Serum Concentrations of the CXC Chemokines Interleukin 8 and Growth-Regulated Oncogene-α Are Elevated in Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** To determine whether serum concentrations of 2 CXC chemokines, interleukin 8 (IL-8) and growth-regulated oncogene-α (GRO-α), which are potent chemoattractants and activators for neutrophils, are elevated and whether they correlate with clinical features in patients with systemic sclerosis (SSc).

Methods. Serum samples from patients with diffuse cutaneous SSc (dSSc; n = 36), limited cutaneous SSc (lSSc; n = 42), systemic lupus erythematosus (SLE; n = 15), dermatomyositis (DM; n = 15), and healthy controls (n = 35) were examined by ELISA.

Results. Serum IL-8 was detected significantly more frequently in patients with dSSc (61%) and lSSc (55%) relative to healthy controls (6%), patients with SLE (7%), and those with DM (13%). Similarly, serum GRO-α concentrations in SSc patients were significantly increased compared with controls, patients with SLE, or those with DM. Elevated IL-8 concentrations significantly correlated with decreased % DLCO and rheumatoid factor positivity, while increased GRO-α levels were significantly associated with decreased % DLCO and % vital capacity, involvement of kidney and muscle, the presence of anti-topoisomerase I antibody, and increased serum IgG levels.

Conclusion. Our results suggest that the elevation of serum levels of the CXC chemokines IL-8 and GRO-α is specific to SSc and that the elevation of CXC chemokines, particularly GRO-α, correlates with the involvement of internal organs, especially pulmonary damage. (J Rheumatol 2003;30:1524–8)

Key Indexing Terms:
SYSTEMIC SCLEROSIS INTERLEUKIN 8 GROWTH-REGULATED ONCOGENE-α PULMONARY DAMAGE

Systemic sclerosis (SSc) is a connective tissue disease characterized by fibrosis and vascular changes in the skin and other visceral organs. Although the pathogenesis of SSc remains unclear, infiltration of specific leukocytes into the tissue plays a critical role in organ involvement[1-3]. Chemokines are the important factors that regulate leukocyte recruitment into the inflamed tissue. Interleukin 8 (IL-8) and growth-regulated oncogene-α (GRO-α) are CXC chemokines whose major role is to attract and activate neutrophils[4]. Several functional responses, including adherence to endothelium, enzyme secretion, and induction of respiratory burst, are observed *in vitro* after CXC chemokine stimulation of neutrophils[4]. In addition, CXC chemokines have been reported to have other target cells than neutrophils: IL-8 is a potent chemoattractant for T cells and attracts and activates eosinophils and basophils[4-6], while GRO-α is chemotactic for basophils[7]. IL-8 and GRO-α are produced by various cell types, including macrophages, neutrophils, epithelial cells, endothelial cells, and fibroblasts[8,9].

Neutrophil recruitment and activation in the lower respiratory tract are involved in lung injury and subsequent fibrosis[10,11]. Fibrosing alveolitis (FA), histologically and radiologically similar to cryptogenic FA (CFA), is observed in most SSc patients (FASSc)[12]. In both CFA and FASSc, neutrophilia in bronchoalveolar fluids are associated with the disease activity and severity[3,13]. IL-8 has been shown to be a key mediator of neutrophil traffic to the lower respiratory tract in both FASSc and CFA[14-16].

We investigated serum concentrations of IL-8 and GRO-α in patients with SSc using sensitive ELISA systems and compared these results with clinical or laboratory findings.

MATERIALS AND METHODS

Patients. Serum samples were obtained from 78 Japanese patients with SSc

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(71 women and 7 men). The patients were aged 10–77 years (mean age 51). Thirty-five age and sex matched healthy individuals (mean age 50, range 13–72 yrs, 32 women and 3 men) were used as controls, and 15 age and sex matched patients with systemic lupus erythematosus (SLE; mean age 46, range 14–70 yrs, 14 women and one man) and 15 patients with dermatomyositis (DM; mean age 51, range 9–75 yrs, 14 women and one man) served as disease controls. Patients with SSc and SLE fulfilled the criteria proposed by the American College of Rheumatology[17,18]. Patients with DM fulfilled the diagnostic criteria proposed by Bohan and Peter[19]. Patients with SSc were grouped according to the classification system proposed by LeRoy, *et al*[20]: 36 (31 women and 5 men) patients had diffuse cutaneous SSc (dSSc) and 42 (40 women and 2 men) patients had limited cutaneous SSc (lSSc). Patients with SSc, SLE, or DM did not receive any treatment, including corticosteroids or immunosuppressive agents. None had a history of infection or other inflammatory diseases. Fresh venous blood samples were centrifuged shortly after clot formation. All samples were stored at –70°C prior to use.

Clinical assessment. Clinical and laboratory data were obtained within 2 weeks of collection of serum samples. All patients had detailed clinical assessment for laboratory findings and involvement of the organ systems. Organ system involvement was defined as described previously with some modifications[21]: (1) lung: bibasilar fibrosis on chest radiography; (2) isolated pulmonary hypertension: clinical evidence of pulmonary hypertension and increased systolic pulmonary arterial pressure (> 35 mm Hg) by Doppler echocardiography, in the absence of severe pulmonary interstitial fibrosis[22]; (3) esophagus: hypomotility shown by barium radiography; (4) joint: inflammatory polyarthralgias or arthritis; (5) heart: pericarditis, congestive heart failure, or arrhythmias requiring treatment; (6) kidney: elevated serum creatinine, decreased glomerular filtration rate (GFR; < 75% of the predicted normal values), or malignant hypertension; (7) muscle: proximal muscle weakness and elevated serum creatine kinase. Pulmonary function tests, including vital capacity (VC) and diffusion capacity for carbon monoxide (DLCO), were also evaulated. When the VC and DLCO were < 80% and <75%, respectively, of the predicted normal values, these results were considered abnormal. The protocol was approved by the Kanazawa University Graduate School of Medical Science, and informed consent was obtained from all patients.

Enzyme linked immunosorbent assay (ELISA). Serum levels of IL-8 or GRO-α were measured with specific ELISA kits (Quantikine ELISAKit, 2nd Generation, R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocol. Each sample was tested in duplicate. The detection limit of each assay was 10 pg/ml for both IL-8 and GRO-α.

Statistical analysis. Statistical analysis was performed using Mann-Whitney U test for comparison between 2 groups, Fisher's exact probability test for analysis of frequencies, and Bonferroni's test for multiple comparisons. Spearman's rank correlation coefficient was used to examine the relationships between 2 continuous variables. Factorial analysis of variance was used for multivariate analysis. A p value < 0.05 was considered statistically significant. All data were shown as mean ± SD.

RESULTS

Serum IL-8 concentrations in SSc. Serum IL-8 levels were detected in more than half of patients with SSc (45/78, 58%), while only 6% (2/35) of controls showed detectable serum IL-8 levels ($p < 0.0001$; Figure 1A). Elevated serum IL-8 levels were observed at significantly higher frequency in SSc patients than in patients with SLE (1/15, 7%; $p < 0.0005$) or those with DM (2/15, 13%, $p < 0.005$). Regarding subgroups of SSc, the frequency of serum IL-8 detection was significantly higher in both dSSc (22/36, 61%) and lSSc (23/42, 55%) patients relative to controls ($p < 0.0001$), patients with SLE ($p < 0.005$), or those with DM ($p < 0.005$). However, there was no significant difference in the frequency of IL-8 detection or detectable IL-8 levels between patients with dSSc and those with lSSc.

Serum GRO-α concentrations in SSc. SSc patients (87.0 ± 15.8 pg/ml) had significantly higher serum GRO-α levels than controls (79.3 ± 6.7; $p < 0.01$), patients with SLE (78.8 ± 8.7; $p < 0.05$), or those with DM (76.2 ± 11.6; $p < 0.001$; Figure 1B). Concerning subgroups of SSc, serum GRO-α levels in patients with dSSc (88.2 ± 18.2) were significantly increased compared with controls ($p < 0.01$), patients with SLE ($p < 0.05$), or those with DM ($p < 0.001$). Serum GRO-α levels in patients with lSSc (85.5 ± 13.5) were significantly elevated in comparison to controls ($p < 0.05$) or patients with DM ($p < 0.005$). Serum GRO-α levels were similar for dSSc and lSSc patients. Patients with DM had significantly lower serum GRO-α levels than controls ($p < 0.05$). There was a positive correlation between serum IL-8 and GRO-α levels ($r = 0.475$, $p < 0.0001$).

Serum GRO-α levels higher than the mean + 2 SD (92.7 pg/ml) of the control serum samples were considered to be elevated. Elevated serum GRO-α levels were observed in 28% (22/78) of patients with SSc, 31% (11/36) of patients with dSSc, and 26% (11/42) of patients with lSSc, while GRO-α levels were not elevated in normal controls, and were increased in only 7% (1/15) of patients with SLE and 7% (1/15) of patients with DM.

Correlation between serum chemokine concentrations and clinical or laboratory features in SSc. Any detectable serum IL-8 level was regarded as elevated, since only 2 out of 35 controls had detectable IL-8 levels. Patients with detectable serum IL-8 levels had increased frequency of decreased % DLCO ($p < 0.05$) and rheumatoid factor positivity ($p < 0.05$) in comparison to those without detectable serum IL-8 levels (Table 1). Serum IL-8 levels correlated inversely with % DLCO, when samples showing undetectable IL-8 activity were taken with a value of 0 ($r = –0.38$, $p < 0.005$; Figure 2A).

When elevated serum GRO-α levels were defined as greater than the mean + 2 SD of the control serum samples, patients with elevated serum GRO-α levels had increased frequency of decreased % VC ($p < 0.05$), decreased % DLCO ($p < 0.05$), kidney involvement ($p < 0.01$), muscle involvement ($p < 0.01$), the presence of anti-topoisomerase I antibody ($p < 0.01$), and increased serum IgG ($p < 0.05$) relative to those with normal GRO-α levels, while patients with normal GRO-α levels had increased frequency of anti-centromere antibody compared with those with elevated GRO-α levels ($p < 0.05$; Table 1). The factorial analysis of variance showed that increased frequency of anti-topoisomerase I antibody positivity in SSc patients with elevated GRO-α levels was not secondary to the increased frequency of % VC and % DLCO. Serum GRO-α levels correlated inversely with % DLCO ($r = –0.40$, $p < 0.005$; Figure 2B) and % VC ($r = –0.34$, $p < 0.01$; Figure 2C).

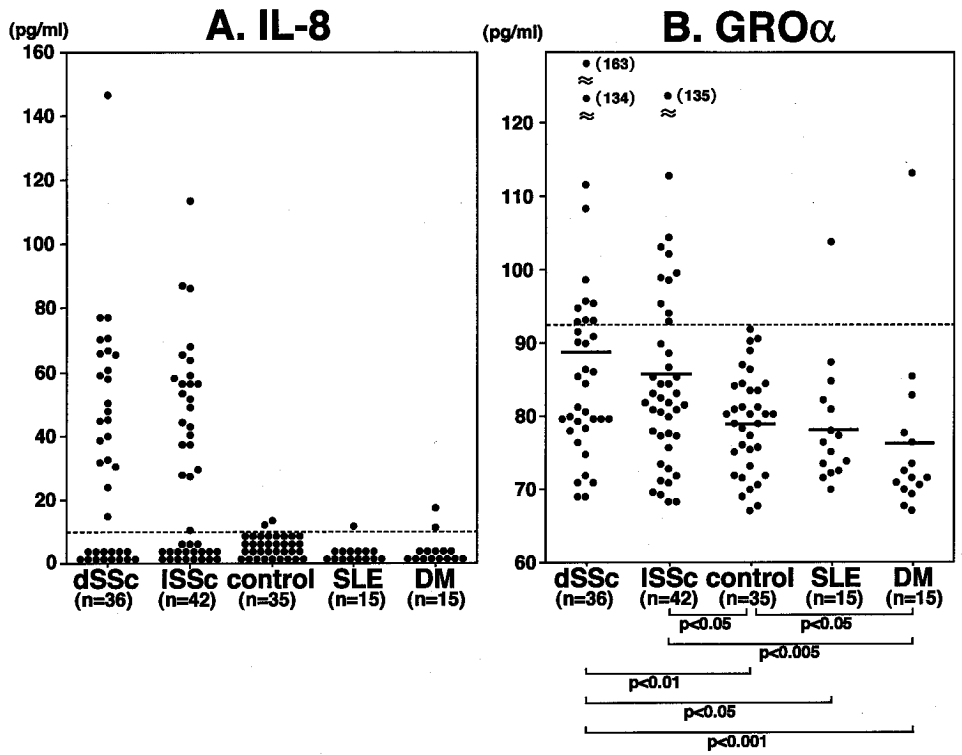

Figure 1. Serum levels of IL-8 (A) and GRO-α (B) in patients with dSSc, lSSc, SLE, DM, and controls. Serum levels of IL-8 and GRO-α were determined by specific ELISA. Broken lines show the detection limit for IL-8 and the cutoff value (mean + 2 SD of control serum samples) for GRO-α. Bars indicate mean values.

Table 1. Clinical and laboratory features of patients with SSc according to serum IL-8 or GRO-α levels. Unless noted otherwise, values are percentages.

	Elevated IL-8 (n = 45)	Normal IL-8 (n = 33)	Elevated GRO-α (n = 22)	Normal GRO-α (n = 56)
Age at onset, yrs, mean ± SD	51 ± 17	51 ± 12	47 ± 19	53 ± 12
Sex, female:male	42:3	29:4	24:4	47:3
Duration, yrs, mean ± SD	7.1 ± 7.5	8.7 ± 10.9	7.1 ± 8.2	8.2 ± 9.5
Clinical features, %				
Pitting scars	42	36	43	38
Short sublingual frenulum	60	41	64	45
Contracture of phalanges	45	58	57	48
Diffuse pigmentation	49	42	50	44
Organ involvement, %				
Pulmonary fibrosis	38	25	40	25
Pulmonary hypertension	18	12	21	13
Decreased %VC	38	20	48*	20
Decreased %DLCO	88*	66	95*	69
Esophagus	61	56	57	59
Heart	33	13	40	16
Kidney	23	9	38**	6
Joint	18	31	28	21
Muscle	27	17	44**	11
Laboratory findings, %				
Anti-topoisomerase I antibody	48	42	59**	18
Anticentromere antibody	34	30	26*	56
Anti-U1RNPantibody	5	3	7	2
Rheumatoid factor	23*	3	15	15
Elevated ESR	37	33	50	28
Elevated CRP	18	18	30	16
Increased IgG	36	25	50*	20

* $p < 0.05$; ** $p < 0.01$ vs normal chemokine levels. ESR: erythrocyte sedimetation rate; CRP: C-reactive protein.

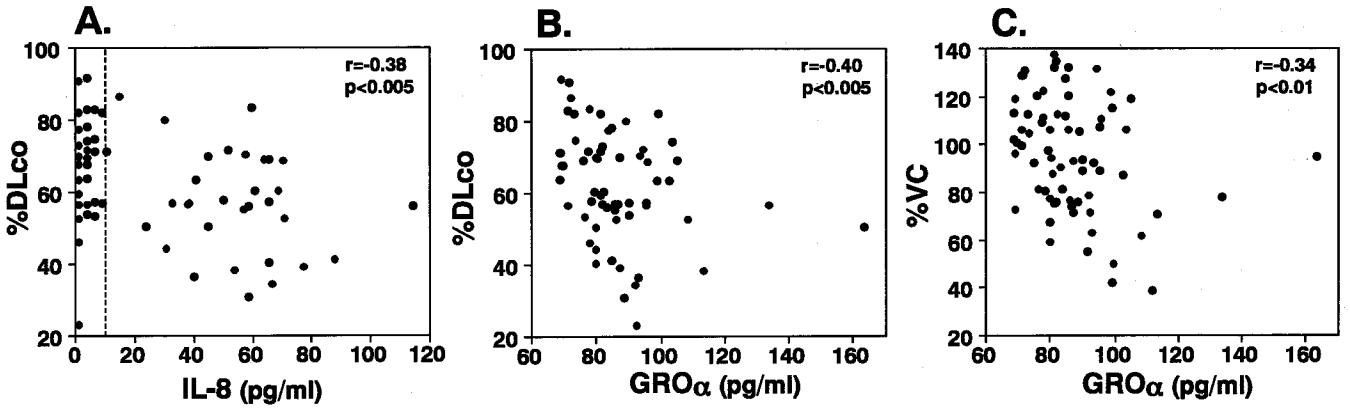

Figure 2. The relationship between serum IL-8 levels and % DLCO (A) and between serum GRO-α levels and % DLCO (B) or % VC (C) in patients with SSc. Serum levels of IL-8 and GRO-α were determined by ELISA. Broken line shows the detection limit for IL-8. Samples with undetectable IL-8 levels were assigned a value of 0.

DISCUSSION

Serum IL-8 concentrations were more frequently detected in patients with SSc than in controls, patients with SLE, or those with DM. Similarly, serum GRO-α levels in SSc patients were increased compared with controls, patients with SLE, or those with DM. Further, elevated IL-8 levels correlated with decreased % DLCO, but not decreased % VC, suggesting only a weak association of IL-8 with pulmonary damage. In contrast, increased GRO-α levels were associated with decreased % DLCO and % VC and involvement of kidney and muscle. Thus, GRO-α correlated more closely with pulmonary damage. Collectively, our results suggest that the elevation of serum IL-8 and GRO-α levels is specific to SSc and that the elevation of CXC chemokines, particularly GRO-α, correlates with the involvement of internal organs, especially pulmonary damage. This is consistent with the notion that fibrosing alveolitis is specific for SSc among these collagen diseases.

Two previous reports that showed the elevation of serum IL-8 levels in SSc failed to detect a correlation between serum IL-8 levels and clinical or laboratory findings[23,24]. In addition to the difference between patients assessed, these discrepant results may be due to the small sample numbers analyzed or low sensitivity of the ELISA system used. Szegedi, *et al* evaluated only 18 patients with SSc[24]. Reitamo, *et al* examined 108 patients with SSc (48 dSSc and 60 lSSc), using the ELISA system with 50 pg/ml of detection limit, far less sensitive than ours (10 pg/ml)[23]. When the cutoff value was set at 50 pg/ml in the current study, the correlation of elevated serum IL-8 levels with decreased % DLCO and the presence of rheumatoid factor was no longer detectable (data not shown). Using a more sensitive ELISA, we analyzed 78 patients with SSc (36 dSSc and 42 lSSc) and showed the correlation of elevated serum IL-8 levels with decreased % DLCO. Further, our study is the first to show that serum GRO-α levels were also elevated in SSc and associated with pulmonary damage.

IL-8 and GRO-α are potent chemoattractants and activators for neutrophils that have been shown to infiltrate mainly the lung in SSc. Augmented production of IL-8 by alveolar macrophages from SSc patients has also been reported[10,25]. Neutrophils infiltrating the lung in response to IL-8 and GRO-α may participate in ongoing fibrosing lung injury, with degranulation and the release of reactive oxygen radicals[14,15,26,27]. Although correlation of elevated IL-8 and GRO-α levels with lung inflammation determined by bronchoalveolar lavage analysis and lung biopsy was not determined in our study, IL-8 and GRO-α might be involved in the progression of lung fibrosis by mediating neutrophil recruitment and activation. In addition to the effects of IL-8 and GRO-α on neutrophils, IL-8 and GRO-α have a wide variety of biological activities. IL-8 induces migratory phenotype of fibroblasts, promotes fibroblast chemotaxis, and thereby may affect the progression of fibrosis[9]. In addition, IL-8 and GRO-α induce migration and proliferation of endothelial cells, resulting in angiogenesis[28,29]. IL-8 and GRO-α are produced by epithelial cells, endothelial cells, fibroblasts, and alveolar macrophages in the lung[8,9]. Thus, the association of elevated IL-8 and GRO-α levels with decreased pulmonary function may be related to the effects of IL-8 and GRO-α on fibrotic process and angiogenesis.

Our results suggest that serum concentrations of IL-8 and GRO-α may reflect the activity or severity of lung fibrosis in SSc. Repeated and sequential analysis of serum levels of IL-8 or GRO-α may clarify their usefulness as a serological marker for the severity or activity of lung injury and may enable us to better understand the pathogenesis of pulmonary damage in SSc.

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