Evaluation of biomarkers related to endothelial dysfunction: proof of vasculopathy in anti-melanoma differentiation-associated gene 5 dermatomyositis

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Abstract

Objective

We aimed to reveal evidence of endothelial dysfunction in the development of anti-melanoma differentiation-associated gene 5 (MDA5) dermatomyositis (DM).

Methods

Thirty anti-MDA5 DM patients were enrolled and compared with patients with polymyositis (PM) (n=10) and healthy controls (n=20). The concentrations of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), endothelin-1 (ET-1) and von Willebrand factor (vWF) as well as interferon-alpha (IFN-α) and Galcetin-9 in the peripheral blood were tested by enzyme-linked immunosorbent assay (ELISA).

Results

Plasma levels of sICAM-1, sVCAM-1, ET-1 and vWF were higher in the anti-MDA5 DM patients than in either the healthy controls or the PM patients. In the anti-MDA5 DM cohort, the ET-1 and vWF levels were significantly lower in the cases without cutaneous ulcers and ILD than the other cases. There was a strong positive relationship between the concentrations of ET-1 and Galectin-9 in the anti-MDA5 DM group.

Conclusion

Our data suggest that endothelial dysfunction may be involved in the development of anti-MDA5 DM.

Key words

dermatomyositis, endothelial dysfunction, melanoma differentiation-associated gene 5, type I interferon, ulcer

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Introduction

Dermatomyositis (DM) is traditionally thought of as a single clinical entity defined by the presence of characteristic cutaneous and muscular abnormalities. Recently, the discovery of myositis-specific autoantibodies (MSAs) has advanced our understanding of the heterogeneous nature of DM (1). These MSAs play an important role in the diagnostic approach, therapeutic choices and prognostic stratification of the patients with DM. An example would be the autoantibody to melanoma differentiation-associated gene 5 (MDA5). Patients with DM and anti-MDA5 autoantibodies usually possess a clinical phenotype characterised by typical cutaneous manifestations and mild or even no myopathy but frequently show interstitial lung disease (ILD) (2, 3). In Asian populations, patients with anti-MDA5 DM have an increased risk of rapidly progressive ILD (RPILD) and a fatal outcome compared to patients with classical DM (4-6). An improvement in the outcomes of patients with anti-MDA5 DM is urgently needed, but the underlying pathogenesis has not been fully elucidated.

Cutaneous ulcer is one of the most distinctive features of anti-MDA5 DM. The prevalence of ulcers in anti-MDA5 DM has been estimated to be 28–80% in studies from Europe and America (7-9) and 60–85% in studies from China (10) and Japan (11). Characteristic locations of the cutaneous ulcers include the extensor surfaces overlying joints (particularly over Gottron's papules), lateral nailfolds and digital pulp. Notably, these cutaneous ulcers have predictive significance for the unfavourable outcome of patients with anti-MDA5 DM. The ILD risk was specifically increased (12) and the prevalence of RPILD can be as high as 71% (13) in patients with anti-MDA5 DM and cutaneous ulcers. In addition, a mortality rate of 33.33% was reported in patients with anti-MDA5 DM and cutaneous ulcers in Cao's study (14).

The precise pathogenic mechanisms of cutaneous ulcers have not been determined. Although vasculitis was previously considered to be a major contributor, pathological evidence of vasculopathy has been found in biopsies of cutaneous ulcers from patients with anti-MDA5 DM. In one study from China (10), the pathological change of obliterative microvasculopathy with sparse inflammatory infiltration was shown in the site of ulceration. Such features have been identified in several case reports with histopathologic examinations of ulcerated lesions in patients with anti-MDA5 DM (7, 14, 15). Based on these previous findings, we postulate the role of endothelial dysfunction in the pathogenesis of anti-MDA5 DM. To date, serum biomarkers of endothelial dysfunction have not been evaluated in patients with anti-MDA5 DM.

In the present study, we investigated endothelial dysfunction involved in the development of anti-MDA5 DM. We also showed the effect of targeted treatment to ameliorate the underlying vasculopathy in two anti-MDA5 DM cases with refractory ulcers.

Materials and methods

Patients

Thirty adult patients with DM positive for anti-MDA5 antibodies were enrolled in the present study. There were also 10 adult patients with polymyositis (PM) and 20 age- and sex-matched healthy subjects enrolled as controls. The diagnosis of DM and PM was based on the Bohan and Peter criteria (16). These enrolled patients were admitted to our hospital (West China Hospital) between June 2017 and January 2019. Patients were excluded from the study if they had concomitant malignancy, overlapping rheumatic disease or diabetes, hypertension, or a history of cardiovascular and cerebrovascular diseases. All plasma samples were taken before administration of high-dose corticosteroid (prednisone <50 mg/d or methylprednisolone <40 mg/d). Moreover, all patients with ILD received oxygen therapy (see Supplementary Table SI for complete therapy information). The baseline demographic, clinical and laboratory data of all patients are listed in Table I. Blood tests, including assays of C-reactive protein (CRP), creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and anti-extractable

Table I. Clinical characteristics of the enrolled patients.

	Anti-MDA5 DM (n=30)		PM (n=10)
	with ulceration (n=10)	without ulceration (n=20)	
Age at onset (years), mean ± SD	52.3 ± 10.6	46.6 ± 16.4	46.5 ± 10.8
Female, n (%)	9 (90)	14 (70)	9 (90)
Disease duration (months), median (IQR)	3.5 (1.8-5.5)	2 (1.3-5.5)	6.5 (2.0-10.5)
Myalgia (%)	5 (50)	17 (85)	10 (100)
Arthralgia/arthritis n (%)	8 (80)	13 (65)	2 (30)
ILD	10 (100)	16 (80)	6 (60)
RPILD	5 (50)	1 (5)	0 (0)
Laboratory data			
CK, IU/L, median (IQR)	93 (35-299)	64 (49-117)	762 (184-2000)
AST, IU/L, median (IQR)	91 (39-253)	92 (50-193)	45 (36-99)
LDH, IU/L, mean±SD	435.2 ± 134.4	347.8 ± 116.1	395.9 ± 220.5
CRP, mg/L, median (IQR)	31.7 (2.48-70.55)	4.08 (2.67-9.25)	6.40 (1.28-16.88)
MAAs profiles, n (%)			
Anti-Ro52 Ab positive	5 (50)	13 (65)	5 (50)
Anti-RNP Ab positive	0 (0)	1 (5)	0 (0)
MSAs profiles, n (%)			
Anti-MDA5 Ab positive	10 (100)	20 (100)	0 (0)
Anti-ARS Ab positive	0 (0)	0 (0)	6 (60)
Anti-SRP Ab positive	0 (0)	0 (0)	3 (30)

IQR: interquartile range; CK: creatine kinase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; CRP: C-reactive protein; MAAs: myositis-associated autoantibodies; RNP: ribonucleoprotein; MDA5: melanoma differentiation-associated gene 5; ARS: aminoacyl-tRNA-synthetase; and SRP: signal-recognition particle.

nuclear antigen (ENA) antibodies, were performed and recorded at the clinical laboratory at West China Hospital. ILD was diagnosed independently by two radiologists based on high-resolution computed tomography findings. Patients who exhibited a radiologic worsening of their ILD with progressive dyspnea and/or hypoxemia within 1 month of respiratory symptom onset were defined as having RPILD (17). Hypoxemia was defined in a previous study as resting oxygen saturation (SpO2) of less than 88% (18). Ethics approval for this study was granted by the medical ethics review board of the West China Hospital, Sichuan University. Patients provided written informed consent/assent according to the standards of the Declaration of Helsinki.

Measurement of cytokine levels

Ethylenediaminetetraacetic acid-anticoagulated peripheral blood samples from all patients were collected at the first visit. Plasma samples were separated into multiple aliquots and stored at -80°C until use. The plasma levels of cytokines, including endothelin-1 (ET-1, R&D Systems, Inc., Minneapolis, MN, USA) von Willebrand factor (vWF, RayBiotech, Norcross, GA, USA), soluble vascular cell adhesion molecule-1 (sVCAM-1, RayBiotech, Norcross, GA, USA), soluble intercellular adhesion molecule-1 (sICAM-1, RayBiotech, Norcross, GA, USA), interferon-alpha (IFN-α, Invitrogen, Carlsbad, CA, USA) and Galectin-9 (RayBiotech, Norcross, GA, USA), were measured by ELISA kits according to the manufacturer's protocols. The minimum detectable doses of ET-1, vWF, sVCAM-1, sICAM-1, Galectin-9 and IFN-α defined by the manufacturer were 0.064 pg/ml, 0.13 ng/ml, 300 pg/ml, 150 pg/ml, 36 pg/ml and 3.2 pg/ml, respectively.

Detection of myositis-specific autoantibodies

Anti-MDA5, anti-aminoacyl-tRNA synthetase (anti-ARS, including anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ and anti-OJ), anti-Mi2, anti-nuclear matrix protein 2 (anti-NXP2), anti-transcriptional intermediary factor 1-γ (anti-TIF1-γ), anti-signal recognition particle (anti-SRP) and anti-small ubiquitin-like modifier activating enzyme 1 (anti-SAE1) autoantibodies were measured using a line blot immunoassay kit (Euroline Myositis Profile; Euroimmun, Lubeck, Germany).

Statistical analysis

Data are expressed as the mean±SD (for data that were normally distributed) or the median and interquartile range (IQR; for data that were not normally distributed). The chi-square test was used to compare the frequencies between two groups of categorical variables. Differences in quantitative parameters between two groups were assessed using the Mann-Whitney U-test. Differences in quantitative parameters between more than two groups were assessed using the Kruskal-Wallis test followed by the Mann-Whitney U-test. Spearman's test was used for correlation analysis as appropriate. Differences were considered significant if the p-value was <0.05. All statistical analyses were performed using Prism 7.0 software (GraphPad Software, La Jolla, CA, USA).

Results

Plasma levels of endothelial markers

To explore whether endothelial dysfunction is involved in the development of anti-MDA5 DM, we examined several related markers in the peripheral blood and compared them among the patients with anti-MDA5 DM, the patients with PM and the healthy controls. The plasma levels of sICAM-1 ($P<0.001$), sVCAM-1 ($p<0.0001$), ET-1 ($p<0.0001$) and vWF ($p<0.0001$) were all higher in the patients with anti-MDA5 DM than in the healthy controls (Fig. 1). We found that 86.7% of the patients had ILD complications in the anti-MDA5 DM group and 60% of the patients had ILD complications in the PM group ($p=0.1709$). Even compared with the patients with PM, the patients with anti-MDA5 DM had obviously higher levels of sICAM-1 (median [IQR]: anti-MDA5 DM: 229.1 [181.5–347.2] ng/ml *vs*. PM: 172.1 [126.2–237.7] ng/ml), sVCAM-1 (median [IQR]: 366.3 [212.1–493.6] ng/ml *vs*. 199.9 [145.9–269.2] ng/ml), ET-1 (median [IQR]: 4.9 [4.143–5.843] ng/ml *vs*. 3.814 [2.83–4.532] ng/ml) and

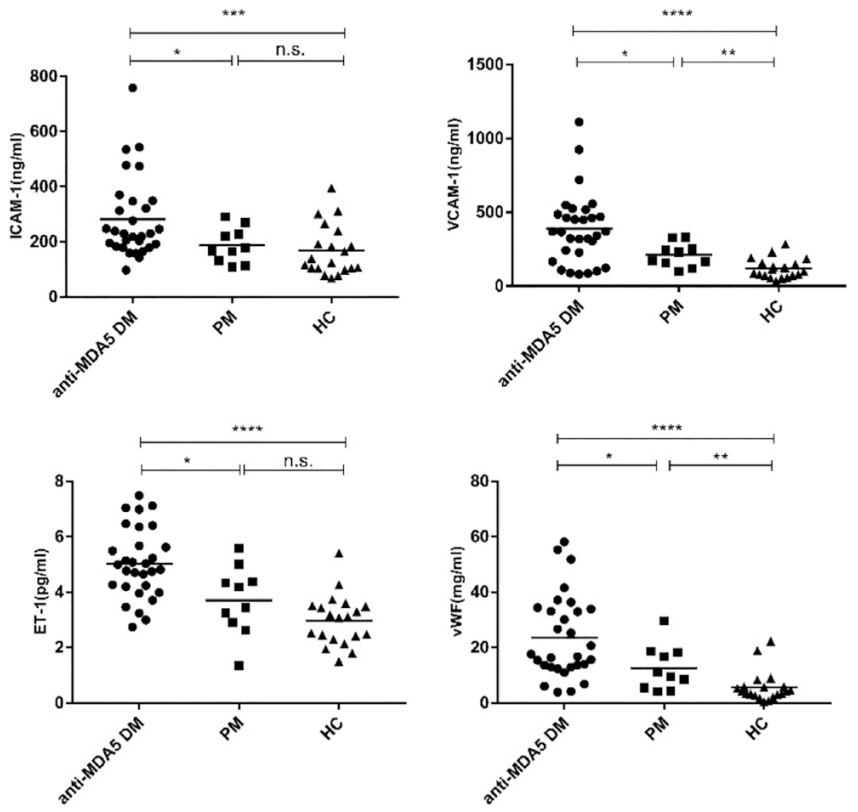

Fig. 1. Plasma levels of endothelial markers in patients with anti-MDA5 DM (n=30), patients with PM (n=10) and healthy controls (n=20). *p<0.05, ***p<0.001, ****p<0.0001. n.s., not significant. sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; ET-1, endothelin-1; vWF, von Willebrand factor.

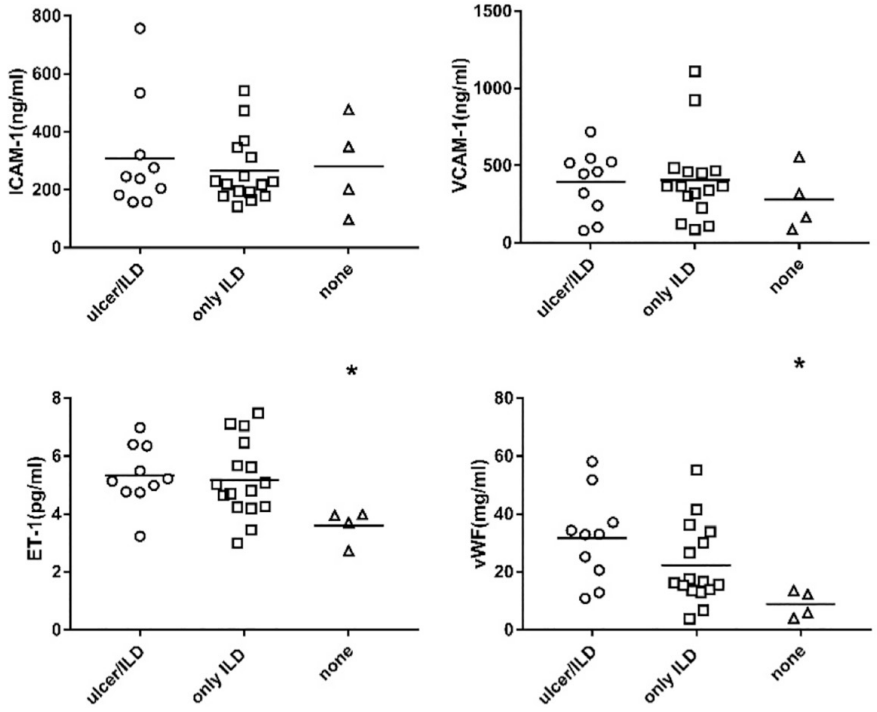

Fig. 2. Plasma levels of endothelial markers in the anti-MDA5 DM cohort. The plasma levels of ICAM-1, VCAM-1, ET-1 and vWF were compared among the anti-MDA5 DM cohort, including the ulcer/ILD subgroup (n=10), only ILD subgroup (n=16), and none subgroup (n=4). *p<0.05. sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; ET-1, endothelin-1; vWF, von Willebrand factor; and ILD, interstitial lung disease.

vWF (median [IQR]: 17.13 [12.92–34.01] ng/ml *vs*. 10.25 [5.132–18.32] mg/ml) (all comparisons, p<0.05).

Plasma levels of endothelial markers analysed in the anti-MDA5 DM cohort

Both cutaneous ulcers and ILD have been proposed to be independent risk factors for poor outcome in patients with anti-MDA5 DM (2). In terms of clinical phenotype in our anti-MDA5 DM cohort, 33.33% of the cases (n=10, ulcer/ILD subgroup) had both cutaneous ulcers and ILD, 53.33% of the cases (n=16, only ILD subgroup) had ILD and no cutaneous ulcers, and 13.33% of the cases (n=4, none subgroup) had neither ulcers nor ILD. Although no differences were shown in the expression of sICAM-1 (p=0.9427) and sVCAM-1 (p=0.6424), the expression levels of ET-1 (median [IQR]: 3.83 [2.978–3.976] ng/ml) and vWF (median [IQR]: 9.139 [4.625–13.31] ng/ml) in the none subgroup were the lowest among the three subgroups (p<0.05 and P<0.05, respectively) (Fig. 2). There were slight elevations in both ET-1 (p=0.6227) and vWF (p=0.1824) expression levels in the ulcer/ILD subgroup compared with the only ILD subgroup.

ET-1 was correlated with Galectin-9 in the patients with anti-MDA5 DM

The type I IFN system has been proposed to play an important role in the pathogenesis of myositis (19). A high type I IFN signature was detected in the serum and skin samples of patients with anti-MDA5 DM (20, 21). Type I IFN can cause endothelial dysfunction, which may contribute to organ damage in lupus patients (22). Indeed, increased levels of IFN-α were found in the plasma samples from the patients with anti-MDA5 DM compared to those from the patients with PM (p<0.01) (Fig. 3A). The expression of Galectin-9, which was demonstrated to be a stable biomarker for evaluating the type I IFN signature (23), was substantially increased (p<0.0001) and well correlated with the expression of IFN-α (rs=0.4652, p=0.0096) in the anti-MDA5 DM group. Moreover, there was a strong positive relationship between the concentrations of ET-1

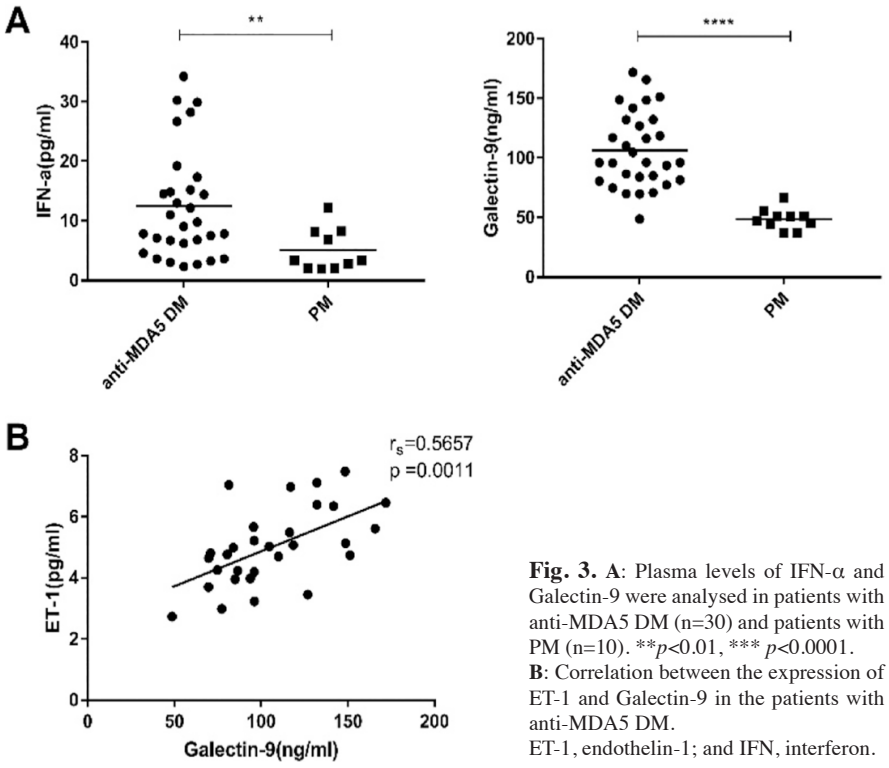

Fig. 3. A: Plasma levels of IFN-α and Galectin-9 were analysed in patients with anti-MDA5 DM (n=30) and patients with PM (n=10). **p<0.01, *** p<0.0001.
B: Correlation between the expression of ET-1 and Galectin-9 in the patients with anti-MDA5 DM.
ET-1, endothelin-1; and IFN, interferon.

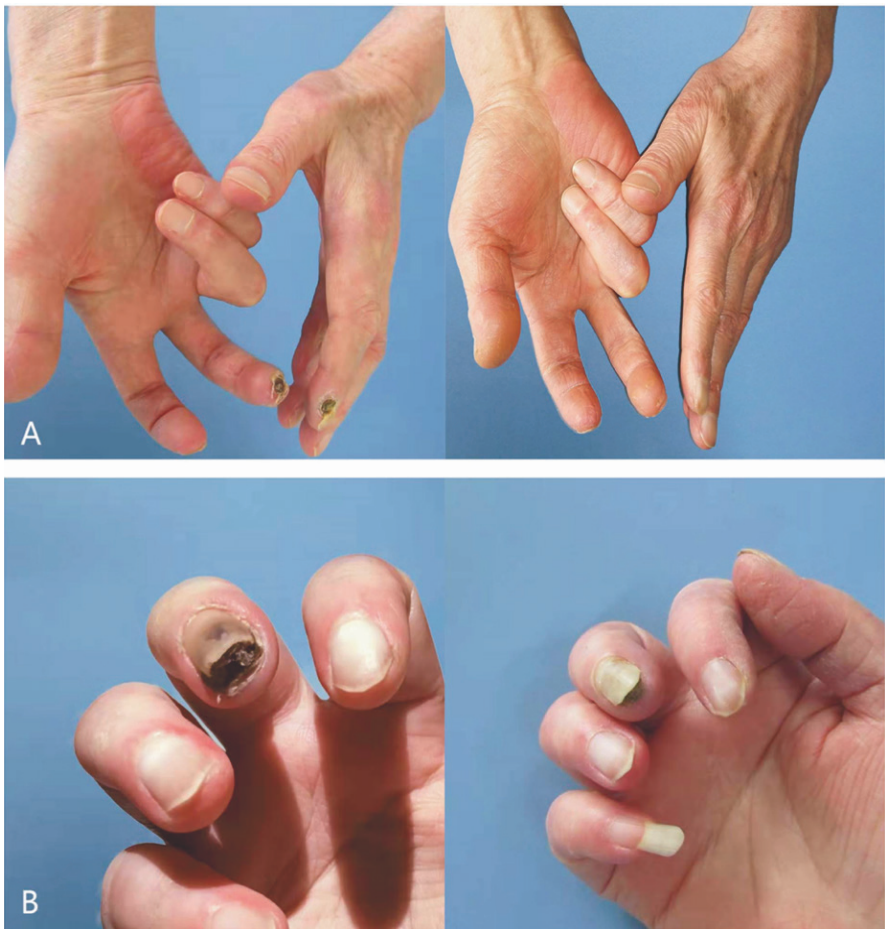

Fig. 4. Digital pulp and periungual ulcers in two patients with anti-MDA5 DM (**A**: male and **B**: female). After 12 weeks of ambrisentan treatment, the cutaneous ulcers had mostly resolved.

and the concentrations of Galectin-9 in the anti-MDA5 DM group (rs=0.5657, p=0.0011, Fig. 3B).

Discussion

Anti-MDA5 DM has characteristic features that are substantially different from those observed in classic DM. In addition to an elevated risk of ILD/RPILD, the unique mucocutaneous features are often striking signs of anti-MDA5 DM. The underlying pathogenesis is not yet well understood. "Cytokines storm" (24, 25) and unrest immune cells, such as T and B lymphocytes (26-30) and neutrophils (31, 32) have been suggested to contribute to the development of this disease. In recent years, vasculopathy was proposed to be another pathogenic process contributing to anti-MDA5 DM, according to limited histological findings. To our knowledge, this is the first study to evaluate biomarkers related to endothelial dysfunction, which may be involved in vasculopathy in anti-MDA5 DM.

In the present study, we selected several common biomarkers relevant to endothelial cell (EC) (dys)function (33): ICAM-1 and VCAM-1 are biomarkers of EC activation, ET-1 is a marker of defective vascular tone control, and vWF is a marker of coagulopathy. We found that sICAM-1, sVCAM-1, ET-1 and vWF were significantly elevated in the plasma from the patients with anti-MDA5 DM, even compared with that from the patients with PM. In our anti-MDA5 DM group, the patients without risk factors, such as cutaneous ulcer and ILD, had obviously lower levels of ET-1 and vWF than the patients with cutaneous ulcer and/or ILD. In addition, we described two anti-MDA5 DM cases with refractory cutaneous ulcers who received treatment targeting ET-1. One case was a man in his 60s with a 33-month course, and the other was a woman in her 50s with a 42-month course. ILD was present in the initial period of their diseases and was improved under immunosuppressive therapy. However, cutaneous ulcers remained intractable problems in their courses and were refractory to immunosuppressive therapy. Off-label use of ambrisentan (5 mg daily), which

is a relatively selective endothelin receptor type A antagonist, was administered after the approval of these two patients. Interestingly, ET-1 targeted therapy rather than more aggressive immunosuppressive therapy relieved the cutaneous ulcers. The therapeutic effects on ulcers in these two anti-MDA5 DM cases are shown in Figure 4. A similar case with anti-MDA5 DM under bosentan treatment was reported by Combalia *et al*. (34). Therefore, together with the occlusive vasculopathy features shown in histological descriptions from Cao's study (10), endothelial dysfunction should be considered another independent mechanism contributing to the development of anti-MDA5 DM. The pathogenic process of vasculopathy could combine with autoimmune inflammation or independently play a role in the different stages of this disorder. Appropriate intervention, not always aggressive immunosuppressive therapy, appears to improve patient outcomes.

Accumulating evidence by several studies has suggested that aberrant activation of the type I interferon system is involved in the pathogenesis of anti-MDA5 DM: i) abnormally high expression levels of type I IFN-induced genes were detected in peripheral blood mononuclear cells (PBMCs) (20, 21); ii) increased levels of IFN-α were demonstrated in the peripheral circulation (21, 35); and iii) strong expression levels of type I IFN-induced proteins, such as Mx1, STAT1 and ISG15 (20, 21), were shown in the affected skin tissues and the vasculature by immunohistochemistry. Several studies have demonstrated in SLE patients that an overactivated type I interferon system could mediate endothelial dysfunction and participate in organ damage (36-38). In our study, we found significantly elevated concentrations of IFN-α and Galectin-9 in the plasma from the patients with anti-MDA5 DM. Moreover, there was a positive correlation between the expression of Galectin-9 and ET-1 in the anti-MDA5 DM group.

In summary, our present findings suggest that endothelial dysfunction may be involved in the development of anti-MDA5 DM. Therapies aimed at ameliorating the underlying vasculopathy, such as endothelin receptor antagonists, deserve attention in addition to extensive immunosuppressive treatment.

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