

The expression of Ets-1 and Fli-1 is associated with interferon-inducible genes in peripheral blood mononuclear cells from Japanese patients with systemic lupus erythematosus

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Abstract

Transcription factors E26 transformation-specific-1 (Ets-1) and Friend leukemia insertion site-1 (Fli-1) and type I interferon (IFN) have been implicated in systemic lupus erythematosus (SLE). We examined the expression of these genes in peripheral blood mononuclear cells (PBMCs) from Japanese patients with SLE and analyzed their association with SLE. We enrolled 53 Japanese patients with SLE, 42 patients with rheumatoid arthritis (RA), and 30 healthy donors (HDs) (as controls) in this study. PBMCs were collected from all participants, and the expressions of Ets-1, Fli-1, and three interferon-inducible genes (IFIGs) (interferon-inducible protein with tetratricopeptide 1 [IFIT1], interferon-inducible protein 44 [IFI44], and eukaryotic translation initiation factor 2 alpha kinase 2 [EIF2AK2]) were measured using real-time polymerase chain reaction (PCR). The relationships of each molecule with clinical symptoms, laboratory data, and treatments were analyzed. The expression of Ets-1 and Fli-1 was significantly lower in the PBMCs from patients with SLE than that in the PBMCs from patients with RA and HDs. The expression of the three IFIGs was significantly higher in the PBMCs from patients with SLE than that in the PBMCs from patients with RA and HDs. For patients with SLE, significantly positive correlations were found between Ets-1 and three IFIGs; a similar trend was observed between Fli-1 and IFIGs. IFIG expression in the PBMCs was significantly higher in patients with SLE than that in other participants, and the expression of Ets-1 and Fli-1 was positively associated with IFN expression. Therefore, it was suggested that Ets-1 and Fli-1 were associated with the pathophysiology of SLE by regulating the type I IFN pathway.

Abbreviations: CD = cluster of differentiation, EIF2AK2 = eukaryotic translation initiation factor 2 alpha kinase 2, Ets-1 = E26 transformation-specific-1, Fli-1 = Friend leukemia insertion site-1, HDs = healthy donors, IFI44 = interferon-inducible protein 44, IFIGs = interferon-inducible genes, IFIT1 = interferon-inducible protein with tetratricopeptide 1, IFN = interferon, IQR = interquartile range, PBMCs = peripheral blood mononuclear cells, PCR = polymerase chain reaction, PDCs = plasmacytoid dendritic cells, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SLEDAI = SLE Disease Activity Index.

Keywords: Ets-1, Fli-1, interferon-inducible gene, systemic lupus erythematosus, type I interferon

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with a wide spectrum of clinical and immunological abnormalities.^[1,2] SLE is characterized by arthritis, glomerulonephritis, vasculitis, and autoantibody production.^[1,2] The pathogenesis of SLE is multifactorial and includes contributions from the environment, stochastic factors, and genetic susceptibility.^[3] The immunological abnormalities of SLE are believed to

include a mechanism in which nuclear antigens are exposed to the immune system for a prolonged period due to phagocytosis of macrophages and other cells, resulting in autoantibody induction, as well as a mechanism in which immune complexes are taken up by plasmacytoid dendritic cells (PDCs) and bind to toll-like receptors 7 and 9, inducing type I interferon (IFN) production, which in turn induces inflammation and autoantibody production.^[3] The production of type I IFNs (with IFN- α as the dominant mediator) was reported to increase in SLE and play

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The corresponding author will provide the datasets produced during and/or analyzed during the current research upon reasonable request.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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an important role in its pathogenesis.^[4–6] Several IFN-targeted therapies for SLE have been examined in clinical trials,^[7–9] and anifrolumab, a fully human immunoglobulin G1 kappa monoclonal antibody that targets several type I IFNs through binding of the type I IFN receptor subunit 1, improved the pathogenesis of SLE and has started to be used as a therapeutic agent.^[10–13]

E26 transformation-specific-1 (Ets-1) and Friend leukemia insertion site-1 (Fli-1) belong to the Ets family of transcription factors, which share a common 85-amino-acid DNA binding domain.^[14,15] Ets transcription factors control a wide variety of cellular processes including cell proliferation and differentiation.^[14,15] The gene expression of Ets-1 was reported to be lower and the gene expression of Fli-1 higher in the peripheral blood mononuclear cells (PBMCs) of patients with SLE.^[16,17] Ets-1 mutant mice were also reported to spontaneously develop SLE-like disease.^[18] The involvement of Fli-1 in lupus was established by Fli-1 transgenic mice with 2- to 3-fold Fli-1 overexpression that developed a lupus-like disease with a progressive immune complex-mediated kidney disease and ultimately died of renal failure.^[19] Fli-1 heterozygous knockout mice of lupus model mice showed significantly reduced SLE-like disease.^[20,21] Two genome-wide association studies of Chinese patients with SLE independently found that genetic variants in Ets-1 are associated with susceptibility to SLE.^[22,23] Ets-1 has also been implicated in numerous cellular abnormalities that are known to participate in the pathogenesis of SLE, playing an inhibitory role in T helper 17 (Th17) cell, B-cell, and T follicular helper type 2 cell differentiation.^[24–27] Based on the results from Fli-1 hetero-knockout and functional domain knockout lupus model mice, Fli-1 modulates T cells, including a cluster of differentiation 3 (CD3+), CD4+, regulatory T cells, and B cells. Fli-1 also modulates mononuclear phagocyte development, including dendritic cells and macrophages.^[15,28–30] Fli-1 also regulates the production of inflammatory cytokines and chemokines including interleukin-6, C-C motif chemokine ligand 5, and monocyte chemoattractant protein-1, which contribute to the pathogenesis of SLE and lupus nephritis.^[15,31–33]

Although type I IFNs and corresponding modulating systems are important in the pathogenesis of SLE, the relationship between Ets-1/Fli-1 and type I IFNs is still unclear. Furthermore, few reports on the expression of Ets-1 and Fli-1 in SLE in clinical practice exist. In this report, we measured the gene expression of Ets-1 and Fli-1 in the PBMCs of patients with SLE and those with rheumatoid arthritis (RA) followed-up by Fukushima Medical University (Japan). We investigated the differential expression of Ets-1, Fli-1, and interferon-inducible genes (IFIGs) among patients with SLE, those with RA, and healthy donors (HDs) and analyzed the associations between those genes and the clinical manifestations including symptoms, disease activity, laboratory results, and IFIGs.

2. Materials and Methods

2.1. Patients and controls

Our cohort included 53 patients with SLE (4 men, 49 women, median age 36.0 years, interquartile range [IQR] 28.0–46.0 years), 42 patients with RA (14 men, 28 women, median age 63.5 years, IQR 57.8–69.3), and 30 HDs (6 men, 24 women, median age 33.5 years, IQR 27.8–40.3). Among the 53 SLE patients, 48 were receiving treatment, and 5 were not. The SLE and RA patients were followed-up at Fukushima Medical University Hospital. The patients with SLE met the American College of Rheumatology revised criteria for the classification of SLE,^[34] and the patients with RA met the American Rheumatism Association 1987 classification of RA^[35] or the 2010 RA classification criteria (American College of Rheumatology/European League Against Rheumatism).^[36] The SLE disease activity score was calculated based on the SLE Disease Activity Index (SLEDAI)-2K instrument.^[37] Written informed consent was obtained from all participants, and the study was approved by the appropriate ethics committee.

2.2. Samples and complementary DNA

Peripheral blood was obtained from all participants. PBMCs were isolated from 10 mL of heparinized peripheral blood via Ficoll–Paque Plus (Amersham Biosciences, Uppsala, Sweden), following the protocol recommended by the manufacturer. One million cells of each sample were spun down to pellets, and total RNA was extracted from the cell pellets using Isogen (Nippon Gene, Tokyo, Japan). Complementary DNA was synthesized using SuperScript™ II Reverse Transcriptase (Thermo Fisher Scientific, Yokohama, Japan), following the instructions provided by the manufacturer. Complementary DNA was used to quantify the expression of several genes. Residual cells were suspended in Cellbanker 2 (Takara Bio Inc., Kusatsu, Japan) and stored at -80 °C before use for flow cytometry.

2.3. Real-time reverse transcriptase-PCR

The complementary DNA samples were amplified with specific primers and fluorescence-labeled probes for the target genes. Amplified product genes were monitored on a StepOne Real-Time PCR system (Thermo Fisher Scientific). TaqMan Fast Universal PCR Master Mix (2×) was purchased from Thermo Fischer Scientific. Mixtures of one pair of primer and fluorescent probe (TaqMan Gene Expression Assays) for Ets-1 (Hs 00428293_m1), Fli-1 (Hs 00956709_m1), IFIT1 (Hs 01911452_s1), IFI44 (Hs 00951349_m1), EIF2AK2 (Hs 00169345_m1), and GAPDH (Hs 02786624_g1) were purchased from Applied Biosystems (South San Francisco, CA). The thermal cycle conditions were as follows: 95 °C for 2 minutes, then 40 cycles at 95 °C for 1 second and 60 °C for 20 seconds. In all measurements, a specific sample was used to adjust the relative amount of gene expression in each measurement. All measurements were performed in triplicate. Each gene expression was corrected via GAPDH gene expression.

2.4. Flow cytometry

Single-cell suspensions were prepared from the PBMCs. The cells were stained with fluorochrome-conjugated antibodies and analyzed on a FACSCanto II flow cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Data were analyzed via FlowJo software (Tomy Digital Biology Co., Ltd, Tokyo, Japan). The antibodies were purchased from BioLegend (San Diego, CA) and eBioscience (San Diego, CA, USA). The following specific antibodies were used to characterize the cell subsets: PDCs [CD45 + lineage (CD2, CD3, CD14, CD16, CD19, CD56, CD235a) – HLA-DR + CD123+].

2.5. Statistical analysis

To determine statistically significant differences between the two groups, we employed the Mann–Whitney *U* test and Fisher exact test when comparing the two categories. A *P* value of <.05 was considered statistically significant. GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA) was applied to perform all of the statistical analyses.

2.6. Ethical approval and consent to participate

The study was approved by the Ethics Committee of Fukushima Medical University (No. 1648), and written informed consent was obtained from all participants.

3. Results

3.1. Patient characteristics

Table 1 lists the participants' demographics. All participants in this study were Japanese; the patients with SLE had a median

Table 1
Baseline characteristics of the participants.

	SLE	RA	HD	SLE vs RAP	SLE vs HDP
Number of subjects	53	42	30		
Median age (IQR), year	36.0 (28.0, 46.0)	63.5 (57.8, 69.3)	33.5 (27.8, 40.3)	<.001†*	.29†
Number of woman (%)	49 (92.5)	28 (66.7)	24 (80)	.003††*	.16††
Median disease duration (IQR), year	8.0 (3.0, 17.0)	7.0 (2.0, 12.8)	NA	.45†	-
Median prednisolone dosage (IQR), mg/day	10.0 (5.0, 15.0)	0 (0, 2.3)	NA	<.001†*	-
Median SLEDAI-2K (IQR)	4.0 (2.0, 8.0)	NA	NA	-	-
Median ESR (IQR), mm/h	7.0 (4.0, 14.0)	8.0 (4.0, 19.0)	NA	.65†	-

ESR = erythrocyte sedimentation rate, HD = healthy donor, IQR = interquartile range, NA = not applicable, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SLEDAI-2K = systemic lupus erythematosus disease activity index 2000.

†Mann-Whitney *U* test.

††Fisher exact test.

*indicates $P < .05$.

age of 36.0 years (interquartile rate [IQR] 28.0–46.0) and were significantly younger than the patients with RA (36 years vs 63.5 years; $P < .001$). No statistically significant difference was found between the patients with SLE and the HDs. Of the patients with SLE, 92.5% were women, a significantly larger proportion than for the patients with RA (66.7%; $P = .003$). For the study patients with SLE, the median disease duration was 8.0 years (IQR 3.0–17.0), the median prednisolone dose was 10.0 mg (IQR 5.0–15.0), and the median SLEDAI-2K score was 4 (IQR 2.0–8.0). Table 2 lists the clinical characteristics of SLE patients. Since the majority of these patients were receiving treatment at the time of the study's admission, their SLEDAI-2K scores were often low.

3.2. Ets-1/Fli-1 expression in the PBMCs of the HDs and patients with SLE or RA

We measured the gene expression of Ets-1 and Fli-1 in the PBMCs from the HDs and patients with SLE or RA. Ets-1 expression in the patients with SLE was significantly lower than in the HDs and patients with RA ($P = .04$ for SLE vs RA; $P < .001$ for SLE vs HD). Fli-1 expression in the patients with SLE was significantly lower than in the HDs and patients with RA ($P = .01$ for SLE vs RA; $P < .001$ for SLE vs HD). A significantly positive correlation was found between the gene expression of Ets-1 and Fli-1 in the patients with SLE ($r = .59$, $P < .001$) (Fig. 1).

3.3. Association between Ets-1 or Fli-1 and clinical findings in the patients with SLE

We analyzed the association between the expression of Ets-1/Fli-1 and clinical findings including symptoms, laboratory data, and treatments for patients with SLE. Significantly negative correlations were found between the expression of Ets-1 and Fli-1 and the amount of prednisolone administered (Ets-1, $r = -.45$, $P < .001$; Fli-1, $r = -.32$, $P < .001$) (Fig. 2A and B). Ets-1 expression was considerably higher in the untreated group than the treatment group (Ets-1: $P = .01$ for untreated vs. treated; Fli-1: $P = .25$ for untreated vs treated) according to a comparison of Ets-1 and Fli-1 expression in the untreated and treated groups (Fig. 2C and D). In the other items (e.g., skin rash, arthritis, renal involvement, neuropsychiatric symptoms, hematological abnormalities, and anti-DNA antibodies), no statistically significant correlations were found with Ets-1 or Fli-1.

3.4. Association of PDCs in PBMCs of patients with SLE and Ets-1 or Fli-1

The PDCs in the PBMCs were detected via flow cytometry and were defined as CD45⁺ lineage (CD2, CD3, CD14, CD16,

Table 2
Characteristics of patients of SLE at study initiation.

Total number of SLE patients	53
Median age (IQR), year	36.0 (28.0, 46.0)
Number of women (%)	49 (92.5)
Median disease duration (IQR), year	8.0 (3.0, 17.0)
Number of untreated patients (%)	5 (9.4)
Median SLEDAI-2K (IQR)	4 (2, 8)
Clinical manifestations during the disease period, number (%)	
Skin rash	32 (60.4)
Oral ulcer	8 (15.0)
Arthritis	35 (66.0)
Serositis	16 (30.2)
Nephritis	28 (52.8)
Neurological disorder	17 (32.1)
Hematological disorder	48 (90.6)
Laboratory characteristics, number (%)	
Leukopenia (<4000/ μ L)	10 (18.9)
Low serum complement level (C3, C4)	37 (69.8)
Increased titer of anti-DNA antibodies	31 (58.5)
Treatment, number (%) (in 48 treated patients)	
No therapy	2 (4.2)
Prednisolone \leq 10 mg/day	26 (49.1)
Prednisolone > 10 mg/day	20 (41.7)
Use of immunosuppressants	14 (26.4)

IQR = interquartile range, SLE = systemic lupus erythematosus, SLEDAI = SLE disease activity index 2000.

CD19, CD56, CD235a)-HLA-DR⁺CD123⁺ cells. Figure 3A shows a representative result. Significantly fewer PDCs were found in the PBMCs of the patients with SLE than in the patients with RA (6.1/ μ L [IQR 2.6–8.5] vs 7.5/ μ L [IQR 6.0–11.3]; $P = .01$) (Fig. 3B). No statistically significant correlation was found between Ets-1 or Fli-1 and the number of PDCs (Ets-1, $r = .16$, $P = .29$; Fli-1, $r = .06$, $P = .72$) (Fig. 3C and D).

3.5. Gene expression of IFIGs and the association with Ets-1 or Fli-1

The gene expression of representative IFIGs (interferon-inducible protein with tetratricopeptide 1 [IFIT1], interferon-inducible protein 44 [IFI44], and eukaryotic translation initiation factor 2 alpha kinase 2 [EIF2AK2]) from PBMCs was measured using real-time PCR. These three IFIGs were selected based on a study conducted by Kirou et al^[4] in the patients with SLE, the gene expression of IFIT1, IFI44, and EIF2AK2 was significantly higher than that in the HDs and patients with RA (Fig. 4A–C). In the patients with SLE, significantly positive correlations were found between Ets-1 and IFIGs (IFIT1, $r = .45$, $P < .001$; IFI44, $r = .32$, $P = .02$; EIF2AK2, $r = .43$, $P = .001$) (Fig. 5A–C), and

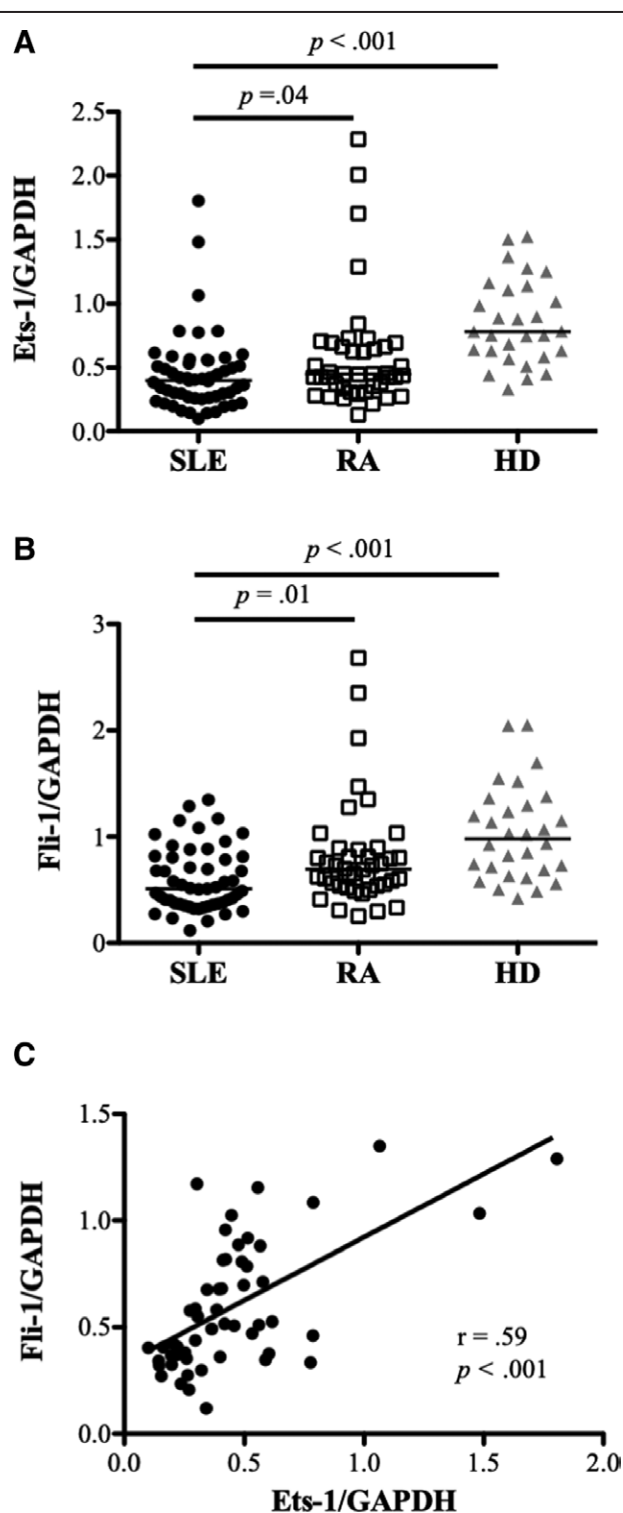


Figure 1. The expression of Ets-1 and Fli-1 in peripheral blood mononuclear cells (PBMCs) from patients with systemic lupus erythematosus (SLE), patients with rheumatoid arthritis (RA), and healthy donors (HDs). Each symbol represents an individual sample; horizontal lines show median values. (A) Relative expression levels of Ets-1 in PBMCs were significantly lower in the patients with SLE ($n = 53$) than in the patients with RA ($n = 42$) and the HDs ($n = 30$). (B) Relative Fli-1 expression levels in PBMCs were significantly lower in the patients with SLE than in the patients with RA and the HDs. (C) Relative Fli-1 expression levels were positively correlated with the relative expression of Ets-1 in the patients with SLE.

significantly positive correlations were found between Fli-1 and IFIGs except for IFI44 (IFIT1, $r = .34$, $P = .01$; IFI44, $r = .25$, $P = .07$; EIF2AK2, $r = .32$, $P = .02$) (Fig. 5D–F).

4. Discussion

In this study, we found that the expression of type I IFN-induced genes was significantly higher in the PBMCs from patients with SLE than from HDs or patients with RA. The gene expression of Ets-1 and Fli-1 was significantly lower in patients with SLE than in the HDs or patients with RA. A positive correlation was found between the gene expression of Ets-1/Fli-1 and several IFIGs in the patients of SLE.

Among the SLE-associated genes that have undergone genome-wide association studies, Ets-1 has been identified along with molecules related to DNA modification and IFN-related genes.^[22,23,38,39] The identification of a large number of IFN-related genes in the pathogenesis of SLE reaffirms the importance of type I IFNs in the pathogenesis of SLE. Previous studies have also reported that Ets-1 is involved in the pathogenesis of SLE. Ets-1 had been reported as a negative regulator of B-cell differentiation and Th 17 cell proliferation, and patients with SLE have demonstrated reduced Ets-1 expression, which might contribute to abnormal B-cell differentiation into immunoglobulin-secreting plasma cells and an increased number of Th 17 cells.^[40] Ets-1 was also reported to inhibit the differentiation of T follicular helper type 2 cells and regulate the development of SLE.^[26] These reports; therefore, suggest that Ets-1 might be involved in the pathogenesis of SLE by regulating the function of B and T cells. Few reports describe a direct relationship between type I IFN and Ets-1. The positive correlation between the expression levels of Ets-1 and the three IFIGs in the patients with SLE in this study suggests that Ets-1 might be associated with the expression of type I IFN (Fig. 5A–C). The results for increased production of IFN signatures include PDC activation, retention of risk alleles, and dysfunction of B cells and natural killer cells.^[41] In this study, we were unable to correlate Ets-1 expression with the number of PDCs, and we did not investigate PDC activation in this report. Previous reports have found an association between Ets-1 and B-cell activation, and Ets-1 is also required for natural killer cell differentiation.^[42] In terms of risk alleles, an association has been suggested between single nucleotide polymorphisms and IFN regulatory factor 5 and Ets-1, molecules involved in innate immunity that are thought to be important in the development of SLE.^[43,44] Rutherford et al showed that Ets-1 competes for the binding site of IFN-stimulated gene factors 3 and IFN-stimulated response element and mentioned the possibility that Ets transcription factors negatively regulate IFN transcriptional activity via an IFN-stimulated response element.^[45] Wen et al reported that in patients with SLE, the DNA containing immune complex induced anti-dsDNA antibodies through the Toll-like receptor 2/MyD88/microRNA-155 pathway, that is, microRNA-155 inhibited the expression of Ets-1, resulting in the reduction of anti-dsDNA antibodies through activating Blip-1.^[46] This mechanism may lead to an increased type I IFN production via activation of PDC by immune complexes containing anti-ds-DNA antibodies. Ets-1 might also be involved in regulating type I IFNs through direct and indirect mechanisms.

In this study, we found that Fli-1 expression in the PBMCs from the patients with SLE was significantly lower than that in the HDs and patients with RA, which contradicts a previous report showing higher Fli-1 expression in PBMCs from patients with SLE compared with healthy controls.^[16] Several possible explanations exist for this difference. First, we found that Ets-1 had significantly lower expression in the PBMCs of our patients with SLE, and Ets-1 was reported to regulate Fli-1 expression.^[47] Thus, the lower Fli-1 expression in the PBMCs in our study might be partially due to Ets-1 expression, which, in a previous report, did not differ between patients with SLE and healthy controls.^[16] Second, we demonstrated that Fli-1 regulates the expression of inflammatory cytokines through type I IFN stimulation. Reduced Fli-1 expression in human mesangial cells resulted in significantly less inflammatory cytokine production following type I IFN stimulation.^[48] High type I IFN in

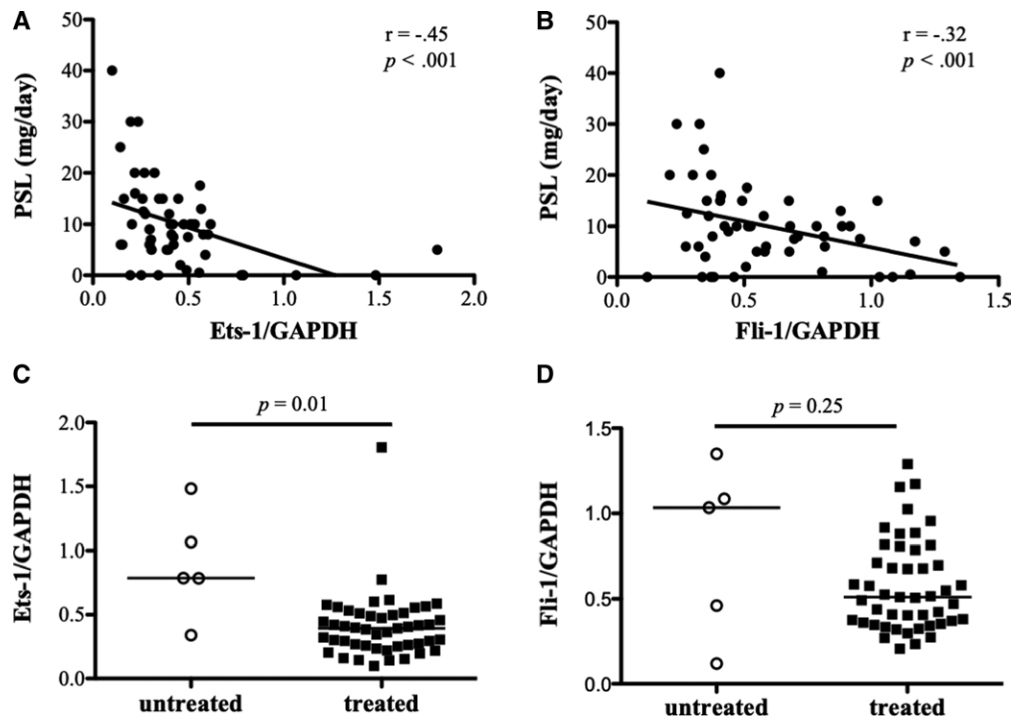


Figure 2. Association between Ets-1/Fli-1 and prednisolone dose in the patients with systemic lupus erythematosus (SLE). (A) The relative expression levels of Ets-1 in peripheral blood mononuclear cells (PBMCs) of the patients with SLE (n = 53) were negatively correlated with the prednisolone (PSL) dose. (B) The relative expression levels of Fli-1 in the PBMCs of the patients with SLE were negatively correlated with the prednisolone dose. (C) Ets-1 gene expression was considerably greater in the untreated group (n = 5) than in the treated group (n = 48). (D) No statistically significant difference between the treated and untreated groups in the Fli-1 gene expression.

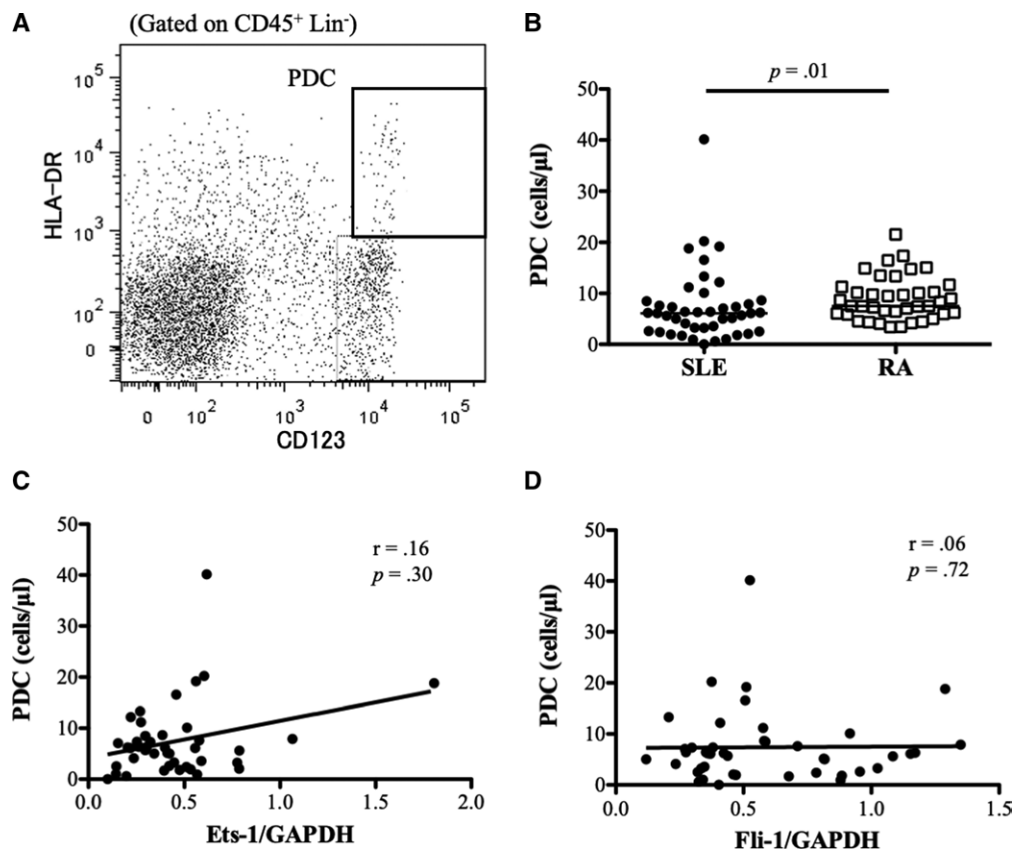


Figure 3. Association between Ets-1/Fli-1 and the plasmacytoid dendritic cells (PDCs) in the peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE). (A) Flow cytometry analysis of PDCs in PBMCs was performed. PDCs were defined as CD45⁺ lineage (CD2, CD3, CD14, CD16, CD19, CD56, CD235a)⁻ HLA-DR⁺CD123⁺. (A) Representative result is shown. (B) Significantly fewer PDCs were found in the PBMCs of the patients with SLE than in patients with rheumatoid arthritis (RA). (C) No correlation was found between Ets-1 and the number of PDCs. (D) No correlation was found between Fli-1 and the number of PDCs.

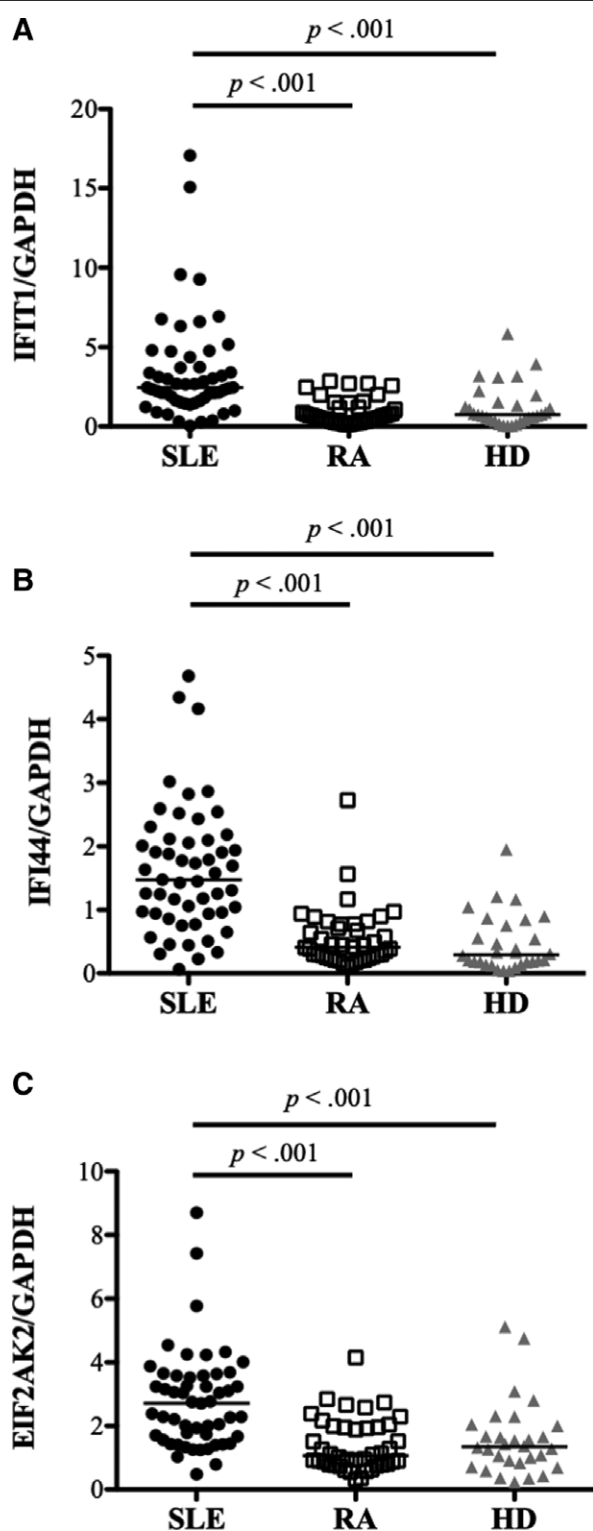


Figure 4. The gene expression of interferon-inducible genes in the peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE), patients with rheumatoid arthritis (RA), and healthy donors (HDs). Each symbol represents an individual sample; horizontal lines show median values. (A) In the patients with SLE, the gene expression of interferon-inducible protein with tetratricopeptide 1 (IFIT1) was significantly higher than that in the HDs and patients with RA. (B) In the patients with SLE, the gene expression of interferon-inducible protein 44 (IFI44) was significantly higher than in the HDs and patients with RA. (C) In the patients with SLE, the gene expression of eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2) was significantly higher than in the HDs and patients with RA.

patients with SLE might have a negative feedback loop to lower Fli-1 expression. Another explanation is the difference between the patients with SLE enrolled in our study and those in previous studies. Conclusively, the various methods utilized in our study and the prior publication to evaluate gene expression may have contributed to the discrepancy in the results. We found that although the level of Fli-1 expression is important for regulating inflammatory cytokines, reduced Fli-1 expression by specific Fli-1 siRNA or Fli-1 inhibitors resulted in significantly decreased inflammatory cytokine production in renal cells and immune cells. Fli-1 is largely activated through posttranslational modifications following stimulation rather than by transcription levels.^[49] (Zhang et al., unpublished data) Interpreting the collaboration between Ets-1 and Fli-1 and prednisolone dose is equally challenging. Furthermore, it was challenging to determine whether the decrease in Ets-1 and Fli-1 expression with increasing doses of prednisone was more of a direct effect of prednisolone or a secondary effect of improved SLE status even though gene expression levels of both were high in the untreated group (Fig. 2C and D).

In terms of IFIG expression, the gene expression of IFIT1, IFI44, and EIF2AK2 was predominantly higher in Japanese patients with SLE than that in HDs or patients with RA. These results were similar to those of a previous report.^[4] The data set used in this study was taken from a study by Panwar et al.^[50] In this study by Panwar et al, the expression of Ets-1 in the PDCs of patients with SLE was low, whereas the expression of Fli-1 was high. The relationship between IFIGs and the expression of Ets-1 or Fli-1 tended to be often negative. The results of Panwar et al study differed from those of our study. There are several explanations for the differences in the results between our study and Panwar et al study, including racial differences, disease condition of patients with SLE (many patients in this study were treated and were relatively less severe), different cell types and PBMCs used, and the effects of steroid and other therapeutic drugs used. Type I IFN and IFIG blood levels are higher in SLE patients, according to previous reports.^[51,52] Studies have reported that Japanese people have distinct genetic polymorphisms than people of other racial groups in relation to IFN regulatory factor 5, a crucial molecule in type I IFN regulation.^[53] The findings imply that ethnic disparities in IFN synthesis and IFIG expression may exist in SLE. Additionally, we were able to demonstrate that patients with SLE had considerably fewer PDCs in their PBMCs than patients with RA. Owing to the fact that activated PDCs appeared to infiltrate inflammatory tissue, the frequency of circulating PDCs appeared to decrease in patients with SLE when compared to healthy controls.^[54,55] Additionally, the PDC frequency in early RA patients is known to be lower than that of healthy controls.^[56] Our search has not yet brought up any reports presenting a comparison of articles comparing PDC counts in patients with SLE and RA's peripheral blood. According to these findings, PDC may play a larger role in the pathogenesis of SLE in the tissues.

The present study has several limitations that should be acknowledged. First, the sample size was relatively small. It is therefore difficult to perform a sufficient statistical analysis. Second, due to the study's retrospective nature, heterogeneity among the patients with SLE could not be ruled out. Owing to differences in the timing of treatments, the treatment regimen varied based on time and pathogenesis. At the time of entry into this study, many of the patients were in remission after a period of high disease activity. Third, we could not rule out the possibility that the high median dose of prednisolone (10 mg) in patients with SLE affected the results for each gene expression. Fourth, although it might be due to the disease characteristics, differences in the sex and age distribution of the patients with RA were found, which might be a potential bias in the comparison. Lastly, the small volume of

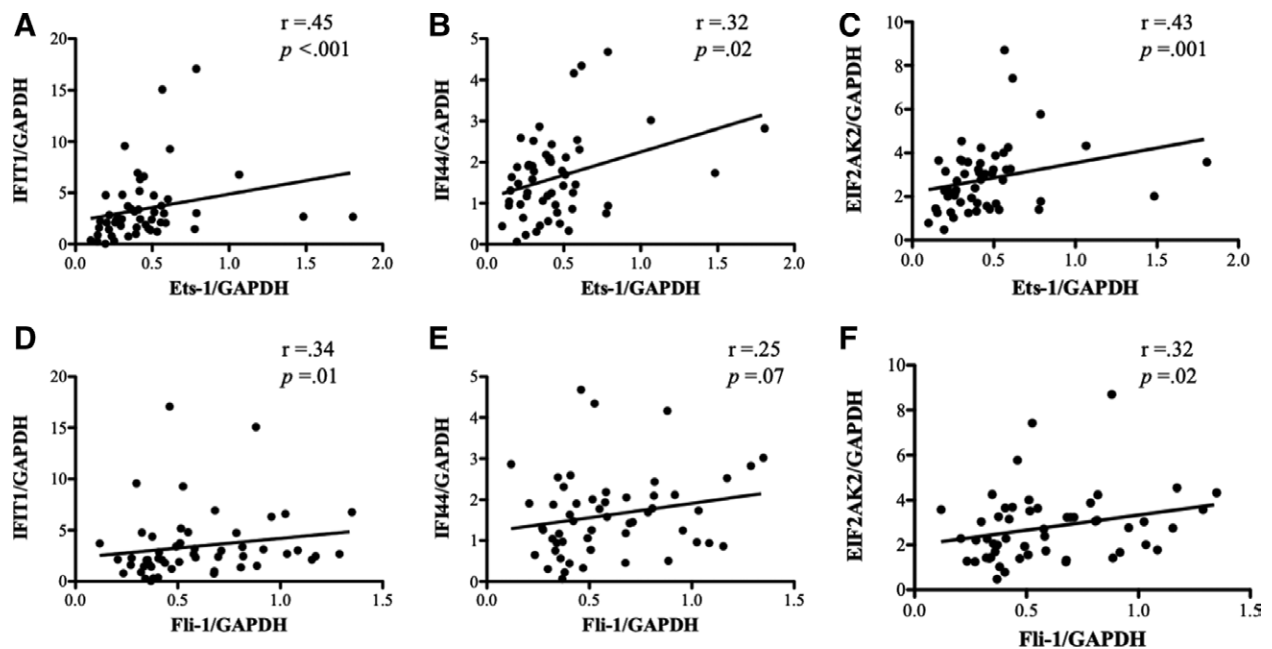


Figure 5. Gene expression of interferon-inducible genes (IFIGs) and association with Ets-1 or Fli-1. (A–C) In the patients with systemic lupus erythematosus (SLE), significantly positive correlations were found between Ets-1 and all IFIGs (interferon-inducible protein with tetratricopeptide 1 [IFIT1], interferon-inducible protein 44 [IFI44], and eukaryotic translation initiation factor 2 alpha kinase 2 [EIF2AK2]). (D–F) In the patients with SLE, significantly positive correlations were found between Ets-1 and IFIGs, except for IFI44.

specimens from each individual did not allow the measurement of the expression of each gene on a segmented PBMC or the measurement of the gene expression of different IFIGs. We were unable to examine the expression levels of various IFIGs and genes after subdividing the PBMCs. A large, multicenter, prospective study of patients before treatments and of patients with relatively similar treatments needs to be conducted to reveal more detailed and accurate information on the mechanisms.

In summary, we found that IFIG expression was significantly higher in the PBMCs from patients with SLE than that from HDs and patients with RA. IFIT1 expression was positively related to the expression of Ets-1 and Fli-1 in the PBMCs from patients with SLE. In addition to B and T cell-mediated pathways as previously reported, Ets-1 and Fli-1 could regulate the pathogenesis of SLE through type I IFNs. The role of Ets-1 and Fli-1 in SLE needs further investigation.

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