Activation of the Interferon Pathway in Peripheral Blood of Patients with Sjögren's Syndrome

OSAMU KIMOTO, JIN SAWADA, KUMIKO SHIMOYAMA, DAISUKE SUZUKI, SATOKI NAKAMURA, HIDEHARU HAYASHI, and NORIYOSHI OGAWA

ABSTRACT. ***Objective.*** DNA microarray analysis and quantitative real-time polymerase chain reaction (PCR) were performed to identify key target genes in peripheral blood from patients with Sjögren's syndrome (SS).

Methods. DNA microarray analysis was performed in 19 patients with SS (all women) and 10 healthy controls (5 men and 5 women) using a low-density DNA microarray system with 778 genes. For confirmation, the expression of upregulated genes was analyzed by quantitative real-time PCR in another 37 SS patients (35 women and 2 men) and 9 healthy controls (8 women and 1 man). Relationships between gene signatures and various clinical measures, such as disease duration, symptoms and signs, complications, immunological findings, and salivary and lacrimal functions, were analyzed.

Results. Interferon-α (IFN-α)-inducible protein 27 (IFI27) showed the most significant difference between SS patients and controls in the microarray screening. We performed quantitative RT-PCR for IFI27. IFI27 gene expression level was increased in patients with SS compared with controls ($p < 0.01$) by real-time PCR, supporting our observations from the microarray data. The level of IFI27 was significantly correlated with serum IgG levels ($r = 0.462$, $p < 0.01$) and $ß_2$-microglobulin ($r = 0.385$, $p < 0.05$), soluble interleukin 2 receptor ($r = 0.473$, $p < 0.01$), erythrocyte sedimentation rate ($r = 0.333$, $p < 0.05$), and antinuclear antibody titer (speckled pattern; $r = 0.445$, $p < 0.01$).

Conclusion. Our results suggest that upregulation of IFN-inducible genes in SS patients is a systemic phenomenon, and IFN may play an important role in the pathogenesis of SS. The expression level of IFI27 could be an effective and specific biomarker associated with SS. (First Release Nov 15 2010; J Rheumatol 2011;38:310–16; doi:10.3899/jrheum.100486)

Key Indexing Terms:
SJÖGREN'S SYNDROME
INTERFERONS
MICROARRAY ANALYSIS
POLYMERASE CHAIN REACTION

Sjögren's syndrome (SS) is characterized by autoimmune exocrinopathy preferentially involving the salivary and lacrimal glands, which causes dry mouth (xerostomia) and dry eyes (xerophthalmia). Extraglandular manifestations are present at varying frequencies and include arthralgia/arthritis, Raynaud's phenomenon, lymphadenopathy, and vasculitis, as well as lung and kidney involvement. Patients with systemic autoimmune disorders other than primary SS, especially rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and dermatomyositis (DM), may also fulfill the criteria for SS and are then defined as secondary SS. The etiology of SS remains unclear, but studies suggest that pathogenesis is associated with both genetic predispositions and environmental factors[1,2].

Recently, gene expression analyses have provided an important perspective on unknown biological phenomena. Microarray analysis for diagnostic and prognostic biomarker signatures was first developed in the field of cancer. Blood profiling studies were extended rapidly to other diseases, and characteristic signatures were reported in patients with autoimmune diseases, including SS, SLE, DM, and RA[3,4]. Global profiling of gene expression in peripheral blood mononuclear cells (PBMC) indicated the upregulation of interferon (IFN)-inducible genes in patients with SLE compared with healthy controls[5]. Pathological overproduction of type I IFN has been noted in SLE and DM[6,7,8]. Further, the magnitude of overexpression of IFN-inducible genes is greater and is correlated with disease activity in SLE and DM[5,9]. In another study, the IFN expression signature was correlated with disease activity of patients with pediatric lupus[10].

From the Third Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan.

Supported by a grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

O. Kimoto, MD; J. Sawada, MD; K. Shimoyama, MD, PhD; D. Suzuki, MD; S. Nakamura, MD, PhD, Assistant Professor; H. Hayashi, MD, PhD, Professor; N. Ogawa, MD, PhD, Senior Assistant Professor, Third Department of Internal Medicine, Hamamatsu University School of Medicine.

Address correspondence to Dr. O. Kimoto, Third Department of Internal Medicine, Hamamatsu University School of Medicine, 1-20-1, Handayama, Higashi-ku, Hamamatsu 431-3192, Japan. E-mail: kimoto@hama-med.ac.jp

Accepted for publication September 20, 2010.

It recently became apparent that SS is also associated with activation of the type I IFN pathway. cDNA microarray analysis revealed that numerous type I IFN-inducible genes were highly expressed in SS salivary glands[11,12,13]. Further, the IFN pathway and inflammation-related genes, including the Toll-like receptor (TLR) family, T-cell receptor, STAT-1, and IP-10, are overexpressed in the SS salivary gland[11,12,14,15]. IFN production is detected in both sera and salivary gland biopsy specimens[16,17]. These findings suggest that IFN-related genes could be useful biomarkers in SS. However, it would be better to use more easily accessible materials than salivary glands for screening.

We examined whether expression levels of these genes are correlated with severity of the disease. We attempted to identify potential biomarkers associated with SS using peripheral blood of Japanese patients with SS.

MATERIALS AND METHODS

Patients and controls. Blood was obtained from 19 SS patients (all women), 10 healthy controls (5 men and 5 women), and 10 disease controls (10 patients with RA) for DNA microarray analysis. Of the 19 SS patients, 5 had secondary SS [SLE 1, scleroderma (SSc) 4] and 2 had been treated with low-dose corticosteroids. For real-time polymerase chain reaction (PCR), another 37 SS patients (35 women and 2 men; no overlap with the microarray group) and 9 healthy controls (8 women and 1 man) were analyzed. Of the 37 patients with SS, 9 had secondary SS (RA 5, mixed connective tissue disease 2, SSc 2) and 8 had been treated with corticosteroids. The profiles of SS patients and controls are shown in Tables 1 and 2. Healthy volunteers with no history of serious illness during the previous 6 months were also recruited as controls. Written informed consent was obtained from all participants. This study was performed in accord with the guidelines of the Independent Ethical Committee of Hamamatsu University School of Medicine, which are consistent with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects.

All SS patients fulfilled the 1999 revised Japanese criteria for classification of SS[18]. All RA patients fulfilled the American College of Rheumatology 1987 revised criteria for RA[19].

cDNA microarray analysis. Total RNA was isolated from 2.5 ml whole blood with a PAXgene Blood RNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. We prepared a low-density DNA microarray for mRNA expression profiling in whole blood. Genes for this microarray were selected from the public database of SAGE results (Department of Molecular Preventive Medicine, School of Medicine, The University of Tokyo [Japanese]; http://133.11.248.12) from activated blood cells, such as T cells, dendritic cells, monocytes, and macrophages. Microarray analysis was performed using a DNA microarray system (Genomessage V2, GEO accession no. GPL5460; Japan Genome Solutions, Tokyo, Japan) with 778 genes. Reference RNA was established from a mixture of whole-blood RNA samples from healthy volunteer donors. Competitive hybridization of Cy3-labeled reference and Cy5-labeled sample cDNA on the microarray was carried out with chamber systems (Agilent Technologies, Palo Alto, CA, USA). The Cy5/Cy3 ratio for each mRNA signal was calculated. The relationships of gene signature with various clinical measures, such as disease duration, symptoms and signs, complications, immunological findings, and salivary and lacrimal functions, were analyzed.

Table 1. Clinical features of 19 patients with SS (all women) and 10 healthy controls (5 men and 5 women) examined by DNA microarray analysis.

Characteristic	SS Patients, n = 19	Controls, n = 10
Sex	19 women	5 women, 5 men
Age, yrs	60.8 ± 10.3	39.3
Primary/secondary disease	14/5 (SLE 1, SSc 4)	
Saxon test, g per 2 min	0.70 ± 0.46	
Focus score	4.8 ± 3.4	
IgG, mg/dl	1878.4 ± 569.1	
Anti-Ro/SSA-positive, n (%)	16 (84.2)	
Anti-La/SSB-positive, n (%)	7 (36.8)	

Table 2. Clinical features of 37 patients with SS (35 women and 2 men) and 9 healthy controls (8 women and 1 man) analyzed by quantitative real-time PCR.

Characteristic	SS Patients, n = 37	Controls, n = 9
Sex	35 women, 2 men	8 women, 1 man
Age, yrs	58.9 ± 14.3	40.4 ± 6.4
Primary/secondary disease	28/9 (RA 5 MCTD 2 SSc 2)	
Saxon test, g per 2 min	0.97 ± 1.3	
Focus score	5.4 ± 4.7	
Steroid therapy (%)	8/37 (21.6)	
ESR, mm/h	37.7 ± 23.0	
IgG, mg/dl	2095.3 ± 765.5	
sIL-2R, U/ml	711.5 ± 568.1	
ß$_2$ MG, μg/ml	2.4 ± 0.70	
Anti-Ro/SSA-positive, n (%)	35 (97.2)	
Anti-La/SSB-positive, n (%)	14 (14.0)	

ESR: erythrocyte sedimentation rate; sIL-2R: soluble interleukin 2 receptor; ß$_2$MG: ß$_2$-microglobulin.

RNA extraction and cDNA synthesis for real-time PCR. Total RNA was isolated from 2.5 ml whole blood with a PAXgene Blood RNA Kit. Quality of RNA was confirmed from the ratio of absorbance at 260 nm to that at 280 nm (A260/A280 ratio), and RNA samples with a ratio > 1.8 were used for quantitative PCR. Samples were quantified based on the A260, and 1000 ng RNA was used as the template for reverse transcription. cDNA was synthesized using a first-strand cDNA Synthesis Kit for RT-PCR (Roche Diagnostics GmbH, Mannheim, Germany).

Quantitative real-time PCR. Relative quantification with real-time PCR was performed using the LightCycler® 2.0 system (Roche Diagnostics). The primer sequences were as follows: IFN-α-inducible protein 27 (IFI27): 5'-TCA CCT CAT CAG CAG TGA-3' (forward) and 5'-CAT CTT GGC TGC TAT GGA-3' (reverse); G3PDH: 5'-TCC CAT CAC CAT CTT CCA-3' (forward) and 5'-CAT CAC GCC ACA GTT TCC-3' (reverse). After an initial denaturation step at 95°C for 10 min, PCR was performed with 40 cycles of denaturation at 95°C for 5 s, annealing at 56°C for 5 s, and extension at 72°C for 20 s. G3PDH was used as an internal standard. All samples were automatically quantified simultaneously during the amplification process by comparison to the standard curve and expressed as a ratio relative to the reference sample.

Statistical analysis. Data were analyzed using SPSS (v 11.0.1; SPSS Inc., Chicago, IL, USA) for Windows. Mann-Whitney U test was carried out to compare IFI27 gene expression levels between patients and controls. Linear regression analysis and correlation analysis using Spearman's correlation coefficients were performed to estimate associations between mRNA expression levels and clinical variables in the patient group. Statistical significance was defined as $p < 0.05$.

RESULTS

cDNA microarray analysis. To identify the putative SS-specific genes in whole peripheral blood of SS patients compared to controls, we performed DNA microarray analysis. Genes that were upregulated by 1.6-fold or more in patients with primary SS relative to controls (the top 30) are shown in Table 3. Among the 10 most highly upregulated genes, at least 6 (60%) were IFN-α/ß-inducible genes. Table 4 shows the genes (top 30) that were upregulated by 1.4-fold or more in patients with secondary SS compared to controls. Similar to patients with primary SS, among the 10 most highly upregulated genes, at least 6 (60%) were IFN-α/ß-inducible genes. A comparison of transcript levels for patients with primary SS and secondary SS and controls revealed that genes induced by IFN-α/ß had the largest fold difference. The magnitude of upregulation was generally higher in primary SS than in secondary SS. In RA patients, among the 10 most highly upregulated genes, 3 (30%) were IFN-α/ß-inducible genes (Table 5). All IFN-inducible genes were upregulated less than 1.8-fold compared to controls. The gene expression pattern of SS was different from that of RA. IFN-inducible genes were upregulated in primary and secondary SS. IFI27 showed the most significant difference between SS patients and healthy controls in microarray screening. In RA patients, IFI27 was slightly upregulated (1.35-fold) compared to healthy controls. IFI27 expression was much higher in SS compared to RA (35.6 ± 31.4 vs 1.35 ± 0.55 fold). These findings suggest that IFI27 could be used as an SS-specific gene marker.

Confirmation of microarray findings by real-time PCR analysis. Based on microarray analysis, to further investigate the significance of IFI27 in SS, we examined IFI27 gene expression levels by real-time PCR. The mean IFI27 relative expression levels in SS patients was 137.2 ± SD 250.2 and that in healthy controls was 9.1 ± 22.7. Quantitative real-time PCR showed that IFI27 gene expression levels were significantly increased in patients with SS compared with healthy controls ($p < 0.01$), supporting our microarray data. Further, the relative IFI27 expression levels in primary SS, secondary SS, and healthy controls were 111.1 ± 212.3, 218.6 ± 346.0, and 9.1 ± 22.7, respectively. IFI27 gene expression levels were significantly increased in patients with primary SS ($p < 0.01$) and secondary SS ($p < 0.05$) compared with healthy controls (Figure 1).

Table 3. Genes showing upregulated expression in peripheral blood from patients with primary SS relative to healthy controls (top 30). The average fold change is shown based on normalized microarray fluorescence data of SS patients compared to controls.

No.	Gene Name	Gene Bank No.	Mean Expression Level
1*	Interferon-α-inducible protein 27 (IFI27; ISG12)	X67325	56.0
2	2-5A synthetase (1.8 kb RNA)	X02875	3.82
3	2-5A synthetase (71 kDa)	M87434	3.52
4*	IFN-induced 17/15 kDa protein (G1P2)	M13755	3.44
5*	IFN-α/ß-inducible p44 (p44) (IFI44)	NM_006417	3.42
6*	MxA (interferon-induced cellular resistance mediator protein)	M30817	3.28
7	2, 5-oligo-adenylate synthetase (2, 5-AS) 40/46 kDa (OAS1)	D00068	2.87
8*	ISG-56K (IFN-inducible 56 k protein)	X03557	2.81
9	Immunoglobulin heavy constant gamma 3	J00230	2.76
10*	RIG-G, interferon-induced protein with tetratricopeptide repeats 4 (IFIT4)	U52513	2.72
11*	IFN-α/ß/γ-inducible IFI-56K (IFI-56K)	M24594	2.40
12	Granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) (GZMB)	NM_004131	2.38
13	Rearranged immunoglobulin mRNA for mu heavy chain enhancer and constant region	X58529	2.21
14*	IFI-6-16, IFN-α-inducible (G1P3)	BC011601	2.11
15	Thymosin ß-10 (TMSB10)	M20259	2.11
16*	Interferon-inducible double-strand RNA-activated protein kinase (p68 kinase) (PKR)	M35663	2.11
17	CD81 (antiproliferative antibody 1); 26 kDa cell surface protein TAPA-1	M33680	2.10
18	Ig-γ chain; Ig rearranged gamma chain, V-J-C region	M63438	1.99
19	CX3C chemokine receptor-1 (CX3CR1)	BC028078	1.88
20*	Interferon regulatory factor 7 (IRF7)	U73036	1.81
21	Mac-2 binding protein (macrophage scavenger receptor family)	L13210	1.78
22*	Interferon-inducible transmembrane protein 9-27	J04164	1.77
23	Immunoglobulin kappa (light chain) variable 1D8	Y14736	1.77
24	Perforin 1 (pore forming protein) (PRF1)	M28393	1.76
25	BST-2 (bone marrow stromal antigen 2, synovial cell cDNA)	D28137	1.75
26	TRIP14 (tyroid receptor interactor), eoxn 3	L40387	1.67
27	TCR ß chain; T-cell receptor beta chain	X00437	1.67
28*	STAT1α, IFN-α stimulated gene, transcription factor ISGF-3 (91 or 84 kD)	M97935	1.65
29	CD2 (p50)	M16445	1.62
30	bc12 homologous antagonist/killer 1 (BAK1)	U23765	1.61

* Genes regulated by type I IFN.

Table 4. Genes showing upregulated expression in peripheral blood from patients with secondary SS relative to controls (top 30).

No.	Gene Name	Gene Bank No.	Mean Expression Level
1*	ISG-56K (IFN-inducible 56k protein)	X03557	2.56
2*	MxA (interferon-induced cellular resistance mediator protein)	M30817	2.31
3*	Interferon-α-inducible protein 27 (IFI27; ISG12)	X67325	2.30
4*	IFN-α/ß/γ-inducible IFI-56K (IFI-56K)	M24594	2.25
5*	RIG-G, IFN-induced protein with tetratricopeptide repeats 4 (IFIT4)	U52513	2.15
6	2, 5-oligo-adenylate synthetase (2, 5-AS) 40/46 kDa (OAS1)	D00068	2.08
7*	IFN-α/ß-inducible p44 (p44) (IFI44)	NM_006417	2.07
8	Ribosomal protein L41	AF02844	2.04
9	HLA-class II, DQ alpha 1 (HLA-DQA1)	M34996	1.96
10	fte-1 (v-fos transformation effector protein), ribosomal protein S3a	M84711	1.95
11	2-5A synthetase (71 kDa)	M87434	1.83
12	Ribosomal protein L7 (RPL7)	X57959	1.72
13	2-5A synthetase (1.8 kb RNA)	X02875	1.70
14	Heat shock protein 90 alpha (HSPCA)	X15183	1.70
15*	IFN-inducible double-strand RNA-activated protein kinase (p68 kinase) (PKR)	M35663	1.69
16	Ribosomal protein L36a (RPL36AL)	M15661	1.66
17	Ribosomal protein S27 (RPS27)	U57847	1.66
18*	IFN-induced 17/15 kDa protein (G1P2)	M13755	1.60
19	CX3C chemokine receptor-1 (CX3CR1)	BC028078	1.57
20	NADH: ubiquinone oxidoreductase MLRQ subunit	U94586	1.55
21*	IFI-6-16, IFN-α-inducible (G1P3)	BC011601	1.52
22	Ribosomal protein L11 (RPL11)	X79234	1.47
23	Immunoglobulin heavy constant gamma 3	J00230	1.46
24	CD81 (antiproliferative antibody 1)	M33680	1.46
25	Pros-27 (prosomal RNA-binding protein p27)	X59417	1.45
26	Thymosin ß-10 (TMSB10)	M20259	1.45
27	Granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	BC015739	1.44
28	Elongation factor 1-α (EEF1A1)	X16869	1.44
29	Neuroleukin (NLK); glucose phosphate isomerase (GPI)	K03515	1.42
30*	STAT1α, IFN-α stimulated gene, transcription factor ISGF-3 (91 or 84 kD)	M97935	1.41

* Genes regulated by type I IFN.

Table 5. Top 10 genes showing upregulated expression in peripheral blood from 10 patients with RA relative to 10 controls, by microarray analysis.

No.	Gene Name	Gene Bank No.	Mean Expression Level
1	Orosomucoid-1	X02544	3.16
2	α-1 acid glycoprotein (Orosomucoid-1)	M13692	2.42
3	S100 calcium binding protein A9 (calgranulin B) (S100A9)	X06233	1.98
4	Interleukin 1 receptor, type II (IL1R2)	U74649	1.95
5	dsRNA adenosine deaminase RNA-specific (ADAR)	U10439	1.80
6*	ISG-56K (IFN-inducible 56k protein)	X03557	1.79
7	AP-1 clathrin adaptor complex, sigma 1B subunit	AB015320	1.72
8*	IFN-inducible double-strand RNA-activated protein kinase (p68 kinase) (PKR)	M35663	1.71
9*	IFN-α/ß/γ-inducible IFI-56K (IFI-56K)	M24594	1.71
10	Glycogen phosphorylase type IV (PYGL)	M36807	1.66

* Genes regulated by type I IFN.

Association between IFI27 expression and clinical variables. The expression level of IFI27 was significantly correlated with serum IgG level ($r = 0.462$, $p < 0.01$; Figure 2). Moreover, IFI27 expression was correlated with serum $ß_2$-microglobulin ($r = 0.385$, $p < 0.05$), soluble interleukin 2 (IL-2) receptor ($r = 0.473$, $p < 0.01$), erythrocyte sedimentation rate (ESR; $r = 0.333$, $p < 0.05$), and antinuclear antibody (ANA) titer (speckled pattern; $r = 0.445$, $p < 0.01$; Table 6). Other manifestations, such as severity of dry mouth and dry eye, rash, arthritis, hematological involvement, occurring currently or previously, were not associated with IFI27 expression.

DISCUSSION

In SS, disease activity cannot be easily defined due to its nonspecific symptoms and the lack of both sensitive and

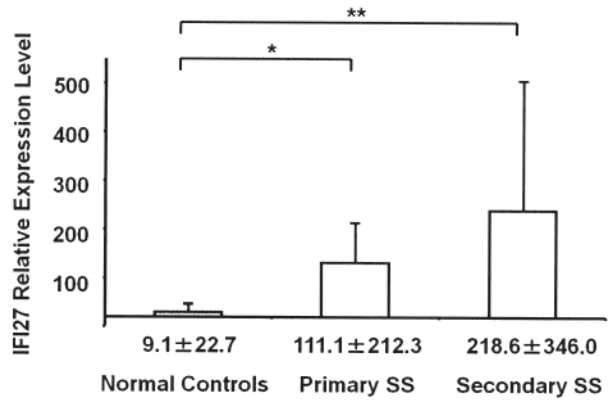

Figure 1. Relative expression of IFI27 in primary SS was 111.1 ± 212.3, in secondary SS 218.6 ± 36.0, and in healthy controls 9.1 ± 22.7. IFI27 gene expression was significantly increased in patients with primary (*p < 0.01) and secondary Sjögren's syndrome (**p < 0.05) compared with controls (Mann-Whitney U test).

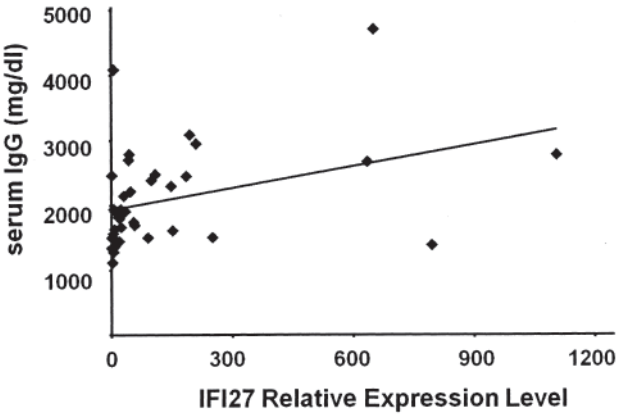

Figure 2. Linear regression analysis showed a significant positive correlation between relative expression of IFI27 and serum IgG levels in patients with Sjögren's syndrome ($r^2 = 0.1316$, $p < 0.0274$).

specific biomarkers. Serum IgG, soluble IL-2 receptor, $ß_2$-microglobulin, anti-Ro/SSA, and anti-La/SSB have been found at elevated concentrations in sera from patients with SS, but none individually was sufficiently sensitive or specific to use for evaluation of disease activity[20,21,22]. To monitor disease activity and to apply more appropriate treatment, there is increasing interest in the identification of biomarkers for SS.

Our study showed that IFN-inducible genes reveal the most significant difference between SS patients and healthy controls in microarray screening, and IFI27 is expressed at the highest level in the peripheral blood of SS patients. The level of IFI27 was significantly correlated with various clinical measures. The number of patients in our experimental groups was too small to discuss the differences between primary and secondary SS. Although IFI27 is reported to be upregulated in patients with SLE and DM[7], IFI27 is not the most upregulated gene in these diseases. Moreover, we

Table 6. IFI27 expression was correlated with serum IgG, $ß_2$-microglobulin ($ß_2$MG), soluble interleukin 2 receptor (sIL-2R), erythrocyte sedimentation rate (ESR), and antinuclear antibody (ANA) titer. Correlation analyses using Spearman's correlation coefficients were performed to estimate associations between mRNA expression levels and clinical variables in the SS patient group.

Indicator	R	p
Saxon test, g per 2 min	0.2094	0.093
Schirmer R, mm/5 min	0.0768	0.709
Schirmer L, mm/5 min	–0.0859	0.677
Focus score	0.3675	0.093
Lymph, per μl	–0.1468	0.400
ESR, mm/h	0.3334	0.047*
IgG, mg/dl	0.4618	0.004*
$ß_2$MG, μg/ml	0.3849	0.022*
sIL-2R, U/ml	0.4734	0.005*
C3, mg/dl	–0.1926	0.260
C4, mg/dl	–0.1593	0.353
ANA (speckled-type)	0.4452	0.007*

* Significant.

showed that IFI27 expression is much higher in SS compared to RA. These findings suggest that IFI27 could serve as an SS-specific biomarker.

Plasmacytoid dendritic cells (pDC) are the main producers of type I IFN. Viruses may initiate the production of IFN; continuous IFN-α synthesis is caused by immune complexes that activate pDC to prolong IFN-α production at the tissue level in patients with SS[17]. As pDC are detectable in the salivary glands of patients with SS, they are considered an important source of type I IFN[14]. The systemic type I IFN signature in patients with SS may be linked to higher systemic pDC activation[23]. Genes involved in IFN signaling are upregulated in labial salivary gland cells of primary SS[13,24]. In response to immunological stimuli such as apoptotic cells or viruses, hyperactive pDC may cause SS in those who have susceptibility for developing autoimmune disorders like Bcl-2 antagonist killer 1 (BAK1) polymorphisms[13,24].

IFN-inducible genes, such as *IP-10*, *Mig*, *I-TAC*, *STAT1*α, and *STAT1*ß, are highly expressed in the salivary gland tissues of patients with SS[11,25,26,27]. Type I IFN stimulation of DC has been reported to increase the production of B cell-activating factor (BAFF) belonging to the tumor necrosis factor (TNF) family[23,28]. BAFF is known to promote B cell survival and Ig class-switching[29,30]. In the SS salivary gland, infiltrating inflammatory cells strongly express BAFF protein[31]. As a result, the elevated serum IgG concentration may be a reflection of enhanced IFN-α activity. Indeed, in our study, the level of serum IgG correlated with expression levels of IFI27. These findings suggest that the IFN pathway is important in the pathogenesis of SS. The mechanism of IFN pathway upregulation remains to be elucidated in future studies.

SS and SLE share some clinical and immunological fea-

tures, including the presence of infiltrating pDC in target organs and activation of IFN pathways. In SLE, the serum concentration of IFN-α is correlated with both disease activity and severity as well as clinical manifestations such as fever and rashes[28,32,33]. One of the major differences between the 2 diseases is the organ specificity of SS. Our data showed that the expression level of IFI27 was not correlated with the sicca symptoms. These observations suggest that expression of IFN-inducible genes may be not only a dynamic component of the SS disease process, but also a relatively stable characteristic. Nine (29%) of 37 patients with SS showed IFI27 gene levels that were significantly increased (above 100-fold); and 6 (66.7%) of the 9 cases were complicated by interstitial nephritis, aplastic anemia, mononeuritis multiplex, primary biliary cirrhosis, liver dysfunction, and hypothyroidism, respectively. Longitudinal analysis of subsets of patients with SS based on IFN-inducible gene levels is necessary to determine whether IFN-inducible genes affect disease progression.

Anti-IFN-α monoclonal antibody can neutralize overexpression of type I IFN-inducible genes in whole blood and lesional skin from SLE patients, and has profound effects on signaling pathways, including BAFF, TNF-α, and IL-10, which may be located downstream of IFN-α[28]. Corticosteroid therapy has previously been shown to downregulate IFN-inducible gene expression[10]. In this study, 8 (21.6%) SS patients received oral corticosteroid therapy (average prednisolone 9.4 mg/day). One, with interstitial nephritis, received 30 mg/day prednisolone. The remaining 7 patients were treated with low-dose prednisolone (2–10 mg/day) as maintenance therapy. The expression of IFI27 was not correlated with the steroid dose. Our data suggest that anti-IFN-α agents may be useful in SS patients who show elevated serum IgG, $ß_2$-microglobulin, soluble IL-2 receptor, ESR, and ANA titer. Treatment of SS with anti-TNF agents has not been successful to date. Interestingly, etanercept was not effective for SS because etanercept exacerbates overexpression of IFN-α and BAFF[34].

Our data suggest an important role of the IFN pathway in the pathogenesis of SS. IFI27 could be an effective and specific biomarker related to disease activity or disease progression in SS. The IFN pathway could be a novel therapeutic target in SS in the future.

ACKNOWLEDGMENT

The authors thank the patients and healthy volunteers who participated in this study.

REFERENCES

1. Anaya JM, Delgado-Vega AM, Castiblanco J. Genetic basis of Sjögren's syndrome. How strong is the evidence? Clin Dev Immunol 2006;13:209-22.
2. Fox RI. Sjögren's syndrome. Lancet 2005;366:321-31.
3. Hu S, Zhou M, Jiang J, Wang J, Elashoff D, Gorr S, et al. Systems biology analysis of Sjögren's syndrome and mucosa-associated lymphoid tissue lymphoma in parotid glands. Arthritis Rheum 2009;60:81-92.
4. Takei M, Shiraiwa H, Azuma T, Hayashi Y, Seki N, Sawada S. The possible etiopathogenic genes of Sjögren's syndrome. Autoimmun Rev 2005;4:479-84.
5. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci USA 2003;100:2610-5.
6. Wenzel J, Zahn S, Bieber T, Tuting T. Type I interferon-associated cytotoxic inflammation in cutaneous lupus erythematosus. Arch Dermatol Res 2009;301:83-6.
7. Walsh RJ, Kong SW, Yao Y, Jallal B, Kiener PA, Pinkus JL, et al. Type I interferon-inducible gene expression in blood is present and reflects disease activity in dermatomyositis and polymyositis. Arthritis Rheum 2007;56:3784-92.
8. Ishii T, Onda H, Tanigawa A, Ohshima S, Fujiwara H, Mima T, et al. Isolation and expression profiling of genes upregulated in the peripheral blood cells of systemic lupus erythematosus patients. DNA Res 2005;12:429-39.
9. Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, et al. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis Rheum 2006;54:2951-62.
10. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med 2003;197:711-23.
11. Wakamatsu E, Nakamura Y, Matsumoto I, Goto D, Ito S, Tsutsumi A, et al. DNA microarray analysis of labial salivary glands of patients with Sjögren's syndrome. Ann Rheum Dis 2007;66:844-5.
12. 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.
13. Perez P, Anaya JM, Aguilera S, Urzua U, Munroe D, Molina C, et al. Gene expression and chromosomal location for susceptibility to Sjögren's syndrome. J Autoimmun 2009;33:99-108.
14. Gottenberg JE, Cagnard N, Lucchesi C, Letourneur F, Mistou S, Lazure T, et al. Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjögren's syndrome. Proc Natl Acad Sci USA 2006;103:2770-5.
15. Hu S, Wang J, Meijer J, Ieong S, Xie Y, Yu T, et al. Salivary proteomic and genomic biomarkers for primary Sjögren's syndrome. Arthritis Rheum 2007;56:3588-600.
16. 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.
17. Bave U, Nordmark G, Lovgren T, Ronnelid J, Cajander S, Eloranta ML, et al. Activation of the type I interferon system in primary Sjögren's syndrome: a possible etiopathogenic mechanism. Arthritis Rheum 2005;52:1185-95.
18. Takashi F, Susumu S, Nobuyuki M, Yoshio H, Kazuo T. Revised Japanese criteria for Sjögren's syndrome (1999): availability and validity. Mod Rheumatol 2004;14:425-34.
19. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
20. Spadaro A, Riccieri V, Benfari G, Scillone M, Taccari E. Soluble interleukin-2 receptor in Sjögren's syndrome: relation to main serum immunological and immunohistochemical parameters. Clin Rheumatol 2001;20:319-23.
21. Maddali Bongi S, Campana G, D'Agata A, Palermo C, Bianucci G. The diagnosis value of beta 2-microglobulin and immunoglobulins in primary Sjögren's syndrome. Clin Rheumatol 1995;14:151-6.

22. Manoussakis MN, Papadopoulos GK, Drosos AA, Moutsopoulos HM. Soluble interleukin 2 receptor molecules in the serum of patients with autoimmune diseases. Clin Immunol Immunopathol 1989;50:321-32.
23. 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.
24. Delgado-Vega AM, Castiblanco J, Gomez LM, Diaz-Gallo LM, Rojas-Villarraga A, Anaya JM. Bcl-2 antagonist killer 1 (BAK1) polymorphisms influence the risk of developing autoimmune rheumatic diseases in women. Ann Rheum Dis;69:462-5.
25. Wakamatsu E, Matsumoto I, Yasukochi T, Naito Y, Goto D, Mamura M, et al. Overexpression of phosphorylated STAT-1 alpha in the labial salivary glands of patients with Sjögren's syndrome. Arthritis Rheum 2006;54:3476-84.
26. Ogawa N, Kawanami T, Shimoyama K, Ping L, Sugai S. Expression of interferon-inducible T cell alpha chemoattractant (CXCL11) in the salivary glands of patients with Sjögren's syndrome. Clin Immunol 2004;112:235-8.
27. Ogawa N, Ping L, Zhenjun L, Takada Y, Sugai S. Involvement of the interferon gamma-induced T cell-attracting chemokines, interferon-gamma-inducible 10-kd protein (CXCL10) and monokine induced by interferon-gamma (CXCL9), in the salivary gland lesions of patients with Sjögren's syndrome. Arthritis Rheum 2002;46:2730-41.
28. Yao Y, Richman L, Higgs BW, Morehouse CA, de los Reyes M, Brohawn P, et al. Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus. Arthritis Rheum 2009;60:1785-96.
29. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity 2001;14:461-70.
30. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol 2002;3:822-9.
31. Lavie F, Miceli-Richard C, Quillard J, Roux S, Leclerc P, Mariette X. Expression of BAFF (BLyS) in T cells infiltrating labial salivary glands from patients with Sjögren's syndrome. J Pathol 2004;202:496-502.
32. Bengtsson AA, Sturfelt G, Truedsson L, Blomberg J, Alm G, Vallin H, et al. Activation of type I interferon system in systemic lupus erythematosus correlates with disease activity but not with antiretroviral antibodies. Lupus 2000;9:664-71.
33. Dall'era MC, Cardarelli PM, Preston BT, Witte A, Davis JC Jr. Type I interferon correlates with serological and clinical manifestations of SLE. Ann Rheum Dis 2005;64:1692-7.
34. Mavragani CP, Niewold TB, Moutsopoulos NM, Pillemer SR, Wahl SM, Crow MK. Augmented interferon-alpha pathway activation in patients with Sjögren's syndrome treated with etanercept. Arthritis Rheum 2007;56:3995-4004.