Increased Serum Soluble OX40 in Patients with Systemic Sclerosis

KAZUHIRO KOMURA, AYUMI YOSHIZAKI, MASANARI KODERA, YOHEI IWATA, FUMIHIDE OGAWA, KAZUHIRO SHIMIZU, TAKAMASA WAYAKU, TORU YUKAMI, MAKI MURATA, MINORU HASEGAWA, MANABU FUJIMOTO, KAZUHIKO TAKEHARA, and SHINICHI SATO

ABSTRACT. ***Objective.*** To determine levels of serum soluble OX40 (also termed CD134, a member of the tumor necrosis factor receptor superfamily) and their clinical associations in patients with systemic sclerosis (SSc).

Methods. Serum soluble OX40 levels were examined by ELISA in 53 patients with SSc, 15 patients with systemic lupus erythematosus (SLE), and 32 healthy individuals.

Results. OX40 levels were significantly elevated in SSc patients (125.7 ± 5.7 pg/ml) compared to patients with SLE (80.7 ± 1.7 pg/ml; $p < 0.005$) and controls (88.2 ± 3.0 pg/ml; $p < 0.0001$). Elevated OX40 levels were found to be associated with disease duration of less than 2 years ($p < 0.05$).

Conclusion. Our results suggest that serum soluble OX40 levels correlate with the early-onset of SSc disease. (First Release Oct 1 2008; J Rheumatol 2008;35:2359–62; doi:10.3899/jrheum.080120)

Key Indexing Terms:
SYSTEMIC SCLEROSIS OX40 T LYMPHOCYTES

Systemic sclerosis (SSc) is a connective tissue disease characterized by systemic extracellular matrix deposition with autoimmune background[1]. It is likely that some T cell-related cytokines, growth factors, and/or chemokines regulate the development of fibrosis by stimulating the synthesis of extracellular matrix components[1,2]. OX40 [also termed CD134, a member of the tumor necrosis factor (TNF) receptor superfamily] has been described as a primary costimulator expressed on activated T cells[3]. OX40/OX40 ligand interactions are crucial for the generation of memory T cells, by promoting the survival of effector T cells. Dysfunction and/or abnormal expression of OX40 has been suggested in several autoimmune diseases, whereas expression of OX40 is unknown in patients with SSc[4-7]. We examined levels of serum soluble OX40 in patients with SSc.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from 53 Japanese patients with SSc (45 female, 8 male). They were all referred to the Department of Dermatology, Kanazawa University, in the period 2000 to 2005. All patients fulfilled the criteria for SSc proposed by the American College of Rheumatology (ACR)[8]. Patients were of ages between 2 and 76 years (mean 54 yrs). They were grouped according to the classification system[8] as follows: 21 patients (all female) had limited cutaneous SSc (lSSc) and 32 (24 female, 8 male) had diffuse cutaneous SSc (dSSc). The disease durations of patients with lSSc and dSSc, defined as the time from the first symptom related with Raynaud's phenomenon, were 5.3 ± 4.7 and 6.1 ± 4.5 years, respectively. Four patients had been treated with low-dose corticosteroids and 3 with low-dose D-penicillamine at the first visit. No patient had received other immunosuppressive therapy, or had a recent history of infection or other inflammatory diseases. We also examined serum samples from 15 patients with systemic lupus erythematosus (SLE) that fulfilled the ACR criteria[9]. Patients with SLE with more than 10 points of the SLE Disease Activity Index were included in this study. As well, 32 serum samples from age and sex matched healthy volunteers were used as normal controls.

Peripheral venous blood samples were drawn into pyogen-free blood collection tubes without additives, immediately immersed in melting ice, and allowed to clot 1 h before centrifugation. All samples were stored at –70°C prior to use.

Clinical assessments. Complete medical histories, physical examinations, and laboratory tests were conducted for all subjects at the first visit as described[10].

The study complied with the Declaration of Helsinki. The protocol was approved by Kanazawa University School of Medicine, Kanazawa University Hospital, and the Nagasaki University Graduate School of Biomedical Sciences.

ELISA. Specific ELISA kits were used for measuring serum soluble OX40 levels (Medsystems Diagnostics, Vienna, Austria), according to the manufacturer's protocol. Each sample was tested in duplicate. The detection limit of soluble OX40 was 1.8 pg/ml.

Statistical analysis. Statistical analysis was performed using the Mann-Whitney U test for comparison of soluble OX40 levels, Fisher's exact probability test for comparison of frequencies, Bonferroni's test for multiple comparisons, and a multiple regression analysis. A p value < 0.05 was considered statistically significant. All data are shown as means ± SD.

RESULTS

Serum soluble OX40 levels were elevated in patients with

From the Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki; and Department of Dermatology, Kanazawa University Graduate School of Medical Science, Ishikawa, Japan.

K. Komura, MD, PhD, Assistant Professor; A. Yoshizaki, MD; Y. Iwata, MD; F. Ogawa, MD, PhD, Assistant Professor; K. Shimizu, MD, PhD, Associate Professor; S. Sato, MD, PhD, Professor, Chairman, Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences; M. Kodera, MD, PhD; T. Wayaku, MD, PhD; T. Yukami, MD, PhD; M. Murata, MD; M. Hasegawa, MD, PhD, Assistant Professor; M. Fujimoto, MD, Associate Professor; K. Takehara, MD, Professor, Department of Dermatology, Kanazawa University Graduate School of Medical Science.

Address reprint requests to Dr. S. Sato, Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. E-mail: s-sato@nagasaki-u.ac.jp

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SSc, and were significantly higher in patients with SSc than in healthy controls (125.7 ± 5.7 pg/ml vs 88.2 ± 3.0 pg/ml, respectively; $p < 0.005$; Figure 1) and patients with SLE (80.7 ± 1.7; $p < 0.05$).

Serum samples were divided into 2 groups, according to a level of OX40 of 119 pg/ml (the mean + 2 SD of control serum sample values) as the cutoff value. Soluble OX40 levels did not correlate with clinical features and laboratory data (Table 1; data not shown).

Elevated soluble OX40 levels were associated with shorter disease duration in SSc. Patients with elevated serum soluble OX40 levels were found to have a shorter duration of disease ($p < 0.05$; Table 1). Moreover, dSSc patients with disease duration < 2 years had significantly elevated soluble OX40 levels compared to those with disease duration of 2–5 years and those with disease duration > 5 years (Figure 2). Patients with lSSc with duration < 2 years also had significantly elevated OX40 levels compared to those with duration of 2–5 years and those with duration > 5 years. A multiple regression analysis was done with OX40 as dependent variable and age and disease duration as independent variables. OX40 was found to be independently correlated with disease duration (r = 0.625, $p < 0.005$, lower confidence value = 0.179, and upper confidence value = 0.541). Thus, serum soluble OX40 levels were elevated in the early phase of SSc.

DISCUSSION

In our study, serum soluble OX40 levels were elevated in patients with SSc. Further, serum soluble OX40 was elevated in the early phase of SSc. Since chronic T cell activation characterized by increased T cell-associated cytokines and chemokines is possibly a contributing factor in tissue inflammation[1,2], OX40 may activate T cells in SSc. Similar to other members of the TNF receptor superfamily, soluble OX40 may be produced through cleavage from cell-surface OX40 and/or alternative splicing[11]. Soluble forms of TNF receptor superfamily have effects antagonistic to membrane-bound receptors[11]. Therefore, it is possible that abnormal activation of T cells may induce alternative splicing of OX40 in patients with SSc. However, the function of soluble OX40 in SSc remains unknown.

There are limitations in our study. A larger study of a longitudinal design will be meaningful. Studies focusing on expression of membrane-bound OX40 and membrane-bound

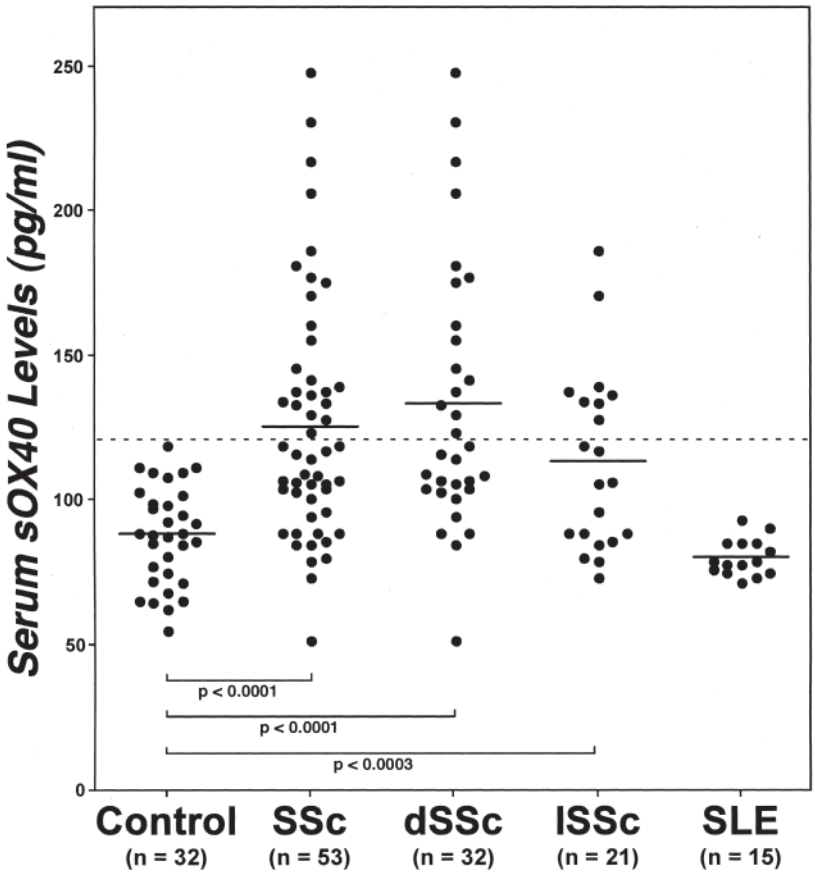

Figure 1. Serum levels of soluble OX40 in healthy controls and patients with diffuse cutaneous SSc (dSSc), limited cutaneous SSc (lSSc), and systemic lupus erythematosus (SLE). OX40 levels were determined by a specific ELISA. Short bars indicate mean values in each group. Broken line indicates the cutoff value (mean + 2 SD of control samples).

Table 1. Clinical and laboratory features of patients with SSc showing elevated serum soluble OX40 levels. Unless noted otherwise, values are percentages.

Characteristic	Elevated OX40, n = 23	Normal OX40, n = 30	p
Age at disease onset, yrs, mean ± SD	43.7 ± 20.5	45.1 ± 15.4	0.79
Male:female	4:19	4:26	0.72
Disease duration, yrs, mean ± SD	3.14 ± 2.3	7.53 ± 6.2	< 0.05
Clinical features			
dSSc (n = 32)	65	35	0.58
lSSc (n = 21)	57	43	0.58
TSS, points, mean ± SD	13.9 ± 9.8	15.0 ± 11.2	0.69
Pitting scars	43	47	> 0.99
Contracture of phalanges	48	47	> 0.99
Diffuse pigmentation	61	53	0.78
Organ involvement			
Pulmonary hypertension	13	10	0.35
Pulmonary fibrosis	39	43	0.78
Decreased %VC	17	37	0.12
Decreased %DLCO	78	56	0.19
Esophagus	74	63	0.24
Heart	9	23	0.12
Kidney	4	3	> 0.99
Joint	17	26	0.44
Muscle	22	20	0.34
Laboratory findings			
Anti-topoisomerase I antibody	52	47	0.9
Anticentromere antibody	26	43	0.25
Increased IgG	30	37	0.42
Elevated ESR	17	30	0.24
Elevated CRP	4	23	0.30

TSS: modified Rodnan total skin thickness score, lSSc: limited cutaneous SSc, dSSc: diffuse cutaneous SSc, VC: vital capacity, DLCO: diffusion capacity for carbon monoxide, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.

OX40 ligand, and intracellular signaling of OX40 in several organs in patients with SSc, would provide more information about associations between SSc and OX40/OX40 ligand interactions. The relationship between T cell-derived cytokines or chemokines and OX40 levels should be analyzed, and this may clarify the relative contribution of each T cell subset. However, our results suggest that regulation of OX40/OX40 ligand interactions may provide a new therapeutic approach for patients with SSc.

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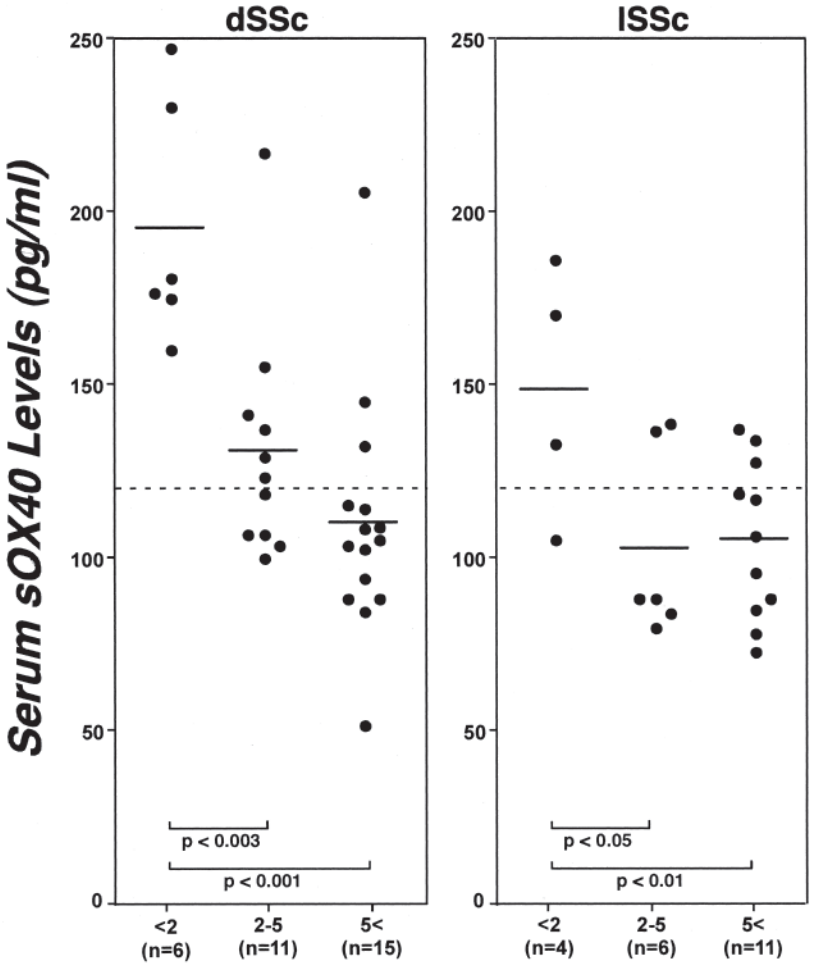

Figure 2. Correlation of disease duration (< 2, 2–5, and > 5 years) with serum soluble OX40 levels in patients with dSSc and lSSc. OX40 levels were determined by a specific ELISA. Broken lines indicate the cutoff value.