Elevated Serum Levels of Soluble CD163 in Polymyositis and Dermatomyositis: Associated with Macrophage Infiltration in Muscle Tissue

Qing-Lin Peng, Yin-Li Zhang, Xiao-Ming Shu, Han-Bo Yang, Lu Zhang, Fang Chen, Xin Lu, and Guo-Chun Wang

ABSTRACT. ***Objective.*** To investigate serum levels of soluble CD163 (sCD163) in patients with polymyositis (PM) and dermatomyositis (DM), and to correlate these to clinical manifestations and laboratory data.

Methods. Serum levels of sCD163 were detected in 24 patients with PM, 84 patients with DM, and 46 healthy controls by using the ELISA method. Immunohistochemistry staining of macrophage infiltration in muscle tissue using anti-CD163 monoclonal antibody was conducted on muscle biopsy specimens from 13 patients with PM and 17 with DM.

Results. Serum levels of sCD163 were significantly increased in patients compared with healthy controls ($p < 0.001$). Patients with interstitial lung disease (ILD) had statistically higher sCD163 levels than patients without ILD ($p < 0.001$). High serum sCD163 levels were associated with increased incidence of antinuclear antibody ($p < 0.05$), higher serum levels of immunoglobulin G ($p < 0.01$) and immunoglobulin A ($p < 0.05$), and increased erythrocyte sedimentation rates ($p < 0.01$). Serum sCD163 levels were inversely correlated with CD3+ T cell counts in peripheral blood of patients ($r = –0.306$, $p < 0.01$). Cross-sectional assessment and longitudinal study revealed a significant correlation between serum sCD163 levels and disease activity. Patients with high serum sCD163 levels showed a higher incidence of CD163+ macrophage infiltration in muscle tissue than patients with normal sCD163 levels (chi-square value = 10.804, $p < 0.01$).

Conclusion. Serum levels of sCD163 were significantly elevated and correlated with disease severity in patients with PM/DM, suggesting serum sCD163 as a promising biomarker in the disease evaluation of PM/DM. Our finding of elevated serum sCD163 levels associated with muscle macrophage infiltration highlights the role activated macrophage plays in the pathogenesis of PM/DM. (First Release April 15 2015; J Rheumatol 2015;42:979–87; doi:10.3899/jrheum.141307)

Key Indexing Terms:
SOLUBLE CD163 MACROPHAGE POLYMYOSITIS DERMATOMYOSITIS

Idiopathic inflammatory myopathies (IIM) are a group of acquired, heterogeneous, systemic diseases that mainly affect skeletal muscle. Polymyositis (PM) and dermatomyositis (DM) are 2 common subsets of IIM characterized by symmetrical proximal muscle weakness, decreased muscle endurance, and inflammatory infiltrates in skeletal muscle tissue[1,2]. According to the muscle biopsy findings in inflammatory myopathies, the inflammatory cells commonly seen in PM and DM include B and T lymphocytes, macrophages, and plasma cells[3]. The precise pathogenic role of inflammatory cells in PM/DM remains to be fully elucidated, and macrophage is of growing interest.

Macrophages are immune cells that play essential roles in both innate and adaptive immune systems[4]. Through both cell-to-cell interactions and the release of proinflammatory cytokines, macrophages participate in key pathogenic events in many pathological conditions, such as sepsis, cancer, inflammatory diseases, and autoimmune diseases[5]. Macrophages are very efficient at scavenging self-tissue following injury through scavenger receptors, and therefore may increase autoimmune diseases by presenting self-antigen to T cells. In addition, macrophages may also exacerbate immune complex-mediated pathology and fibrosis[6].

CD163 is a type I transmembrane protein with about 130 kDa molecular weight that was first recognized as the macrophage scavenger receptor for haptoglobin-hemoglobin complexes[7]. Further, CD163 was found to exert other functions including erythroblast adhesion[8], immune sensing of bacteria[9], and binding of tumor necrosis factor-like weak inducer of apoptosis (TWEAK)[10]. The expression of human CD163 is restricted to monocyte/macrophage lineage, and is

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regulated by a variety of factors[11]. CD163 can be cleaved by an ADAM17 (ADAM metallopeptidase domain 17)/TACE (tumor necrosis factor-α convertase)-dependent mechanism, consequently forming soluble CD163 (sCD163)[12]. Intriguingly, sCD163 has emerged as a marker of macrophage activation in various diseases and pathological conditions[11].

In a previous study, we found increased serum levels of sCD163 in patients with PM and DM, as well as an inverse correlation between serum levels of sCD163 and serum levels of TWEAK[13]. However, the clinical significance of serum sCD163 levels and their relationship with macrophage infiltration in muscle tissue remains unclear.

Therefore, we set out to investigate serum levels of sCD163 in patients with PM and DM, and to analyze their correlations with clinical variables and muscle macrophage infiltration.

MATERIALS AND METHODS

Study population. From the inpatients and outpatients who visited the China-Japan Friendship Hospital between 2010 and 2013, 108 patients with probable or definite PM/DM according to the diagnostic criteria of Bohan and Peter[14,15] were recruited for our study. Patients with the following conditions were excluded: (1) inclusion body myositis according to the diagnostic criteria proposed by Griggs, *et al*[16]; (2) immune-mediated necrotizing myopathy according to the European Neuromuscular Centre criteria[17]; (3) a malignancy diagnosed within 1 year of myositis diagnosis; (4) complications with other connective tissue diseases; and (5) having received immunosuppressive therapies or steroids in the past 3 months before serum sampling. In addition, 46 age- and sex-matched healthy volunteers were selected to be the healthy control group during the same time period. Magnetic resonance imaging–directed muscle biopsies were carried out for patients with active disease. This study was performed with the approval of the Human Ethics Board of the China-Japan Friendship Hospital (Beijing, China). Written informed consent was obtained from all participating individuals.

Measurement of serum sCD163 levels and serum TWEAK concentrations. Fresh venous blood samples were centrifuged shortly after clot formation, and serum samples were collected. All samples were stored at –70°C before use. Serum levels of sCD163 were detected using a commercially available ELISA kit (R&D Systems) according to the manufacturer's protocol. Briefly, 100 μl assay diluent were added to microplates coated with a mouse monoclonal antibody against human CD163. Following this step, 50 μl of standard, control, and diluted samples, in duplicate, were added to the designated wells. After incubating at room temperature for 2 h, the plates were washed and incubated with 200 μl of CD163 conjugate for 2 h. After washing the plates 4 times, substrate solution was added. About 30 min later, stop solution was added and absorbance was measured using the ELISA reader at 450 nm with 570 nm as the reference wavelength. A standard curve for each assay was generated, and serum sCD163 concentration was calculated. Each sample was analyzed in triplicate.

Serum TWEAK concentrations were detected using an ELISA kit (Bender MedSystems). The assays were performed according to the manufacturer's protocol.

Assessment of disease activity. Myositis Disease Activity Assessment Visual Analog Scales (MYOACT), established by the International Myositis Assessment and Clinical Studies (IMACS) group[18], were used to evaluate the disease activity of patients with PM/DM.

Immunohistochemistry staining of macrophage infiltration. Eight-μm–thick unfixed cryostat muscle sections from patients with PM/DM were applied for immunohistochemistry staining of macrophage infiltration in muscle tissue. Mouse anti-human CD163 monoclonal antibody (clone number RM3/1, Cat. No. ab17051; Abcam) was used as primary antibodies at a working concentration of 4 μg/ml. Horseradish peroxidase-conjugated anti-mouse immunoglobulin G (IgG; Santa Cruz Biotechnology) was used as a secondary antibody. Mouse IgG1 isotype control (Abcam) was used as a negative control for the primary antibody. Immunohistochemistry staining was conducted according to the following steps: muscle sections were fixed with precooled acetone at room temperature for 10 min and then rinsed 4 times in phosphate-buffered solution. Then the tissue sections were blocked in 10% normal serum with 1% bovine serum albumin in tris-buffered saline (TBS) for 2 h at room temperature. Primary antibodies diluted in TBS were applied to the tissue sections after draining for a few seconds. After incubating overnight at 4°C, the tissue sections were treated with 0.3% H_2O_2 for 15 min, following secondary antibodies for 1 h at room temperature. The tissue sections were washed 3 times. As a chromogenic reagent, 3,3′-diaminobenzidine was used. After mounting on glass slides, the stained tissue sections were visualized under an Olympus optical microscope. The immunohistochemistry staining sections were assessed by conventional microscopy on 2 different occasions by 2 independent observers who were blinded to the identity of the specimens. For conventional microscopic evaluation of the number of CD163-positive macrophages, we examined at least 10 visual fields on each section and the mean number of CD163-positive macrophages was calculated per visual field at a magnification of 200×. A number of ≥ 25 CD163-positive macrophages in muscle tissue area examined at a magnification of 200× was considered as positive infiltration with CD163+ macrophages.

Statistical analyses. Statistical analyses were performed using GraphPad Prism V.4.03 (GraphPad Software) and SPSS V.16.0 (SPSS). Quantitative variables were described as mean ± SD, and the normal distribution data were compared by independent Student t test. Nonparametric distribution data were expressed as median values and interquartile ranges, and the data of unpaired samples were analyzed by using the Mann-Whitney U test. Spearman correlation analysis was used to test for correlations. The Wilcoxon signed-rank test and chi-square test were used when appropriate. A p value equal to or less than 0.05 was considered statistically significant.

RESULTS

Clinical characteristics and treatment of patients with PM/DM. As shown in Table 1, among the 108 patients enrolled in the study, 24 had PM (22.2%) and the remaining 84 had DM (77.8%), according to the criteria of Bohan and Peter. Women outnumbered men in the cohort (76 women/32 men). The mean age of onset was 45.1 years. The mean disease duration of the patients with PM/DM was 2.3 years (range from 0–25.0 yrs). The clinical and laboratory characteristics of the enrolled patients are summarized in Table 1.

Because the expression of CD163 can be induced by glucocorticoids[19,20], we included only patients who were not treated with steroids and immunosuppressants in the past 3 months before serum sampling. After being included in our study, the patients received various treatments according to the severity of their disease. All the patients received corticosteroid at doses between 0.5 mg/kg and 1 mg/kg as part of their initial therapy. Meanwhile, about 89% of our patients (96 out of 108) also received at least 1 or more immunosuppressants, among them methotrexate, cyclophosphamide, azathioprine, intravenous immunoglobulin, hydroxychloroquine, or mycophenolate mofetil.

Serum concentrations of sCD163 in patients with PM/DM. A commercially available ELISA kit was used to measure the serum levels of sCD163, and the results showed that the

Table 1. Demographic, clinical, and laboratory characteristics of patients with PM/DM. Forty-six healthy volunteers were involved as healthy controls (31 women, 15 men). The age of the control group was 42.7 ± 11.3 (21–76) years. Values are mean ± SD (range) or n (%) unless otherwise specified.

Characteristics	Patients with PM/DM
Female/male ratio	76/32
Age at onset, yrs	45.1 ± 14.2 (18–77)
No. patients, PM/DM	24/84
Disease duration, yrs	2.3 ± 4.0 (0–25.0)
Clinical features	
Interstitial lung disease	43 (39.8)
Oropharyngeal dysphagia	26 (24.1)
Raynaud phenomenon	25 (23.2)
Mechanic hands	19(17.6)
Arthritis	39 (36.1)
Calcinosis	3 (2.8)
Laboratory variables	
ANA-positive	54 (50.0)
Anti-Jo1–positive	10 (9.3)
Anti-Ro antibody–positive	31 (28.7)
MAA/MSA positive, data available for 58 patients	29 (50)
CK levels, IU/l, mean ± SD	1356.7 ± 2941.8

PM: polymyositis; DM: dermatomyositis; ANA: antinuclear antibodies; MAA: myositis-associated antibodies (including anti-Ro, anti-Ku, anti-PM-Scl100, anti-PM-Scl75 antibodies); MSA: myositis-specific antibodies (including anti-Mi–2, anti-Jo1, antisignal recognition particle, antithreonyl-tRNA synthetase, antialanyl-tRNA synthetase, antiglycyl-tRNA synthetase, antiisoleucyl-tRNA synthetase antibodies); CK: creatine kinase.

median value of serum sCD163 levels in patients with PM/DM was 785.6 ng/ml (range 212.6–2869.5 ng/ml), while that in healthy controls was 491.2 ng/ml (range 209.0–783.9 ng/ml). Therefore, the serum levels of sCD163 in patients with PM/DM were significantly higher than those in healthy controls ($p < 0.001$). In addition, the serum sCD163 levels of patients with DM (median value 830.5 ng/ml, range 212.6–2869.5 ng/ml) were statistically increased compared with that of patients with PM (median value 607.3 ng/ml, range 317–1815.3 ng/ml, $p < 0.05$; Figure 1A).

We analyzed the associations between serum sCD163 levels and clinical features in patients with PM/DM, and serum levels of sCD163 were significantly elevated in patients with PM/DM with interstitial lung disease (ILD) compared with patients without ILD ($p < 0.001$; Figure 1B). However, no significant differences were observed when serum sCD163 levels were compared in PM/DM subgroups based on the disease duration, the presence of oropharyngeal dysphagia, Raynaud phenomenon, mechanic hands, and arthritis (Table 2). Additionally, by using the Mann-Whitney U test, the serum sCD163 levels of 41 previously untreated patients (median value 854.3 ng/ml, range 317–2392.2 ng/ml) showed no significant difference with that of patients who were treated previously (median value 764.1 ng/ml, range 212.6–2869.5 ng/ml, $p > 0.05$). Intriguingly, we found a significantly increased incidence of ILD (chi-square value =

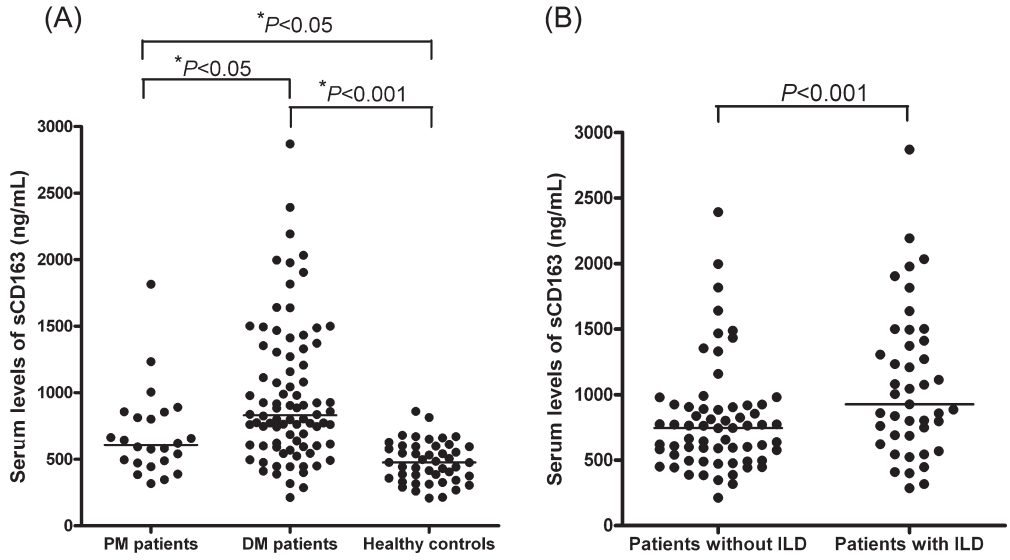

Figure 1. Serum concentrations of sCD163 in patients with PM/DM. Serum levels of sCD163 were detected in 24 patients with PM, 84 patients with DM, and 46 healthy controls using ELISA method. The results showed that (A) serum levels of sCD163 in patients with PM and DM were significantly higher than those in healthy controls. (B) Serum levels of sCD163 were significantly elevated in patients with PM/DM with ILD compared with patients without ILD. * The displayed p values have been adjusted by Bonferroni correction and are from 1-way ANOVA tests of logarithmically transformed data. The data of unpaired samples were analyzed using the Mann–Whitney U test. PM: polymyositis; DM: dermatomyositis; ILD: interstitial lung disease; sCD163: soluble CD163.

Table 2. Comparison of demographic, clinical, and laboratory features of patients with PM/DM with normal and high serum sCD163 levels. The high concentration of sCD163 was defined as a value greater than the mean plus 2 SD of the value in healthy controls. Values are mean ± SD or n/total (%) unless otherwise specified.

Characteristics	Normal sCD163 Levels, n = 54	High sCD163 Levels, n = 54	p
Age, yrs	42.8 ± 13.9	47.2 ± 14.2	NS
Disease duration, yrs	2.2 ± 4.1	2.4 ± 4.0	NS
ILD	14/54 (25.9)	29/54 (53.7)	p < 0.01
Oropharyngeal dysphagia	12/54 (22.2)	14/54 (25.9)	NS
Raynaud phenomenon	11/54 (20.4)	14/54 (25.9)	NS
Mechanic hands	16/54 (29.6)	13/54 (24.1)	NS
Arthritis	20/54 (38.9)	19/54 (35.2)	NS
ANA-positive	22/54 (40.7)	32/54 (59.3)	p < 0.05
Anti-Jo1–positive	4/54 (7.4)	6/54 (11.1)	NS
Anti-Ro antibody–positive	13/54 (24.1)	18/54 (33.3)	NS
MAA/MSA-positive*	14/29 (48.3)	15/29 (51.7)	NS
IgG, mg/dl**	1056.3 ± 380.7	1433.8 ± 784.6	p < 0.01
IgA, mg/dl**	197.9 ± 96.2	248.4 ± 117.6	p < 0.05
IgM, mg/dl**	128.7 ± 85.8	158.0 ± 116.5	NS
CD3+ T cell count, cell/mm^3^	1031.2 ± 581.1	748.8 ± 406.5	p < 0.01
CD3+CD4+ T cell count, cell/mm^3^	607.5 ± 412	445.1 ± 395	p = 0.063
CD3+ CD8+ T cell count, cell/mm^3^	375.6 ± 209.7	276.2 ± 242	p < 0.01
CD19+CD5– B cell count, cell/mm$^{3\#}$	197.4 ± 161.4	172.1 ± 192.4	NS
CD19+CD5+ B cell count, cell/mm$^{3\#}$	62.6 ± 94.2	68.1 ± 94.8	NS
CK, IU/l	1144.5 ± 2185.9	1569.0 ± 3550.0	NS
ESR, mm/h	13.5 ± 15.8	22.7 ± 26.7	p < 0.01
CRP, mg/dl	0.52 ± 0.71	0.96 ± 1.51	NS

* Data available for 58 patients. ** Data available for 107 patients. ^ Data available for 98 patients. # Data available for 95 patients. PM: polymyositis; DM: dermatomyositis; sCD163: soluble CD163; ILD: interstitial lung disease; ANA: antinuclear antibodies; MAA: myositis-associated antibodies (including anti-Ro, anti-Ku, anti-PM-Scl100, anti-PM-Scl75 antibodies); MSA: myositis-specific antibodies (including anti-Mi-2, anti-Jo1, antisignal recognition particle, antithreonyl-tRNA synthetase, antialanyl-tRNA synthetase, antiglycyl-tRNA synthetase, antiisoleucyl-tRNA synthetase antibodies); IgG: immunoglobulin G; IgA: immunoglobulin A; IgM: immunoglobulin M; CK: creatine kinase; ESR: erythrocyte sedimentation rate; CRP: C-reaction protein; NS: not significant (p > 0.05).

11.071, p < 0.01) in patients with positive anti-Ro antibody (20/31, 64.5%) compared with that in patients with negative anti-Ro antibody (23/77, 29.9%), which is in line with previously published studies[21,22].

The associations between serum sCD163 concentrations and laboratory variables were also evaluated. Patients with PM/DM with positive antinuclear antibody (ANA; median value 855.7 ng/ml, range 212.6–2869.5 ng/ml) showed increased serum sCD163 levels over patients with negative ANA (median value 752.2 ng/ml, range 317–1640.7 ng/ml, p < 0.05). Interestingly, we found that serum levels of sCD163 inversely correlated with CD3+ T cell counts in peripheral blood (r = –0.306, p < 0.01). We defined a cutoff value of serum sCD163 levels as the mean plus 2 SD of the control subjects, which was 788.2 ng/ml. Compared with patients with normal serum sCD163 levels, we found that patients with PM/DM who had high serum sCD163 levels (above 788.2 ng/ml) had significantly higher serum levels of IgG (p < 0.01) and IgA (p < 0.05). Additionally, patients with PM/DM with high serum sCD163 levels had higher erythrocyte sedimentation rates (ESR) than patients with normal serum sCD163 levels (p < 0.01), as shown in Table 2. No further associations or correlations were found between serum sCD163 levels and other laboratory factors (Table 2).

We further performed multiple linear regression analysis that included all the factors listed in Table 2. The results showed that CD3+ T cell count (β = –0.304, SE = 0.091, p < 0.01) and serum IgG level (β = 0.34, SE = 0.073, p < 0.01) were significantly associated with serum sCD163 levels.

Correlation between serum levels of sCD163 and disease activity. To analyze the correlation between serum levels of sCD163 and disease activity of patients with PM/DM, we first conducted a cross-sectional study in a cohort including 108 patients with PM/DM. Disease activity was evaluated at the time of blood sampling. By using Spearman correlation analysis, the result showed that serum levels of sCD163 were significantly correlated with the global disease activity scores of patients with PM/DM (r = 0.519, p < 0.001; Figure 2A). In addition, a weak correlation was found between serum levels of sCD163 and muscle disease activity scores (r = 0.314, p < 0.01). Moreover, we analyzed the correlation between sCD163/soluble TWEAK (sTWEAK) ratio and

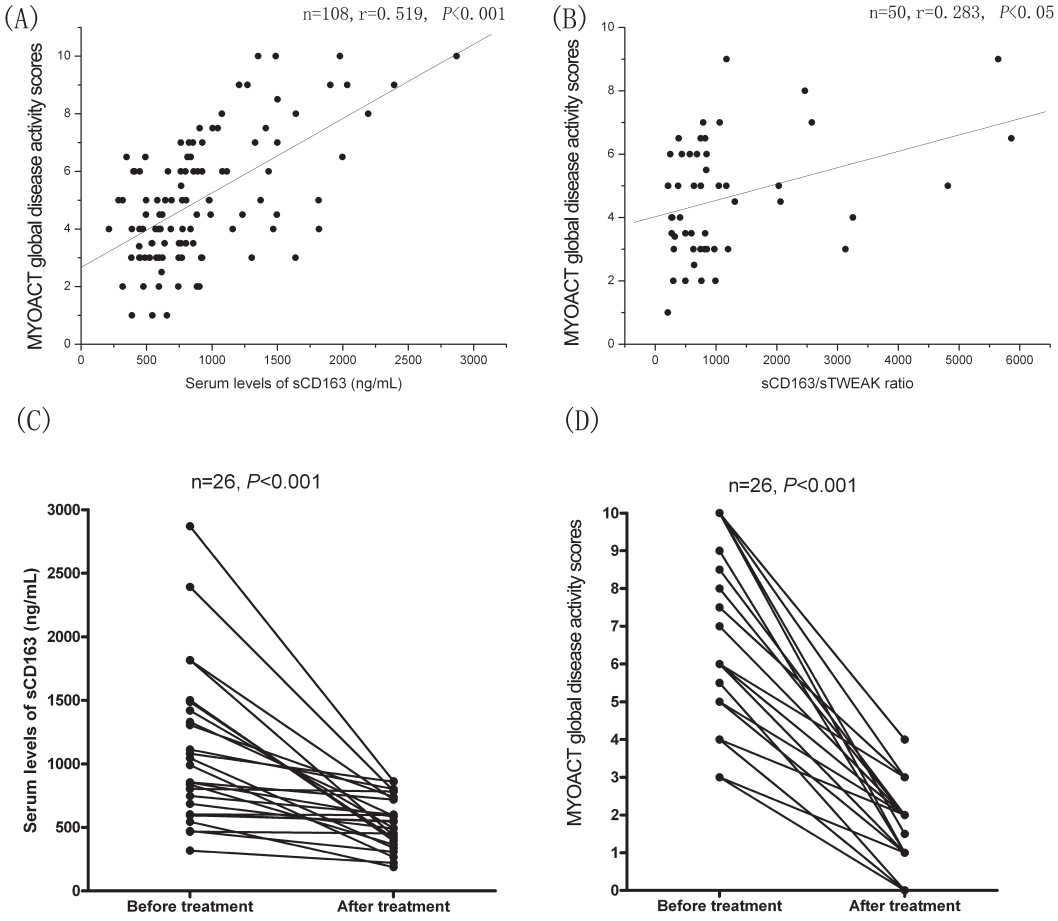

Figure 2. Correlations between serum levels of sCD163 and disease activity in patients with PM/DM. (A) Serum levels of sCD163 correlated with MYOACT global disease activity scores. (B) sCD163/sTWEAK ratio positively correlated with MYOACT global disease activity scores. (C) Longitudinal study showed that serum levels of sCD163 significantly decreased after treatment, along with the MYOACT global disease activity scores (D). Spearman correlation analysis was adopted to test for correlations. The Wilcoxon signed-rank test was used on paired data. sCD163: soluble CD163; PM: polymyositis; DM: dermatomyositis; MYOACT: Myositis Disease Activity Assessment Visual Analog Scales; sTWEAK: soluble tumor necrosis factor-like weak inducer of apoptosis.

disease activity, and the results revealed a weak correlation between sCD163/sTWEAK ratio and global disease activity scores ($r = 0.283$, $p < 0.05$; Figure 2B). However, sCD163/sTWEAK ratio did not correlate with muscle disease activity ($p > 0.05$).

We further designed a longitudinal study to investigate the variation in serum levels of sCD163 over time and in relation to disease activity. In our cohort, 26 newly diagnosed patients with PM/DM were included in the longitudinal study. The serum samples and clinical data of these patients before and after treatment were collected. By applying the Wilcoxon signed-rank test, serum sCD163 levels were found to be significantly decreased after treatment compared with the levels before treatment ($p < 0.001$), as shown in Figure 2C. In addition, the global disease activity scores were decreased along with the serum levels of sCD163 ($p < 0.001$; Figure 2D). Moreover, the change in serum levels of sCD163 correlated with the change in global disease activity scores ($r = 0.67$, $p < 0.001$). Interestingly, after treatment, the serum sCD163 levels of patients with PM/DM in remission stage were found to be comparable with that of healthy controls (542.4 ± 199.6 ng/ml and 477.8 ± 155.1 ng/ml, respectively, $p > 0.05$).

Association between serum levels of sCD163 and macrophage infiltration in muscle tissue. Muscle biopsy sections from 13 patients with PM and 17 patients with DM were applied for immunohistochemistry staining to investigate macrophage infiltration in muscle tissue. The results showed that CD163+ macrophage infiltrations were observed in 5 out of 13 patients with PM and 13 out of 17 patients with DM. Figure 3A shows representative results of immunohistochemistry staining of the muscle biopsies from patients with CD163+ macrophage infiltration; a large number of scattered or focal macrophages are obvious. Immunostaining results

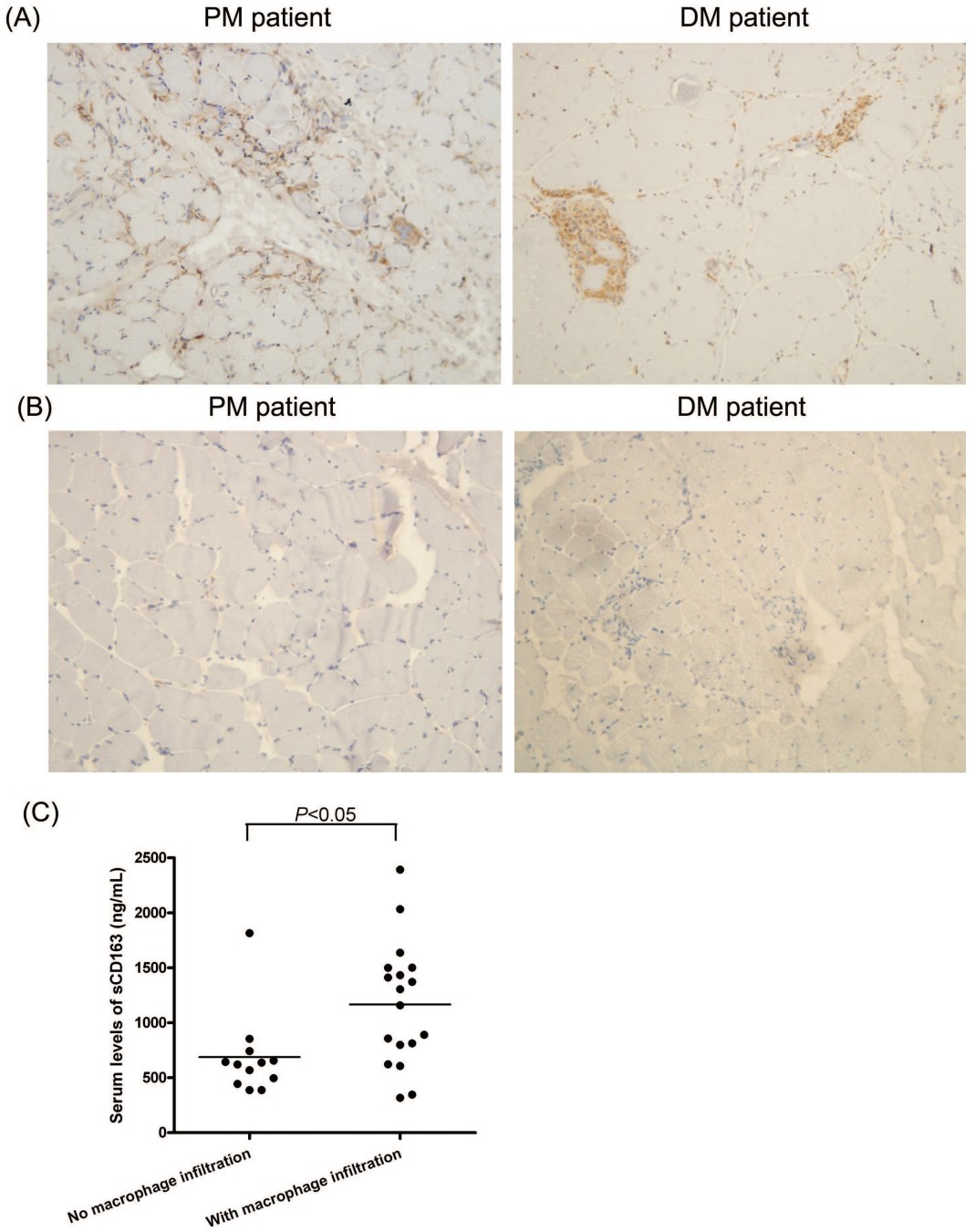

Figure 3. Serum levels of sCD163 associated with macrophage infiltration in muscle tissue. Immunohistochemistry staining was applied to identify macrophage infiltration in muscle tissue of patients with PM/DM. (A) Muscle biopsies from a patient with PM and a patient with DM showed obvious macrophage infiltration in muscle biopsy. (B) Representative patients with PM/DM without macrophage in muscle tissue. (C) Patients with PM/DM with CD163+ macrophage infiltration in muscle tissue showed significantly higher serum levels of sCD163 compared with patients without macrophage infiltration. Magnification is 200×. sCD163: soluble CD163; PM: polymyositis; DM: dermatomyositis.

of patients without CD163+ macrophage infiltration are representatively shown in Figure 3B.

Intriguingly, we found that patients with PM/DM with CD163+ macrophage infiltration in muscle tissue had significantly increased serum levels of sCD163 compared with patients without macrophage infiltration (1166.4 ± 559.7 ng/ml and 687.9 ± 381.7 ng/ml, respectively, $p < 0.05$), as shown in Figure 3C. In addition, by subgrouping these 30

patients into 2 groups based on the cutoff value (788.2 ng/ml), we found that in patients with high serum sCD163 levels, 14 out of 16 (87.5%) were CD163+ macrophage–positive in muscle tissue, while 4 out of 14 patients (28.6%) with normal serum sCD163 levels showed CD163+ macrophage in muscle tissue. Therefore, the patients with high serum sCD163 levels showed a significant higher incidence of macrophage infiltration than patients with normal serum sCD163 levels (chi-square value = 10.804, $p < 0.01$).

DISCUSSION

In our study, we found significantly elevated serum levels of sCD163 in patients with PM/DM compared with healthy individuals in a large myositis cohort. Subgrouping patients according to clinical phenotype and laboratory data revealed that high serum levels of sCD163 were associated with an increased incidence of ILD and ANA, decreased CD3+ T cell count in peripheral blood, higher serum levels of IgG and IgA, increased ESR, and CD163+ macrophage infiltration in muscle tissue. In addition, serum levels of sCD163 correlated with disease severity in patients with PM/DM, suggesting serum sCD163 as a promising biomarker in the disease evaluation of PM/DM.

The soluble form of CD163 ectodomain has been found in normal plasma, and increased serum levels of sCD163 are observed in diseases relating to macrophage activity, including acute and chronic inflammations[23]. Several rheumatic diseases where inflammation is a characterized component of the pathogenesis also show increased serum levels of sCD163, such as rheumatoid arthritis[24], systemic lupus erythematosus[25], and systemic sclerosis (SSc)[26,27]. However, there is a paucity of information regarding the expression of sCD163 in patients with PM and DM. In the study conducted by Shimizu, *et al*, 20 patients with DM were included as a control group, and their serum levels of sCD163 were found to be significantly higher compared with healthy subjects[26]. Our study confirmed the increased serum sCD163 levels in a much larger cohort of patients with PM/DM that was also in line with our previous findings[13]. Notably, we also revealed a positive correlation between serum sCD163 levels and disease activity in patients with PM/DM, suggesting that serum sCD163 may be a useful biomarker for PM/DM disease activity. Despite the increased ESR found in patients with high serum sCD163 levels compared with patients with normal serum sCD163 levels, we found no associations between sCD163 and standard biomarkers of the diseases [creatine kinase (CK) and C-reactive protein (CRP)] in our present study. More importantly, our results showed significant correlation between sCD163 and MYOACT score, which is considered as a more reliable and valid tool for evaluating myositis disease activity[28]. Although CK and CRP are commonly used as standard markers of disease, there are some limitations when they are applied to evaluate myositis disease activity because they frequently do not reflect disease activity. Further validation of myositis markers, including serum sCD163, are quite necessary, as described by the IMACS Group[18,29].

Studies have found that sCD163 is able to specifically bind and neutralize TWEAK[10], and the clinical significance of sCD163/sTWEAK ratio has been clarified in several diseases, including peripheral artery disease[30], pulmonary arterial hypertension[31], and SSc[32]. Especially in patients with SSc, compared to high sCD163 levels, a much stronger association was found between high sCD163/sTWEAK ratio and lower risk of digital ulcers[32]. In contrast to the findings in SSc, our study did not observe a higher correlation coefficient between disease activity and sCD163/sTWEAK ratio compared with that between disease activity and serum sCD163 levels. These differences may indicate the diverse roles of sCD163 and TWEAK in the pathogenesis of PM/DM relative to SSc.

An increasing number of studies have suggested macrophages as significant contributors in the pathogenesis of PM/DM. Macrophages expressed increased MRP8/MRP14 that directly inhibit the proliferation of myoblasts and differentiation to myotubes *in vitro*[33], indicating that infiltrated macrophages could promote destruction and impair the regeneration of myocytes through the secretion of MRP8/MRP14[33]. In addition, Rostasy, *et al* revealed that late-activated macrophages in PM/DM were capable of producing inflammatory molecules such as inducible nitric oxide synthase and transforming growth factor-β, suggesting an active part that late-activated macrophages exert in the disease process[34]. Moreover, Lidbury, *et al* demonstrated that after Ross River virus infection, macrophage-depleted mice only developed mild disease with no inflammation in the synovial and muscle tissue[35]. Because sCD163 was considered to be a marker of macrophage activation[11,36,37,38], our finding of elevated serum sCD163 levels associated with macrophage infiltration in muscle tissue provides strong evidence for macrophage activation in patients with PM/DM. Together with the positive correlation between serum levels of sCD163 and disease activity, the current study underlined the role of macrophage activation in PM/DM.

Intriguingly, our study demonstrated an association between the increment of serum sCD163 levels and ILD presence in patients with PM/DM, indicating that active macrophage may take part in the pathogenesis of ILD in PM/DM. Because of the lack of lung tissue specimen, we did not analyze the presence of macrophage in the lung tissue of patients with PM/DM. Thus, the results of our study did not allow us to make firm conclusions regarding the association of macrophage infiltration with ILD in patients with PM/DM. However, there is growing evidence exhibiting the pathogenic role of active macrophage in lung fibrosis, including idiopathic pulmonary fibrosis[39,40] and scleroderma-related ILD[41,42]. The current finding of an association between high

serum sCD163 levels and ILD presence provides clues for further study to clarify the potential function of macrophage in ILD of PM/DM.

We acknowledge that our study has some limitations. First, we did not examine the presence of CD163+ macrophage infiltration in the muscle tissue of patients with PM/DM in the remission stage. This was because it is not necessary to take additional muscle biopsies for patients in the remission stage, and thus the muscle specimens were unavailable. Another limitation was that the infiltrated macrophages in lung tissue were not investigated, as discussed above. In addition, there was a lack of knowledge about the subsets of patients with DM in our study that prevented a firm conclusion on whether elevated sCD163 levels were present in all DM subsets, especially patients with clinical amyopathic DM.

Our study revealed significantly increased serum levels of sCD163 and a positive correlation between serum sCD163 levels and disease severity in patients with PM/DM. In the light of reported evidence showing sCD163 as a marker of macrophage activation, including the present finding of elevated serum sCD163 levels in association with macrophage infiltration in muscle tissue, our data highlight the role of macrophage activation in the pathogenesis of PM/DM.

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