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Original Research Article

The sCD163/sTWEAK ratio in the peripheral blood mononuclear cells cultures correlates with disease severity in early systemic sclerosis

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ABSTRACT

Introduction: CD163 is a scavenger receptor expressed exclusively on monocytes/macrophages and a decoy receptor for a TNF-related weak inducer of apoptosis (TWEAK), a multifunctional cytokine involved in the regulation of inflammatory response, angiogenesis and connective tissue remodeling. However, very little is known about the significance of CD163 and TWEAK interactions *in vivo*.

Aim: We hypothesized that CD163 and TWEAK interactions might play a role in the pathogenesis of systemic sclerosis (SSc).

Material and methods: The concentrations of soluble CD163 (sCD163) and soluble TWEAK (sTWEAK) were measured by the enzyme immunoassays in the supernatants of cultured peripheral blood mononuclear cells (PBMC) from 25 patients with early SSc and 16 healthy controls (HC). The sCD163/sTWEAK ratio was calculated and its association with disease parameters was assessed.

Results and discussion: The production of sTWEAK was comparable between SSc patients and HC ($p > .05$). The mean concentration of sCD163 and the mean sCD163/sTWEAK ratio were significantly greater in SSc patients as compared with HC ($p < .05$ for both). However, only sCD163/sTWEAK ratio, but not sCD163 or sTWEAK alone, correlated with the modified Rodnan skin score (Spearman $R = 0.46$) and, inversely, with forced vital capacity ($R = -0.49$) and diffusing capacity of the lungs for carbon monoxide ($R = -0.50$) in SSc patients.

Conclusions: We showed that there is an imbalance in the production of sCD163 and sTWEAK by the PBMC from SSc patients resulting in increased sCD163/sTWEAK ratio. Correlation of sCD163/sTWEAK ratio in PBMC supernatants with the severity of skin and

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lung involvement indicates that interaction of these two molecules might affect the development of SSc.

1. Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disease affecting skin and internal organs. Pathogenesis of SSc is not fully understood. However, activation of inflammatory cells, vascular injury and increased production of connective tissue by activated fibroblast are considered key events in the development of SSc.[1]

CD163 is a scavenger receptor for haptoglobin–hemoglobin complexes and is expressed solely on macrophages and monocytes.[13] Proteolytic shedding of the membrane-bound CD163 produces soluble CD163 (sCD163) which can be detected in body fluids.[21] A role of sCD163 molecule is not well understood; however available data indicate that it may play a role in the regulation of inflammatory and immune reactions.[8,13,21] It has been shown that concentration of sCD163 increases in the peripheral blood of patients with inflammatory conditions such as sepsis, asthma or autoimmune diseases including rheumatoid arthritis and SSc.[12,13,17,21,24,25] Recently, it has been shown that CD163 can bind and neutralize a tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK).[5] TWEAK is a member of the TNFalfa superfamily of cytokines and has been shown to exert a broad range of biological activities generally of proinflammatory nature.[7,11,28] TWEAK has also been shown to regulate angiogenesis and to affect connective tissue remodeling.[6,7,28] Binding of TWEAK by CD163 may modulate its biological functions. Indeed, it has recently been shown that sCD163/sTWEAK ratio is a better predictor of subclinical arteriosclerosis as compared with sCD163 or sTWEAK alone, indicating that CD163/TWEAK interactions may play an important role *in vivo*.[20]

It has recently been shown that the peripheral blood mononuclear cells (PBMC) isolated from patients with SSc *in vitro* spontaneously release significantly greater amounts of sCD163 as compared with healthy controls (HC) but the release of sTWEAK by PBMC was not different between SSc patients and HC.[3,4]

2. Aim

The aim of this study was to investigate the mutual relations of *in vitro* sCD163 and sTWEAK production by PBMC of patients with early SSc in relation to its clinical features.

3. Material and methods

3.1. Patients

In this study 25 patients with SSc (19 women and 6 men) were investigated. Since pathogenic events, which could also represent potential therapeutic targets, are believed to take place early in the evolution of the disease, only patients with early SSc were selected for this study.[2,18] In agreement with generally accepted criteria, early SSc was defined as shorter than 3 years in diffuse SSc (dSSc) patients or shorter than 5 years duration in patients with limited SSc (lSSc) measured from the first non-Raynaud's phenomenon clinical symptom attributable to SSc, which corresponds to the early phase of overt disease.[19] All patients selected for the study fulfilled the ACR classification criteria for SSc and/or the criteria of early SSc as proposed by LeRoy et al.[15,26] Only patients who had not taken any immunosuppressive therapies or in whom immunosuppressive therapies had been stopped at least 6 months before blood collection were considered eligible.

SSc patients were evaluated as described in previous studies.[3,4] Shortly, clinical assessment included evaluation of the subtype of the disease, dSSc or lSSc, as defined by LeRoy et al.[14]; duration of Raynaud's phenomenon and duration of the disease (calculated from the time of the first non-Raynaud's symptom attributable to SSc), extent of skin involvement (using the modified Rodnan skin score, mRSS), the presence of scleroderma interstitial lung disease (ILD), pulmonary hypertension, digital ulcers and scleroderma renal crisis. The severity of lung involvement was assessed by measurement of forced vital capacity (FVC) and diffusing capacity of the lungs for carbon monoxide (DLCO). Laboratory assessment consisted of evaluation of the presence of antinuclear antibodies (ANA), anticentromere antibodies (ACA) and anti-topoizomerase I antibodies (anti-topo I) as well as measurement of erythrocyte sedimentation rate (ESR) and serum concentration of C-reactive protein (CRP). Clinical characteristics of the patients are shown in Table 1.

Control group consisted of 16 age- and sex-matched healthy controls (HC): 12 women and 4 men. The mean (±SD, range) age of the HC was 41 (±14, 20–70) years. There were no significant differences in the sex or age between SSc patients and the HC.

Study protocol was approved by the local ethics committee and all patients gave appropriate informed consent.

3.2. PBMC cultures and measurements of sCD163 and sTWEAK in supernatants

Peripheral blood mononuclear cells (PBMC) were isolated from the whole blood using density gradient centrifugation on Histopaque and incubated in RPMI medium supplemented with 5% fetal calf serum at a density of 10^5 cells/mL and cultured at 37°C under 5% CO_2 for 24 h as described elsewhere.[3,4] Subsequently the cells were centrifuged and supernatants were collected and frozen at –80°C until measurements were performed. The concentrations of sCD163 and sTWEAK in the PBMC supernatants were measured using commercially available enzyme immunoassay (ELSA) kits

Table 1 – Clinical characteristics of the patients with SSc.

Parameter	SSc patients ($n=25$)
Female/Male, no.	19/6
Age, mean±SD (range), yr	47±14 (25–72)
Disease duration, mean±SD (range), yr	1.49±1.35 (0–4.50)
Duration of Raynaud's phenomenon, mean±SD (range), yr	4.57±4.06 (0.30–15.00)
dSSc/lSSc, no.	12/13
ANA positive, no. (%)	25 (96)
Anti-topo I positive, no. (%)	13 (52)
ILD in HRCT, no. (%)	13 (52)
ACA positive, no. (%)	7 (28)
Raynaud's phenomenon, no. (%)	25 (100)
Pulmonary hypertension, no. (%)	2 (0.80)
Digital ulcers, no. (%)	4 (1.60)

Comments: ACA – anticentromere antibodies, ANA – antinuclear antibodies, dSSc – diffuse systemic sclerosis, HRCT – high resolution computed tomography, lSSc – limited systemic sclerosis, ILD – scleroderma interstitial lung disease.

(sCD163: R&D Systems, Inc., Minneapolis, MN, USA, and sTWEAK: Bender MedSystems).

3.3. *Statistical analysis*

For the assessment of between-group comparisons the ANOVA Kruskall–Wallis test and the Mann–Whitney *U* test were used, when appropriate. Correlations were assessed using the Spearman correlation test. The differences were considered significant at $p<.05$. All results are expressed as mean±standard deviation (SD), unless stated otherwise.

4. Results

4.1. *CD163 in PBMC cultures from SSc patients and HC*

There were no differences in the total number of leukocytes or differential cell count numbers between SSc patients and the HC (data not shown).

The mean concentration of sCD163 in PBMC cultures was significantly greater in SSc patients (2794.16±690.94 pg/mL) as compared with HC (2294.88±282.34 pg/mL, $p<.05$). In SSc patients concentrations of sCD163 significantly correlated with the serum concentration of CRP ($R=0.53$, $p<.01$). Any other significant associations or correlations between concentration of sCD163 and clinical or laboratory parameters could not be found in SSc patients.

There were no significant differences in the mean sTWEAK concentrations between the SSc patients (28.66±7.14 pg/mL) and HC (28.81±6.50 pg/mL, $p>.05$). There were no significant associations or correlations between concentration of sTWEAK and clinical or laboratory parameters in SSc patients.

4.2. *sCD163/sTWEAK ratios in PBMC cultures in SSc patients and HC*

The mean sCD163/sTWEAK ratio in SSc patients (102.00±33.00) was significantly higher as compared with HC (83.04±15.00, $p<.05$). The sCD163/sTWEAK ratio was significantly greater in patients with dSSc (118.56±37.66) as compared with patients with the lSSc (86.70±18.68, $p<.05$), and in patients with ILD (119.72±37.12) as compared with those without ILD (82.80±10.00, $p<.01$) (Fig. 1). Accordingly, in SSc patients the sCD163/sTWEAK ratio correlated with the mRSS ($R=0.46$, $p<.05$), and inversely with FVC ($R=-0.49$, $p<.05$) and DLCO values ($R=-0.50$, $p<.05$). In addition, there was a significant correlation between sCD163/sTWEAK ratio and laboratory markers of inflammation (ESR: $R=0.49$, $p<.05$, and serum concentration of CRP: $R=0.52$, $p<.01$). No significant associations could be found between the sCD163/sTWEAK ratio and the presence of digital ulcers or the presence of specific autoantibodies (ACA, anti-Scl-70). Since there were no patients with scleroderma renal crisis and only 2 patients had pulmonary hypertension in echo, no associations between these severe vascular complications and the sCD163/sTWEAK ratio could be assessed.

5. Discussion

The results of the present study confirm in the larger, homogenous population of early SSc patients, previous findings concerning release of sCD163 and sTWEAK by PBMC reported in a more general SSc population (including both early and late SSc).[3,4] Indeed, similar to the previous study, we found that release of sCD163 is significantly greater in SSc patients than in HC and that the concentration or sCD163 in PBMC cultures significantly correlates with the serum concentration of CRP.[4] We did not find significant differences in the concentrations of sTWEAK between SSc patients and HC which is again in line with the previous report.[3] Unlike in the previous study we could not demonstrate significant correlation between levels of sTWEAK and duration of Raynaud's phenomenon. This could be explained by the fact that in the present study only patients with early SSc were selected in whom duration of Raynaud's phenomenon is generally much shorter.

Our findings are also in agreement with the work by Highashi-Kuwata et al.[9] who showed that the number of CD163 positive macrophages and CD163 positive peripheral blood monocytes is increased in patients with SSc. Moreover, our observations are in agreement with the results of the study by Juniantito et al.[10] who showed that expression of CD163 increases during development of experimental skin fibrosis in mice. However none of the previous studies investigated relationships between CD163 and TWEAK in SSc patients. In

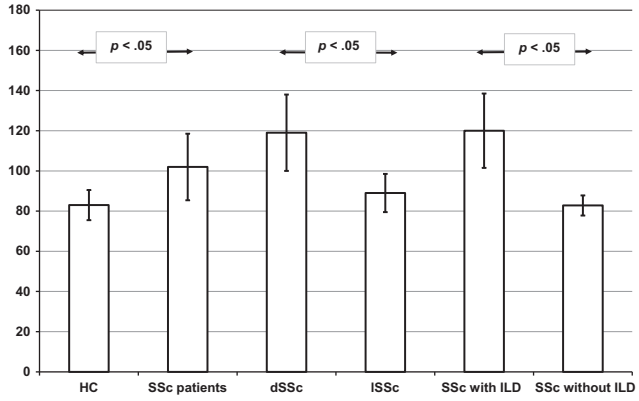

Fig. 1 – The sCD163/sTWEAK ratio. Hc – healthy controls, SSc – all patients with SSc, dSSc – patients with diffuse SSc, lSSc – patients with limited form of SSc, SSc–SLD – SSc patients with SLD, SSc without SLD – without SLD.

our study, sCD163/sTWEAK ratio was significantly higher in SSc patients as compared with HC. Interestingly, sCD163/sTWEAK ratio, but not sCD163 or sTWEAK alone, correlated with the severity of skin and lung involvement in SSc patients. This observation suggests that CD163 and TWEAK interactions might play an important role in the pathogenesis of skin and lung fibrosis in SSc. Our findings seem intriguing since available evidence indicates that both CD163 and TWEAK are involved in the regulation of connective tissue remodeling. It has been shown that CD163-postive macrophages release profibrotic cytokines such as transforming growth factor beta, and that the number of CD163-postive cells increases during development of tissue fibrosis in the skin and the heart.[10,16,22] It has also been shown that TWEAK activates fibroblasts *in vitro* and that elevated levels of sTWEAK augment, through interaction with TWEAK receptor, the development of cardiac fibrosis in mice.[6,23] On the contrary, elevated expression of TWEAK was associated with lower risk of development of ILD in SSc and preceded improvement of liver fibrosis in rats.[27,29] These potentially contradictory data suggest that the role of TWEAK in the pathogenesis of fibrosis is complex and might depend on specific site, time and interaction with other factors. Neutralization of TWEAK by CD163 may represent potential mechanism involved in the regulation of the role of TWEAK in the remodeling of connective tissue. This hypothesis appears very interesting from both pathophysiological and therapeutic points of view. We could not however identify a single study which would address the role of CD163 and TWEAK interactions in the development of fibrosis so far. Indeed, further studies are required to elucidate whether CD163 and TWEAK interactions play a role in the regulation of connective tissue metabolism and which is the exact role of CD163-TWEAK pathway in the development of fibrosis in SSc.

6. Conclusions

In conclusion, our major finding is that there is an imbalance in the production of sCD163 and sTWEAK by the PBMC from SSc patients resulting in increased sCD163/sTWEAK ratio in SSc. Moreover, we found that sCD163/sTWEAK ratio in PBMC cultures, but not sCD163 or sTWEAK alone, correlates with more severe disease in SSc patients, indicating that CD163 and TWEAK interactions might play an important role in the development of fibrosis in SSc. In addition sCD163/sTWEAK ratio may serve as a new biomarker of the severity of SSc-related organ involvement.

Conflict of interest

None declared.

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