Clinical association of serum interleukin-17 levels in systemic sclerosis: Is systemic sclerosis a Th17 disease?

Letter to the Editor

Clinical association of serum interleukin 17 levels in systemic sclerosis: Is systemic sclerosis a Th17 disease?

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Footnotes: IL-17 in systemic sclerosis

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Systemic sclerosis (SSc) is a connective tissue disorder characterized by fibrosis and vascular changes in the skin and internal visceral organs, with an autoimmune background. The increase of the collagen deposition is characteristic with SSc and depends on various cytokines. Among them, transforming growth factor-β (TGF- β) is considered to play a central role in the pathogenesis of SSc[1].

TGF- β may be involved not only fibrosis but also in autoimmune response in SSc. Recently, a subset of interleukin（IL)-17-producing helper T cells (Th17 cells) distinct from Th1 or Th2 cells have been shown to play a crucial role in the induction of autoimmune tissue injury. IL-17 is a member of a newly identified cytokine family comprising IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F. IL-17 acts as a potent inflammatory cytokine in vitro and in vivo, with pleiotropic activities. IL-17 regulates tissue inflammation by inducing the expression of proinflammatory cytokines such as IL-6 and TNFα, chemokines such as KC, MCP-1 and MIP-2, and matrix metalloproteases, which mediate tissue infiltration and tissue destruction. In addition, IL-17 costimulates T cells and enhances the maturation of dendritic cells. Importantly, TGF−β and IL-6 induce development of the Th17 cell lineage [2], while TGF- β alone induces $CD4^+CD25^+Fop3^+$ regulatory T (Treg) cells. Since both TGF- β and IL-6 have been considered as crucial cytokines in SSc [1, 3], IL-17 and the Th17 response may be profoundly involved in the pathogenesis of SSc. Indeed, Kurosawa et al. have assessed 16 SSc patients, and have demonstrated that serum IL-17 levels are increased in SSc patients. They also reported that IL-17 was overproduced by T cells in early stage of SSc [4]. However, IL-17 levels and their clinical correlations have not been investigated in a large SSc population. Therefore, in this study, we examined the correlation of clinical and laboratory features with serum IL-17 levels in SSc patients.

Serum samples from 59 consecutive SSc patients referred to our hospital (12 men and 47 women; median age, 49 years) were assessed for IL-17 levels. All patients fulfilled the criteria proposed by American College of Rheumatology. They were

classified into 28 patients with diffuse cutaneous SSc (dcSSc) and 31 limited cutaneous SSc (lcSSc) patients. No patients had been treated with corticosteroid or other immunosuppressive therapies. In a retrospective longitudinal analysis, we examined serum samples from 25 SSc patients (5 men and 20 women) whose sera were serially obtained for more than two consecutive years. During the follow-up period 20 patients had corticosteroid therapy. Fifteen healty donors (3 men and 12 women; median age, 47 years) were included as controls. Complete medical histories, physical examinations, and laboratory tests including high resolution computed tomography of the chest were conducted for all patients at the first visit and during follow-up. Organ system involvement was defined as described previously [5]. 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. Serum IL-17 levels were measured using an ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturer's protocol. Each sample was tested in duplicate. The protocol was approved by Kanazawa University Hospital, and informed consent was obtained from all patients. Statistical analysis was performed using Mann-Whitney U test for comparison of values, and Fisher's exact probability test for comparison of frequencies. *P* values less than 0.05 were considered statistically significant.

Serum IL-17 levels were significantly higher in SSc patients compared with normal controls (4.96 pg/ml vs. 1.15 pg/ml; $p<0.0001$, Fig. 1a), while there was no significant difference between dcSSc and lcSSc. When values higher than the mean + 2SD (2.44 pg/ml) of the control serum samples were considered to be elevated, serum IL-17 levels were elevated in 85% (50/59) of SSc patients. The correlation between IL-17 levels and disease duration was not also recognized, although patients with normal serum IL-17 levels showed significantly higher modified Rodnan total skin thickness score (TSS) compared with those with elevated IL-17 levels (20.2 vs. 10.8, $p<0.05$, Table 1). There were no significant differences in frequencies of visceral

involvement including the esophagus, kidney, heart, and lung. Additionally, anti-topoisomerase I antibodies were positive in patients with normal serum IL-17 at a significantly higher rate than those with elevated serum IL-17 (78% vs. 35%, $p<0.05$).

These results confirm that serum IL-17 levels are elevated in most SSc patients, suggesting that IL-17 is involved in a common pathway of SSc development regardless of the disease subset. Our results that elevated IL-17 levels correlated with lower TSS may indicate that IL-17 transiently increases in the earlier phase of the disease, which may be consistent with the report by Kurosawa et al. Furthermore, in our longitudinal analysis using serial sera, the tendency that serum IL-17 levels attenuate during the follow-up period was observed (Fig. 1b).

TGF-β is conventionally regarded as an anti-inflammatory cytokine, while it paradoxically plays a major role in SSc, which is considered as an autoimmune disease. TGF- β alone induces Treg cells that inhibit autoimmunity and protect against tissue injury [6 7], but induces Th17 cells in the presence of IL-6 in mice. However, TGF- β may be dispensable in Th17 induction in human, where IL-1β has been demonstrated to play a dominant role in driving Th17 differentiation [8]. Nonetheless, the current study possibly proposes a hypothesis that the dysregulated production of TGF-β and IL-6 may lead to the imbalance of Th17 vs. Treg in SSc. Furthermore, IL-17 induces IL-6 and IL-8 production and enhances the surface expression of intracellular adhesion molecule-1 in human fibroblasts [9]. Alternatively, since IL-17 induces the expression of matrix metalloproteases that facilitate collagen metabolism [10], augmented IL-17 production in SSc may serve as a protective factor against fibrotic process. Collectively, in addition to the recent identification of Th17 involvement in multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases, Th17 cells may also be involved in the pathogenesis of SSc.

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Table 1. Clinical features of SSc patients with or without elevated IL-17 levels

	Elevated IL-17 (n=50)	Normal IL-17 (n=9)	P
Sex, male:female	8:42	4:5	NS
Disease duration (years)	5.2±7.1	4.6±6.8	NS
Clinical features			
Total skin thickness score	10.8±11	20.2±12	<0.05
Pitting scar	39% (19/49)	44% (4/9)	NS
Nailfold bleeding	74% (37/50)	78% (7/9)	NS
Organ involvement			
Lung	42% (21/50)	78% (7/9)	NS
%VC	103±21%	96±18%	NS
%DLco	63±18%	60±15%	NS
Pulmonary hypertension	8% (4/49)	22% (2/9)	NS
Heart	16% (8/49)	0% (0/9)	NS
Kidney	2% (1/48)	0% (0/9)	NS
Arthritis	38% (19/50)	44% (4/9)	NS
Esophagus	46% (23/50)	44% (4/9)	NS
Muscle	6% (3/50)	0% (0/9)	NS
Laboratory data			
Positive anti-topoisomerase I antibody	35% (17/49)	78% (7/9)	<0.05
Positive anticentromere antibody	38% (19/49)	11% (1/9)	NS
Positive anti-U1RNP antibody	4% (2/49)	22% (2/9)	NS

NS = not significant (P>0.05)

Figure Legends

Figure 1. (A) Serum IL-17 levels in patients with SSc and normal controls. Serum samples were obtained from 59 SSc patients and 15 normal controls. Serum IL-17 levels were measured by an enzyme immunoassay. The cut-off value for serum IL-17 levels was arbitrarily determined as the mean + 2SD (2.44 pg/ml; indicated by a dotted line) level in normal controls.

(B) Serial changes in serum IL-17 levels during the follow-up period in 25 SSc patients. 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. Patients with serum IL-17 levels being higher than the mean + 2SD of normal controls (horizontal dotted line) at more than one point were shown by line plot.

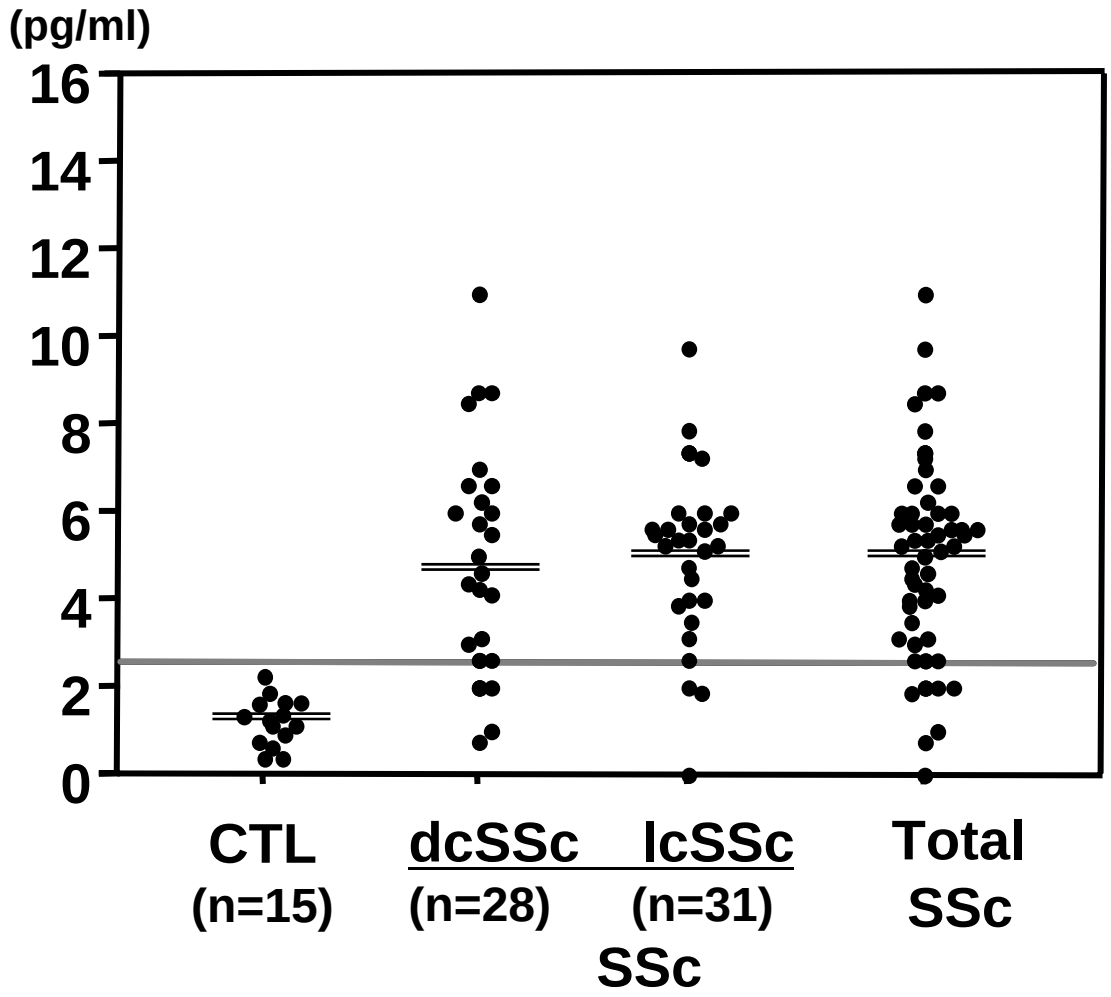

Figure 1A
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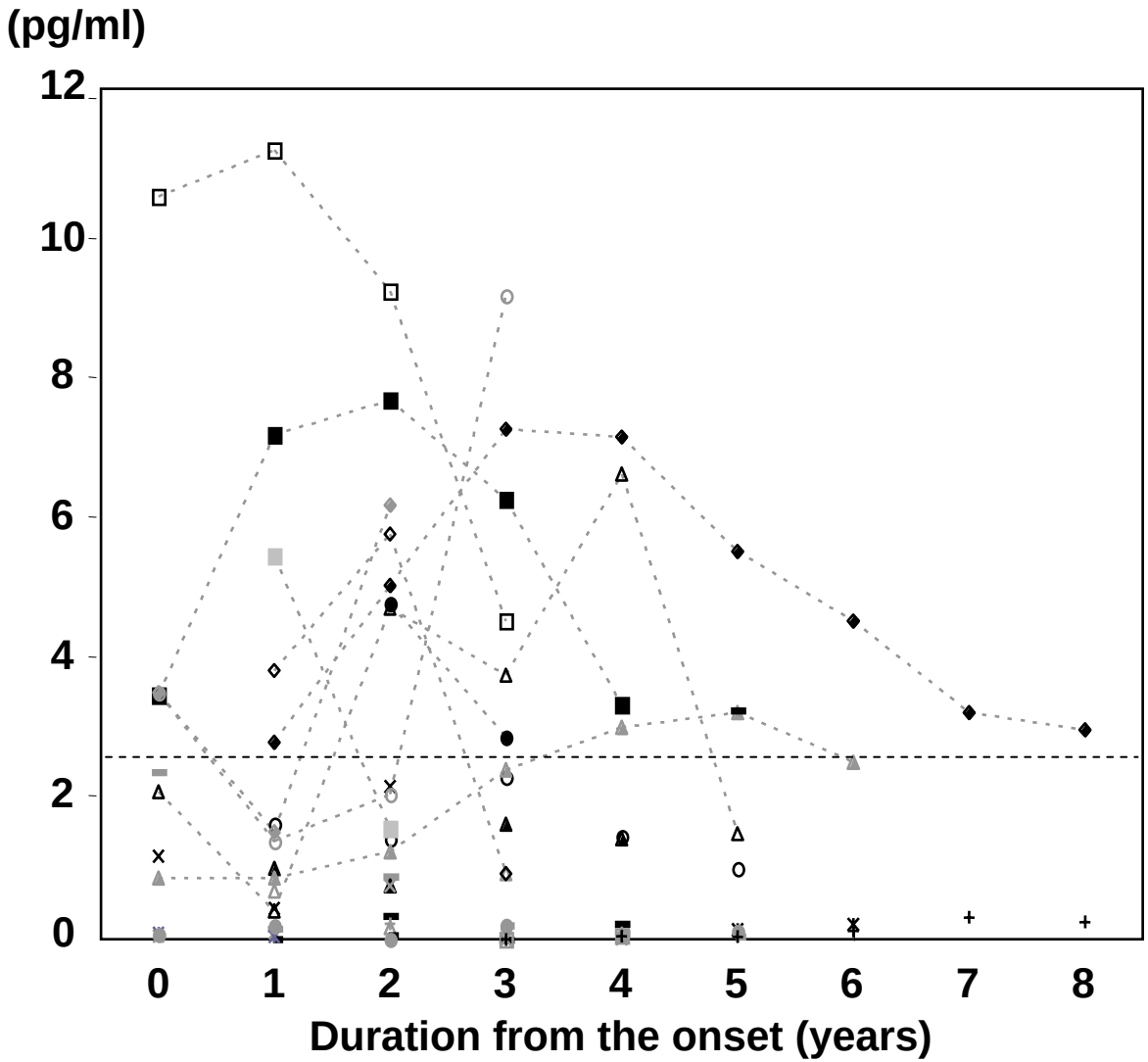

Figure 1B
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