Elevated Serum Insulin-like Growth Factor (IGF-1) and IGF Binding Protein-3 Levels in Patients with Systemic Sclerosis: Possible Role in Development of Fibrosis

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ABSTRACT. ***Objective.*** To examine serum concentrations of insulin-like growth factor (IGF-1) and IGF binding protein (IGFBP-3), a major carrier protein for IGF-1, in patients with systemic sclerosis (SSc); and to relate the results to clinical features in SSc.

Methods. Serum IGF-1 and IGFBP-3 levels in 92 Japanese patients with SSc were measured by ELISA. Expression of IGF-1 and IGFBP-3 messenger RNA (mRNA) in the skin was quantified by real-time reverse transcription-polymerase chain reaction.

Results. Serum IGF-1 and IGFBP-3 levels were significantly elevated in patients with SSc compared with patients with systemic lupus erythematosus or healthy controls. IGF-1 levels were higher in patients with diffuse cutaneous SSc (dcSSc) than in patients with limited cutaneous SSc (lcSSc). Patients with increased IGF-1 levels had more severe skin involvement and pulmonary fibrosis. IGF-1 mRNA was upregulated in the affected skin of patients with SSc. There were no significant differences in serum IGFBP-3 levels between dcSSc and lcSSc. IGFBP-3 levels were not associated with skin thickness and pulmonary fibrosis. Patients with increased IGF-1 or IGFBP-3 had lower frequency of telangiectasia than patients with normal levels.

Conclusion. These results suggest that both IGF-1 and IGFBP-3 are involved in the development of SSc. The role of IGF-1 appears to be different from that of IGFBP-3. (First Release Nov 1 2008; J Rheumatol 2008;35:2363–71; doi:10.3899/jrheum.080340)

Key Indexing Terms:
SYSTEMIC SCLEROSIS — INSULIN-LIKE GROWTH FACTOR
INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN — TOTAL SKIN THICKNESS SCORE

Systemic sclerosis (SSc) is a connective tissue disorder characterized by microvascular damage and excessive fibrosis of the skin and various internal organs with an autoimmune background. Although the pathogenesis of SSc remains unknown, immunologic abnormalities have been suggested to play an important role. Most of the infiltrating cells in the skin of patients with SSc are activated T lymphocytes with a predominant CD4-positive phenotype[1]. Cytokines play a major role in regulating extracellular matrix deposition by fibroblasts as well[2]. It has been reported that Th2 response is predominant in SSc, and a shift from a Th2 response to a Th1 response is associated with the improvement of cutaneous involvement in diffuse cutaneous SSc (dcSSc)[3,4]. However, little is known about a role of mediators of the endocrine system in the development of SSc.

Insulin-like growth factor-1 (IGF-1) is a single-chain polypeptide consisting of 70 amino acids, and it shares an approximate 50% homology with insulin[5,6]. Systemic IGF-1 concentrations are mainly determined by production in the liver; however, most cells throughout the body, including fibroblasts, can synthesize this growth factor[7]. Growth hormone is the primary inducer of IGF-1 synthesis by the liver and a central regulator of the concentration of circulating IGF-1. Most functions of IGF-1 are mediated by the IGF-1 receptor (IGF-1R), a highly specific membrane receptor associated with intracellular tyrosine kinase and homologous to the insulin receptor[8]. The bioactivity of IGF-1 is modulated by 6 binding proteins, the IGF binding proteins (IGFBP) 1 to 6. IGFBP-3 is a predominant carrier protein in sera and binds to 80%–95% of the circulating IGF-1 in a stable ternary complex that consists of an additional protein, termed the acid-labile subunit[9]. This complex is considered a circulating store for IGF-1.

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IGF-1 has a broad range of physiological functions from early embryonic development to the whole period of life. Generally, IGF-1 is an endocrine mediator of growth and development under physiological conditions. IGF-1 enhances cell-cycle progression and mitosis[10]. IGF-1 can act as a survival factor by decreasing apoptosis in diverse cells[11]. IGF-1 also plays a prominent role in the regulation of immunity and inflammation[7]. For example, tumor necrosis factor-α and prostaglandin E_2 induce IGF-1 production in macrophages[12]. T and B cells and natural killer cells are influenced by the effect of IGF-1, as these cells express the IGF-1R[13,14]. These data imply a role of regulation by IGF-1 in innate immunity as well as acquired immunity.

It has been described that IGFBP, not only IGFBP-3, have diverse biological activities in addition to the simple role as a carrier protein for IGF-1. There are reports elucidating a role of IGFBP-3 and IGFBP-5 in fibrosis; IGFBP-5 mRNA levels in fibroblasts from SSc patients were increased[15]. Increased IGFBP-3 and IGFBP-5 mRNA and protein levels were observed *in vivo* in lung tissue from patients with idiopathic pulmonary fibrosis and *in vitro* in primary fibroblasts from skin and lung tissue from patients with SSc[16]. Overexpression of IGFBP-3 and IGFBP-5 in primary human fibroblasts increased the expression of extracellular matrix[16]. These results suggest that IGFBP-3 is involved in the development of fibrosis in SSc. However, serum levels of IGFBP-3 in SSc patients have not been reported to date. Therefore, it is of significant importance to examine levels of IGF-1 and IGFBP-3 in sera from SSc patients to determine the contribution of IGF-1 and IGFBP-3 in SSc.

There are a few studies describing the contribution of the IGF system to rheumatic diseases[17]. It has been reported that C-reactive protein concentrations are significantly associated with IGF-1, IGF-2, and IGFBP-3 levels in patients with rheumatoid arthritis (RA)[18]. In contrast, Denko and Malemud reported no significant differences in basal serum IGF-1 levels between RA patients and age-matched healthy subjects[19]. In patients with chronic juvenile arthritis, serum IGF-1 levels were lower than in the control group[20]. Neidel reported that IGFBP-3 and IGFBP-2 levels were elevated, but the molar ratio of IGF-1/IGFBP-3 was significantly reduced, in patients with clinically active RA[21]. Matsumoto and Tsurumoto described significantly lower serum IGF-1 but higher IGFBP-3 levels in RA patients and that the ratio of IGF-1 to IGFBP-3 in RA patients was significantly lower than that in controls, suggesting that an inappropriate balance of IGF-1 and IGFBP-3 levels reduces the availability of IGF-1 and is involved in the pathogenesis of RA[22]. On the other hand, no significant differences in serum IGF-1 levels were found in women with active systemic lupus erythematosus (SLE) compared with a group of age-matched healthy women[23]. In a study in SSc, serum IGF-1 levels in all 13 SSc patients were within normal limits, and no correlation was found between serum IGF-1 levels and clinical features[24]. Since this may be due to the small number of patients, an additional study will be required to clarify the role of the IGF system in the development of SSc.

We examined serum levels of IGF-1 and IGFBP-3 in patients with SSc to investigate the involvement of the IGF system in SSc.

MATERIALS AND METHODS

Patients. Serum samples were obtained from 92 Japanese patients with SSc (75 women, 17 men), median age 46 years (range 13–76 yrs), with median disease duration of 6.1 years (range 0.2–35). All patients fulfilled the criteria for SSc proposed by the American College of Rheumatology (ACR)[25]. These patients were grouped according to the classification system proposed by LeRoy, *et al*[26]: 50 patients (34 women, 16 men) had dcSSc and 42 (40 women, 2 men) had limited cutaneous SSc (lcSSc). Antitopoisomerase I antibody was positive in 36 patients, anticentromere antibody (ACA) was positive in 29, and anti-RNA polymerase I and III antibody was positive in 8. Disease duration was calculated from the time of the first clinical event (other than Raynaud's phenomenon) that was clearly a manifestation of SSc.

At the first visit, 5 patients were receiving low-dose steroids (prednisolone, 5–20 mg/day), while 2 patients were being treated with low-dose penicillamine (100–300 mg/day). No SSc patient was receiving other immunosuppressive therapy or had a recent history of infection or other inflammatory diseases. Serving as disease controls, we also examined serum samples from 14 patients with SLE who fulfilled the ACR criteria[27]. Twenty age and sex-matched Japanese individuals (16 women, 4 men; median age 52 yrs, range 23–72 yrs) were recruited as healthy controls. Fresh venous blood samples were centrifuged shortly after clot formation. All samples were stored at –70°C before use.

All participants provided written informed consent. The protocol was approved by the Kanazawa University Graduate School of Medical Science and Kanazawa University Hospital.

Clinical assessment. Complete medical histories were obtained, and physical examinations and laboratory tests were conducted for all patients at their first visit, with limited evaluations during followup visits. Skin thickness was scored according to the modified Rodnan method[28]. Organ system involvement was defined as described[29,30]: lung: bibasilar fibrosis on chest radiography and high resolution computed tomography; esophagus: hypomotility shown by barium radiography; joint: 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; and muscle: proximal muscle weakness and elevated serum creatine kinase concentration. Pulmonary function tests, including vital capacity (VC) and diffusion capacity for carbon monoxide (DLCO), were performed as well. Abnormal values for VC and DLCO were considered to be < 80% and < 70%, respectively, of predicted normal values.

ELISA. Specific ELISA kits were used for measuring serum levels of IGF-1 (BioSource, Camarillo, CA, USA) and IGFBP-3 (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocol. The IGF-1 ELISA kit detects all IGF-1 in sera, and includes an extraction step in which IGF-1 is separated from its binding protein. Immunoglobulins were absorbed from the serum sample using a commercial protein A-coated plate (Protein A HP MultiTrap™, Amersham Biosciences, Piscataway, NJ, USA). Briefly, 100 μl of serum was applied to each well and incubated 60 min at room temperature. Then the serum with absorption of immunoglobulins was harvested in the collection tube for ELISA. Each sample was tested in duplicate.

Real-time RT-PCR. Skin biopsy specimens were obtained from the forearm of 9 SSc patients (5 dcSSc and 4 lcSSc; 7 women, 2 men; median age 54

yrs, range 26–68). Five age and sex-matched healthy individuals (4 women, 1 man, age 48 yrs, range 25–58) served as controls. All skin samples were snap-frozen in liquid nitrogen and stored at –80°C before use. Total RNA were extracted from injured skin samples using Qiagen RNeasy spin columns (Qiagen Ltd., Crawley, UK) and digested with DNase I (Qiagen) to remove chromosomal DNA in accord with the manufacturer's protocols. Total RNA was reverse-transcribed to cDNA using a reverse transcription system with random hexamers (Promega, Madison, WI, USA). Real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed using the TaqMan® system (Applied Biosystems, Foster City, CA, USA) on an ABI Prism 7000 Sequence Detector (Applied Biosystems) according to the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize mRNA, and probe and primers were from Pre-Developed TaqMan® Assay Reagents (Applied Biosystems). Relative expression of RT-PCR products was determined using the $\Delta\Delta C_T$ technique (Applied Biosystems user bulletin no. 2) as described[31]. A comparative threshold cycle (C_T) was used to determine gene expression relative to the normal control (calibrator). We normalized each set of samples with the housekeeping gene (GAPDH) using the formula $\Delta C_T = C_{T\ sample} - C_{T\ GAPDH}$. One of the control samples was then chosen as a calibrator, and relative mRNA levels were calculated by the expression $2^{-\Delta\Delta CT}$, where $\Delta\Delta C_T = \Delta C_{T\ sample} - \Delta C_{T\ calibrator}$. Hence, IGF-1 or IGFBP-3 mRNA levels were expressed as an n-fold difference relative to the calibrator. Each reaction was done in at least triplicate.

Statistical analysis. Statistical analysis was performed using the Mann-Whitney U test for determining the level of significance of differences between sample means, Fisher's exact probability test for comparison of frequencies, and Bonferroni's test for multiple comparisons. Spearman's rank correlation coefficient was used to examine the relationship between 2 continuous variables. A p value < 0.05 was considered statistically significant. The data were shown as the median (range) unless otherwise indicated.

RESULTS

Serum IGF-1 levels in SSc. Serum IGF-1 levels were significantly higher in patients with SSc (median 163 ng/ml, range 49–576) than in those with SLE (median 50 ng/ml, range 39–138; $p < 0.0001$) or in healthy controls (median 86 ng/ml, range 41–198; $p < 0.0005$; Figure 1). Concerning the SSc subgroups, IGF-1 levels in patients with dcSSc (median 202 ng/ml, range 78–576) and those with lcSSc (median 117 ng/ml, range 49–313) were increased compared with those in patients with SLE ($p < 0.0001$, $p < 0.05$, respectively). While serum IGF-1 levels in patients with dcSSc were significantly higher than in healthy controls ($p < 0.0001$), no significant differences in IGF-1 levels were observed between patients with lcSSc and healthy controls. Further, serum IGF-1 levels were significantly elevated in patients with dcSSc relative to those with lcSSc (p <

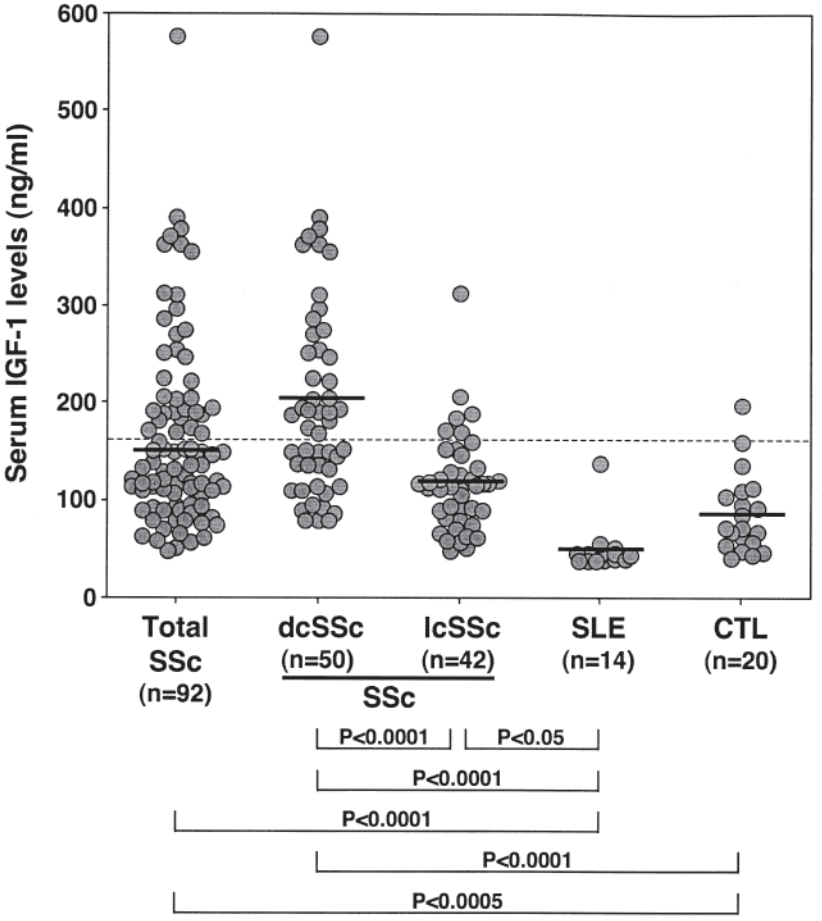

Figure 1. Serum IGF-1 levels in patients with autoimmune diseases at the first evaluation. IGF-1 levels were determined by a specific ELISA in serum samples from total patients with SSc, with dcSSc, with lcSSc, with SLE, and healthy controls (CTL). Broken line indicates the cutoff value (mean + 2 SD of control samples).

0.0001). Thus, IGF-1 levels were increased in SSc and higher in dcSSc than in lcSSc.

Clinical association of serum IGF-1 levels in SSc. Clinical and laboratory measures obtained at the first evaluation were compared between SSc patients with elevated serum IGF-1 levels and those with normal IGF-1 levels (Table 1). In this study, values higher than the mean plus 2 SD (169 ng/ml) of the control serum samples were considered to be elevated. IGF-1 levels were elevated in 29% (27/92) of all patients with SSc, in 44% (22/50) of patients with dcSSc, and in 12% (5/42) of patients with lcSSc. There was no significant difference in disease duration between patients with elevated IGF-1 levels and those with normal levels (Table 1). In addition, IGF-1 levels did not correlate with disease duration in patients with dcSSc, in those with lcSSc, or in the total number of patients with SSc (data not shown). Examining demographic features, the frequency of male patients with elevated IGF-1 levels (9/27) was higher than for those with normal levels (8/65; $p < 0.05$). SSc patients with elevated IGF-1 levels had significantly higher modified Rodnan total skin thickness scores (TSS) compared to patients with normal IGF-1 levels ($p < 0.05$); thus, the frequency of dcSSc was higher in patients with elevated IGF-1 levels than in those with normal levels ($p < 0.01$). IGF-1 levels correlated positively with modified Rodnan TSS in patients with SSc ($p < 0.05$, $r = 0.261$; Figure 2). SSc patients with elevated IGF-1 levels had telangiectasia less frequently ($p < 0.05$), but had pulmonary fibrosis more frequently ($p < 0.05$; Table 1). In all 27 patients with elevated IGF-1 levels, 18 (67%) were positive for antitopoisomerase I antibody and only 2 (7%) were positive for ACA (Table 1). However, IGF-1 levels did not correlate significantly with antitopoisomerase I antibody or ACA levels, as determined by ELISA (data not shown).

To determine whether IGF-1 acts as a marker for dcSSc or as a marker for skin sclerosis in patients with dcSSc (and other manifestations, such as pulmonary fibrosis), clinical features were compared in dcSSc patients with elevated serum IGF-1 levels and those with normal levels. The modified Rodnan TSS was similar in dcSSc patients with elevated IGF-1 levels (median 21, range 5–41) compared to TSS in those with normal levels (median 21, range 6–49). In addition, the frequency of lung involvement in dcSSc patients with elevated IGF-1 levels was similar to the frequency in those with normal levels (73% vs 64%). Thus, elevated IGF-1 levels were generally associated with dcSSc, rather than increased severity of skin sclerosis and lung fibrosis, in SSc.

IGF-1 mRNA expression in skin from patients with SSc. To assess local IGF-1 expression in affected skin from patients with SSc, IGF-1 mRNA expression was quantified by real-time RT-PCR. The expression of IGF-1 mRNA was significantly upregulated (1.9-fold) in skin from patients with SSc

Table 1. Clinical and laboratory characteristics of patients with systemic sclerosis (SSc), according to IGF-1 level. Unless noted otherwise, values are percentages. All clinical and laboratory measures and serum IGF-1 levels were determined at the first evaluation.

Characteristic	Elevated IGF-1, n = 27	Normal IGF-1, n = 65
Age at onset, median (range) yrs	43 (14–65)	48 (13–76)
Women/men	18/9†	57/8
Disease duration, median (range) yrs	5.3 (0.2–33)	6.6 (0.2–35)
SSc subtype, no. with dcSSc/lcSSc	22/5††	28/37
Median (range) modified Rodnan TSS	18 (1–41)†	12 (1–49)
Clinical features		
Digital pitting scars or ulcers	33	32
Contracture of phalanges	40	43
Diffuse pigmentation	59	48
Telangiectasia	22†	57
Organ involvement		
Lung		
Pulmonary fibrosis	65†	41
Decreased % VC	22	19
Decreased % DLCO	63	66
Esophagus	69	72
Heart	8	14
Kidney	0	3
Joint	19	28
Muscle	11	17
Laboratory findings		
Antitopoisomerase I antibody	67††	29
Anticentromere antibody	7††	45
Anti-RNA polymerase I and III antibody	11	9
Elevated ESR	41	29
Elevated C-reactive protein	23	19
IgG, median (range) μg/ml	1513 (907–2440)	1699 (821–5350)
IgA, median (range) μg/ml	257 (134–497)	283 (95–583)
IgM, median (range) μg/ml	157 (72–374)	202 (36–829)

TSS: total skin thickness score; VC: vital capacity; DLCO: diffusing capacity for carbon monoxide; ESR: erythrocyte sedimentation rate. † $p < 0.05$ versus patients with normal IGF-1 levels. †† $p < 0.01$ versus patients with normal IGF-1 levels.

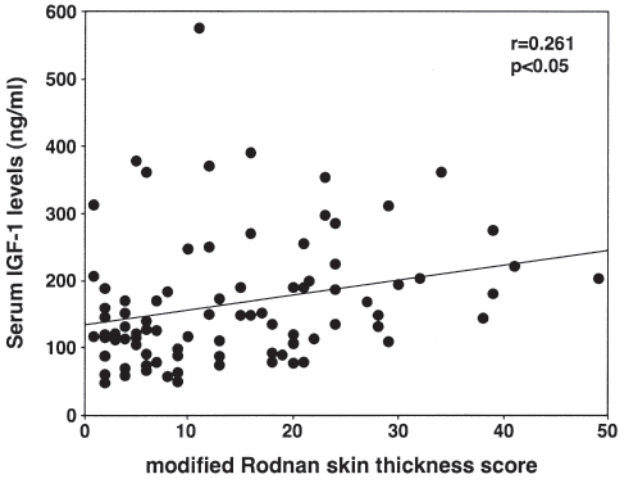

Figure 2. Correlation of serum IGF-1 levels against the modified Rodnan total skin thickness score in patients with SSc at the first evaluation. Serum IGF-1 levels were determined by a specific ELISA.

compared with skin from healthy subjects (2.02 ± 0.65 vs 1.05 ± 0.63; $p < 0.02$; Figure 3). Thus, IGF-1 mRNA expression in lesional skin from SSc patients was increased.

Serum IGFBP-3 levels in SSc. Since it has been reported that IGFBP-3 has diverse biological activities, such as a positive regulator of fibrosis, in addition to the role of carrier protein for IGF-1[16], we measured serum IGFBP-3 levels as well. Serum IGFBP-3 levels correlated positively with serum IGF-1 levels ($p < 0.0001$, $r = 0.313$; Figure 4). There were no significant differences in the ratio of IGF-1 to IGFBP-3 levels in serum between patients with SSc and SLE or controls, or between dcSSc and lcSSc (data not shown). Serum IGFBP-3 levels were significantly higher in patients with SSc (median 1187 ng/ml, range 39–3825) than in those with SLE (median 131 ng/ml, range 54–934; $p < 0.005$) or healthy controls (median 530 ng/ml, range 88–1759; $p < 0.01$; Figure 5). IGFBP-3 levels in patients with dcSSc (median 1237 ng/ml, range 50–3825) and with lcSSc (median 1095 ng/ml, range 39–3059) were increased compared to patients with SLE ($p < 0.005$ and $p < 0.01$, respectively) and healthy controls ($p < 0.01$ and $p < 0.05$). In contrast to serum IGF-1 levels, no significant difference in serum IGFBP-3 levels was observed between patients with dcSSc and those with lcSSc. Thus, like IGF-1, IGFBP-3 levels were increased in SSc, but no difference was found between dcSSc and lcSSc.

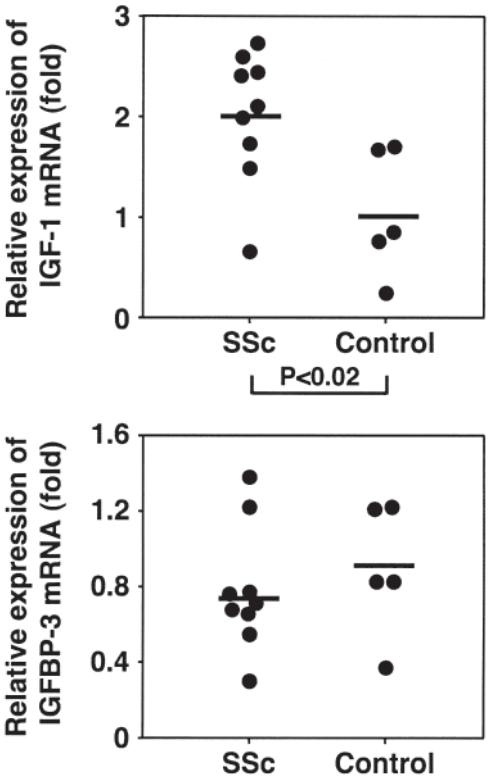

Figure 3. IGF-1 and IGFBP-3 mRNA expression in skin from patients with SSc was quantified by real-time RT-PCR. IGF-1 and IGFBP-3 mRNA expression relative to a calibrator, one of the control samples, is expressed as $2^{-\Delta\Delta CT}$ (fold) and shown as a dot. Horizontal lines indicate the median.

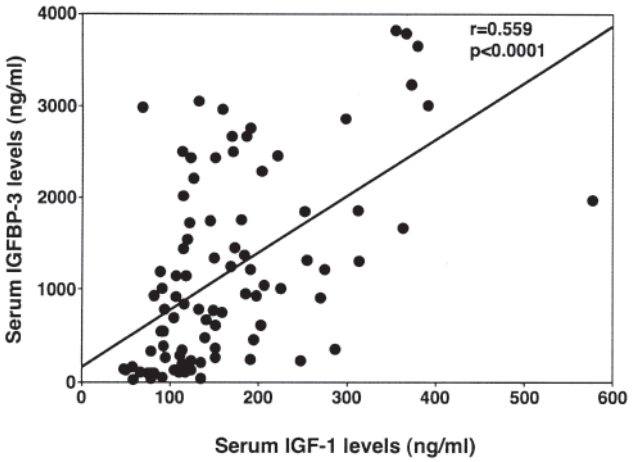

Figure 4. Correlation of serum IGF-1 levels against serum IGFBP-3 levels in patients with SSc at first evaluation. Serum IGF-1 and IGFBP-3 levels were determined by specific ELISA.

Clinical associations of serum IGFBP-3 levels in SSc. Clinical and laboratory measures at the first evaluation were compared between SSc patients with elevated serum IGFBP-3 levels and those with normal IGFBP-3 levels (Table 2). Values higher than the mean + 2 SD (1536 ng/ml) of the control serum samples were considered to be elevated. IGFBP-3 levels were elevated in 30% (28/92) of all patients with SSc, in 30% (15/50) of patients with dcSSc, and in 30% (13/42) with lcSSc. There were no significant differences in disease duration, gender, and modified Rodnan TSS between patients with elevated IGFBP-3 levels and those with normal levels (Table 2). The frequency of telangiectasia and muscle involvement in SSc patients with elevated IGFBP-3 levels was significantly lower than in those with normal levels ($p < 0.05$ and $p < 0.05$, respectively). The frequency of pulmonary fibrosis was similar between patients with elevated IGFBP-3 levels and normal levels (44% vs 50%). IGFBP-3 levels did not correlate significantly with antitopoisomerase I antibody or ACA levels, as determined by ELISA (data not shown). Thus, unlike IGF-1, IGFBP-3 levels were not associated with skin and pulmonary fibrosis.

IGFBP-3 mRNA expression in skin from patients with SSc. To assess local IGFBP-3 expression in affected skin from patients with SSc, IGFBP-3 mRNA expression was quantified by real-time RT-PCR as well. Expression of IGFBP-3 mRNA was not upregulated in affected skin from patients with SSc compared with skin from healthy subjects (0.78 ± 0.33 vs 0.91 ± 0.40; Figure 3). Thus, IGFBP-3 mRNA expression in lesional skin from SSc patients was not increased compared to IGF-1.

DISCUSSION

This is the first report to reveal significantly elevated serum

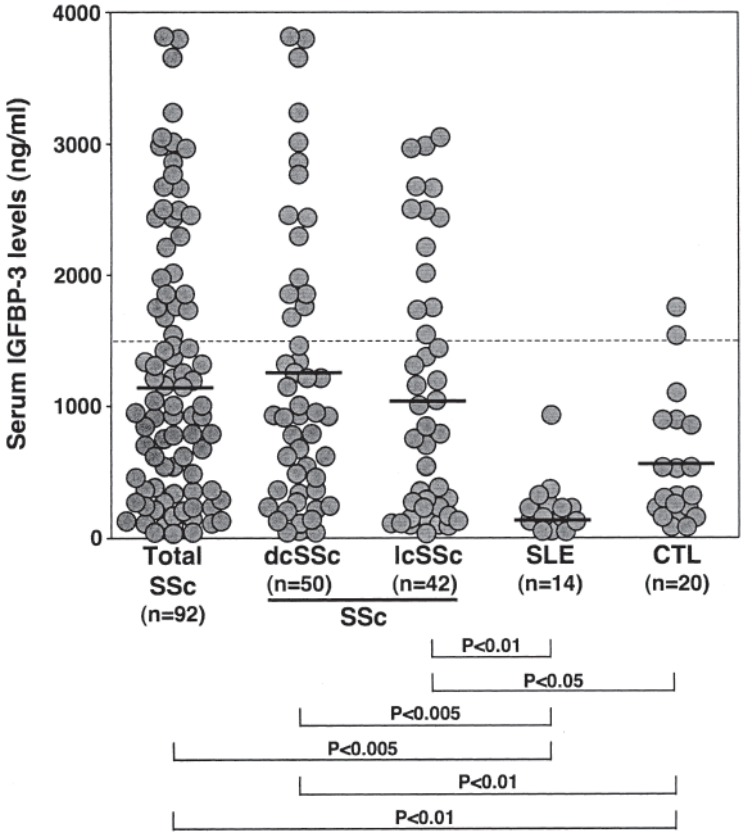

Figure 5. Serum IGFBP-3 levels in patients with autoimmune diseases at the first evaluation. IGFBP-3 levels were determined by a specific ELISA in serum samples from total patients with SSc, with dcSSc, with lcSSc, with SLE, and healthy controls (CTL). Broken line indicates the cutoff value (mean + 2 SD of control samples).

IGF-1 and IGFBP-3 concentrations in patients with SSc, compared to levels in SLE and healthy controls. The IGF system, which contains IGF-1 and IGFBP-3, is involved in both innate and acquired immunity and hence appears to work as an immunoregulator[7]. Inappropriate balance or abnormal function of the IGF system has been reported in autoimmune disorders such as RA. Our finding that IGF-1 and IGFBP-3 levels in patients with SSc were increased support the hypothesis that the IGF system may contribute in part to the development of SSc.

Clinical features of patients with elevated IGF-1 levels were different from those of patients with elevated IGFBP-3 levels. Patients with dcSSc had higher IGF-1 levels than those with lcSSc. Moreover, patients whose IGF-1 levels increased had higher modified Rodnan TSS and had pulmonary fibrosis more frequently than those with normal IGF-1 levels. In addition, IGF-1 mRNA expression was increased in affected skin from patients with SSc. These results suggest that IGF-1 is strongly associated with the development of skin and lung fibrosis. On the other hand, there were no significant differences of IGFBP-3 levels between dcSSc and lcSSc patients, although IGFBP-3 levels in patients with SSc were significantly increased compared to patients with SLE and controls. Further, IGFBP-3 was not associated with severity of skin thickness and frequency of pulmonary fibrosis, and expression of IGFBP-3 mRNA was not upregulated in SSc-affected skin. These findings raise the possibility that IGF-1 and IGFBP-3 are independently involved in the development of SSc, even though both belong to the IGF system. Alternatively, available IGF-1 that is not bound to IGFBP-3 in sera might be associated with fibrosis. However, it is difficult to confirm this hypothesis, as total IGF-1, not free IGF-1, levels in sera were measured in this study. In addition, we cannot exclude the possibility that changes in serum IGF-1 and IGFBP-3 levels may just reflect responses to alterations in systemic inflammation, since various proinflammatory cytokines are potent inducers of the IGF-1/IGFBP-3 axis[7].

Fibroblasts derived from patients with SSc exhibit the promotion of collagen synthesis and the dysregulation of extracellular matrix, resulting in excess deposition of extracellular matrix in internal organs. Transforming growth factor-ß (TGF-ß), platelet-derived growth factor (PDGF), interleukin 4 (IL-4), and IL-6 are known as regulators of collagen metabolism. Progression of fibrosis by the PDGF pathway is considered one of the major mechanisms of fibrosis

Table 2. Clinical and laboratory characteristics of patients with systemic sclerosis (SSc), according to IGFBP-3 level. Unless noted otherwise, values are percentages. All clinical and laboratory measures and serum IGF-1 levels were determined at the first evaluation.

Characteristic	Elevated IGFBP-3 (n = 28)	Normal IGFBP-3 (n = 64)
Age at onset, median (range) yrs	44 (14–61)	48 (13–76)
Women/men	21/7	54/10
Disease duration, median (range) yrs	4.9 (0.4–35)	6.8 (0.2–33)
SSc subtype, no. with dcSSc/lcSSc	15/13	35/29
Median (range) modified Rodnan TSS	13 (1–41)	15 (1–49)
Clinical features		
Digital pitting scars or ulcers	36	31
Contracture of phalanges	29	48
Diffuse pigmentation	46	53
Telangiectasia	29†	55
Organ involvement		
Lung		
Pulmonary fibrosis	44	50
Decreased % VC	15	22
Decreased % DLCO	63	66
Esophagus	70	71
Heart	4	16
Kidney	4	2
Joint	18	28
Muscle	4†	20
Laboratory findings		
Antitopoisomerase I antibody	46	29
Anticentromere antibody	29	45
Anti-RNA polymerase I and III antibody	4	9
Elevated ESR	39	29
Elevated C-reactive protein	18	19
IgG, median (range) μg/ml	1538 (985–2302)	1691 (821–5350)
IgA, median (range) μg/ml	253 (95–418)	286 (105–583)
IgM, median (range) μg/ml	173 (50–478)	195 (36–829)

TSS: total skin thickness score; VC: vital capacity; DLCO: diffusing capacity for carbon monoxide; ESR: erythrocyte sedimentation rate. † $p < 0.05$ versus patients with normal IGFBP-3 levels.

in SSc[32]. IGF-1 may be related to this PDGF-induced fibrosis, since it has been reported that PDGF can induce fibroblast proliferation by IGF-1 via upregulation of expression of IGF-1R on fibroblasts[33].

Cytokines play a major role in regulating extracellular matrix deposition by fibroblasts[2]. It has been suggested that T helper 1 (Th1) cytokines generally decrease extracellular matrix deposition, whereas Th2 cytokines increase it[2]. Serum concentrations of Th2 cytokines, such as IL-4, IL-6, IL-10, and IL-13, are increased in SSc[34-37]. Th2 cytokines such as IL-4 and IL-13 can induce macrophage-derived IGF-1 expression[38], and macrophage-derived IGF-1 protects myofibroblasts from apoptosis[39]. Thus, increased IGF-1 levels in our study may result from a cytokine balance shifted to Th2.

IGFBP have diverse biological activities in addition to the role as a carrier protein for IGF-1[40]. Recently, it has been shown that expression of IGFBP-3 and IGFBP-5 is increased in skin[41] and lung[42] in patients with SSc. IGFBP-5 may be more strongly involved in the fibrosis than IGFBP-3, as IGFBP-5 induces more fibroblast proliferation and extracellular matrix deposition. IGFBP-3 may inhibit the production of extracellular matrix, since IGFBP-3 enhances signal transducer and activator of transcription-1 protein expression, which can prevent activation of the TGF-ß-dependent fibrosis pathway[43]. However, the role of IGFBP-3 in the process of fibrosis remains controversial, as IGFBP-3 is capable of both increasing and decreasing cell proliferation[40]. IGFBP-3 protects the vasculature from damage and promotes proper vascular repair after hyperoxic insult[44]. In our study, IGFBP-3 was increased with no association with the disease subset and the degree of fibrosis in SSc patients, indicating that IGFBP-3 might be increased to restore the vascular damage that is one of the representative impairments in SSc.

The frequency of telangiectasia was lower in patients with elevated IGF-1 or IGFBP-3 levels. We have reported that patients with elevated soluble endoglin (sEndoglin) had telangiectasis more frequently than those with normal sEndoglin levels[45]. Co-cultures of hematopoietic stem cells

with IGF-2 *in vitro* resulted in the reduction of endoglin expression[46]. Thus, inappropriate increases of IGF-1 or IGFBP-3 might influence the expression of endoglin on endothelial cells, leading to lower frequency of telangiectasia.

It appears that both IGF-1 and IGFBP-3 are involved in the development of SSc. However, the contribution of IGF-1 to SSc seems different from that of IGFBP-3. It has been reported that most biological activities of IGF-1 and IGFBP-3 are tissue and time-specific. Our model could provide a useful tool to determine the cause and effect relationship between IGF-1 and SSc. Our study has a potential weakness, in the lack of mechanistic experiments. For example, the best possible study would examine IGF-1 levels in the lungs of SSc patients, which could provide direct evidence that the IGF system is closely associated with the development of pulmonary fibrosis. It is apparent that this issue brings the difficulty of making solid conclusions in our study. Further studies will be required to determine whether an increase in IGF-1 leads to SSc, or that SSc results in increased IGF-1.

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