Elevation of Serum Lymphotactin Levels in Patients with Systemic Sclerosis

EIJI MUROI, FUMIHIDE OGAWA, KAZUHIRO SHIMIZU, KAZUHIRO KOMURA, MINORU HASEGAWA, MANABU FUJIMOTO, and SHINICHI SATO

ABSTRACT. ***Objective.*** To determine serum concentrations of lymphotactin, a Th1 chemokine, and their clinical association in patients with systemic sclerosis (SSc).

Methods. Lymphotactin levels were examined in serum samples from patients with SSc (n = 68), systemic lupus erythematosus (SLE; n = 42), or dermatomyositis (DM; n = 29), and healthy controls (n = 18) by enzyme linked immunosorbent assay.

Results. Serum lymphotactin levels were significantly elevated in SSc patients compared to patients with SLE or DM as well as controls. Serum lymphotactin levels were similar in patients with limited cutaneous SSc and diffuse cutaneous SSc (dSSc). Clinical correlation of elevated lymphotactin levels was not detected in the total group of patients with SSc, while elevation of lymphotactin levels was significantly associated with higher percentage vital capacity and percentage diffusing capacity of carbon monoxide, lower lung severity grade and serum IgG levels, and less frequent presence of short sublingual frenulum in patients with dSSc.

Conclusion. Our results indicate that elevated serum lymphotactin levels correlate with relatively milder manifestations in dSSc, especially lower severity of lung involvement, suggesting that lymphotactin may play a role in the development of dSSc. (First Release Mar 1 2008; J Rheumatol 2008;35:834–8)

Key Indexing Terms:

SYSTEMIC SCLEROSIS	LYMPHOTACTIN	CHEMOKINE
PULMONARY FIBROSIS	IMMUNOGLOBULIN G	TH1 CELLS

Systemic sclerosis (SSc) is a multisystem disorder of connective tissue characterized by excessive fibrosis and vascular changes in the skin and various internal organs, such as the lungs, kidneys, esophagus, and heart. Although the pathogenesis of SSc remains unknown, it has been suggested that immunological abnormalities have a critical role[1-3]. Most of the infiltrating cells in the skin of patients with SSc are activated T lymphocytes with a predominant CD4+ phenotype[4]. Hyperactivity of circulating CD4+ T cells has also been detected in patients with SSc[5]. Cytokines play a major role in regulating extracellular matrix deposition by fibroblasts[6]. Stimulated naive T cells then differentiate into memory/effector T cells that are classified into T helper 1 (Th1) and Th2 subsets based on their profiles of cytokine production[7]. Imbalance between Th1 and Th2 immune responses is considered to play an important role in autoimmune and allergic diseases[8,9]. Although Th1/Th2 imbalance in SSc appears to be complicated, accumulating evidence has shown that SSc is generally a Th2-dominant autoimmune disease, especially in the early phase of the disease[10-12]. It has been reported that serum concentrations of Th2 cytokines, such as interleukin 4 (IL-4), IL-6, IL-10, and IL-13, were increased in patients with SSc[10,11,13]. Th2 cytokine production by stimulated peripheral blood lymphocytes was also elevated in SSc[13]. Further, patients with SSc exhibited Th2 cytokine production by cultured CD4+ T cells isolated from skin lesions[14].

Lymphotactin[15] was independently detected by 3 groups and denoted with 2 different names: single C motif 1[16] and activation-induced, T cell–derived, and chemokine-related molecule[17]. This chemokine is structurally related to the CC chemokine subfamily that lacks the first and third cysteine residues, and it is thus considered to represent the C chemokine subfamily. Lymphotactin is selectively expressed in activated CD8+ T cells and in a small proportion of activated CD4+ T cells[17], thymocytes[15], intraepithelial T cells[18], mast cells[19], and natural killer (NK) cells[20]. Lymphotactin acts via a unique G protein–coupled receptor XCR1[21,22]. The full spectrum of biologic functions of lym-

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

E. Muroi, MD, PhD, Clinical Fellow; F. Ogawa, MD, PhD, Assistant Professor; K. Shimizu, MD, PhD, Associate Professor; K. Komura, MD, PhD, Assistant Professor; S. Sato, MD, PhD, Professor and Chairman, Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences; M. Hasegawa, MD, PhD, Assistant Professor; M. Fujimoto, MD, PhD, Associate 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

Accepted for publication December 27, 2007.

photactin is still unknown, but the functional properties that have been detected include chemotactic activity for both CD4+ and CD8+ T cells[23,24] and induction of migratory responses in NK cells after activation with IL-2[25]. Moreover, a recent study using a murine model of listeriosis has shown that lymphotactin, macrophage inflammatory protein 1α and 1ß, and regulated on activation, normal T cell expressed and secreted (RANTES) are cosecreted with interferon-γ (IFN-γ) by activated NK cells, CD8+ T cells, and CD4+ Th1 cells, and function as Th1 cytokines by upregulating CD40, IL-12, and tumor necrosis factor by macrophages[26].

Lymphotactin expression has been studied in several clinical and experimental models of inflammatory diseases, such as acute allograft rejection[27,28], autoimmune diabetes[29], encephalomyelitis[30], experimental crescentic glomerulonephritis[31], chronic inflammatory bowel disease[32], and rheumatoid arthritis[33]. Results of these studies support the concept of a potential role of lymphotactin in Th1-type inflammatory processes. To evaluate a role of lymphotactin in the development of SSc, we investigated lymphotactin levels in serum samples and their clinical correlation.

MATERIALS AND METHODS

Patients and samples. Serum samples were obtained from 68 Japanese patients with SSc (58 women, 10 men). All patients fulfilled the criteria proposed by the American College of Rheumatology[34]. Patients were grouped according to the classification system proposed by LeRoy, *et al*[35]: 37 patients (29 women, 8 men) had diffuse cutaneous SSc (dSSc) and 31 (29 women, 2 men) had limited cutaneous SSc (lSSc). The age of patients with SSc (mean ± SD) was 49.4 ± 16.8 years old. Patients with dSSc were 43.3 ± 15.6 years old, while those with lSSc were 57 ± 9.4 years old. The disease duration of patients with dSSc and lSSc was 3.61 ± 4.28 and 9.24 ± 9.62 years, respectively. Antinuclear antibody (ANA) was determined by indirect immunofluorescence and autoantibody specificities were further assessed by enzyme linked immunosorbent assay (ELISA) and immunoprecipitation. Anticentromere antibody was positive in 21 patients, anti-topoisomerase-1 antibody in 24, anti-U1RNP in 5, anti-U3RNP in 2, anti-RNA polymerases I and III in 3, and Th/To in 1. Seven patients had ANA; however, their specificities were not identified. The remaining 5 patients were negative for autoantibody. Forty-two patients with systemic lupus erythematosus (SLE), who fulfilled American College of Rheumatology criteria[36], were examined as disease controls. In addition, 29 patients with dermatomyositis (DM) that fulfilled the Bohan and Peter criteria[37,38] were included as disease controls. Eighteen age- and sex-matched Japanese healthy individuals were used as normal controls. Fresh venous blood samples were centrifuged shortly after clot formation. All samples were obtained before treatment and stored at –70°C prior to use. The protocol was approved by the Kanazawa University Graduate School of Medical Science and Kanazawa University Hospital, and informed consent was obtained from all patients.

Clinical assessment. Complete medical histories, examinations, and laboratory tests were conducted for all patients at their first visit. Skin score was measured with the modified Rodnan total skin thickness score[39]. Organ involvement was defined as described[40]: lung = bibasilar fibrosis on chest radiography and high resolution computed tomography; esophagus = hypomotility by barium radiography; joint = inflammatory polyarthralgias or arthritis; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment; and muscle = proximal muscle weakness and elevated serum creatinine kinase. Since there were no definite criteria regarding the length of short sublingual frenulum, it was defined as white and hypertrophic sublingual frenulum. Isolated pulmonary hypertension was defined as 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. Pulmonary function testing, including vital capacity (VC) and diffusion capacity for carbon monoxide (DLCO), was conducted to evaluate the severity of lung involvement. When the DLCO and VC were < 75% and < 80%, respectively, of the predicted normal values, they were considered to be abnormal. SSc patients with a smoking habit or other respiratory disorders, which could have affected %DLCO or %VC, were excluded. The lung severity was graded using the lung severity scale in SSc[41].

ELISA for lymphotactin. Specific ELISA kits were used for measuring serum lymphotactin levels (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocol. Each sample was tested in duplicate. The detection limit of this assay was 62.5 pg/ml.

Statistical analysis. The Mann-Whitney U-test was used to compare lymphotactin levels, Fisher's exact probability test to compare frequencies, and Bonferroni's test for multiple comparisons. Spearman's rank correlation coefficient was used to examine the relationship between 2 continuous variables. All p values less than 0.05 were considered statistically significant.

RESULTS

Serum lymphotactin levels in SSc. Serum levels of lymphotactin in patients with SSc and healthy controls are shown in Figure 1. For comparison, patients with DM or SLE were included. Serum lymphotactin levels were significantly elevated in total patients with SSc (median 3.28 ng/ml, range 2.88–3.85) compared with healthy controls (median 2.71, range 2.09–3.28; $p < 0.0001$), patients with SLE (median 2.58, range 1.99–3.06; $p < 0.0001$), and patients with DM (median 2.78, range 2.36–3.44; $p < 0.0001$). Lymphotactin levels in patients with SLE or DM were similar to those in healthy controls; however, lymphotactin levels in patients with SLE were significantly decreased relative to those in patients with DM ($p < 0.0001$). As for subgroups of SSc, lymphotactin levels in patients with lSSc (median 3.23 ng/ml, range 2.88–3.79) and dSSc (median 3.39, range 2.93–3.85) were significantly higher than those in normal controls ($p < 0.0001$ and $p < 0.0001$, respectively), in patients with SLE ($p < 0.0001$ and $p < 0.0001$), and in patients with DM ($p < 0.0001$ and $p < 0.0001$). No significant difference between patients with lSSc and those with dSSc was observed in serum lymphotactin levels. Thus, elevated serum lymphotactin levels were specific to patients with SSc.

Clinical correlation. Values higher than the mean + 2 SD (3.45 ng/ml) of the control serum samples were considered to be elevated in our study. Elevated lymphotactin levels were observed in 37% (25/68) of total patients with SSc, in 41% (15/37) of patients with dSSc, and in 32% (10/31) of patients with lSSc. By contrast, elevated lymphotactin levels were not detected in any patients with SLE or DM or any healthy individuals.

In total patients with SSc, no clinical correlation of elevated lymphotactin levels was detected. Then we examined their clinical correlation in patients with dSSc. As shown in

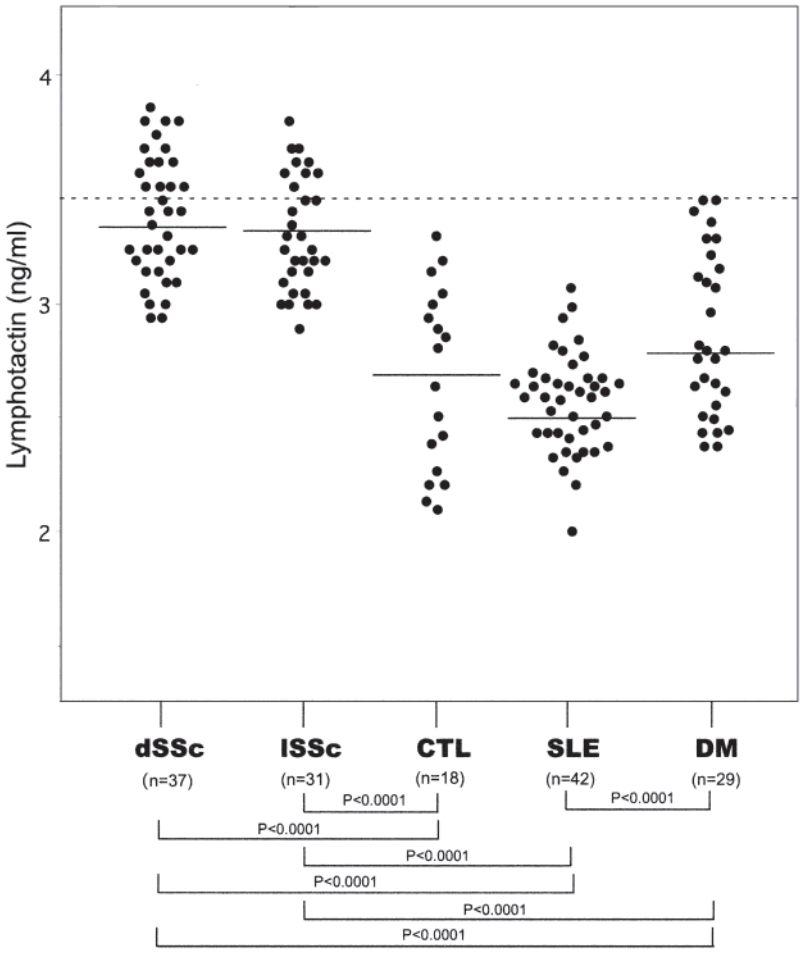

Figure 1. Levels of lymphotactin in serum samples from patients with dSSc, lSSc, SLE, and DM and normal controls (CTL). Serum lymphotactin levels were determined by ELISA. Horizontal lines show mean values. Broken line indicates the cutoff value (mean + 2 SD of the control samples).

Table 1, %VC and %DLCO in dSSc patients with elevated lymphotactin levels were significantly higher than in those with normal lymphotactin levels ($p < 0.05$ and $p < 0.05$, respectively). The lung severity grades in dSSc patients with elevated lymphotactin levels (median 1, range 0–2) were significantly lower than in those with normal lymphotactin levels (median 2, range 0–3; $p < 0.05$). In addition, dSSc patients with elevated lymphotactin levels had short sublingual frenulum less frequently than those with normal lymphotactin levels (36% vs 73%; $p < 0.05$). Serum IgG levels in dSSc patients with elevated lymphotactin levels were significantly decreased compared to those with normal lymphotactin levels ($p < 0.05$). Further, lymphotactin levels correlated negatively with serum IgG levels in patients with dSSc ($r = –0.357$, $p < 0.05$). To determine correlation of serum lymphotactin levels with disease activity, we tried to analyze lymphotactin levels between dSSc patients with < 3 years disease duration and those with disease duration > 3 years, and between lSSc patients with < 9 years disease duration and those with disease duration > 9 years. However, we did not observe any significant difference (data not shown). Thus, elevated lymphotactin levels were associated with the lower severity of lung involvement, lower prevalence of short sublingual frenulum, and decreased serum IgG levels. By contrast, no clinical correlation of elevated lymphotactin levels was detected in patients with lSSc.

DISCUSSION

In our study, serum lymphotactin levels were significantly elevated in SSc patients compared with normal controls. The elevation of lymphotactin levels was specific to patients with SSc, since lymphotactin levels in SLE or DM patients were similar to those found in normal controls. The elevation of serum lymphotactin levels was associated with higher %VC and %DLCO, lower lung severity grade and frequency of short sublingual frenulum, and lower serum IgG levels in patients with dSSc, suggesting that elevated lym-

Table 1. Clinical and laboratory data in dSSc patients with elevated serum lymphotactin levels.

	Elevated Lymphotactin, n = 15	Normal Lymphotactin, n = 22
Sex, male:female	4:11	4:18
Age, mean ± SD, yrs	43.1 ± 16.1	43.5 ± 15.7
Disease duration, mean ± SD, yrs	4.1 ± 5.4	3.3 ± 3.4
Modified Rodnan TSS, mean ± SD	16.9 ± 9.9	15.5 ± 7.8
SSF, n	36*	73
Organ involvement		
Lung severity grade, median (range)	1 (0–2)*	2 (0–3)
Pulmonary fibrosis	67	68
%VC, mean ± SD	96.1 ± 22.9*	86.9 ± 25.4
%DLCO, mean ± SD	72.9 ± 24.9*	56.7 ± 16.2
Pulmonary hypertension	13	14
Esophagus	77	82
Heart	0	0
Joint	20	36
Muscle	13	36
Laboratory findings		
IgG, mean ± SD, mg/dl	1573.3 ± 457.8	1945.5 ± 720.0
IgA, mean ± SD, mg/dl	238.4 ± 81.1	284.2 ± 103.7
IgM, mean ± SD, mg/dl	174.6 ± 103.2	171.2 ± 157.9

Unless noted otherwise, values are percentages. TSS: total skin thickness score; VC: vital capacity; DLCO: diffusion capacity for carbon monoxide; SSF: short sublingual frenulum. * $p < 0.05$, vs dSSc patients with normal serum lymphotactin levels.

photactin levels are related to relatively milder manifestations, especially lower severity of lung involvement, in dSSc.

Lymphotactin is produced mainly by activated T cells and NK cells, and its biological functions as well as its pathological roles remain to be elucidated. Lymphotactin has been reported to be induced by stimulation through T-cell receptors in Th1 cells, but not in Th2 cells, and cosecreted to a high degree with IFN-γ by activated Th1 cells[2,26]. Thus, lymphotactin is a chemokine related to Th1 immune responses. In general, Th1 cells producing IFN-γ and IL-12 limit the development of tissue fibrosis, whereas Th2 cells producing IL-4 and IL-13 exaggerate tissue fibrosis[42-44]. Further, a recent study has shown that a shift from Th2 to Th1 response correlates with improvement of skin fibrosis in dSSc during the disease course[12]. Collectively, correlation of lymphotactin levels with milder manifestation in patients with SSc may be mediated by its Th1 activity. However, IFN-γ, a Th1 cytokine, is not detected in the sera from patients with SSc even at the later, regression stage of skin fibrosis[12]. This finding was not consistent with a shift to a Th1 profile at the later stage. However, serum IL-12 levels were increased and correlated with the later, regression stage of skin fibrosis in SSc. Although the reasons for this discrepancy remain unknown, SSc appeared to exhibit the activation of some Th1 cytokines, at least IL-12, at the later phase of the disease. Alternatively, we recently found that serum levels of IL-23, an important cytokine for inducing Th17 cells, were elevated in patients with SSc (unpublished data). This suggests that the activation of Th17 may affect IFN-γ production and influence fibrosis in SSc, since IL-23 or IL-17 can suppress Th1 cell differentiation in the presence of IL-12[45]. Thus, cytokine activation and its association with the disease activity may be complicated in SSc.

In our study, the disease duration and extent of skin fibrosis were similar between both lSSc and dSSc patients with elevated lymphotactin levels and those with normal levels, suggesting that unlike serum IL-12 levels, lymphotactin is not associated with the improvement of skin fibrosis. Moreover, there was no significant difference in lymphotactin levels between dSSc patients with < 3 years disease duration and those with disease duration > 3 years. This finding suggests that induction of lymphotactin is not related to the later, stable phase of less disease activity. However, lack of association of lymphotactin levels with the disease activity might be due to the heterogeneity of the timepoint when the immune response shifts to Th1. To address the correlation of lymphotactin levels with disease activity and Th1 response, a prospective longitudinal study of serum lymphotactin levels in SSc will be required.

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