Elevated serum interleukin-27 levels in patients with systemic sclerosis: association with T cell, B cell and fibroblast activation

Ayumi Yoshizaki,1 Koichi Yanaba,1 Yohei lwata,1 Kazuhiro Komura,2 Asa Ogawa,1 Eiji Murao,1 Fumihide Owaga,1 Motoi Takenaka,1 Kazuhiro Shimizu,1 Minoru Hasegawa,2 Manabu Fujimoto,2 Shinichi Sato1,3

ABSTRACT

Objective To determine serum levels of interleukin (IL)-27 in patients with systemic sclerosis (SSc) and relate the results to the clinical features of SSc.

Methods Serum levels of IL-27 in 91 patients with SSc and the production of IL-27 by isolated monocytes were examined by ELISA. The expression of IL-27 receptor in the skin fibroblasts, B cells and T cells was quantified by real-time PCR. The effect of IL-27 on immunoglobulin G (IgG) production of B cells, IL-17 production of CD4 T cells and proliferation and collagen synthesis of fibroblasts was also analysed.

Results Serum IL-27 levels were raised in patients with SSc compared with healthy controls and correlated positively with the extent of skin and pulmonary fibrosis and immunological abnormalities. IL-27 levels also correlated positively with serum levels of hyaluronan, recently identified as an endogenous ligand for Toll-like receptors. The retrospective longitudinal analysis showed a tendency for serum IL-27 levels to be attenuated during the follow-up period. IL-27 production by cultured monocytes was increased by hyaluronan stimulation. IL-27 receptor expression was upregulated in the affected skin fibroblasts, B cells and CD4 T cells of patients with SSc. Moreover, IL-27 stimulation increased IgG production of B cells, IL-17 production of CD4 T cells and proliferation and collagen synthesis of fibroblasts in patients with SSc compared with those in healthy controls.

Conclusion These results suggest that IL-27 and its signalling in B cells, T cells and fibroblasts contributes to disease development in patients with SSc.

INTRODUCTION

Systemic sclerosis (SSc) is a multisystem disorder of connective tissue characterised by excessive accumulation of extracellular matrix in the skin and various internal organs.1 It is accompanied by a number of immunological abnormalities including autoantibody production and elevated levels of several cytokines such as tumour necrosis factor (TNF)α and interleukin (IL)-1β, IL-4, IL-6, IL-10 and IL-17.2–4 Furthermore, previous studies have shown that serum levels of endogenous ligands for Toll-like receptors (TLR) such as hyaluronan are increased in SSc.5–8 In our previous study we showed that serum hyaluronan levels are associated with disease severity and immunological abnormalities.5 Although these abnormalities were associated with skin fibrosis and involvement of several organs, the mechanism and pathogenesis of SSc remain unknown.9

IL-27 is a new member of the IL-12 and IL-6 families which consists of an IL-12 p40-related protein and a newly discovered IL-12 p35-related protein.10 Examination of cDNA libraries has indicated that human IL-27 is highly induced in activated antigen-presenting cells such as monocytes.11–18 Although there are no studies of the regulation of IL-27 expression, signalling via TLR4 activated by lipopolysaccharide (LPS) is the key inducer of IL-27.14–16 IL-27 receptor complex comprises IL-27R (also called WSX-1) and glycoprotein 130 (gp130).10 IL-27 is the only known ligand for IL-27R. IL-27R and gp130 are found to be coexpressed by a large variety of cells including monocytes, T cells, B cells and fibroblasts, indicating that IL-27 may display pleiotropic functions.17

Previous studies have shown that the role of IL-27 in the regulation of immune responses is somewhat controversial. Some studies have shown that IL-27 promotes naïve T cell proliferation and initiates Th1 immune responses.11 18 However, other studies have shown that IL-27 suppresses the expansion of effector and memory T cells and inhibits different cytokine secretion, suggesting anti-inflammatory functions of IL-27.19 20 The dual role of IL-27 in vitro has also been demonstrated on in vivo infectious and autoimmune inflammatory models. IL-27 neutralisation suppressed inflammation in rodent adjuvant arthritis.21 In contrast, IL-27R knockout mice displayed deterioration of inflammation in autoimmune encephalomyelitis models.20 22 However, at present, our understanding of the role of IL-27 in SSc is limited. The objective of this study is therefore to assess the role of IL-27 in SSc.

PATIENTS AND METHODS

Serum samples Serum samples were obtained from 91 Japanese patients with SSc (81 women and 10 men) at the time of diagnosis. All patients fulfilled the criteria proposed by the American College of Rheumatology.23 The duration of the disease was calculated from the time of onset of the first clinical event (other than Raynaud’s phenomenon) that was a clear manifestation of SSc.24–26 Patients were grouped according to the classification system proposed by LeRoy et al27: 46 patients (44 women and 2 men) had limited cutaneous SSc (IscSc) and 45 patients (37 women
and 8 men) had diffuse cutaneous SSc (dSSc). The mean±SD age of the patients was 47±16 years (dSSc: 48±18, lSSc: 45±17) and the disease duration of patients with dSSc and lSSc was 8.5±9.5 and 3.1±2.5 years, respectively. None of the patients was treated with corticosteroid or other immunosuppressive therapy at the evaluation. Antinuclear antibody (Ab) was determined by indirect immunofluorescence using Hep-2 cells and specificities were further assessed by ELISA and immunoprecipitation. Anti-topoisomerase I Ab was positive for 41 (34 dSSc and 7 lSSc), anticientromere Ab for 37 (2 dSSc and 35 lSSc), anti-U1RNP Ab for 2 (all lSSc), anti-U3RNP Ab for 1 (dSSc), anti-RNA polymerases I and III Ab for 7 (all dSSc) and Th/To Ab for 1 (lSSc). The remaining two patients were negative for autoantibodies. Twenty age- and sex-matched healthy Japanese individuals (17 women and 3 men; age 48±15 years) were used as normal controls. In a retrospective longitudinal analysis we examined serum samples from 10 patients with SSC (5 dSSc and 5 lSSc).

Clinical assessment
Complete medical histories, physical examinations and laboratory tests including vital capacity (VC) and diffusion capacity for carbon monoxide (Dlco) were conducted for all patients. When the Dlco and VC were <75% and <80%, respectively, of the predicted normal values, they were considered to be abnormal. Skin score was measured by the modified Rodnan total skin thickness score (TSS).28 Organ involvement was defined by rheumatologists, neurologists, nephrologists and radiologists as described previously.24-26: pulmonary fibrosis=bibasilar fibrosis on chest radiography and high-resolution CT; oesophagus=hypomotility shown by barium radiography; joints=inflammatory polyarthralgias or arthritis; heart=pericarditis, congestive heart failure or arrhythmias requiring treatment; kidney=malignant hypertension and rapidly progressive renal failure with no other explanation; muscle=proximal muscle weakness and elevated serum creatine kinase.

IL-27R expression in sclerotic skin
Immunohistochemistry for IL-27R was performed as previously described.29 Skin tissues were obtained from the forearms of 10 patients with dSSc (5 women and 5 men; median age 42 years, range 28–53) and 5 normal controls. Sections were incubated with a primary monoclonal Ab to human IL-27R (10 µg/ml; Abcam, Cambridge, Massachusetts, USA). Concentration matched monoclonal mouse immunoglobulin G (IgG) (Abcam) was used as isotype control staining. The reaction products were visualised using diaminobenzidine (Dako, Carpinteria, California, USA) with methyl green as a counterstain. Each section was examined independently by two investigators (AY and SS) in a blinded manner. Expression levels of IL-27R and IL-27 were also analysed using a real-time PCR quantification method as described previously.5 Each sample was tested in duplicate.

ELISAs for serum IL-27, interferon-γ, IL-4, IL-10, IL-17 and hyaluronan levels
ELISAs for serum levels of IL-27 (ID Labs, London, Ontario, Canada), interferon-γ (R&D Systems, Minneapolis, Minnesota, USA), IL-4 (R&D Systems), IL-10 (Immunotech, Munster, Germany), IL-17 (BioSource, Fleurus, Belgium) and hyaluronan (Echelon Biosciences, Salt Lake City, Utah, USA) were performed as described using specific ELISAs.5 30 31 Each sample was tested in duplicate.

Monocyte, T cell and B cell purification and stimulation
Heparinised blood samples were obtained from 25 patients with dSSc (21 women and 4 men; median age 47 years, range 21–68) and 10 healthy individuals. Peripheral blood monocytes, CD4 T cells or B cells were enriched with each isolation kit using AutoMACS isolator (Miltenyi Biotec, Bergisch Gladbach, Germany), respectively. A total of >99% of these cells were CD14, CD4 or CD19 (data not shown). To analyse mRNA expression of IL-27R, total RNA was isolated from CD4 T cells and B cells with RNAeasy spin columns (Qiagen, Crawley, UK). Purified monocytes, CD4 T cells or B cells (1 × 10^6) were cultured in RPMI 1640 containing 10% heat-inactivated fetal calf serum (Gibco Life Technologies, Paisley, UK). Cells were serum-starved for 12 h and then stimulated with 0.2 ng/ml human recombinant IL-27 (R&D Systems) with or without 50 ng/ml low molecular weight hyaluronan (15–40 kDa; R&D Systems) or LPS (Sigma-Aldrich, St Louis, Missouri, USA). IL-27, hyaluronan and LPS were dissolved in phosphate-buffered saline (PBS). Monocytes or T cells were cultured for 72 h and IL-27 or IL-17 concentrations in the culture medium were measured by ELISA. In addition, B cells were cultured for 8 days and IgG concentrations in the culture medium were measured by ELISA (Bethyl Laboratories, Montgomery, Texas, USA). Each sample was performed in triplicate.

Fibroblast proliferation and collagen synthesis with IL-27 stimulation
Human dermal fibroblasts were obtained by skin biopsy from the forearms of six patients with dSSc (3 women and 3 men; median age 41 years, range 28–53) and six healthy individuals. Primary explant cultures were established.30 Fibroblasts were serum-starved for 12 h and then cultured for 24 h with or without rIL-27 (0.2 ng/ml) and/or rIL-17 (0.2 ng/ml; R&D Systems). Expression of IL-27R was analysed using western blot assay.33 The protein was subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred onto membranes for immunoblotting. These membranes were incubated for 1 h at room temperature with 1:200 dilutions of the anti-IL-27 Ab (Santa Cruz Biotechnology, Santa Cruz, California, USA). We processed anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) Ab (Cell Signaling Technology, Beverly, Massachusetts, USA) as an indicator of the amounts of protein loaded. The intensities of IL-27R band and GAPDH band in each lane were quantified with an automated gel digitising system (Un-Scan-It; Silk Scientific, Orem, Utah, USA). Proliferation of cultured dermal fibroblasts was quantified by a colorimetric 5-bromo-2-deoxyuridine (BrDU) cell proliferation ELISA kit (Roche Applied Science, Indianapolis, Indiana, USA). Type I collagen, the major fibre-forming collagen of the skin, is the product of the procollagen I (α1) collagen type I (COL1A1) and procollagen II (α2) collagen (COL1A2) genes.34 Therefore, to assess the collagen synthesis activity in fibroblasts, COL1A1 and COL1A2 were analysed using real-time PCR. Type 1 collagen protein levels were also assessed using a specific ELISA kit (Applied Cell Biotechnologies, Yokohama, Japan). Each sample was performed in triplicate.

Statistical analysis
The Statview III program (Abacus Concepts, Berkeley, California, USA) was used for statistical analyses. Statistical analysis was performed using the Mann–Whitney U test for determining the level of significance of differences between sample means, the Fisher exact probability test for comparison of frequencies and the Bonferroni test for multiple comparisons. Spearman rank
RESULTS
Serum IL-27 levels in SSc
The levels of IL-27 in serum samples from patients with SSc and controls were assessed by ELISA (figure 1). Serum IL-27 levels were significantly elevated in patients with SSc (median 74.1 pg/ml (range 21.5–188.8)) compared with controls (median 41.8 pg/ml (range 28.7–65.6); p<0.005). For the SSc subgroups, IL-27 levels in both patients with dSSc (median 83.0 pg/ml (range 37.1–188.8)) and those with lSSc (median 65.3 pg/ml (range 21.5–112.1)) were elevated compared with those in controls (p<0.001 and p<0.01, respectively). Furthermore, serum IL-27 levels were significantly elevated in patients with dSSc relative to those with lSSc (p<0.05). Values higher than the mean +2SD (62.9 pg/ml) of the control serum samples were found in 58% (53/91) of all patients with SSc, in 71% (32/45) of patients with dSSc and in 46% (21/46) of patients with lSSc. By contrast, only 5% (1/20) of controls had elevated IL-27 levels.

Clinical features of patients with SSc with IL-27 overproduction
We assessed the clinical features of patients with SSc with increased IL-27 production compared with patients with SSc with normal IL-27 production (table 1). The duration of disease was significantly shorter (p<0.01) in patients with SSc with increased IL-27 production (3.0±3.2 years, n=52) than in those with normal IL-27 production (7.2±8.1 years, n=39). Moreover, the retrospective longitudinal analysis in this study showed a tendency for serum IL-27 levels to be attenuated during the follow-up period (figure 2).

In addition, as shown in table 1, patients with SSc with elevated serum levels of IL-27 had significantly higher modified Rodnan TSS points (total SSc, p<0.05; dSSc, p<0.01; lSSc, correlation coefficient was used to examine the relationship between two continuous variables. A p value <0.05 was considered statistically significant.

### Table 1 Clinical and laboratory features of patients with SSc

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Elevated IL-27 (n=52)</th>
<th>Normal IL-27 (n=39)</th>
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<tr>
<td>Sex, M/F</td>
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<td>4/35</td>
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<tr>
<td>Age at onset, mean±SD (years)</td>
<td>47±14</td>
<td>46±17</td>
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<td>Disease duration, mean±SD (years)</td>
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<td>7.2±8.1</td>
</tr>
<tr>
<td>Disease pattern, number with dSSc/lSSc</td>
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<td>13/26</td>
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<tr>
<td>Clinical features</td>
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<tr>
<td>Modified Rodnan TSS, mean±SD (points)</td>
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<td>11.9±8.6</td>
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<td>Pitting scar/ulcer</td>
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<td>Pulmonary fibrosis</td>
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<td>31</td>
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<td>%VC, mean±SD</td>
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<td>%Dlco, mean±SD</td>
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<td>Serum IgG, mean±SD (mg/dl)</td>
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<td>Serum hyaluronan, mean±SD (mg/ml)</td>
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<td>Serum IFNγ, mean±SD (pg/ml)</td>
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<td>Serum IL-10, mean±SD (pg/ml)</td>
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<td>Serum IL-17, mean±SD (pg/ml)</td>
<td>26.9±8.1**</td>
<td>14.3±5.8</td>
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Values are percentages unless stated otherwise.
*p<0.05, **p<0.01 vs patients with SSc with normal IL-27 levels.

CRP, C reactive protein; Dlco, diffusion capacity for carbon monoxide; dSSc, diffuse cutaneous SSc; IFNγ, interferon-γ; IL, interleukin; lSSc, limited cutaneous SSc; SSc, systemic sclerosis; TSS, total skin thickness score; VC, vital capacity.

![Figure 1](image1.png)  
**Figure 1**  
Serum levels of interleukin 27 (IL-27) in patients with systemic sclerosis (SSc), diffuse cutaneous SSc (dSSc) or limited cutaneous SSc (lSSc) and healthy controls (CTL). Serum IL-27 levels were determined by a specific ELISA. Data are presented as box plots, where the lines inside the boxes indicate the medians, the boxes represent the 25th and 75th percentiles and the lines outside the boxes represent the 10th and 90th percentiles. Broken line indicates the cut-off value (mean +2SD of healthy control samples).

![Figure 2](image2.png)  
**Figure 2**  
Serial changes in serum interleukin 27 (IL-27) levels during the follow-up period in 10 patients with systemic sclerosis (SSc) patients (5 with diffuse cutaneous SSc (dSSc) and 5 with limited cutaneous SSc (lSSc)). This retrospective longitudinal analysis showed the tendency for serum IL-27 levels to be attenuated during the follow-up period.
Hyaluronan was 50 μg/ml. The minimal effective dose of hyaluronan was significantly higher than that of normal fluid. IL-27 production by cultured SSc monocytes also correlated positively with levels of serum IgG (total SSc, p<0.05; dSSc, p<0.05; lSSc, p<0.05) and IL-17 (total SSc, p<0.01; dSSc, p<0.01; lSSc, p<0.01) than those with normal levels (table 1). Moreover, serum IL-27 levels correlated positively with levels of serum IgG (total SSc, r=0.48, p<0.0001; dSSc, r=0.42, p<0.0001; lSSc, r=0.45, p<0.0001) and IL-17 (total SSc, r=0.39, p<0.0005; dSSc, r=0.41, p<0.0001; lSSc, r=0.35, p<0.0005; figure 3). However, serum IL-27 levels did not correlate with any other clinical parameters.

With regard to correlation of serum IL-27 levels with immunological parameters, patients with SSc with elevated IL-27 levels had significantly higher frequency of elevated levels of serum IgG (total SSc, p<0.05; dSSc, p<0.05; lSSc, p<0.05) and IL-17 (total SSc, p<0.01; dSSc, p<0.01; lSSc, p<0.01) than those with normal levels (table 1). Moreover, serum IL-27 levels correlated positively with levels of serum IgG (total SSc, r=0.48, p<0.0001; dSSc, r=0.42, p<0.0001; lSSc, r=0.45, p<0.0001) and IL-17 (total SSc, r=0.39, p<0.0005; dSSc, r=0.41, p<0.0001; lSSc, r=0.35, p<0.0005; figure 3). However, serum IL-27 levels did not correlate with any other clinical parameters.

IL-27 production by cultured SSc monocytes

Unstimulated dSSc monocytes produced higher levels of IL-27 relative to normal monocytes (p<0.05, figure 4A). When monocytes were stimulated with LPS or hyaluronan, IL-27 production by both dSSc and normal monocytes was significantly increased (p<0.05). In addition, IL-27 production by LPS- or hyaluronan-stimulated dSSc monocytes was higher than those of normal monocytes (p<0.01). Similar results were obtained using lSSc samples (data not shown). The minimal effective dose of hyaluronan was 50 μg/ml.

Effect of IL-27 on SSc T cells and B cells

The levels of IL-27R expression on dSSc B cells and CD4 T cells were 5.6- and 3.1-fold higher than those observed in healthy controls (p<0.005 and p<0.001, respectively; figure 4B). In the absence of IL-27, the production of IgG by hyaluronan-treated dSSc B cells was higher than that of hyaluronan-stimulated healthy B cells (p<0.005, figure 4C). Healthy B cells treated with hyaluronan and IL-27 decreased production of IgG compared with those treated with hyaluronan alone (24% decrease, p<0.01), while dSSc B cells treated with hyaluronan and IL-27 increased production of IgG compared with those treated with hyaluronan alone (31% increase, p<0.01; figure 4C). Similar to IgG production by B cells, IL-27 treatment reduced IL-17 production by hyaluronan-stimulated healthy CD4 T cells (41% decrease, p<0.01), while dSSc CD4 T cells treated with both hyaluronan and IL-27 increased production of IL-17 (38% increase, p<0.01; figure 4D). Similar results were obtained using lSSc samples (data not shown).

IL-27R expression in the fibrotic skin

IL-27R expression was only faintly detected in the cell membrane and cytoplasm of healthy skin fibroblasts (figure 5A). In contrast, patients with dSSc had higher membrane and cytoplasmic expression of IL-27R in skin fibroblasts (figure 5A). While IL-27R expression was also detected in keratinocytes, endothelial cells and perivascular infiltrated and/or resident mononuclear cells, the expression levels of IL-27R in these cells were not different between dSSc and normal skin. IL-27R expression in sclerotic skin of patients with lSSc was similar to that of patients with dSSc (data not shown). Furthermore, IL-27R expression levels of total skin extract were confirmed using the real-time PCR quantification method (p<0.05, figure 5B). We also assessed IL-27 expression levels in skin samples. IL-27 expression levels in patients with dSSc were significantly higher than those in healthy controls (p<0.01, figure 5B).

Fibroblast proliferation and collagen synthesis with IL-27 stimulation

Stimulation of dSSc or healthy fibroblasts with rIL-17 increased expression of IL-27R compared with PBS alone (p<0.05, figure 5C). Similarly, stimulation of fibroblasts with a combination of rIL-17 and rIL-27 increased IL-27R expression compared with PBS alone (p<0.05). Furthermore, the expression levels of IL-27R in dSSc fibroblasts treated with both rIL-17 and rIL-27 were 3.6- and 3.1-fold higher than those observed in healthy controls (p<0.01). Decreased %VC (total SSc, p<0.05; dSSc, p<0.01; lSSc, p<0.05), decreased %Dlco (total SSc, p<0.05; dSSc, p<0.01; lSSc, p<0.05), elevated levels of serum hyaluronan (total SSc, p<0.05; dSSc, p<0.01; lSSc, p<0.05), and more frequent involvement of pulmonary fibrosis (total SSc, p<0.01; dSSc, p<0.01; lSSc, p<0.01) than those with normal levels. Serum IL-27 levels also correlated inversely with %VC (total SSc: r=−0.29, p<0.005; dSSc: r=−0.34, p<0.001; lSSc: r=−0.28, p<0.005) or %Dlco (total SSc: r=−0.32, p<0.005; dSSc: r=−0.36, p<0.001; lSSc: r=−0.29, p<0.005; figure 3). Furthermore, serum IL-27 levels correlated positively with the modified Rodnan TSS (total SSc: r=0.36, p<0.0005; dSSc: r=0.44, p<0.001; lSSc: r=0.31, p<0.0005) and serum hyaluronan levels (total SSc: r=0.45, p<0.0001; dSSc: r=0.47, p<0.0001; lSSc: r=0.44, p<0.0001; figure 3). However, serum IL-27 levels did not correlate with any other clinical parameters.

With regard to correlation of serum IL-27 levels with immunological parameters, patients with SSc and fibrotic skin.

Figure 3 Correlations of serum interleukin 27 (IL-27) levels with modified Rodnan total skin thickness score (TSS), percentage vital capacity (%VC), percentage diffusion capacity for carbon monoxide (%Dlco) and serum levels of hyaluronan, IgG and IL-27 in patients with systemic sclerosis. Serum IL-27, hyaluronan and IL-17 levels were determined by specific ELISAs.
LPS is an exogenous ligand for TLR4 which strongly induces IL-27 secretion from monocytes (figure 4A), as previously described. Recently, many studies have identified various endogenous ligands for TLR4, such as hyaluronan which regulates inflammatory responses. In our study, hyaluronan treatment enhanced IL-27 production by both healthy and SSC monocytes (figure 4A). Recent studies have shown that, in patients with SSC, monocyte activation may be maintained by one or more enhancing signals such as IL-6 and TNF-α which are increased in patients with SSC and are known to trigger the activation of TLR4 expression. This may explain why IL-27 production by SSC monocytes was higher than by healthy monocytes (figure 4A). Thus, IL-27 production is strongly induced by hyaluronan stimulation in SSC monocytes compared with healthy monocytes.

IL-27R is expressed by a wider range of cells, especially activated CD4 T cells and B cells, which may explain the pleiotropic role of IL-27. IL-27 is mainly secreted from activated antigen-presenting cells such as monocytes by stimulation with their TLR. LPS is an exogenous ligand for TLR4 which strongly induces IL-27 secretion from monocytes (figure 4A), as previously described. Recently, many studies have identified various endogenous ligands for TLR4, such as hyaluronan which regulates inflammatory responses. In our study, hyaluronan treatment enhanced IL-27 production by both healthy and SSC monocytes (figure 4A). Recent studies have shown that, in patients with SSC, monocyte activation may be maintained by one or more enhancing signals such as IL-6 and TNF-α which are increased in patients with SSC and are known to trigger the activation of TLR4 expression. This may explain why IL-27 production by SSC monocytes was higher than by healthy monocytes (figure 4A). Thus, IL-27 production is strongly induced by hyaluronan stimulation in SSC monocytes compared with healthy monocytes.

DISCUSSION

This study is the first to show that IL-27 levels are elevated in serum samples from patients with SSC compared with normal controls (figure 1). We also showed that IL-27 levels correlated positively with modified Rodnan TSS (figure 3 and table 1). Furthermore, elevation of IL-27 levels was accompanied by the presence of pulmonary fibrosis and decreased %VC and %Dlco, indicating that IL-27 levels correlated with the severity of lung fibrosis. Furthermore, retrospective longitudinal analysis showed that there was a tendency for serum IL-27 levels to be attenuated during the follow-up period (figures 2 and 3 and table 1). Collectively, these results may suggest that overproduction of IL-27 has an important role in the pathogenesis of SSC, especially in the earlier phase of the disease.

Figure 4  (A) Interleukin 27 (IL-27) production by monocytes, (B) expression of IL-27R on B cells and CD4 T cells and production of (C) IgG and (D) IL-17 by B cells and CD4 T cells, respectively. Monocytes, B cells and CD4 T cells were obtained from peripheral blood of 25 patients with diffuse cutaneous systemic sclerosis (SSc) and 10 healthy controls (CTL). For production of IL-27, purified monocytes (1×10^5) were stimulated with hyaluronan (HA) or lipopolysaccharide (LPS). IL-27R mRNA expression was analysed using real-time PCR. For production of IgG and IL-17, purified B cells (1×10^5) and CD4 T cells (1×10^5) were stimulated by HA with or without IL-27. Culture supernatants were analysed by ELISA to determine the amount of IL-27, IgG and IL-17. In (A), (C) and (D), bars show the mean and SD. In (B), the data are presented as box plots where the lines inside the boxes indicate the medians, the boxes represent the 25th and 75th percentiles and the lines outside the boxes represent the 10th and 90th percentiles. *p<0.01, **p<0.005.
Thus, IL-27 may have a role in the development of SSc by amplifying collagen synthesis and increasing fibroblast proliferation. Although further studies are required to clarify the role of IL-27 in the development of SSc, it may be a useful serological marker for disease severity and a new therapeutic target in SSc.

**Acknowledgements** The authors thank Ms M Yozaki, A Usui and K Shimoda for technical assistance.

**Funding** This work was supported by a grant for Research on Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan (to Ay and SS).

**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** This study was conducted with the approval of the Nagasaki University Hospital.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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**Figure 5** (A) Immunohistochemical analysis of fibrotic skin using interleukin 27 receptor (IL-27R) mouse monoclonal antibody compared with concentration-matched mouse monoclonal antibody IgG isotype control (CTL). (B) IL-27R and IL-27 mRNA expression of healthy control (n=8) and systemic sclerosis (SSc) (n=8) skin analysed using real-time PCR. (C) Western blot analysis of IL-27R protein in stimulated fibroblasts with or without rIL-27 and/or rIL-17. Fibroblasts were grown to confluence and culture medium was replaced with serum-free modified Eagle’s medium. After further incubation for 24 h, fibroblasts were stimulated with each concentration of rIL-27 for 24 h. After incubation, collagen type I alpha1 (COL1A1) and COL1A2 mRNA expression and type I collagen protein production were analysed by real-time PCR and ELISA, respectively. In proliferation assay, 5-bromo-2-deoxyuridine (BrdU) (10 µm) was added to each well and incubated for 24 h. BrdU incorporation in proliferating cells was quantified by ELISA. In (B), data are presented as box plots, where the lines inside the boxes indicate the medians, the boxes represent the 25th and 75th percentiles and the lines outside the boxes represent the 10th and 90th percentiles. In (C) and (D), bars show the mean and SD. *p<0.05, **p<0.01 vs each fibroblast cultured with vehicle alone. Original magnification ×100.
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Elevated serum interleukin-27 levels in patients with systemic sclerosis: association with T cell, B cell and fibroblast activation

Ayumi Yoshizaki, Koichi Yanaba, Yohei Iwata, et al.

Ann Rheum Dis 2011 70: 194-200 originally published online August 12, 2010
doi: 10.1136/ard.2009.121053

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