupon drugs targeting type I IFN signaling (JAK-STAT signals) were recently shown to be effective in several animal studies (3,4). Tofacitinib (JAK1/3 inhibitor) treatment alleviated the symptoms of systemic lupus erythematosus (SLE) by reducing numbers of CD8⁺ T cells, double-negative T cells, and the formation of neutrophil extracellular trap (NET) (3). Filgotinib (JAK1 inhibitor) suppressed IFN-induced BAFF in primary salivary gland epithelial cells from SS patients and mitigated salivary flow rate and lymphocytic infiltration in the salivary glands of an SS animal model (4). Furthermore, in clinical trials, JAK inhibitors targeting type I IFN signaling are being investigated for immune-mediated inflammatory diseases (5).

Inflammasome is activated by several factors (6). Once inflammasome is activated, apoptosis-associated speck-like protein (ASC) specks are generated and pro-caspase-1 is proteolytically cleaved (6). Activated caspase-1 cleaves IL-1 β or IL-18 into the mature form, which is secreted from the cells (6). Activated caspase-1 also cleaves gasdermin D (GSDMD), and GSDMD membrane pore drives pyroptosis, a type of cell death, which leads to the secretion of inflammatory cytokines and DAMPs (6). Recently, there have been many reports of increased NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in rheumatoid arthritis, systemic sclerosis, SLE and SS (7). An increase in NLRP3 inflammasome activation was also observed in minor salivary glands, peripheral blood, tears, and the ocular surface from SS patients (8-11). In salivary gland epithelial cells (SGECs) from SS patients, activated absent in melanoma 2 (AIM2) inflammasome is observed (12).

Type I IFN is known to positively or negatively affect inflammasome activity depending on context (13-16). As already noted, type I IFN and inflammasomes have been well studied in SS, but their relationship has not been investigated. Here we present, for the first time, the association between type I IFN and inflammasome-associated pyroptosis of salivary glands in SS patients.

MATERIALS AND METHODS

Patients

Patients were diagnosed as having primary SS according to either the American-European Consensus Group criteria (17) or the 2012 American College of Rheumatology classification criteria (18). Informed consent was obtained from all patients in accordance with the principles of the Declaration of Helsinki. Serum/saliva and/or minor salivary gland (MSG) biopsy tissues were obtained from control patients with sicca symptoms as well as from patients with primary SS. Serum/saliva were also obtained from age- and sex-matched healthy volunteers serving as controls. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (approval No. KC13ONMIO646).

Cell stimulation

Human salivary gland epithelial cell line (HSG) was maintained in DMEM (Gibco™, Thermo Fisher Scientific, Waltham, MA, USA) containing 10% FBS (Thermo Fisher Scientific). HSG cells were stimulated with 1,000 U/ml recombinant IFN- α (R&D Systems, Minneapolis,

MN, USA) or 1,000 U/ml IFN- β (R&D Systems) in DMEM containing 10% FBS. For AIM2 inflammasome activation, HSG cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) reagents in Opti-MEM media (Thermo Fisher Scientific). After washing with Opti-MEM media, cells were stimulated with poly(dA:dT) 2 μ g/ml (Invitrogen) for 6 h to 2 days in Opti-MEM media. For NLRP3 inflammasome, HSG cells were stimulated with Nigericin 10 μ M (Sigma-Aldrich, St. Louis, MO, USA) for 12 h to 2 days in DMEM media (Thermo Fisher Scientific). Culture supernatants were collected, and cells were used for western blot or flow cytometry analysis. Caspase-1 inhibitors, VX765 (InvivoGen, San Diego, CA, USA), Caspase-3 inhibitor, Z-DEVD-FMK (R&D Systems), and Caspase-8 inhibitor, Z-IETD-FMK (R&D Systems) were pretreated for 1 h before poly(dA:dT) and Nigericin stimulation.

Confocal microscopy

Tissue sections were immersed in antigen retrieval solution containing citrate (Dako, Glostrup, Denmark), then heated to 115°C for 15 min in a TintoRetriever pressure cooker (Bio SB, Santa Barbara, CA, USA). Tissues were stained with antibodies to Caspase-1 (Abcam, Cambridge, MA, USA), GSDMD (Proteintech, Chicago, IL, USA) and Cytokeratin 19 (Biolegend, San Diego, CA, USA) at 4°C for overnight, followed by incubation with secondary antibodies conjugated to Alexa Fluor 488 and Alexa Fluor 594 (Invitrogen). Nuclei were stained with DAPI (Invitrogen). Isotype control staining was conducted via probing with rabbit/mouse IgG rather than with primary antibodies. Confocal images were acquired using an LSM 710 confocal microscope (Zeiss, Oberkochen, Germany).

Real-time quantitative PCR (qPCR)

Total RNA of HSG cells or tissues was collected using RNAiso Plus reagent (Takara, Kusatsu, Japan). Up to 2 μ g of total RNA was converted to complementary DNA (cDNA) using a Transcriptor First-Strand cDNA Synthesis kit (Roche, Basel, Switzerland). A LightCycler 96 instrument (Roche) was used for PCR amplification and analysis. All reactions were performed using a SYBR Green I Master kit according to the manufacturer's instructions. Primers were designed using the web tool from GenScript (<http://www.genscript.com>). Primer sequences were as follows: human *GAPDH*, 5'-CCCCACTTGATTTTGAGGGA-3' (forward) and 5'-AGGGCTGCTTTAACTCTGGT-3' (reverse); *GSDMD*, 5'-GTGTACGTGGTACTGAGGT-3' (forward) and 5'-CCTCTGCTTCTATCCGGA-3' (reverse); *GSDME*, 5'-GGAGGATGGGAATGTCACCA-3' (forward) and 5'-TCTTGTCTCGAAGCCACCT-3' (reverse); *IFI44L*, 5'-CAGCTGTACCCTCCCACAAT-3' (forward) and 5'-TGCCTACTGCACTTCCTG-3' (reverse); *IFIT3*, 5'-GGGCAGACTCTCAGATGCTC-3' (forward) and 5'-CAGTTGTGTCCACCCTTCCT-3' (reverse); *RSAD2*, 5'-TTTCTGAAGCGAGGAGGAAA-3' (forward) and 5'-TTTTCCCACTGGTGTGTGA-3' (reverse); *ISG15*, 5'-ACTCATCTTTGCCAGTACAG-3' (forward) and 5'-CAGCTCTGACACCGACATG-3' (reverse); AIM2, 5'-TGACAACTTTGGGATCAGCCT-3' (forward) and 5'-TGGGCATGCTCTCCTGAGTCCT-3' (reverse); *CASP1*, 5'-AAATCGAACCTTGCAGAAA-3' (forward) and 5'-GCTTTCTGCTCTCCACACC-3' (reverse); *PYCARD*, 5'-TTTTCAAGCTGGCTTTTCGT-3' (forward) and 5'-AGTTTCACACCAGCCTGGAA-3' (reverse); *IL-18*, 5'-GCATCAACTTTGTGGCAATG-3' (forward) and 5'-CCTCTAGGCTGGCTATCTTT-3' (reverse); and *IL-1 β* , 5'-CCCAACTGGTACATCAGCAC-3' (forward) and 5'-GGAAGACACAAATGTCATGG-3' (reverse).

All messenger RNA (mRNA) expression levels were normalized to the expression of *GAPDH*. Relative fold induction was calculated using the equation $2^{-\Delta\Delta Cq}$ or $2^{-\Delta\Delta Cq}$, where Cq is the cycle at which the threshold is crossed, ΔCq is $Cq_{(target)} - Cq_{(GAPDH)}$, and $\Delta\Delta Cq$ is $\Delta Cq_{(stimulated)} - \Delta Cq_{(unstimulated)}$. PCR product quality was monitored using post-PCR melting curve analysis.

ELISA

Serum, saliva, and culture supernatants were used to determine the levels of IL-18, caspase-1, IL-1 β , and ISG15. Human total IL-18 DuoSet ELISA and IL-1 β DuoSet ELISA were purchased from R&D Systems. A Human caspase-1 antibody pair kit was purchased from Abcam. Measurements were performed in accordance with the manufacturer's instructions. ISG15 (Human) ELISA Kit was purchased from Abnova (Taipei, Taiwan).

Pyroptosis assay

Pyroptosis was determined using a propidium iodide (PI) staining method in accordance with the manufacturer's instructions (eBioscience, Thermo Fisher Scientific). Pyroptosis was evaluated by measuring the total amount of PI⁺ cells in 3 independent experiments.

Western blot analysis

Total cellular protein was extracted using radioimmunoprecipitation assay (RIPA) buffer containing Halt protease/phosphatase inhibitor cocktail (Thermo Fisher Scientific). For immunoblotting, 20 μ g of protein was separated using 10% and 12% SDS-PAGE, then transferred onto a polyvinylidene fluoride membrane (Bio-Rad, Hercules, CA, USA) and probed with the following antibodies: anti-caspase-1 p48 (Cell Signaling Technology, Danvers, MA, USA), anti-caspase-1 p20 (R&D Systems), anti-GSDMD p53/p32 (Proteintech), anti-AIM2 p39 (Abcam), anti-NLRP3 p110 (Cell Signaling Technology), anti-ASC p23 (Cell Signaling Technology), anti-IL-18 p24/p18 (R&D Systems), anti-alpha amylase p57 (Abcam), anti-GAPDH p36 (Abcam), and anti- β -actin p42 (Sigma-Aldrich). Subsequently, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Thermo Fisher Scientific) or goat anti-mouse IgG (Thermo Fisher Scientific) or mouse anti-goat IgG (Santa Cruz Biotechnology, Dallas, Texas, USA). Reactive proteins on the membrane were visualized using SuperSignal West Pico Chemiluminescent substrate (Thermo Fisher Scientific), and the membrane was exposed to an Amersham Imager 600 (GE Healthcare, Chicago, IL, USA).

Statistical analysis

Statistical analyses were performed using GraphPad Prism software, version 5. Statistical significance was determined by the Mann-Whitney *U* test or one-way ANOVA with Tukey's multiple comparison test. Correlations were analyzed by Spearman's rank correlation. The *p*-values less than 0.05 were considered significant.

RESULTS

IL-18, cleaved-caspase-1, and ISG15 secretion are increased in SS patients

To verify inflammasome activation and type I IFN expression in SS patients, levels of IL-18, IL-1 β , caspase-1, and ISG15 were measured in serum/saliva from SS and healthy controls by ELISA (**Fig. 1A and B**). Consistent with previous findings, we confirmed that secretions of IL-18 were significantly increased in saliva/serum (**Fig. 1A**). Caspase-1 secretion was significantly increased in saliva from SS patients compared with controls (**Fig. 1A**). Western blot was performed in saliva to analyze the levels of cleaved caspase-1 (**Fig. 1C**). Cleaved caspase-1 was increased in SS patients compared with controls (**Fig. 1C and D**). Also, ISG15 which is known to be secreted by type I IFN stimulation (19), was increased in saliva and serum from SS patients compared with healthy controls (**Fig. 1A and B**). These findings confirm that inflammasome activation and type I IFN expression were present in our study population.

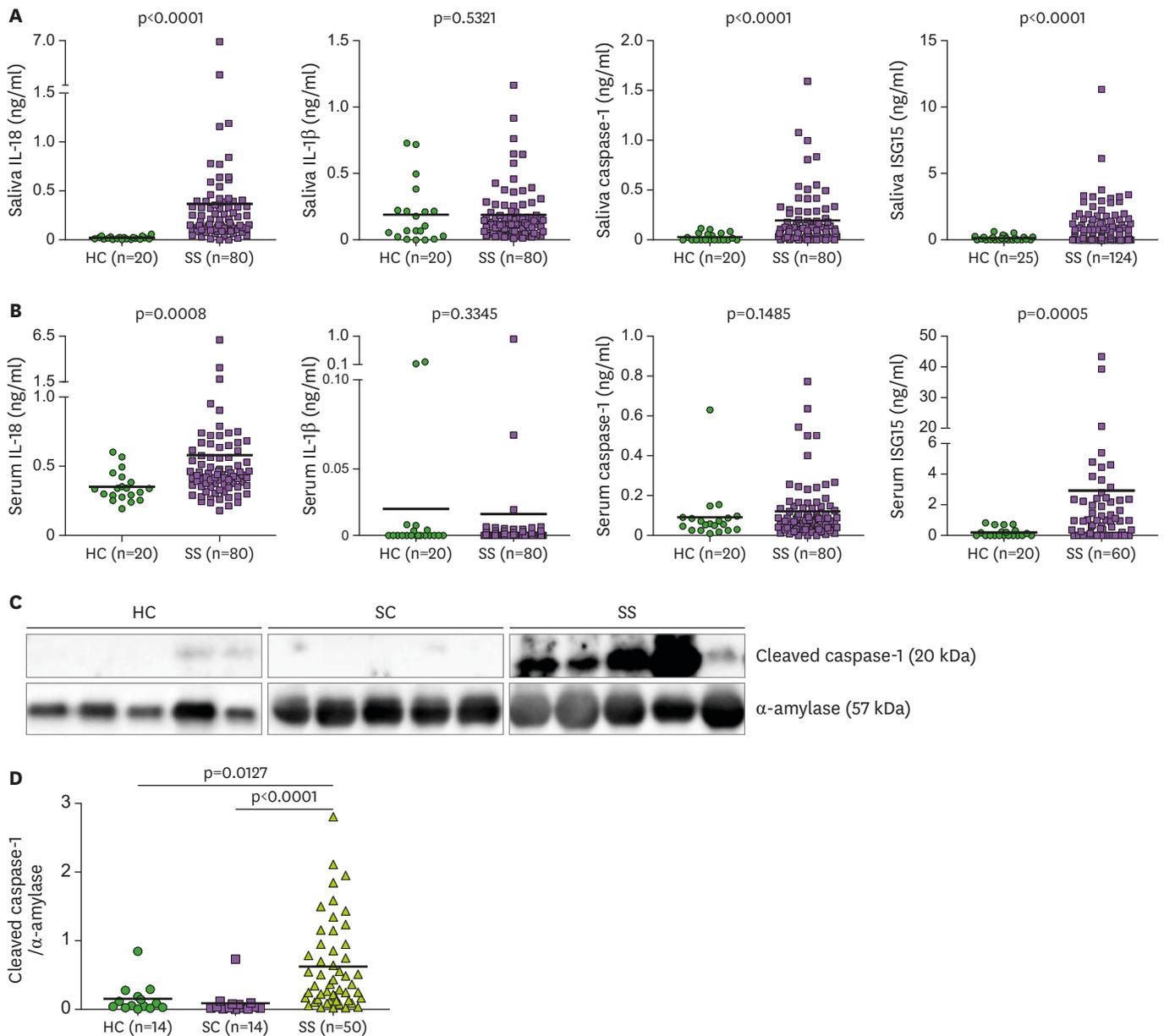


Figure 1. IL-18, cleaved-caspase-1, and ISG15 secretion are increased in SS patients.

IL-18, IL-1 β , caspase-1, and ISG15 from saliva (A) and serum (B) were measured by ELISA. Bars show the mean value. (C) Cleaved caspase-1 from saliva was measured by western blotting. (D) Graphical presentation shows the relative abundance of cleaved caspase-1 after normalization with α -amylase. Bars show the mean value. HC, healthy control; SC, sicca control.

Caspase-1 and GSDMD expression are positively correlated with type I IFN signature genes in the MSG of SS patients

Next, we examined if the salivary gland cells of the SS patients showed increased pyroptosis, which may contribute to gland dysfunction. The mRNA expressions of inflammasome (*CASP1*, *AIM2*, *NLRP3*, *IL18*, *IL1B*, *PYCARD*; PYD and CARD Domain Containing protein) and pyroptosis (*GSDMD*, gasdermin E [*GSDME*]) components from MSG were analyzed (Fig. 2A). We observed that the expressions of *CASP1*, *GSDMD*, *AIM2*, and *IL18* were significantly increased in SS patients compared with sicca controls (Fig. 2A). In addition, the expressions of type I IFN signature genes (*IFI44L*; Interferon induced protein 44 like, *IFIT3*; Interferon induced protein

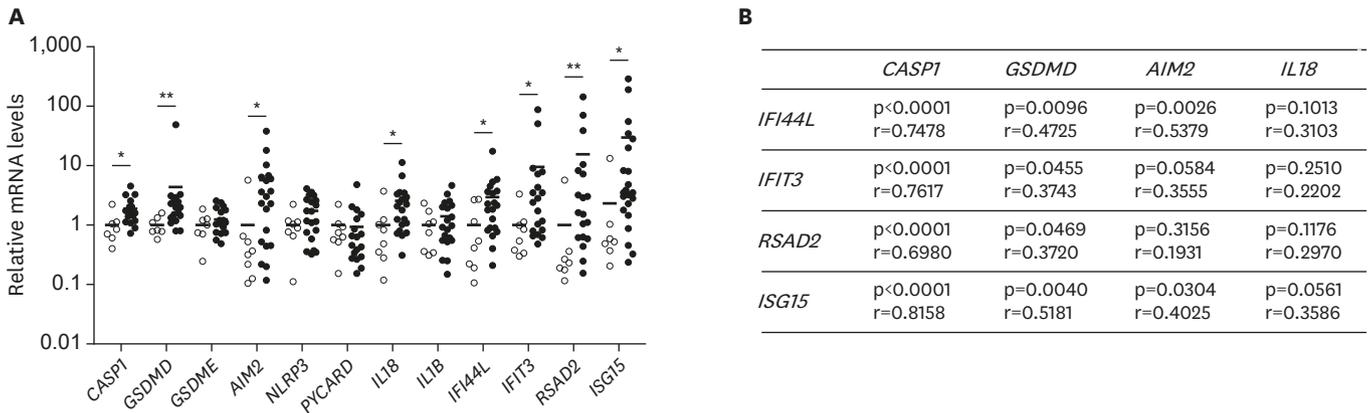


Figure 2. Caspase-1 and GSDMD expression are positively correlated with type I IFN signature genes from MSG. (A) mRNA expressions of inflammasome components (*CASP1*, *AIM2*, *NLRP3*, *PYCARD*, *IL18*, *IL1B*), pyroptosis components (*GSDMD*, *GSDME*), and type I IFN signature genes (*IFI44L*, *IFIT3*, *RSAD2*, *ISG15*) in the MSG of SS patients (closed circles, n=18–20) or sicca controls (open circles, n=7–8) were analyzed by qPCR. Bars show the mean value. (B) Correlation data between type I IFN signature genes and both inflammasome and pyroptosis components, statistically evaluated by Spearman's rank correlation. *p<0.05; **p<0.01.

with tetratricopeptide repeats 3, *RSAD2*; radical SAM domain-containing 2, *ISG15*) in SS were also increased (Fig. 2A). Correlation analysis between the expressions of type I IFN signature genes and both inflammasome and pyroptosis components indicated that the expressions of *CASP1* and *GSDMD* were correlated with *IFI44L*, *IFIT3*, *RSAD2*, and *ISG15* (Fig. 2B).

Caspase-1 and GSDMD expression are elevated in SGECs from SS patients

To verify the mRNA expression results, caspase-1 and GSDMD protein expression were analyzed by confocal staining in salivary glands from SS patients and sicca controls (Fig. 3A and B). Intriguingly, caspase-1 and GSDMD were not only expressed in infiltrated leukocytes, but also in SGECs (Fig. 3A and B). Furthermore, expressions of caspase-1 and GSDMD in SGECs were higher in SS patients (Fig. 3A–D).

Type I IFN stimulation increases caspase-1 and GSDMD expression in SGECs

To examine the relationship between type I IFN and both inflammasome and pyroptosis in vitro, we performed experiments using an immortalized human salivary gland epithelial cell line (HSG cells). Although there was a study showing that HSG cells were contaminated with Hela cells (20), confocal staining with the SGEC marker identified that our HSG cells consisted only of SGECs (data not shown). mRNA levels of *CASP1*, *GSDMD*, and *AIM2* were significantly increased by type I IFN stimulation in HSG cells (Fig. 4A). However, at the protein level, only caspase-1 and GSDMD were upregulated by type I IFN stimulation (Fig. 4B). Furthermore, the increased caspase-1 expression by type I IFN was inhibited by Jak1 inhibitor (Supplementary Fig. 1), Filgotinib which had previously been shown to inhibit IFN signaling (4).

Type I IFN stimulation accelerates inflammasome activation of SGECs

To observe the extent of inflammasome activation in type I IFN-stimulated SGECs, HSG cells were pretreated with or without type I IFN and stimulated with poly(dA:dT) for AIM2 inflammasome activation and stimulated with Nigericin for NLRP3 inflammasome activation (Fig. 5A–F). Stimulation with poly(dA:dT) or Nigericin after type I IFN exposure resulted in greater IL-18 secretion and acceleration of pyroptosis (Fig. 5A–F). Furthermore, caspase-1, -3, -8, and GSDMD activation were higher in HSG cells pretreated with type I IFN (Fig. 5A and D). Secretion of IL-18 and pyroptosis were reduced by the caspase-1, caspase-3, and caspase-8 inhibitor (Fig. 5B, C, E,

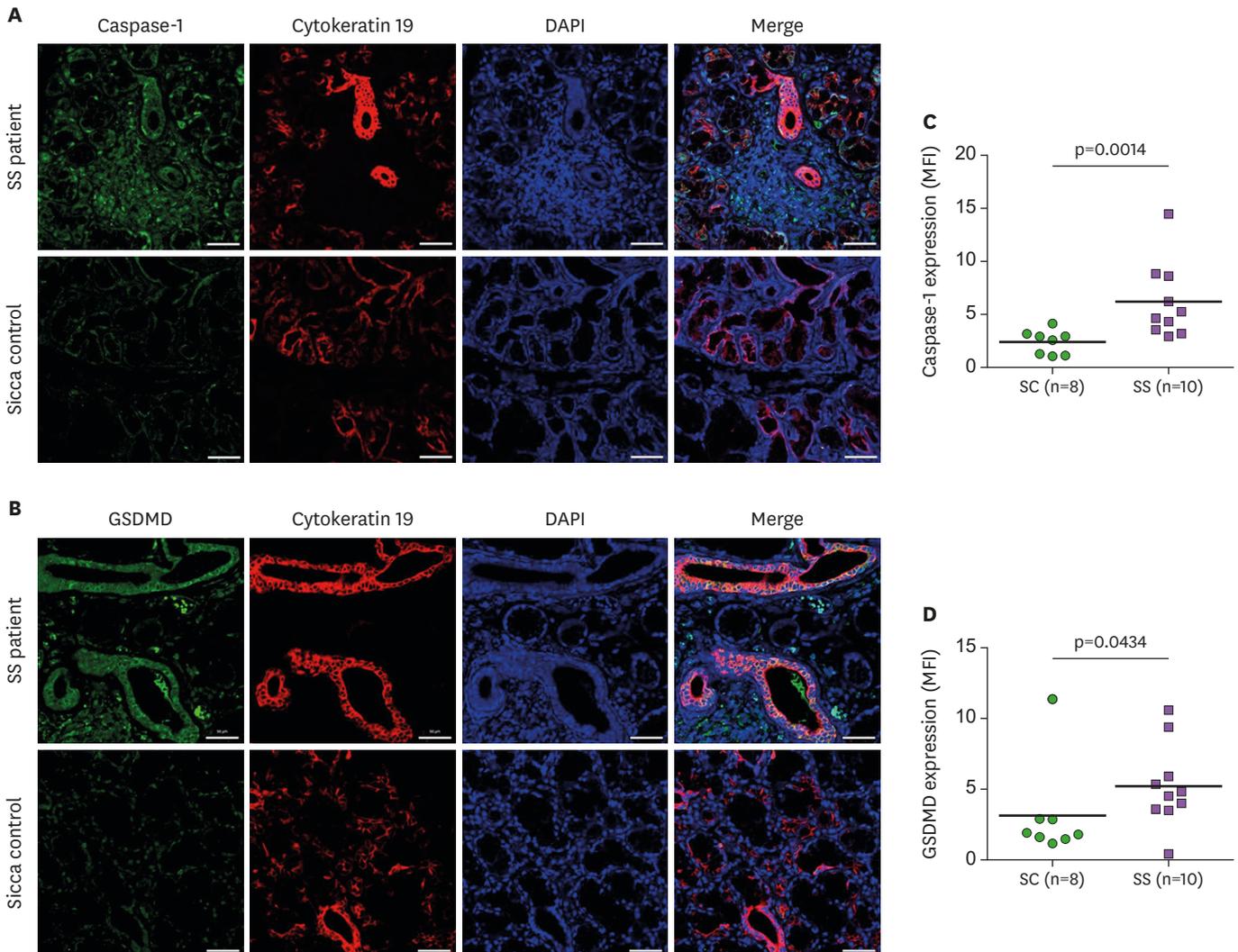


Figure 3. Caspase-1 and GSDMD are increased in salivary gland epithelial cells of SS patients. Confocal microscopy images show immunofluorescent staining for caspase-1 (A) (green) or GSDMD (B) (green), cytokeratin 19 (red), and DAPI (blue) in the MSG of SS patients or sicca controls (scale bar=50 μ m). Expression of caspase-1 (C) and GSDMD (D) in salivary gland epithelial cells were quantified with confocal microscopy, and the results were expressed as mean fluorescence intensity.

and F). High mobility group box 1 (HMGB1) is a damage-associated molecular pattern released by pyroptosis (6). Extracellular HMGB1 was analyzed in culture supernatants by western blot (Fig. 5A and D). Type I IFN-pretreated HSG cells secreted higher levels of HMGB1. Consequently, our results showed that type I IFNs might accelerate inflammasome activation by increasing the expression of caspase-1 and GSDMD protein in SGECs.

DISCUSSION

In this study, we found that IL-18, cleaved-caspase-1, and ISG15 secretion were increased in SS patients. The expression of caspase-1 and GSDMD was increased in SGECs of SS patients and correlated with the expression of type I IFN signature genes. The results of in vitro experiments also showed that caspase-1 and GSDMD expression were increased by type I IFN treatment in HSG cells. Overall, it signified that the increase of caspase-1 and GSDMD in SGECs of SS patients might be due to type I IFN. In addition, IL-18 secretion and pyroptosis

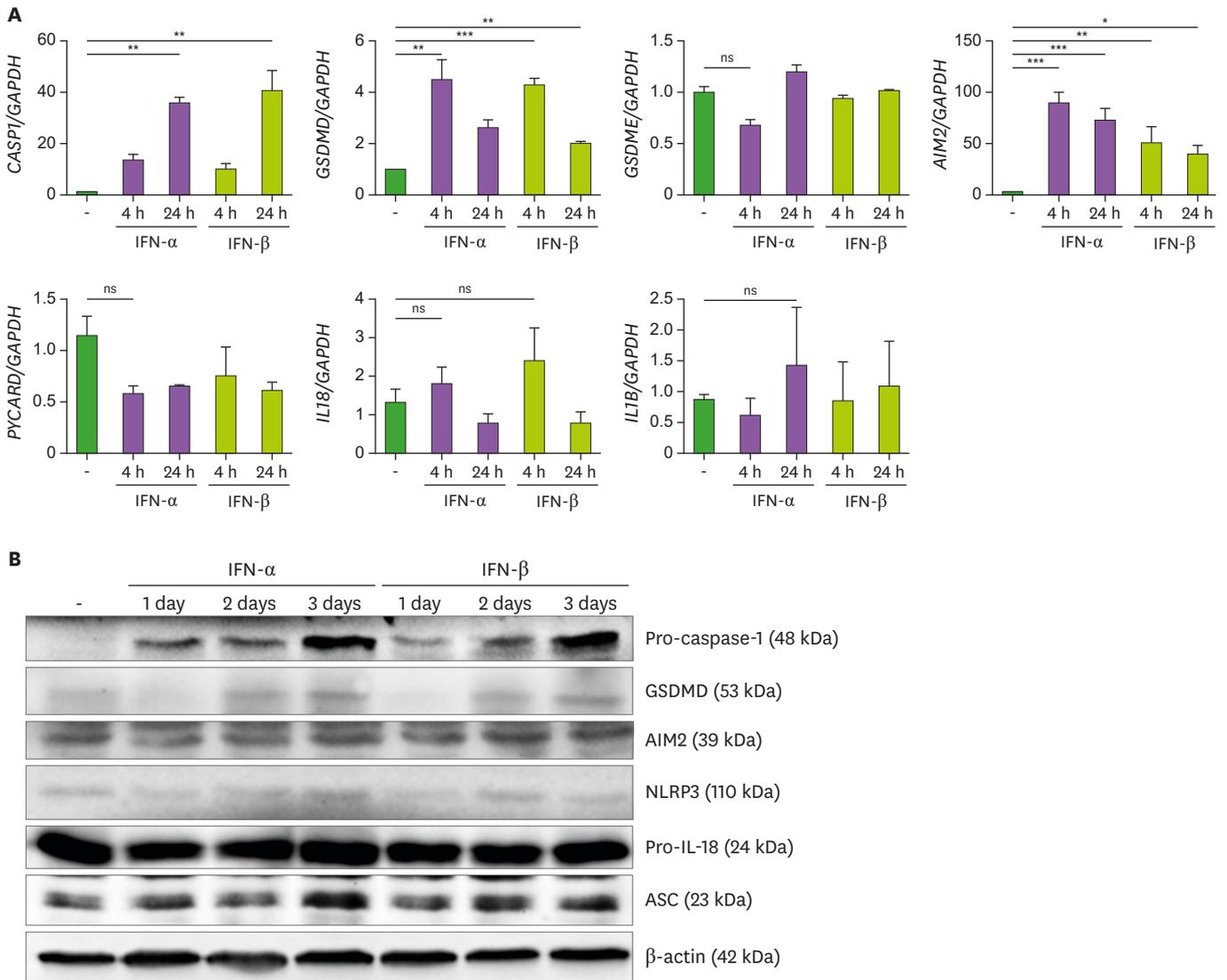


Figure 4. Type I IFN stimulation increases caspase-1 and GSDMD expression in SGECs.

(A) mRNA levels of inflammasome and pyroptosis components were analyzed in HSG cells stimulated with or without 1,000 U/ml IFN- α or IFN- β for 4–24 h using qPCR. (B) Western blot analysis of inflammasome components and GSDMD in lysates of HSG cells stimulated with 1,000 U/ml IFN- α or IFN- β for 1–3 days. β -actin served as a loading control. All data were representative of 3 independent experiments and presented as means \pm SEMs.

ns, not significant.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

were increased by pretreatment of type I IFN (**Fig. 5A**). This might be due to increased caspase-1 and GSDMD by type I IFN.

In our results, the mRNA and protein levels of AIM2 did not match. This meant that there was post-translational mechanism in the regulation of AIM2 protein level. There are 2 different repressor that regulate AIM2 protein, TRIM11 (21) and miR-223 (22). In particular, since miRNA-223 is increased by type I IFN (23), it was possible that miRNA-223 increased by type I IFN regulated AIM2. However, for exact reasons, more experimentation is required.

Our results showed that both AIM2 and NLRP3 inflammasome activity were increased by type I IFN. This suggests that SGEs of SS patients may be susceptible to inflammasome activity

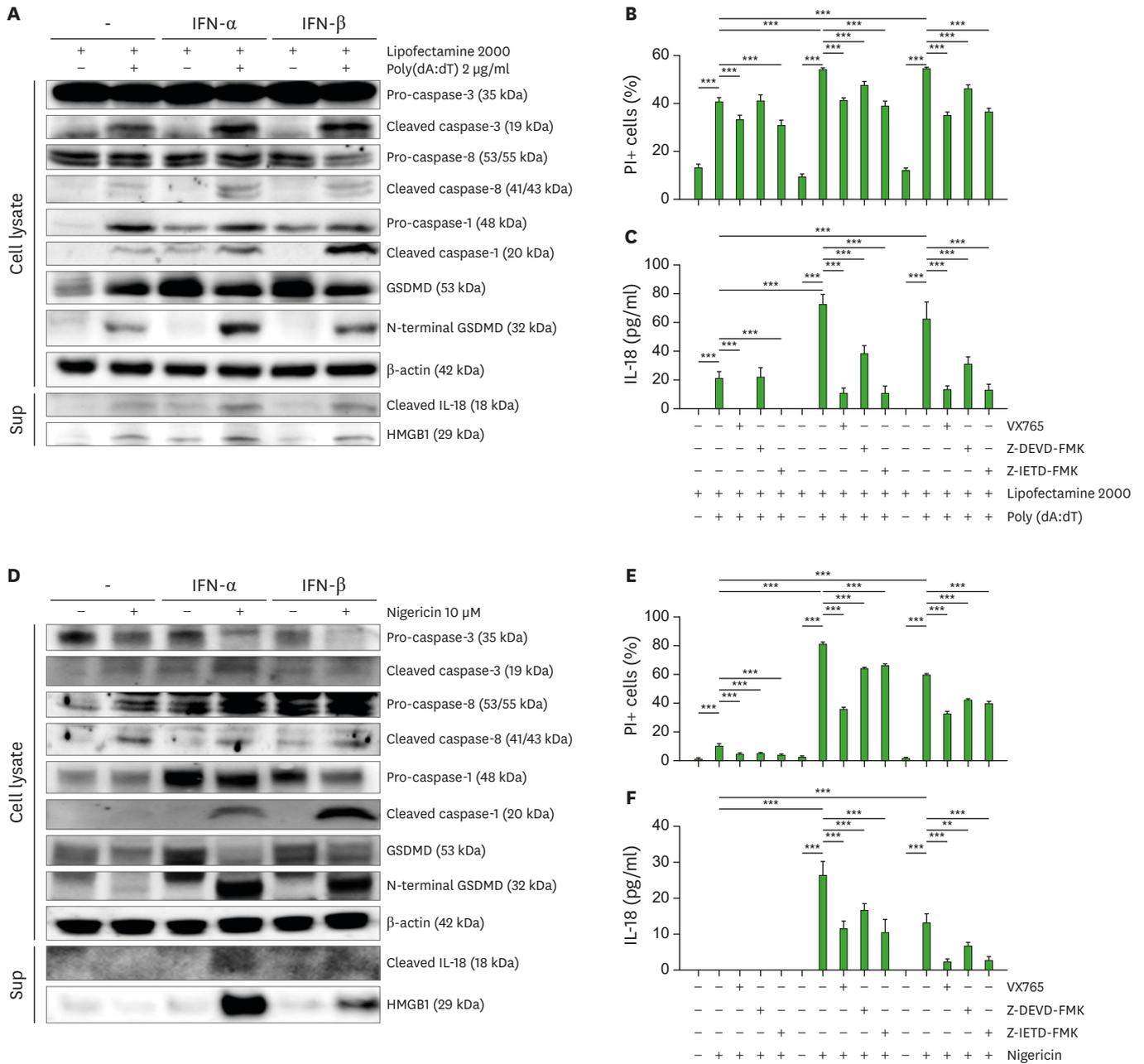


Figure 5. Type I IFN stimulation accelerates inflammasome activation of SGECs. (A) HSG cells were treated with IFN- α or IFN- β for 3 days, followed by stimulation of poly(dA:dT) for 6 h; β -actin served as a loading control. (B) Frequency of PI+ cells of HSG cells treated with IFN- α or IFN- β for 3 days, followed by stimulation of poly(dA:dT) for 2 days. HSG cells were pretreated with or without VX765 (100 μ M), Z-DEVD-FMK (100 μ M), or IETD-FMK (100 μ M) before poly(dA:dT) stimulation. (C) IL-18 levels in supernatant of (B) experiments were analyzed. (D) HSG cells were treated with IFN- α or IFN- β for 3 days, followed by stimulation of Nigericin for 12 h; β -actin served as a loading control. (E) Frequency of PI+ cells of HSG cells treated with IFN- α or IFN- β for 3 days, followed by stimulation of Nigericin for 2 days. HSG cells were pretreated with or without VX765 (100 μ M), Z-DEVD-FMK (100 μ M), or IETD-FMK (100 μ M) before Nigericin stimulation. (F) IL-18 levels in supernatant of (A) experiments were analyzed. All data were representative of 3 independent experiments and presented as means \pm SEMs. ** p <0.01; *** p <0.001.

and pyroptosis. AIM2 inflammasome is known to activate in SGECs of SS patients (12), but NLRP3 inflammasome in SGECs is not known yet. Contrary to AIM2 expression in SGECs, confocal image analysis of salivary gland tissue showed that there was no expression of NLRP3 in SGECs from control and SS patients (12). However, NLRP3 expression is observed

in SGEC lines, which is lower than AIM2 (12). Further research is needed to determine whether the NLRP3 inflammasome occurs in SGECs of SS patients.

Caspase-3 and -8 are well known to cause apoptosis, but they also participate in pyroptosis (24-26). Caspase-8 is involved in forming inflammasome by binding with ASC, activating caspase-1, and cleaving IL-18 (24). In our experimental results, treatment with caspase-1, -3, and -8 inhibitors reduced cell death and IL-18 secretion. Therefore, it meant that caspase-1, -3, and -8 activation were all involved in pyroptosis. Caspase-3 is known to inhibit GSDMD-pore formation (27), and induce pyroptosis through GSDME (25). Further research is needed to determine whether caspase-3 inhibitor regulates GSDME-pore formation, thereby reducing pyroptosis in HSG cells.

There have been several studies of the relationship between type I IFN and both inflammasome activation and pyroptosis (13-16,28,29). Depending on the circumstance, type I IFN enhances or inhibits inflammasome activation (13-16,28,29). For AIM2 inflammasome, type I IFN enhances inflammasome activity by producing the proteins needed to release DNA from bacteria (15). Also, AIM2 inflammasome could be enhanced by type I IFN by increasing the level of AIM2 protein (30). In our results, however, there was no difference in AIM2 protein level in type I IFN-treated HSG cells. Inflammasome activity is also enhanced in SLE patients, and these authors explain that this is due to type I IFN (13). Type I IFN increases the transcription factor IRF-1, and IRF-1 increases caspase-1, which enhances inflammasome activity (13). Type I IFN also increases the GSDMD (28,29), which could potentially contribute to pyroptosis (28). We found that type I IFN increased caspase-1 and GSDMD and enhanced inflammasome activity in *in vitro* experiments using HSGs. Likewise, in SGECs of SS, type I IFN might increase caspase-1 and GSDMD and the possibility of undergoing inflammasome-induced pyroptosis.

Pyroptosis acts not only to protect against external invasion (31), but also plays a pathological role in several diseases (32) including autoimmune diseases (33,34). In lupus, P2X purinoceptor 7 is restrained, which suppresses pyroptosis in T follicular helper cells, thereby increasing survival of T follicular helper cells and contributing to lupus disease (34). MRE11A deficiency in T cells from rheumatoid arthritis causes pyroptosis and induces tissue inflammation (33). There are many studies of inflammasome in SS (8-12), but little is known about pyroptosis. Inflammasome-induced pyroptosis of SGECs may be pathogenic in SS. First, death of saliva secreting cells by pyroptosis can induce xerostomia. Second, the secretion of cytokines and DAMPs can lead to the infiltration and activation of immune cells and induce dysfunction of surrounding SGECs. Thus, pyroptosis in SGECs of SS patients may contribute to SS pathogenesis.

In conclusion, we report here that type I IFN has a relationship with inflammasomes and pyroptosis in SGECs. As *in vitro* experiments showed that type I IFN accelerated inflammasome-induced pyroptosis, type I IFN might lead to inflammasome-induced pyroptosis in the SGECs of SS patients. Our study adds another pathogenic role of type I IFN in SS, suggesting the need for treatment to target type I IFN.

ACKNOWLEDGEMENTS

We thank Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript. This study was supported by a grant from the Korean Health Technology R&D Project of the Ministry of Health & Welfare, Republic of Korea (HI13C0016), and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI15C1062).

SUPPLEMENTARY MATERIAL

Supplementary Figure 1

Jak1 inhibitor regulates caspase-1 expression increased by type I IFN.

[Click here to view](#)

REFERENCES

- Li H, Ice JA, Lessard CJ, Sivils KL. Interferons in Sjögren's syndrome: genes, mechanisms, and effects. *Front Immunol* 2013;4:290.
[PUBMED](#) | [CROSSREF](#)
- Crow MK, Olfertiev M, Kirou KA. Type I interferons in autoimmune disease. *Annu Rev Pathol* 2019;14:369-393.
[PUBMED](#) | [CROSSREF](#)
- Furumoto Y, Smith CK, Blanco L, Zhao W, Brooks SR, Thacker SG, Abdalrahman Z, Sciumè G, Tsai WL, Trier AM, et al. Tofacitinib ameliorates murine lupus and its associated vascular dysfunction. *Arthritis Rheumatol* 2017;69:148-160.
[PUBMED](#) | [CROSSREF](#)
- Lee J, Lee J, Kwok SK, Baek S, Jang SG, Hong SM, Min JW, Choi SS, Lee J, Cho ML, et al. JAK-1 inhibition suppresses interferon-induced BAFF production in human salivary gland: potential therapeutic strategy for primary Sjögren's syndrome. *Arthritis Rheumatol* 2018;70:2057-2066.
[PUBMED](#) | [CROSSREF](#)
- Fragoulis GE, McInnes IB, Siebert S. JAK-inhibitors. New players in the field of immune-mediated diseases, beyond rheumatoid arthritis. *Rheumatology (Oxford)* 2019;58 :i43-i54.
[PUBMED](#) | [CROSSREF](#)
- Kolb JP, Oguin TH 3rd, Oberst A, Martinez J. Programmed cell death and inflammation: winter is coming. *Trends Immunol* 2017;38:705-718.
[PUBMED](#) | [CROSSREF](#)
- Deuteraiou K, Kitas G, Garyfallos A, Dimitroulas T. Novel insights into the role of inflammasomes in autoimmune and metabolic rheumatic diseases. *Rheumatol Int* 2018;38:1345-1354.
[PUBMED](#) | [CROSSREF](#)
- Baldini C, Rossi C, Ferro F, Santini E, Seccia V, Donati V, Solini A. The P2X7 receptor-inflammasome complex has a role in modulating the inflammatory response in primary Sjögren's syndrome. *J Intern Med* 2013;274:480-489.
[PUBMED](#) | [CROSSREF](#)
- Niu L, Zhang S, Wu J, Chen L, Wang Y. Upregulation of nlrp3 inflammasome in the tears and ocular surface of dry eye patients. *PLoS One* 2015;10:e0126277.
[PUBMED](#) | [CROSSREF](#)
- Baldini C, Santini E, Rossi C, Donati V, Solini A. The P2X7 receptor-NLRP3 inflammasome complex predicts the development of non-Hodgkin's lymphoma in Sjogren's syndrome: a prospective, observational, single-centre study. *J Intern Med* 2017;282:175-186.
[PUBMED](#) | [CROSSREF](#)
- Vakrakou AG, Boiu S, Ziakas PD, Xingi E, Boleti H, Manoussakis MN. Systemic activation of NLRP3 inflammasome in patients with severe primary Sjögren's syndrome fueled by inflammagenic DNA accumulations. *J Autoimmun* 2018;91:23-33.
[PUBMED](#) | [CROSSREF](#)

12. Vakrakou AG, Svolaki IP, Evangelou K, Gorgoulis VG, Manoussakis MN. Cell-autonomous epithelial activation of AIM2 (absent in melanoma-2) inflammasome by cytoplasmic DNA accumulations in primary Sjögren's syndrome. *J Autoimmun* 2020;108:102381.
[PUBMED](#) | [CROSSREF](#)
13. Liu J, Berthier CC, Kahlenberg JM. Enhanced inflammasome activity in systemic lupus erythematosus is mediated via type I interferon-induced up-regulation of interferon regulatory factor 1. *Arthritis Rheumatol* 2017;69:1840-1849.
[PUBMED](#) | [CROSSREF](#)
14. Kopitar-Jerala N. The role of interferons in inflammation and inflammasome activation. *Front Immunol* 2017;8:873.
[PUBMED](#) | [CROSSREF](#)
15. Man SM, Karki R, Malireddi RK, Neale G, Vogel P, Yamamoto M, Lamkanfi M, Kanneganti TD. The transcription factor IRF1 and guanylate-binding proteins target activation of the AIM2 inflammasome by Francisella infection. *Nat Immunol* 2015;16:467-475.
[PUBMED](#) | [CROSSREF](#)
16. Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Förster I, Farlik M, Decker T, Du Pasquier RA, Romero P, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity* 2011;34:213-223.
[PUBMED](#) | [CROSSREF](#)
17. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, 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;61:554-558.
[PUBMED](#) | [CROSSREF](#)
18. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, Schiødt M, Umehara H, Vivino F, Zhao Y, et al. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res (Hoboken)* 2012;64:475-487.
[PUBMED](#) | [CROSSREF](#)
19. D'Cunha J, Ramanujam S, Wagner RJ, Witt PL, Knight E Jr, Borden EC. *In vitro* and *in vivo* secretion of human ISG15, an IFN-induced immunomodulatory cytokine. *J Immunol* 1996;157:4100-4108.
[PUBMED](#)
20. Lin LC, Elkashty O, Ramamoorthi M, Trinh N, Liu Y, Sunavala-Dossabhoy G, Pranzatelli T, Michael DG, Chivasso C, Perret J, et al. Cross-contamination of the human salivary gland HSG cell line with HeLa cells: a STR analysis study. *Oral Dis* 2018;24:1477-1483.
[PUBMED](#) | [CROSSREF](#)
21. Liu T, Tang Q, Liu K, Xie W, Liu X, Wang H, Wang RF, Cui J. TRIM11 suppresses AIM2 inflammasome by degrading AIM2 via p62-dependent selective autophagy. *Cell Reports* 2016;16:1988-2002.
[PUBMED](#) | [CROSSREF](#)
22. Yang F, Lou G, Zhou X, Zheng M, He J, Chen Z. MicroRNA-223 acts as an important regulator to Kupffer cells activation at the early stage of Con A-induced acute liver failure via AIM2 signaling pathway. *Cell Physiol Biochem* 2014;34:2137-2152.
[PUBMED](#) | [CROSSREF](#)
23. Chen L, Song Y, He L, Wan X, Lai L, Dai F, Liu Y, Wang Q. MicroRNA-223 promotes type I interferon production in antiviral innate immunity by targeting forkhead box protein O3 (FOXO3). *J Biol Chem* 2016;291:14706-14716.
[PUBMED](#) | [CROSSREF](#)
24. Gurung P, Kanneganti TD. Novel roles for caspase-8 in IL-1 β and inflammasome regulation. *Am J Pathol* 2015;185:17-25.
[PUBMED](#) | [CROSSREF](#)
25. Yu J, Li S, Qi J, Chen Z, Wu Y, Guo J, Wang K, Sun X, Zheng J. Cleavage of GSDME by caspase-3 determines lobaplatin-induced pyroptosis in colon cancer cells. *Cell Death Dis* 2019;10:193.
[PUBMED](#) | [CROSSREF](#)
26. Sarhan J, Liu BC, Muendlein HI, Li P, Nilson R, Tang AY, Rongvaux A, Bunnell SC, Shao F, Green DR, et al. Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during *Yersinia* infection. *Proc Natl Acad Sci U S A* 2018;115:E10888-E10897.
[PUBMED](#) | [CROSSREF](#)
27. Taabazuing CY, Okondo MC, Bachovchin DA. Pyroptosis and apoptosis pathways engage in bidirectional crosstalk in monocytes and macrophages. *Cell Chem Biol* 2017;24:507-514.e4.
[PUBMED](#) | [CROSSREF](#)

28. Li Y, Guo X, Hu C, Du Y, Guo C, Di Wang, Zhao W, Huang G, Li C, Lu Q, et al. Type I IFN operates pyroptosis and necroptosis during multidrug-resistant *A. baumannii* infection. *Cell Death Differ* 2018;25:1304-1318.
[PUBMED](#) | [CROSSREF](#)
29. Kayagaki N, Lee BL, Stowe IB, Kornfeld OS, O'Rourke K, Mirrashidi KM, Haley B, Watanabe C, Roose-Girma M, Modrusan Z, et al. IRF2 transcriptionally induces *GSDMD* expression for pyroptosis. *Sci Signal* 2019;12:eaax4917.
[PUBMED](#) | [CROSSREF](#)
30. Duan X, Ponomareva L, Veeranki S, Panchanathan R, Dickerson E, Choubey D. Differential roles for the interferon-inducible IFI16 and AIM2 innate immune sensors for cytosolic DNA in cellular senescence of human fibroblasts. *Mol Cancer Res* 2011;9:589-602.
[PUBMED](#) | [CROSSREF](#)
31. Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol Rev* 2017;277:61-75.
[PUBMED](#) | [CROSSREF](#)
32. Ma Y, Jiang J, Gao Y, Shi T, Zhu X, Zhang K, Lu K, Xue B. Research progress of the relationship between pyroptosis and disease. *Am J Transl Res* 2018;10:2213-2219.
[PUBMED](#)
33. Li Y, Shen Y, Jin K, Wen Z, Cao W, Wu B, Wen R, Tian L, Berry GJ, Goronzy JJ, et al. The DNA repair nuclease MRE11A functions as a mitochondrial protector and prevents T cell pyroptosis and tissue inflammation. *Cell Metab* 2019;30:477-492.e6.
[PUBMED](#) | [CROSSREF](#)
34. Faliti CE, Gualtierotti R, Rottoli E, Gerosa M, Perruzza L, Romagnani A, Pellegrini G, De Ponte Conti B, Rossi RL, Idzko M, et al. P2X7 receptor restrains pathogenic Tfh cell generation in systemic lupus erythematosus. *J Exp Med* 2019;216:317-336.
[PUBMED](#) | [CROSSREF](#)