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2. 学会発表

なし

G. 知的財産権の出願・登録状況
（予定を含む）

1. 特許取得
該当なし

2. 実用新案登録
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3. その他
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Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

【雑誌】欧文

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Ota Y, **Kawaguchi Y**, Takagi K, Ichida H, Gono T, Hanaoka M, Higuchi T, Yamanaka H	Ghrelin attenuates collagen production in lesional fibroblasts from patients with systemic sclerosis.	Clinical Immunology	147	71-78	2013
Katsumata Y, **Kawaguchi Y**, Baba S, Hattori S, Tahara K, Ito K, Iwasaki T, Yamaguchi N, Hattori H, Nagata K, Okamoto Y, Yamanaka H, Hara M	Serum antibodies against the 70k polypeptides of the U1 ribonucleoprotein complex are associated with psychiatric syndromes in systemic lupus erythematosus: a retrospective study.	Modern Rheumatology	23	71-80	2013
Hanaoka M, Gono T, **Kawaguchi Y**, Uchida K, Koseki Y, Katsumata Y, Kaneko H, Takagi K, Ichida H, Nitta K, Yamanaka H	Urinary free light chain is a potential biomarker for ISN/RPS class III/IV lupus nephritis.	Rheumatology	52	2149-2157	2013

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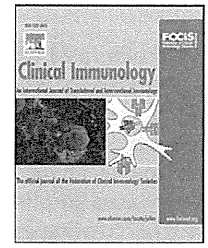

Ghrelin attenuates collagen production in lesional fibroblasts from patients with systemic sclerosis☆

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Abstract Systemic sclerosis (SSc) is a connective tissue disease characterized by thickening of the skin and tissue fibrosis of the internal organs. Ghrelin is primarily described as a gut hormone, and many studies currently indicate that ghrelin has protective effects in different organs, including the heart, pancreas, lung and liver, resulting from its anti-fibrotic properties. We found decreased levels of ghrelin in the plasma from patients with SSc compared with those from healthy controls. In skin fibroblast cultures, recombinant ghrelin diminished the production of collagen type I. In addition, the mRNA levels of *COL1A2* and *TGFB* genes were significantly decreased by the stimulation of ghrelin. We showed that ghrelin may exert anti-fibrotic effects in the skin fibroblasts from patients with SSc. Because the plasma levels of ghrelin are low in SSc, the administration of ghrelin could be a new strategy for the treatment of SSc.

1. Introduction

Systemic sclerosis (SSc) is a multisystem disorder of connective tissue characterized by the excessive accumulation of extracellular matrix in the skin and various internal organs [1,2]. Although the pathogenesis of SSc is not fully understood, autoimmunity may be involved because the production of SSc-specific autoantibodies has been well established for the past two decades, including anti-topoisomerase I antibody, anti-centromere antibody, and anti-RNA polymerase I/III antibody [3]. Fibrosis and endothelial injury are the central manifestations of SSc [4]. Skin fibrosis, interstitial lung disease, gastrointestinal involvement, and cardiac involvement may result from the fibrosis [5]. The pathological examinations of the tissue fibrosis revealed the abnormality of extracellular matrix production in the lesional tissues of patients with SSc [6]. More than 90% of patients with SSc develop gastrointestinal tract involvement [7], but severe manifestations such as pseudo-obstruction, severe constipation, megacolon and malabsorption syndrome are rarely observed in patients with SSc. Although intestinal involvement is believed to result from smooth muscle fibrosis [8], the pathophysiology of the intestinal involvement is not fully understood. Unfortunately, intestinal involvement is often detected when severe complications have already occurred, and it is irreversible and difficult to manage [9]. Therefore, finding new treatments for the organ fibrosis associated with SSc is extremely important.

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Ghrelin, a 28-amino-acid peptide with n-octanoylation indispensable for binding to the growth hormone secretagogue receptor (GHSR), was originally discovered in human and rat stomachs in 1999 [10]. Ghrelin molecules are present as two major endogenous forms, an acylated ghrelin and a desacylated ghrelin [11]. The modification is highly susceptible to circulating esterases, which can convert the active form of ghrelin (acylated form) to the desacylated form [12]. This conversion can occur within minutes after sample collection and can lead to variability and distracting artifacts in acylated ghrelin measurements. In addition to potent growth hormone-releasing effects, ghrelin has been found to have other peripheral effects. Although ghrelin mRNA is particular abundant in the stomach and intestine, it is also expressed at low levels in other organs, notably, the brain, pituitary gland, heart, lung, pancreas, kidney, and placenta [13]. One of the most important biological activities of ghrelin is the stimulation of food intake during the long-term regulation of body weight [14]. The wide distribution of the GHSR in various organs suggests a potentially broad array of actions for ghrelin. Recently, in addition to growth hormone-releasing effects, peripheral effects such as cytoprotection, vasodilatation, and anti-inflammation have been attributed to ghrelin [15,16].

Plasma ghrelin levels increase upon fasting and insulin-induced hypoglycemia and decrease in response to the oral or intravenous administration of glucose. The fasting plasma ghrelin level is elevated in anorexia nervosa and cachexia and is reduced in obesity [17,18]. In autoimmune diseases, the serum ghrelin levels have been measured in patients with rheumatoid arthritis and ANCA-associated vasculitis [19,20]. In patients with rheumatoid arthritis, the serum ghrelin levels did not differ from those of the healthy group. Conversely, the serum ghrelin levels were significantly elevated in patients with ANCA-associated vasculitis compared with the healthy controls (HCs), and the levels were correlated with disease activity.

In 2008, rikkunshito, an herbal medicine, was shown to suppress cisplatin-induced anorexia in rats by increasing the plasma acylated ghrelin. Recent studies also indicated that recombinant ghrelin was successfully administered to patients with a variety of disorders, such as anorexia, caquexia, and gastroparesis [21]. Moreover, ghrelin improves the functional capacity in elderly patients with congestive heart failure and chronic obstructive pulmonary disease, which might be due to its anti-fibrotic effects.

The aim of this study was to investigate the levels of acylated and desacylated ghrelin in the plasma of patients with SSc. We also estimated the association between the plasma ghrelin levels and disease phenotypes, such as organ involvement, limited or diffuse cutaneous types and SSc-specific autoantibodies. In addition, we evaluated the biological functions of ghrelin in skin fibroblasts derived from patients with SSc.

2. Patients and methods

2.1. Patients

Blood samples were obtained from 45 Japanese patients with SSc (38 female and 7 male). All patients fulfilled the criteria for SSc proposed by the American College of Rheumatology [22]. Patients were grouped according to the classification system proposed by LeRoy et al. [23]. The baseline characteristics are shown in Table 1. Twenty seven healthy Japanese individuals were enrolled in the present study as HCs (22 female and 5 male; median age: 52 years old). Informed consent was obtained from each patient and each healthy donor.

2.2. Assessment of clinical characteristics

Skin thickness was quantified using the modified Rodnan total skin thickness score (TSS, maximum possible score: 51) [24]. Interstitial lung disease (ILD) was assessed by chest radiography and high-resolution computed tomography (HRCT). Using HRCT, we defined the presence of the following as indicating ILD: a ground-glass appearance, a reticular pattern, and a honeycomb pattern. All patients were assessed by echocardiography, and all patients with a right ventricular systolic pressure of greater than 40 mm Hg underwent right ventricular catheterization. Pulmonary arterial hypertension (PAH) was defined as a mean pulmonary artery pressure of 25 mm Hg or greater and a pulmonary capillary wedge pressure of 15 mm Hg or less at rest [25]. Scleroderma renal crisis (SRC) was defined as malignant arterial hypertension, rapidly progressive renal failure and/or microangiopathic hemolytic anemia (MHA). Patients with hypertension of recent onset without increases in serum creatinine levels or MHA were not categorized as having SRC. A digital ulcer (DU) was defined as a fingertip or toe ulceration that occurred more than once a year. Esophagus involvement was defined as hypomotility shown by barium radiography or esophageal reflux shown by FGS. Intestinal involvement was defined as the presence of malabsorption syndrome, episodes of pseudo-obstruction and/or the need for parenteral hyperalimentation.

Table 1 Baseline characteristics of the patients and healthy controls.

	SSc (n = 45)	HC (n = 27)
Age, years, median (range)	57 (18–73)	52 (32–70)
Gender, female:male	38:7	22:5
Clinical features		
dcSSc, no (%)	36 (80)	
ILD, no (%)	30 (67)	
PAH, no (%)	8 (18)	
SRC, no (%)	3 (7)	
Esophageal reflux, no (%)	37 (82)	
Intestinal involvement, no (%)	2 (4)	
DU, no (%)	16 (36)	
Autoantibodies		
ANA, no (%)	44 (98)	ND
Topo, no (%)	24 (53)	ND
U1RNP, no (%)	8 (18)	ND
CENP, no (%)	9 (20)	ND

SSc, systemic sclerosis; HC, healthy controls; dcSSc, diffuse cutaneous SSc; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; SRC, scleroderma renal crisis; DU, digital ulcer; ANA, anti-nuclear antibody; Topo, anti-topoisomerase I antibody; CENP, anti-centromere antibody; U1RNP, anti-U1RNP antibody.

2.3. Detection of autoantibodies

The presence of CENP was determined by the distinctive indirect immunofluorescence pattern of HEp-2 cells and by an enzyme immunoassay (MESACUP-2 Test, Medical & Biological Lab, Nagoya, Japan). Both Topo I and U1RNP were determined by double immunodiffusion against calf thymus extracts with commercially available kits (Medical & Biological Lab).

2.4. Measurement of plasma acylated ghrelin and desacylated ghrelin

Blood samples were immediately transferred to chilled polypropylene tubes containing EDTA-2Na (1 mg/ml) and aprotinin (500 U/ml) and centrifuged at 4 °C. Hydrochloride acid (1 N) was added to samples at 10% of the plasma volume immediately after the separation of the plasma, and the plasma sample was stored at −80 °C until assayed. The levels of acylated and desacylated ghrelin were measured using two commercially available enzyme-linked immunosorbent assay (ELISA) kits (Mitsubishi Chemical Medience, Tokyo, Japan). The minimal detection limits of acylated ghrelin and desacylated ghrelin in this assay were 2.5 and 12.5 fmol/ml, respectively.

2.5. Cell culture

Skin biopsies from the dorsal forearm were performed as a diagnostic procedure in 5 patients with SSc. No medical interventions, such as corticosteroids or immunosuppressive agents, were performed before the skin biopsy. The fibroblasts were cultured in Dulbecco's Modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Invitrogen). Dermal fibroblasts from passages 3–5 were used for the experiments.

2.6. Measurement of type I collagen

Cultured fibroblasts were prepared at a density of 2×10^4 cells/well in 24-well culture plates with DMEM plus 10% FBS. After 24 h of culture, the medium was removed, and the cells were cultured in serum-free medium (QBSF-51, Sigma-Aldrich, St. Louis, MO). To assess the effect of ghrelin, skin fibroblasts were cultured with recombinant human ghrelin (Peptide Inc., Minoh, Japan) dissolved in phosphate-buffered saline at concentrations of 10–1000 nM for 24–72 h. The supernatants were collected and stored at −80 °C. Procollagen type I C-peptide was measured using an ELISA kit (Takara Shuzo, Kyoto, Japan).

2.7. Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

RNAs were extracted using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. For the real-time PCR analysis, total RNA was treated with DNase I (Invitrogen), and cDNA was generated using SuperScript III (Invitrogen) with oligo dT primers. The real-time PCR analysis was conducted on Chromo4 (Bio-Rad, Hercules, CA) using the TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) for *GHSR1A*, *COL1A2*, *TGFB* and *GAPDH*.

2.8. GHSR1 expression in SSc fibroblasts

GHSR1 expression was analyzed by immunohistochemical staining. Briefly, SSc skin fibroblasts were plated on an 8-chamber slide, cultured for 72 h and used for immunostaining. The samples were washed three times with PBS and fixed in 2% paraformaldehyde (PFA) (Alfa Aesar, Ward Hill, MA) and 0.1% Triton X-100 (Sigma-Aldrich) in PBS for 1 h. After washing three times with PBS, the cells were incubated with a blocking solution (Dako Japan, Tokyo, Japan) for 20 min at room temperature. Excess blocking solution was drained, and the samples were incubated with anti-GHSR1a antibodies (Phoenix Pharmaceuticals Inc., Burlingame, CA) for 30 min at room temperature. The samples were then rinsed with PBS and incubated with a biotinylated secondary antibody (Vector Lab, Burlingame, CA) for 30 min. The staining was developed using the diaminobenzidine substrate (Dako Japan), and counterstaining was performed with hematoxylin. The samples were photographed with a photomicroscope (Olympus, Tokyo, Japan).

2.9. Statistical analysis

A *student's t*-test was used to compare plasma acylated ghrelin and desacylated ghrelin levels, and Fisher's exact probability test was used to compare the frequencies of 2 groups. The Mann–Whitney U test was used to compare the total skin thickness score (TSS) between the 2 groups of patients with SSc. A probability (P) value of <0.05 was considered significant.

3. Results

3.1. Plasma ghrelin levels in SSc

The levels of acylated ghrelin and desacylated ghrelin in the plasma samples from patients with SSc and the HCs were assessed by ELISA (Figs. 1A and B). The levels of plasma acylated ghrelin were 13.6 ± 6.3 fmol/ml in the patients with SSc and 22.7 ± 8.8 fmol/ml in the HCs. The levels of plasma desacylated ghrelin were 72.8 ± 38.6 fmol/ml in the patients with SSc and 152.9 ± 84.5 fmol/ml in the HCs. Both acylated ghrelin and desacylated ghrelin concentrations were significantly lower in the patients with SSc than in the HCs ($P < 0.0001$). The ratio of acylated ghrelin and desacylated ghrelin was not different between the patients with SSc and the HCs (Fig. 1C).

In the SSc subgroups, the levels of acylated ghrelin in the patients with dcSSc ($P < 0.0001$) and lcSSc ($P = 0.035$) were significantly lower than those in the HCs (Fig. 2A). There were no significant differences in the plasma acylated ghrelin levels between the patients with dcSSc and those with lcSSc ($P = 0.27$, Fig. 2A).

3.2. Association between the clinical features of patients with SSc and plasma ghrelin levels

Fibrosis and endothelial injury are the central manifestations of SSc. To investigate which of these manifestations is associated with ghrelin levels, we estimated the association

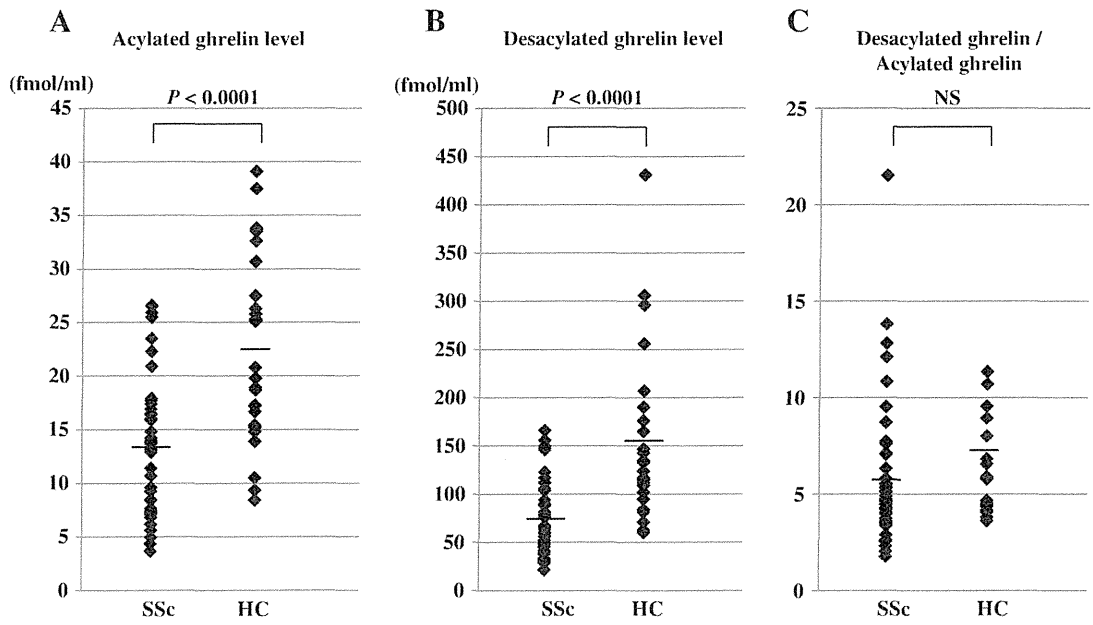

Figure 1 Levels of acylated ghrelin (A) and desacylated ghrelin (B) and the ratio of acylated to desacylated ghrelin (C) in patients with systemic sclerosis (SSc). The levels of both acylated and desacylated ghrelins were significantly decreased in patients with SSc ($P < 0.0001$) compared with healthy controls (HC). The ratio of acylated to desacylated ghrelin in patients with SSc was similar to that in HC.

between the acylated ghrelin level and the clinical manifestations, such as skin fibrosis, ILD, PAH, SRC, esophageal reflux, intestinal involvement, DU, and autoantibodies. Values lower than the mean −1 SD (13.9 fmol/l) of the levels of acylated ghrelin in healthy individuals were considered to be an abnormally low range. We assessed the clinical features between the low and normal levels of acylated ghrelin in patients with SSc. As shown in Table 2, decreased acylated ghrelin levels

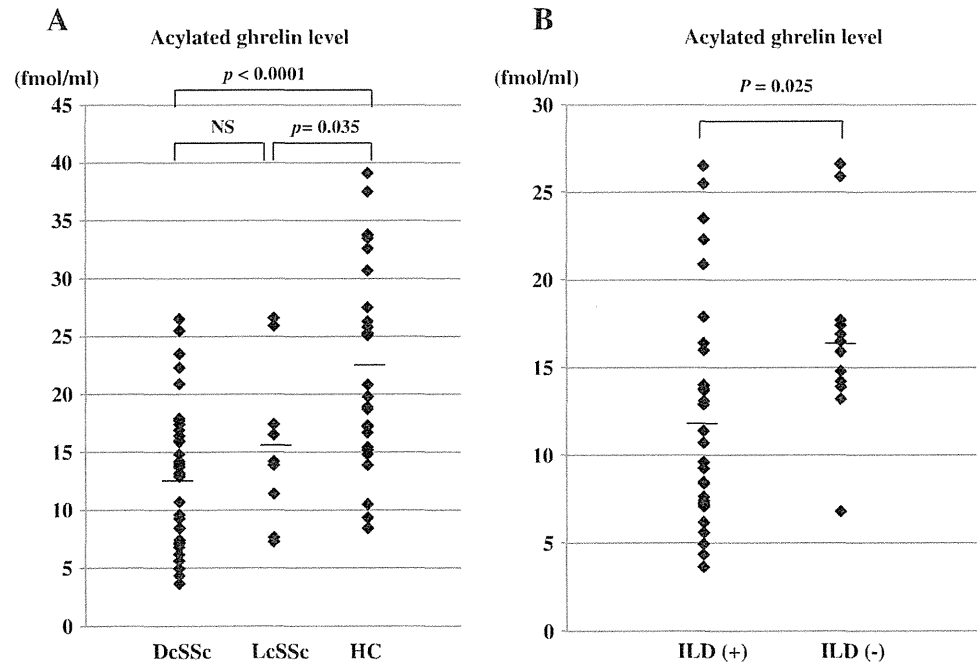

Figure 2 Levels of acylated ghrelin in patients with systemic sclerosis (SSc). (A) The concentrations of acylated ghrelin were measured in both diffuse cutaneous type (dcSSc) and limited cutaneous type (lcSSc). There was no significant difference in the levels of acylated ghrelin between dcSSc and lcSSc. (B) The concentrations of acylated ghrelin were measured in SSc patients with and without interstitial lung disease (ILD). The levels of acylated ghrelin in patients with SSc and ILD were significantly higher than those in patients with SSc in the absence of ILD ($P = 0.025$).

Table 2 Clinical and laboratory findings among SSc patients with elevated acylated ghrelin levels.

	Acylated ghrelin ≤13.9 fmol/ml	Acylated ghrelin >13.9 fmol/ml	*P*	OR	95% CI
Number of patients	24	21			
Sex, M:F	5:19	2:19			
Disease pattern, D:L	20:4	16:5	0.55	1.6	0.4–6.8
Clinical features					
TSS, mean ± SD	22 ± 9	16 ± 9	0.081		
Organ involvement					
ILD, no (%)	21 (88)	9 (43)	0.0015	9.3	2.1–41.3
PAH, no (%)	6 (25)	2 (10)	0.18	3.2	0.6–17.8
SRC, no (%)	2 (8)	1 (5)	0.63	1.8	0.2–21.6
Esophagus, no (%)	20 (83)	17 (81)	0.83	1.2	0.3–5.4
Intestinal involvement, no (%)	2 (8)	0 (0)	0.18	–	–
DU, no (%)	7 (29)	9 (43)	0.45	0.6	0.2–2.2
Autoantibodies					
ANA, no (%)	24 (100)	20 (95)	0.28	–	–
Topo, no (%)	14 (58)	10 (48)	0.47	1.5	0.5–5.0
U1RNP, no (%)	4 (17)	4 (19)	0.83	0.9	0.2–3.9
CENP, no (%)	3 (13)	6 (29)	0.18	0.4	0.1–1.7

TSS, total skin thickness score; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; SRC, scleroderma renal crisis; DU, digital ulcer; ANA, anti-nuclear antibody; Topo, anti-topoisomerase I antibody; CENP, anti-centromere antibody; U1RNP, anti-U1RNP antibody.

were observed in 53% (24/45) of all SSc patients, 56% (20/36) of dcSSc patients, and 56% (4/9) of lcSSc patients. The frequency of ILD in patients with decreased acylated ghrelin levels was significantly higher than that in patients with normal acylated ghrelin levels (88% versus 43%; $P = 0.0015$, OR: 9.3; 95% CI: 2.1–41.3).

Consistent with these observations, the plasma levels of acylated ghrelin were significantly lower in SSc patients with ILD than those without ILD ($P = 0.025$, Fig. 2B). Additionally, the TSS values in patients with decreased acylated ghrelin levels showed a tendency to be higher than those in patients with normal acylated ghrelin levels ($P = 0.081$, Table 2).

3.3. GHSR1A expression in skin fibroblasts of patients with SSc

To estimate the expression of GHSR1a (the growth hormone secretagogue receptor 1a), which is a ligand of acylated ghrelin in skin fibroblasts, we examined the mRNA and protein levels. As shown in Fig. 3A, the *GHSR1A* was expressed at the transcriptional level in skin fibroblasts from both SSc patients and normal individuals, and there was no difference in the expression levels between the two groups. Immunohistochemical staining using a specific anti-GHSR1a antibody revealed that the expression of GHSR1a was detected in cultured skin fibroblasts from both SSc patients and normal controls (Fig. 3B).

3.4. Effects of ghrelin on collagen production in skin fibroblasts derived from patients with SSc

Thus, we explored the effects of ghrelin on collagen production in skin fibroblasts derived from patients with SSc. Skin fibroblasts derived from 5 patients with SSc were used in the present study. In the experiments in vitro, procollagen type I C-peptide production was significantly suppressed by 100 nM and 1 μM ghrelin in cultured SSc fibroblasts for 72 h ($P < 0.05$, Fig. 4). To determine whether the inhibitory effect of ghrelin on collagen type I occurred at the transcription or post-transcription level, real-time RT-PCR was performed. The levels of *COL1A2* mRNA were significantly decreased by ghrelin stimulation (Fig. 5A). Because intrinsic TGF-β signal transduction contributes to the development of tissue fibrosis in SSc, the effects of ghrelin on *TGFB1* expression were investigated. The analysis of real-time RT-PCR using specific primers for *TGFB1* revealed the inhibitory effects of ghrelin on the mRNA levels of *TGFB1* (Fig. 5B).

4. Discussion

Ghrelin is a gut hormone that is also produced by extra-intestinal tissues and exerts a variety of pleiotropic effects in parenchymal cells [26]. We measured the plasma ghrelin levels (both acylated and desacylated) in patients with SSc. The present study was the first to show that both acylated and desacylated ghrelin levels were significantly decreased in the plasma from patients with SSc compared with those from healthy donors. The ratio of acylated to desacylated ghrelin was not different between patients with SSc and healthy donors. These findings suggest that the production of total ghrelin in SSc patients is diminished but that enzymatic acylation after translation may be normally regulated in SSc patients as well as healthy individuals. We also showed that the patients with low levels of acylated ghrelin had a significantly higher rate of ILD and that the levels of TSS tended to be associated with low acylated ghrelin levels. These results indicated that the levels of acylated ghrelin were inversely correlated with tissue fibrosis.

Ghrelin is predominantly produced by a distinct type of endocrine cell in the gastric oxyntic glands [27]. Involvement

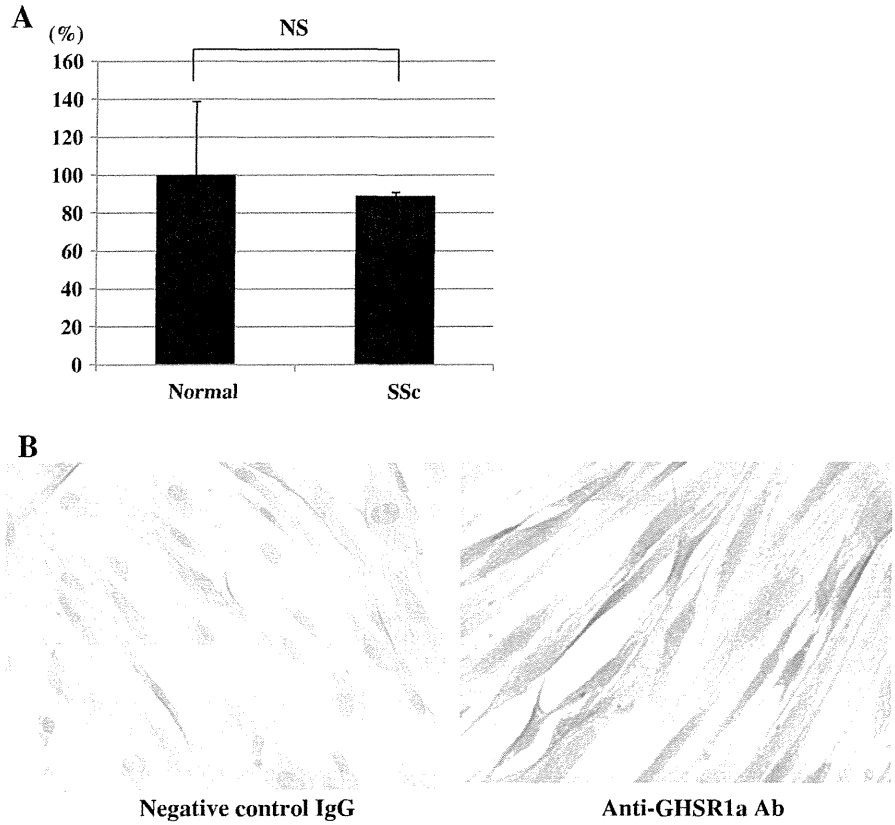

Figure 3 Expression of growth hormone secretagogue receptor (GHSR)1 mRNA and protein in skin fibroblasts. (A) RT-PCR demonstrated the presence of *GHSR1A* mRNA in skin fibroblasts from both systemic sclerosis (SSc) patients and normal individuals, and there was no difference in the expression levels between the two groups. (B) The expression of GHSR1a in skin fibroblasts derived from patients with SSc was estimated by immunohistochemistry. The left panel shows the staining using the negative control IgG, and the right panel shows positive staining using the anti-GHSR1a antibody.

of the upper intestinal tracts, including the esophagus and stomach, is a common complication in patients with SSc [8]. The clinical features of the stomach are gastroparesis and telangiectasia, resulting in atrophy of the acid-secreting mucosa with a resultant loss of feedback to antral gastrin release [28]. These patients frequently had atrophy of the

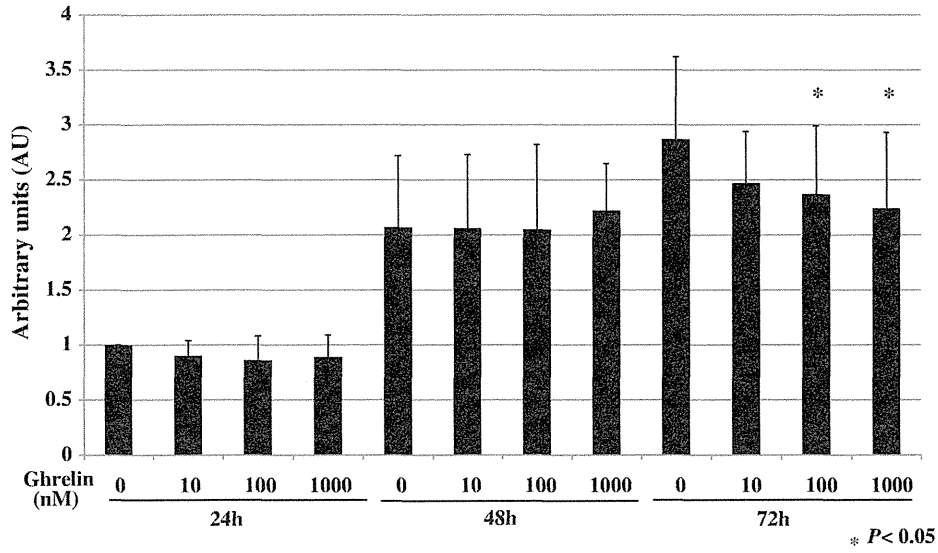

Figure 4 Effect of ghrelin on collagen production in cultured skin fibroblasts from patients with systemic sclerosis (SSc). The fibroblasts were exposed to 0–1000 nM ghrelin for 24–72 h. In 72 h, recombinant ghrelin diminished the production of collagen type I in a dose-dependent manner.

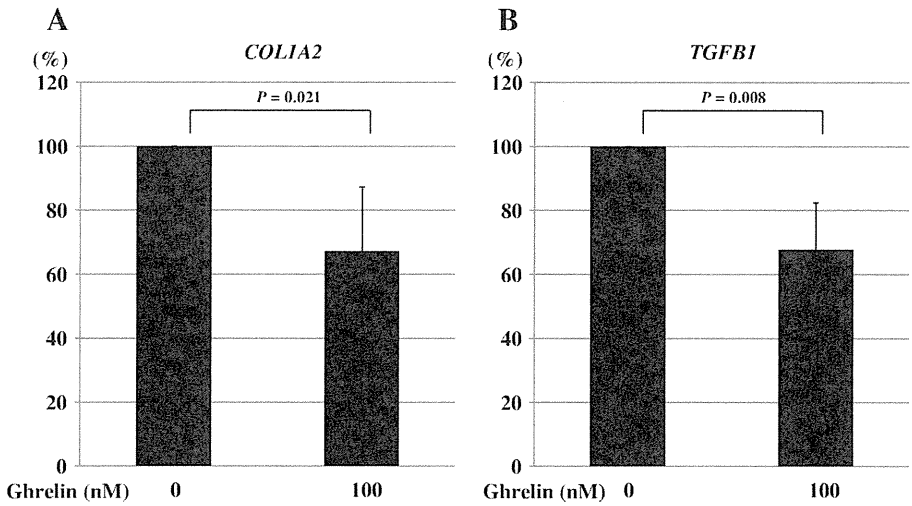

Figure 5 Expression of COL1A2 (A) and *TGFB1* (B) mRNAs in skin fibroblasts exposed to 100 nM ghrelin for 24 h. Recombinant ghrelin exerted the inhibitory effects in the transcription of *COL1A2* and *TGFB1*.

oxyntic gland mucosa. This clinical background in patients with SSc could explain the strikingly low levels of ghrelin in the blood.

In in vitro experiments, we demonstrated for the first time that recombinant ghrelin inhibited collagen type I production in skin fibroblasts. We provided evidence that ghrelin directly reduces collagen synthesis at the mRNA level in cultured skin fibroblasts. However, our observations indicated that ghrelin also exerted inhibitory effects on TGF-β1 expression, which may play a central role in the tissue fibrosis in SSc [29–31]. Taken together, ghrelin may be a potent regulator to suppress fibrosis directly and through the inhibition of TGF-β1.

In 2010, Moreno and colleagues reported that ghrelin reduced the expression of collagen type I and TGF-β1 in hepatic stellate cells, suggesting that ghrelin may be a candidate to treat hepatic fibrosis [32]. More recently, ghrelin was reported to ameliorate bleomycin-induced acute lung injury, which is a mouse model of lung fibrosis [33]. That report indicated that ghrelin suppressed various cytokines and growth factors and prolonged the survival of the bleomycin-treated mice. The authors suggested a novel attractive therapeutic strategy for the treatment of acute lung injury.

In conclusion, the levels of plasma ghrelin might be well inversely correlated to the severity of the fibrosis. Ghrelin exhibited an anti-fibrotic effect in skin fibroblasts in vitro. The administration of ghrelin could be a novel strategy for the treatment of skin and lung fibrosis in patients with SSc.

Conflict of interest statement

The author(s) declare that there are no conflicts of interest.

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ORIGINAL ARTICLE

Serum antibodies against the 70k polypeptides of the U1 ribonucleoprotein complex are associated with psychiatric syndromes in systemic lupus erythematosus: a retrospective study

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Abstract
Objectives We assessed the association between serum autoantibodies against the 70-kDa polypeptide of the U1-ribonucleoprotein (RNP) complex (U1-70k) and the central nervous system (CNS) syndromes in systemic lupus erythematosus (SLE) patients.
Methods We studied 106 hospitalized patients with active SLE, comparing those with ($n = 32$) and without ($n = 74$) CNS syndromes. CNS syndromes were further classified into neurologic ($n = 21$) and psychiatric ($n = 15$) disorders. Immunoglobulin G (IgG) anti-U1-70k antibodies were measured by enzyme-linked immunosorbent assay (ELISA) using recombinant antigens. IgG antibodies against whole U1-RNP were measured using commercial ELISA kits.
Results Although there was no significant difference in the levels of serum anti-U1-70k antibodies in SLE patients with or without CNS syndromes ($p = 0.83$), the levels were significantly elevated in SLE patients compared with patients without psychiatric syndromes ($p = 0.030$). In contrast, no significant difference was observed in the levels of serum anti-U1-RNP antibodies in SLE patients with or without psychiatric syndromes ($p = 0.555$).
Conclusions These results indicate that serum anti-U1-70k antibodies are associated with psychiatric syndromes in SLE but that they are not associated with CNS syndromes as a whole or with neurologic syndromes. The anti-U1-70k antibodies might be involved in the pathological mechanisms of psychiatric syndromes in SLE.

Keywords Autoantibody · Central nervous system · Systemic lupus erythematosus

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Introduction

Central nervous system (CNS) lupus is a serious and potentially life-threatening manifestation of systemic lupus erythematosus (SLE) and occurs in 37–95 % of cases and is associated with an increased risk of death [1]. Despite the frequency and severity of CNS lupus, the lack of a diagnostic gold standard hinders the differentiation of primary CNS lupus from secondary neuropsychiatric (NP) manifestations that are unrelated to SLE at their onset [1–3]. The American College of Rheumatology (ACR) developed a standardized nomenclature system to provide case definitions for 19 NP syndromes associated with SLE, including reporting standards and recommendations for laboratory and imaging tests [2]. Although this standardized nomenclature has helped clarify a complicated situation, its usefulness as a clinical diagnostic instrument remains to be determined. A significant number of reports, including our own, found an association between NP manifestations of SLE and the presence of autoantibodies,

although contradictory data are reported in some cases [1, 4–11]. Although most of these autoantibodies may merely be an epiphenomenon [7], there is some evidence that supports their pathogenic roles. The induction of depression-like behavior was demonstrated by an intracerebroventricular injection of antiribosomal P into mice [12]. It was also reported that an integral membrane protein of the neuronal cell surface was a target for antiribosomal P autoantibody and that these antibodies could cause a rapid and sustained increase in calcium (Ca^{2+}) influx in cultured neurons through interaction with this neuronal surface P antigen, resulting in apoptotic cell death [13]. Furthermore, we reported that anti-*N*-methyl-D-aspartate receptor subunit 2A reactive antibodies decreased cell viability by Ca^{2+} influx in SLE by inhibiting the binding capacity of zinc [14]. In this context, identification and characterization of novel specific autoantibodies could help elucidate the etiology of NP manifestations that accompany SLE and open new avenues of research for devising more effective diagnostic and therapeutic strategies.

Antibodies against the U1-ribonuclear protein (U1-RNP) complex are found in serum obtained from 25 % to 47 % of SLE patients, and high titers of these antibodies are diagnostic of mixed connective tissue disease (MCTD) [15]. Anti-U1-RNP antibodies are not disease specific but, rather, are associated with manifestations such as Raynaud's phenomenon, hand swelling, synovitis, myositis, and sclerodactyly [16]. Anti-U1-RNP immunity has been implicated in the disease pathogenesis of SLE and MCTD through several methods in serial works by Greidinger and others [17–20]. U1-RNP is a nuclear protein-RNA complex that is important in the processing of messenger RNA (mRNA) [17]. It is composed of U1-RNA and contains a partially double-stranded secondary structure as well as polypeptides that are specific to the U1-RNP complex, including U1-A, U1-C, and 70-kDa polypeptide of the U1-RNP complex (U1-70k) [17]. Other proteins, such as Sm, may be associated with U1-RNP but are not specific to the U1-RNP complex [17]. The 70-kDa (70k) polypeptide is one of the major determinants of antibody response to U1-RNP. Anti-U1-70k antibodies often develop early in the U1-RNP antibody response and contribute to the development of antibodies against other proteins in the U1-RNP complex through the so-called epitope-spreading process [21].

Okada et al. [22] reported positive tests for anti-U1-RNP antibodies in the serum of 13 of the 14 patients with aseptic meningitis [eight with SLE, six with MCTD or undifferentiated connective tissue disease, and one with Sjögren's syndrome (SS)] among 1,560 patients with connective tissue disease, suggesting that anti-U1-RNP antibodies may be linked to CNS syndromes [22]. Sato et al. [23] reported that anti-U1-RNP antibodies in cerebrospinal fluid (CSF), as determined by an RNA immunoprecipitation assay and/or an elevation in the anti-U1-RNP index, are more specific markers for CNS lupus than are indices of CSF-derived interleukin-6 (IL-6) and immunoglobulin G (IgG) in the serum of anti-U1-RNP-antibody-positive patients with SLE or MCTD. We assessed the association of serum anti-U1-70k antibodies with CNS syndromes in SLE patients.

Patients and methods

Study participants and sample collection

From 1994 through 2007, serum samples from 106 patients with active SLE were obtained using the Tokyo Women's Medical University SLE Database. All patients were positive for four or more of the revised ACR (formerly the American Rheumatism Association) criteria for SLE [24, 25] and gave informed consent for participation. Because we wanted to compare recently diagnosed active CNS lupus patients to non-NP-SLE patients, those who had non-SLE-related NP manifestations arising from infection, uremia, electrolyte imbalance, hypoxia, brain tumors, trauma, primary mental diseases, drug use, or past histories of NP involvement were excluded because unrelated conditions could have affected current symptoms or laboratory findings. At the time of the serum collection, each patient completed a standardized medical history and was given a physical examination that included neurologic and rheumatologic assessments. Full psychiatric battery recommended by the ACR [2, 26, 27] was employed when psychiatric syndromes were clinically obvious or suspected. Serology profiling for each patient was performed using standard immunoassays. Patients were assigned to the CNS or the non-CNS group according to the presence or absence of active CNS syndromes. The detailed diagnostic criteria for these groups are described below. Control sera were obtained from age- and sex-matched healthy donors and from patients with rheumatoid arthritis (RA), systemic sclerosis (SSc), SS, and multiple sclerosis (MS), all of which were diagnosed using standard criteria [28–31]. This study was approved by the Ethical Committee of our institution, and the principles of the Declaration of Helsinki were followed throughout the study.

Diagnosis of CNS lupus

Final clinical diagnoses and classifications of the various NP syndromes for inclusion in the study were made by an experienced rheumatologist (MH) and psychiatrist (KN) according to the standardized ACR nomenclature and case definitions for NP lupus syndromes [2]. Although the ACR nomenclature and case definitions include 12 CNS and

seven peripheral nervous system syndromes [2, 26, 27], we used only the 12 CNS syndromes for the inclusion criteria for active CNS lupus because of the substantial differences between the central and peripheral nervous systems in anatomy, function, and clinical characteristics. Slight or mild cognitive dysfunction without significant clinical impairment, as indicated only by detailed neuropsychological testing (which was sometimes performed for another study), was excluded from the CNS syndromes used in our study. Tension headache and episodic tension-type headache were also excluded. CNS syndromes were further classified into neurologic disorders (aseptic meningitis, cerebrovascular disease, demyelinating syndrome, headache, movement disorders, myelopathy, and seizure disorders) and psychiatric disorders (acute confusional state, anxiety disorders, clinically evident cognitive dysfunction, mood disorders, and psychosis) [2].

Production of recombinant U1-70k protein

Recombinant U1-70k protein was produced in *Escherichia coli*, as previously described [11]. The purity of the recombinant protein was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and its reactivity against autoantibodies was assessed by Western blot analysis using serum samples from SLE patients and controls, as described below.

Serum self-IgG reactivities against the recombinant U1-70k polypeptide by Western blot

Reactivities of serum IgG autoantibodies against the recombinant U1-70k polypeptide in serum samples from some of the SLE patients were examined by standard Western blot analysis, as described previously [11].

Quantification of autoantibodies against the recombinant U1-70k polypeptide in serum samples from patients and controls by enzyme-linked immunosorbent assay (ELISA)

Next, autoantibodies against the recombinant U1-70k polypeptide in serum samples from patients with SLE and other autoimmune diseases and from the normal healthy controls (NHCs) were quantified through solid-phase, direct ELISAs using the recombinant U1-70k polypeptide, as previously described [32] with slight modifications. Purified autoantigen [0.5 μg/ml in phosphate-buffered saline (PBS)] was coated onto the wells of a microtiter plate (Nunc, Roskilde, Denmark) through overnight incubation at 4 °C. The wells were then blocked for 2 h using 200 μl of PBS containing 1 % bovine serum albumin (BSA) at room temperature. After the plate was washed five times with 400 μl of PBS containing 0.1 % Tween 20 (PBS-T), 100 μl of the diluted serum samples (1:200 in PBS-T containing 0.3 % BSA) were incubated at room temperature for 2 h. After the plate was washed, 100 μl of diluted horseradish-peroxidase-(HRP)-conjugated sheep anti-human IgG (1:20,000 in PBS-T containing 0.3 % BSA) was added to each well and incubated at room temperature for 1 h. After the plate was washed, 100 μl of substrate solution (0.04 % *o*-phenylenediamine dihydrochloride with 0.012 % H_2O_2) was added to each well and incubated at room temperature for 30 min. The reaction was stopped by the addition of 100 μl of 2 N H_2SO_4. The absorbance was measured at 492 nm using a microplate reader. Autoantibody titers were expressed as the mean optical density (OD) values of the triplicate wells. All samples were assayed at the same time with the same incubation and development time. In addition, we coated a few wells of each plate with nonspecific human IgG as controls to check the stability of assays across experiments so that data could be compared across experiments. Samples with positive results by ELISAs were double checked for their reactivities to the recombinant U1-70k polypeptide by Western blot. Nonspecific reactions in ELISAs were examined using a plate coated with 0.5 μg/ml BSA in PBS with the same method as above.

Quantification by ELISA of the anti-U1-RNP antibodies in serum samples from patients and controls

Levels of serum anti-U1-RNP antibodies were also determined by commercial ELISA kits using mixed antigens, including recombinant U1-70k polypeptide, A and C polypeptides, and in-vitro-transcribed U1 RNA, in addition to recombinant RNP (Mesacup® RNP-II test; Medical and Biological Laboratories, Nagoya, Aichi, Japan), according to the manufacturer's protocol [23, 33]. This ELISA kit has been shown to exhibit the same sensitivity and specificity for detecting anti-U1-RNP antibodies as the double immunodiffusion method [33]. The numbers of NHC samples examined varied among anti-U1-70k-antibody and anti-U1-RNP-antibody ELISAs because of sample and antigen availability.

Statistical analyses

Results of ELISAs were correlated with the final clinical diagnosis for each patient. Two-group comparisons were analyzed using Fisher's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables. Three (or more) group comparisons were analyzed by the Kruskal–Wallis test, the Steel multiple comparison test, and the Steel–Dwass multiple comparison test for continuous variables. Relationships between antibody levels and

other continuous variables were analyzed using Spearman's rank correlation. Values of $p < 0.05$ were considered statistically significant, and all tests were two tailed. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the antibodies were also calculated. All statistical analyses were performed using the JMP statistical software (version 9.0; SAS Institute, Cary, NC, USA).

Results

Clinical characteristics of SLE patients

Of the 106 patients with SLE enrolled in the study, 100 were women and six were men. Median patient age was 31 (range 16–68) years. Median disease duration after SLE diagnosis was 1 (range 0–20) years. Patients were all Japanese, except for a woman who was Chinese. The median score of Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)–Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [34] of the patients was 9.5 (range 0–31). Recently (i.e., within the previous month) diagnosed, active CNS syndromes were observed in 32 patients (CNS group), whereas the remaining 74 patients showed neither current CNS syndromes nor a history of CNS syndromes (non-CNS group). A neurologic disorder was diagnosed in 21 participants, a psychiatric disorder in 15, and both in four. The final clinical diagnoses and classifications of the various NP syndromes for inclusion in the study were made according to the standardized ACR nomenclature and case definitions for NP-SLE [2, 26, 27]. No significant differences in other clinical parameters, such as sex, age, and disease duration, were observed between the CNS and non-CNS groups ($p = 0.78$, 0.78, and 0.68, respectively). Antiphospholipid antibodies have been the most widely investigated antibodies in NP-SLE, and there is abundant research on their association with focal (stroke, seizures, epilepsy, and migraine headaches) and diffuse neurological manifestations, including cognitive impairment [4]. However, we found no significant association between antiphospholipid antibodies and CNS syndromes, neurologic disorders, or psychiatric disorders among SLE patients ($p = 0.79$, 0.73, and 0.76, respectively).

Western blot analysis of serum self-IgG reactivities against recombinant U1-70k polypeptide

Reactivities of the serum IgG autoantibodies against recombinant U1-70k polypeptide in serum samples from some SLE patients in the CNS group ($n = 27$) were investigated by Western blot analysis (Fig. 1a). The positive samples all yielded positive ELISA results, and the samples generating negative results all produced negative ELISA results. In addition, we performed Western blot analysis for specificity controls using serum samples from the NHCs ($n = 10$) and patients with RA ($n = 12$), SSc ($n = 11$), SS ($n = 12$), and MS ($n = 11$) and observed positive results, with a small percentage of patients with other diseases, as previously reported [20] (Fig. 1b–f, respectively).

Anti-U1-70k antibody titers as measured using ELISA were higher in patients with active SLE than in NHCs or patients with RA, SSc, SS, or MS

Serum samples from 106 SLE patients, including 32 with active CNS syndromes, and from 135 NHCs measured using anti-U1-70k-antibody ELISAs, and 28 NHCs using anti-U1-RNP-antibody ELISAs, were subjected to ELISA to quantify the autoantibodies reactive against these antigens. The original serum samples used in verifying the recombinant U1-70k polypeptide by Western blot analysis, as described above, were included in these final samples. OD values of SLE patient serum samples were significantly higher than those of NHCs in ELISAs performed using U1-70k and U1-RNP ($p < 0.0001$ and $p < 0.0001$, respectively) (Figs. 2 and 3a, b). Samples with positive results by ELISA were also reconfirmed for their reactivities to the recombinant U1-70k polypeptide by Western blot. To assess disease specificity for SLE of anti-U1-70k antibodies, we also performed ELISAs using serum samples from patients with RA, SSc, SS, and MS. ELISA-generated U1-70k OD values for serum samples from SLE patients were significantly higher than those from patients with the other autoimmune diseases and from NHCs ($p < 0.05$ for SLE vs. all of the other controls using the Steel multiple comparison test; Fig. 2). As summarized in Tables 1 and 2, anti-U1-70k antibodies were detected in serum samples of patients exhibiting various clinical manifestations of active SLE. Prevalence and titers of anti-U1-70k antibodies were not significantly associated with SLE-specific manifestations. There was no significant correlation between levels of serum anti-U1-70k antibodies and SELENA–SLEDAI scores ($p = 0.19$ by Spearman's rank correlation). Established relationships, such as those with Raynaud's phenomenon, hand swelling, myositis, sclerodactyly, and pulmonary hypertension [16], were not assessed to avoid increasing the incidence of α errors.

Comparisons of antibody titers against U1-70k and U1-RNP among CNS and non-CNS groups using ELISA

No OD values were significantly different between the SLE patients with and without active CNS syndromes in

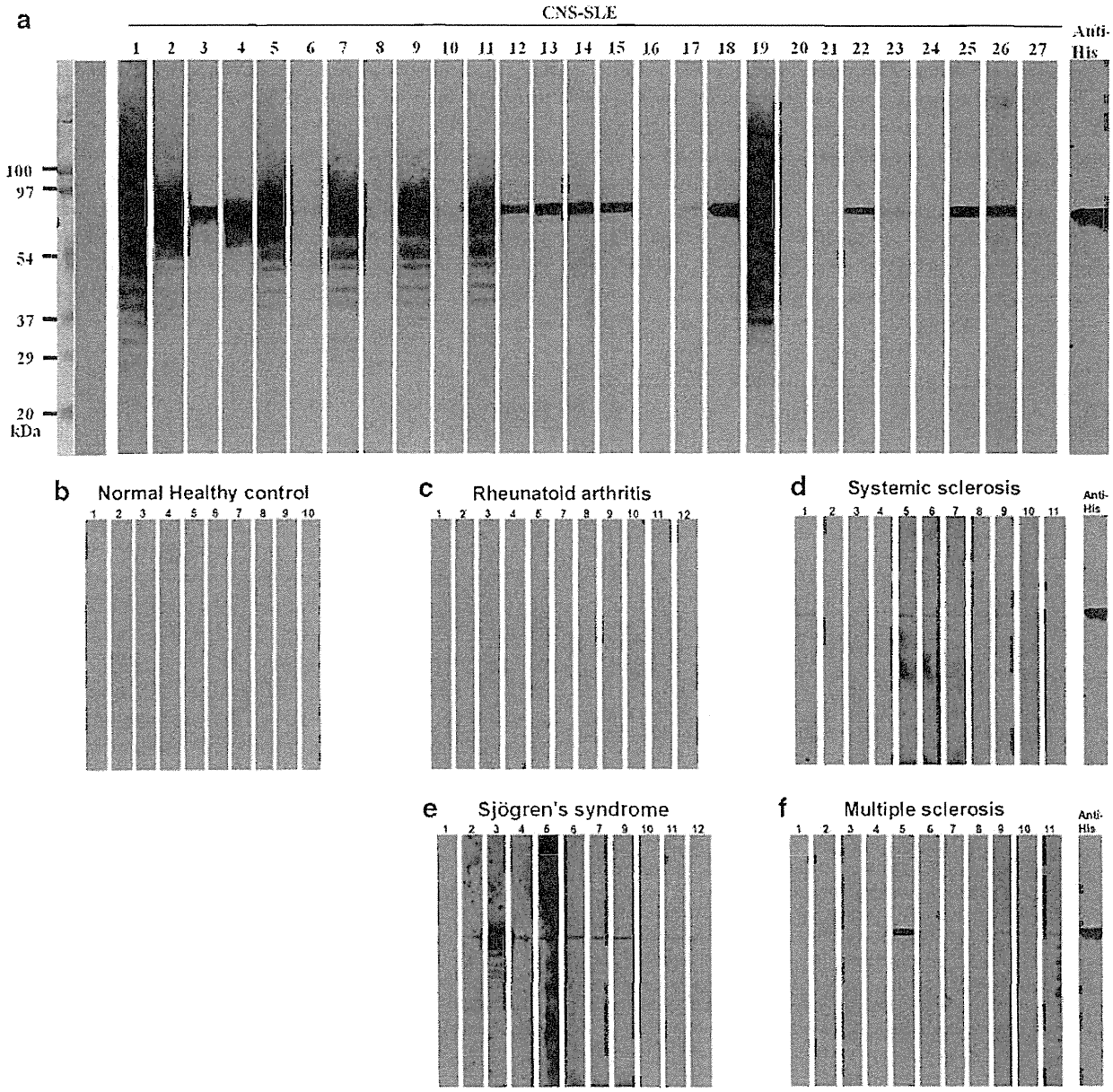

Fig. 1 Representative results of Western blot analysis of reactivities of serum immunoglobulin G (IgG) autoantibodies against the recombinant 70-kDa polypeptide of the U1-ribonucleoprotein (RNP) complex (U1-70k) in serum samples derived from a subset of systemic lupus erythematosus (SLE) patients from the central nervous system (CNS) group (**a** $n = 27$). All positive samples yielded positive results in enzyme-linked immunosorbent assays (ELISAs), and all samples producing negative results in the Western blot analysis had negative results in ELISAs. *Anti-His* results for anti-His-Tag antibodies that recognize His-Tags placed in the fusion recombinant proteins. *CNS-SLE* samples from SLE patients in the CNS group, who exhibited active CNS syndromes related to SLE. In addition, we performed Western blot analysis using serum samples from normal healthy controls (**b** $n = 10$) and patients with rheumatoid arthritis (**c** $n = 12$), systemic sclerosis (**d** $n = 11$), Sjögren's syndrome (**e** $n = 12$), and multiple sclerosis (**f** $n = 11$) and observed positive results with a small percentage of patients with other diseases

ELISAs for the following autoantibodies: U1-70k ($p = 0.83$) and U1-RNP ($p = 0.96$) (Fig. 3a, b, respectively). However, when CNS groups with psychiatric disorders and with neurologic disorders were separately compared with SLE patients without those disorders, anti-U1-70k antibody levels were significantly elevated in SLE patients with psychiatric disorders than levels in SLE patients without psychiatric disorders ($p = 0.030$ by the Steel–Dwass multiple comparison test; Fig. 4a). By contrast, there was no significant difference in levels of serum

anti-U1-RNP antibodies in SLE patients with or without psychiatric syndromes ($p = 0.555$, Fig. 4b). In addition, there were no significant differences in the levels of serum anti-U1-70k antibodies or anti-U1-RNP antibodies in SLE patients with or without neurologic syndromes ($p = 0.61$ and 0.51, Fig. 5a, b, respectively). When samples from SLE patients with and without psychiatric syndromes were compared using a theoretical cutoff titer of 0.83 (OD) for anti-U1-70k antibodies as the NHC mean + 3 standard deviations (SD), sensitivity, specificity, PPV, and NPV determined for the diagnosis of psychiatric SLE were 67 %, 59 %, 21 %, and 92 %, respectively. In addition, the prevalence of psychiatric SLE among samples with high titers of anti-U1-RNP antibodies in ELISA and of anti-U1-70k antibodies in ELISA was two times that of samples with high titers of anti-U1-RNP antibodies in ELISA but without anti-U1-70k antibodies in ELISA (21 % vs. 10 %, respectively), although this relationship did not reach statistical significance ($p = 0.66$ by Fisher's exact test), probably due to the limited sample size. In addition, we found no significant difference in levels of serum anti-U1-70k antibodies in SLE patients with or without antiphospholipid antibodies or those with or without psychiatric syndromes ($p = 0.34$, 0.47, and 0.23).

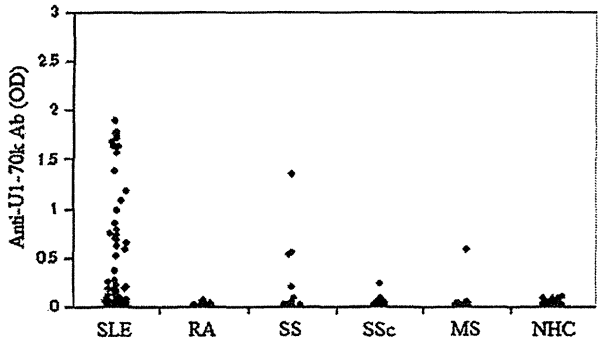

Fig. 2 Serum samples from 106 systemic lupus erythematosus (SLE) patients, including 32 with active central nervous system (CNS) syndromes, and 135 normal healthy controls (NHCs) were subjected to enzyme-linked immunosorbent assay (ELISA) to quantify the anti-70-kDa polypeptide of the U1-ribonucleoprotein (RNP) complex (U1-70k) antibodies (Abs). Optical density (OD) values of SLE patient serum samples were significantly higher than those of NHCs in the U1-70k ELISAs ($p < 0.0001$). To assess disease specificity for SLE of the anti-U1-70k antibodies, we also performed ELISAs using serum samples from patients with rheumatoid arthritis (RA), systemic sclerosis (SSc), Sjögren's syndrome (SS), and multiple sclerosis (MS) ($n = 20$, 20, 20, and 20, respectively). U1-70k ELISA OD values for serum samples from SLE patients were significantly higher than those from patients with the other diseases ($p < 0.05$ for SLE vs. the other diseases using the Steel multiple comparison test)

Discussion

Major findings of this study were the following: (1) no significant difference was observed in the levels of serum anti-U1-70k antibodies in SLE patients with or without CNS syndromes; (2) levels were significantly elevated in SLE patients with vs. those without psychiatric syndromes; and (3) no significant difference was observed in the levels of serum anti-U1-RNP antibodies in SLE patients with or without psychiatric syndromes. These observations indicate that anti-U1-70k but not anti-U1-RNP antibodies in serum might be involved in the pathological mechanisms of psychiatric syndromes, but not of whole CNS or neurologic syndromes, in patients with SLE.

Serum anti-U1-70k but not serum anti-U1-RNP antibody levels were significantly elevated in SLE patients compared with those without psychiatric syndromes. In addition, the prevalence of psychiatric SLE among samples with high titers of both anti-U1-RNP and anti-U1-70k antibodies measured with ELISAs was twice as high as those among samples with high titers of anti-U1-RNP antibodies but without anti-U1-70k antibodies, although this relationship did not reach statistical significance,

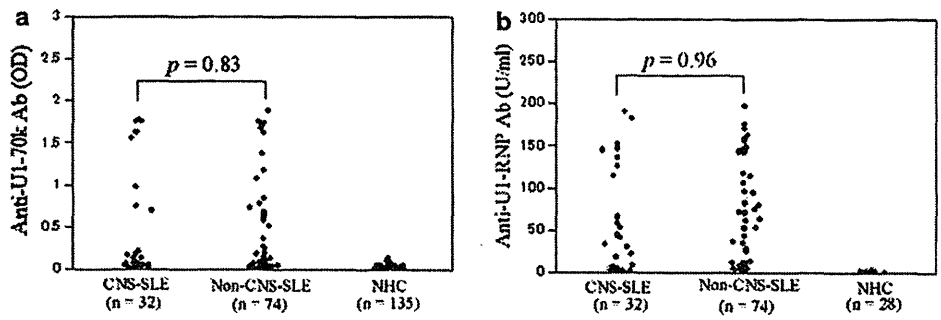

Fig. 3 Comparisons of enzyme-linked immunosorbent assay (ELISA) autoantibody titers against 70-kDa polypeptide of the U1-ribonucleoprotein (RNP) complex (U1-70k) (**a**) and U1-ribonucleoprotein (RNP) (**b**) between the central nervous system (CNS) and non-CNS groups ($n = 32$ and 74, respectively) and between 135 NHCs with anti-U1-70k antibody ELISAs and 28 NHCs with anti-U1-RNP antibody ELISAs. No optical density (OD) values in ELISAs of these antibodies (Abs) differed significantly between SLE patients with and without active CNS syndromes ($p = 0.83$ for U1-70k and $p = 0.96$ for U1-RNP by the Steel–Dwass multiple comparison test)

Table 1 Associations between systemic lupus erythematosus (SLE)-related features and anti-70-kDa polypeptide of the U1-ribonucleoprotein (RNP) complex (U1-70k) antibodies (Abs)

	Anti-U1-70k Abs		P^a	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive ($n = 47$)	Negative ($n = 59$)					
Malar rash/discoid rash	13 (28 %)	19 (32 %)	0.674	41	54	28	68
Oral or nasal ulcers	3 (6 %)	4 (7 %)	1.000	43	56	6	93
Serositis	5 (6 %)	3 (6 %)	0.533	55	57	13	92
Arthritis	13 (28 %)	13 (22 %)	0.650	50	58	28	78
Active nephritis	14 (30 %)	17 (29 %)	1.000	45	56	30	71
CNS lupus	15 (32 %)	17 (29 %)	0.832	47	57	32	71
Thrombocytopenia	5 (11 %)	3 (5 %)	0.462	63	57	11	95
Leukopenia	10 (21 %)	19 (4 %)	0.808	48	56	21	81
Positive anti-dsDNA Ab	22 (47 %)	28 (47 %)	1.000	44	55	47	53
Antiphospholipid Abs[b]	8 (17 %)	11 (19 %)	1.000	42	55	17	81
Positive ANA	47 (100 %)	58 (98 %)	1.000	45	100	100	2

CNS central nervous system, *dsDNA* double-stranded DNA, *ANA* antinuclear antibody, *PPV* positive predictive value, *NPV* negative predictive value

[a] *P* values were determined by Fisher's exact test

[b] Antiphospholipid antibodies include lupus anticoagulant, anti-cardiolipin antibodies, and anti-β2GPI antibodies

Table 2 Associations between systemic lupus erythematosus (SLE)-related features and titers of anti-70-kDa polypeptide of the U1-ribonucleoprotein (RNP) complex (U1-70k) antibodies (Abs)

Feature	Median anti-U1-70k Ab (OD)		P^a
	Present	Absent	
Malar rash/discoid rash	0.06	0.07	0.912
Oral or nasal ulcers	0.03	0.07	0.546
Serositis	0.11	0.06	0.265
Arthritis	0.14	0.06	0.269
Active nephritis	0.07	0.07	0.830
CNS lupus	0.07	0.06	0.568
Thrombocytopenia	0.17	0.06	0.258
Leukopenia	0.07	0.06	0.563
Positive anti-dsDNA Ab	0.07	0.06	0.159
Antiphospholipid Abs[b]	0.07	0.07	0.339
Positive ANA	0.07	0.04	–

CNS central nervous system, *dsDNA* double-stranded DNA, *ANA* antinuclear antibody, *OD* optical density

[a] *P* values were determined by the Mann–Whitney *U* test

[b] Antiphospholipid Abs include lupus anticoagulant, anti-cardiolipin antibodies, and anti-β2GPI antibodies

probably due to the limited sample size. We speculate that patients with high titers of anti-U1-RNP antibodies measured with ELISA but without anti-U1-70k antibodies possess antibodies against U1-A, U1-C, or U1-RNA, although we did not directly measure them. Associations between antibodies against individual U1-RNP proteins and symptoms and/or organ involvement, such as decreased lung diffusion and the presence of antibodies against U1-A and U1-C, have been reported [35]. Sato et al. [23] also reported that the anti-U1-70k index was higher than the anti-U1-A and anti-U1-C indices in the CSF of anti-U1-RNP-antibody-positive SLE patients with CNS syndromes.

Relationships with anti-U1-70k antibodies were different between psychiatric syndromes alone and CNS syndromes as a whole in SLE. Because it is unlikely that a single pathogenic mechanism is responsible for all of these syndromes, different antibodies could be responsible for different NP syndromes in SLE. In fact, diffuse NP manifestations that are practically equivalent to the psychiatric syndromes described in this study have been reported to be linked with other antibodies, such as anti-*N*-methyl-D-aspartate receptor subunit NR2 antibodies and anti-phosphorylated ribosomal (P ribosomal) antibodies [4, 9].

There are a few mechanisms that may explain how serum autoantibodies can be pathogenic in CNS syndromes in SLE patients. Autoantibodies can enter the CSF of SLE patients by passive transfer from the circulatory system through a permeabilized blood–brain barrier [1]. The blood–brain barrier may be permeabilized by factors attributable to SLE (e.g., immune complex deposition and cytokines) or independent of SLE (e.g., smoking and hypertension) [6]. For example, potential interactions between serum anti-P ribosomal antibodies and CSF antineuronal antibodies in the pathogenesis of NP-SLE have been suggested [1, 36]. Antibodies to U1-RNP interact with both mononuclear and endothelial cells [37–39]. These interactions can provide multiple putative pathways for mediation of tissue injury [20]. In this case, therefore, anti-U1-70k antibodies themselves may not be