

Serum levels of soluble CD163 in patients with systemic sclerosis

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Abstract Macrophages may play a role in the pathogenesis of systemic sclerosis (SSc), and CD163-positive M2 macrophages are potentially important source for fibrosis-inducing cytokines. However, no link between M2 macrophages and SSc has been established. The aim is to evaluate the possibility that serum levels of soluble CD163 (sCD163) can be a useful marker for SSc, reflecting M2 activation of macrophages in this disease. Serum sCD163 levels of 43 patients with SSc, 10 patients with scleroderma spectrum disorder (SSD), and 12 healthy controls were measured with specific enzyme-linked immunosorbent assays. SSc patients had significantly higher serum sCD163 levels than healthy controls. The sCD163 levels in SSD patients were higher than healthy controls and lower than SSc patients. Significantly higher right ventricular systolic pressure and lower % DLco levels, and shorter duration of disease were seen in SSc patients with elevated serum sCD163 levels than those with normal levels. These results suggest that sCD163 levels may be increased in proportion to the progression of this disease, indicating the involvement of CD163 in the pathogenesis of SSc. Furthermore, serum sCD163 levels may be a marker of pulmonary hypertension at the early stage in patients with SSc.

Keywords Macrophage · Collagen disease · Fibrosis · Pulmonary hypertension

Introduction

Systemic sclerosis (SSc) or scleroderma is a connective tissue disease, which is characterized by fibrosis of the skin and internal organs. Although the pathogenesis of this disease is still unclear, inflammation, autoimmune attack, and vascular damage lead to activation of fibroblasts and abnormal accumulation of extracellular matrix, mainly collagen. Histopathological hallmarks of SSc are inflammatory infiltrates in early disease stages and accumulation of extracellular matrix proteins resulting in tissue fibrosis. Inflammatory infiltrates are dominated by macrophages and T cells [1, 2].

Heterogeneity of macrophages has been discussed with regard to different responses to various microenvironmental stimuli. Macrophages are classically activated toward M1 phenotype by microbial products or interferon (IFN- γ). M1 macrophages have the IL-12^{high}, IL-23^{high}, IL-10^{low} phenotype and produce nitrogen intermediates and inflammatory cytokines such as IL-1 β , tumor necrosis factor (TNF- α), and IL-6 to promote active inflammation [3–5]. In contrast, macrophages are alternatively activated toward M2 phenotype by stimulation with IL-4, IL-13, and IL-10 [3, 4, 6]. These M2 macrophages have the IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype and express several receptors such as hemoglobin scavenger receptor (CD163) [3, 7, 8]. They are reported to be associated with a high degree of vascularization and wound repair [9]. Also, these macrophages can play a role in certain fibrotic diseases by producing transforming growth factor (TGF- β) [10].

Several investigators have reported the increased expression levels of IL-4, IL-13, and IL-10 in sera of patients with SSc [11–13]. They are responsible cytokines for activation of macrophages toward M2 phenotype as described earlier, suggesting possible involvement of M2

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macrophages in the pathogenesis of SSc. However, no link between M2 macrophages and SSc has been established.

In the present study, we investigated serum levels of soluble CD163 (sCD163) in SSc patients. Full-length CD163 is a transmembrane protein and restricted to cell surface, while sCD163 is shed by proteases at or near the cell surface as reported for the shedding of many other membrane protein ectodomains and can be secreted into serum [11, 14]. To date, clinical significance of serum sCD163 is still unknown. sCD163 has been detected in the plasma in vivo, with the highest levels in patients with myelomonocytic leukemias, pneumonia/sepsis, rheumatoid arthritis, and fulminant hepatic failure [15–18]. As described earlier, sCD163 expression may be associated with activity or pathogenesis of SSc. Thus, in this study, we try to evaluate the possibility that serum levels of sCD163 can be a useful marker for this disease.

Patients and methods

Clinical assessment and patient material

Serum samples were obtained from 43 patients with SSc (9 men and 34 women; age range, 7–85 years; mean, 57.4 years). All patients fulfilled the criteria proposed by the American College of Rheumatology and were grouped according to the classification system proposed by LeRoy et al. [19]. Twenty patients had diffuse cutaneous SSc (dcSSc), and 23 patients had limited cutaneous SSc (lcSSc), as described previously [20]. Clinical and laboratory data reported in this study were obtained at the time of serum sampling. Patients were evaluated for the presence of gastrointestinal, pulmonary, cardiac, or renal involvement, as described previously [20]. Control serum samples were also collected from healthy age- and sex-matched volunteers. Ten patients with scleroderma spectrum disorder (SSD), who did not fulfill the ACR criteria of SSc but were thought to develop SSc in the future based on the criteria proposed by Ihn et al., were also included in this study [14, 21, 22]. Institutional review board approval and written informed consent were obtained before patients, and healthy volunteers were recruited into this study according to the Declaration of Helsinki. All serum samples were stored at -80°C prior to use.

Antinuclear antibodies

Antinuclear antibodies (ANA) were detected by indirect immunofluorescence using HEp-2 cells as the substrate and double immunodiffusion, as described previously [21].

Echocardiographic evaluation

Right ventricular systolic pressure (RVSP) was measured by Doppler echocardiography, as described previously [23].

Measurement of sCD163 concentrations

Levels of serum sCD163 were measured with a specific ELISA kit (R&D Systems). Briefly, anti-CD163 monoclonal antibodies were precoated onto microtiter wells. Aliquots of serum were added to each well, followed by peroxidase-conjugated antibodies to CD163. Color was developed with hydrogen peroxide and tetramethylbenzidine peroxidase, and the absorbance at 450 nm was measured. Wavelength correction was performed by absorbance at 540 nm. The concentration of CD163 in each sample was determined by interpolation from a standard curve.

Statistical analysis

Statistical analysis was carried out with a Mann–Whitney test for the comparison of means, and Fisher's exact probability test for the analysis of frequency. *P* values less than 0.05 were considered significant.

Results

Serum concentrations of sCD163

The serum sCD163 levels in patients with SSc and in the healthy control subjects are shown in Fig. 1. Serum samples were obtained from 43 patients with SSc. Twelve healthy control subjects and 10 SSD patients, who did not fulfill the criteria of SSc but were thought to develop SSc in the future, were also included in this study [14, 21, 22].

Mean serum levels were significantly higher in SSc patients than in those with the healthy control subjects (85.7 ± 29.3 vs. 57.8 ± 20.6 ng/ml, $P = 0.0014$). When SSc patients were classified into lcSSc and dcSSc as described in 'Patients and Methods', both lcSSc patients and dcSSc patients had significantly higher sCD163 levels than health controls (78.9 ± 30.0 vs. 57.8 ± 20.6 ng/ml, $P = 0.03662$; 93.6 ± 27.1 vs. 57.8 ± 20.6 ng/ml, $P < 0.0001$), whereas there was no significant difference between the sCD163 levels in lcSSc patients and those in dcSSc patients.

Although sCD163 levels in SSD patients were higher than those in healthy controls, there was no significant difference (74.4 ± 24.6 vs. 57.8 ± 20.6 ng/ml). Also, patients with lcSSc (78.9 ± 30.0 ng/ml) or dcSSc

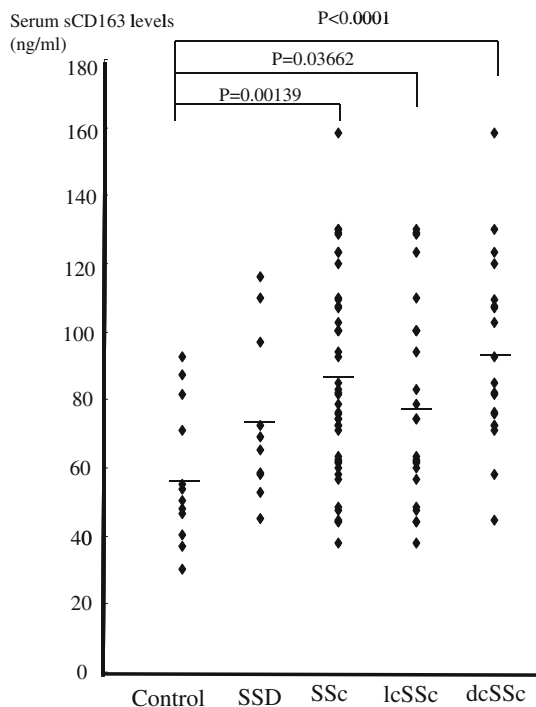


Fig. 1 Serum concentrations of soluble CD163 (sCD163) in patients with systemic sclerosis (SSc) or scleroderma spectrum disorder (SSD) and healthy control subjects (HC). Serum concentrations of sCD163 determined by ELSA are shown on the ordinate; the *horizontal dotted line* indicates the cut-off levels. *Bars* show means. *P* values determined using Mann–Whitney test. *dcSSc* diffuse cutaneous SSc; *lcSSc* limited cutaneous SSc

(93.6 ± 27.1 ng/ml) had higher sCD163 levels than those with SSD (74.7 ± 24.6 ng/ml), we could not find any significant difference.

When the cut-off value was set at 119.5 ng/ml (mean \pm 3SD of the controls), the values in all healthy controls and SSD were below the cut-off line, whereas increased serum concentrations of sCD163 were found in 8 of the 43 SSc patients (19%).

Correlation of serum sCD163 levels with clinical manifestations and laboratory data in patients with SSc

Table 1 shows the association of serum sCD163 levels with the clinical and laboratory features in patients with SSc. There was significant difference between patients with elevated sCD163 levels and those with normal levels in terms of duration of disease (between symptom onset and first visit to the hospital) (88.1 vs. 23.6 months, $P < 0.05$). In addition, elevated RVSP (>30 mmHg) was found at the significantly higher prevalence in patients with elevated serum levels than those without (71.4 vs. 28.6%, $P < 0.05$). Significantly lower %DLCO value was also seen in SSc patients with elevated sCD163 levels than in those with normal levels (73.2 vs. 86.6%, $P < 0.05$). There

was no statistically significant difference in the incidence of other clinical or laboratory features. Taken together, SSc patients with increased serum sCD163 levels tend to visit hospital earlier and have higher RVSP and lower %DLCO value.

Discussion

In this study, we have presented two major findings. First, there was significant difference in serum sCD163 levels between healthy controls and SSc patients. To note, we also found a significant difference between lcSSc and healthy control subjects. The diagnosis of SSc presents little problem when the clinical features have fully developed. However, it may be difficult to diagnose lcSSc, because its skin sclerosis is sometimes not apparent in lcSSc, especially in very early stage [21]. Serum levels of sCD163 may be useful for the differentiation of lcSSc from healthy individuals. Furthermore, sCD163 levels in SSD patients were higher than those in healthy controls and lower than those in lcSSc or dcSSc. Considering that patients with dcSSc had higher sCD163 level than those with lcSSc and that SSD is the condition that is thought to develop SSc in the future, sCD163 levels may be increased proportionally to the progression of this disease, indicating the involvement of CD163 in the pathogenesis.

Second, in SSc patients with elevated serum sCD163 levels, disease duration was significantly shorter, suggesting that these patients may have severe symptoms. Furthermore, the RVSP value was significantly higher, and %DLco was significantly lower in patients with higher sCD163 levels than those with normal levels. Because %DLco is thought to be one of the most sensitive markers for detection of pulmonary hypertension [24], both the higher RVSP and lower %DLco value in patients with elevated serum sCD163 levels may predict the development of pulmonary hypertension in these patients. Although pulmonary hypertension is an important prognostic factor of SSc, early detection may be difficult because it is sometimes asymptomatic clinically. Serum levels of sCD163 may be useful to detect the pulmonary hypertension at the early stage. To note, although pulmonary hypertension is known to be more common in lcSSc, the ratio of lcSSc:dcSSc was not increased in patients with elevated sCD163 compared with those with normal levels in our study. Thus, sCD163 levels are likely to be directly correlated with the presence of pulmonary hypertension. Our results are consistent with the previous reports that macrophages are involved in the pathogenesis of various types of pulmonary hypertension. The pathological changes of hypertensive pulmonary arteries include endothelial injury, proliferation, hypercontraction of vascular smooth muscle cells, and migration of macrophages [25, 26]. Pulmonary macrophages can

Table 1 Correlation of serum soluble CD163 (sCD163) levels with clinical and serological features in patients with systemic sclerosis (SSc)

	Patient with elevated sCD163 levels (<i>n</i> = 8)	Patient with normal sCD163 levels (<i>n</i> = 35)
Age at the time of serum sampling (mean years)	66.9	55.0
Duration of disease (mean months)	23.6*	88.1
Type (diffuse:limited)	4:4	16:19
m-TSS	11.7	11.2
Clinical features		
Pitting scars/ulcers	60.0	38.2
Nailfold bleeding	50.0	53.6
Raynaud's phenomenon	100.0	87.9
Telangiectasia	33.0	23.3
Contracture of phalanges	100.0	85.7
Calcinosis	33.3	0
Diffuse pigmentation	100.0	22.7
Short SF	75.0	75.0
Sicca symptoms	60.0	52.4
Organ involvement		
Pulmonary fibrosis	28.6	37.5
Mean %VC (%)	94.6	102.8
Mean %Dlco (%)	73.2*	86.6
Elevated RVSP (>30 mmHg)	71.4*	28.6
Esophagus	14.3	24.1
Heart	57.2	26.5
Kidney	0	0
Joint	60.0	52.6
Thrombosis	0	0
ANA specificity		
Anti-topo I	25.0	32.2
Anti-centromere	62.5	46.4
Anti-U1 RNP	37.5	14.3

Unless indicated values are percentages

m-TSS modified Rodnan total skin thickness score, *SF* sublingual frenulum, *VC* vital capacity, *DLco* diffusion capacity for carbon monooxidase, *RVSP* right ventricular systolic pressure, *ANA* antinuclear antibodies, *Anti-topo I* anti-topoisomerase I antibody, *Anticentromere*, anticentromere antibodies

* *P* < 0.05 versus patients with normal sCD163 levels using Fisher's exact probability test or Mann–whitney test

produce many vasoactive, mitogenic, and proinflammatory cytokines that have been implicated in tissue injury [27, 28]. Taken together, our study suggests that M2 macrophages play a major role in the development of pulmonary hypertension associated with SSc.

However, there are some limitations to our results. First, Doppler echocardiography as employed in our study is reported to be a reliable method for detecting pulmonary hypertension, and no less reliable than right heart catheterization [29], but there is a possibility that we missed some cases of pulmonary hypertension. Second, CD163 has been detected in both membrane-bound and secreted forms, and it is presently unknown whether its major biological functions are carried out as a secreted protein or as a cell surface receptor [30]. Additionally, it is possible that the shed molecule serves as an inhibitor of the membrane-bound form. These points should be clarified in the future.

In conclusion, we found (1) significant difference in the serum sCD163 levels between control subjects and SSc patients. (2) SSc patients with elevated serum sCD163

levels tend to develop pulmonary hypertension. Our study suggested that serum sCD163 levels are useful for diagnosis and can be a marker of pulmonary hypertension in patients with SSc. Although the detailed mechanisms of the development of pulmonary hypertension in SSc are still unknown, the M2 macrophage/CD163 signaling system may play a role in the pathogenesis of SSc.

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