Role of Endothelial Damage in the Pathogenesis of Interstitial Pneumonitis in Patients with Polymyositis and Dermatomyositis

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ABSTRACT. ***Objective.*** Polymyositis and dermatomyositis (PM/DM) are often complicated by interstitial pneumonitis (IP), which is an important cause of death. It has been reported that blood concentration of transforming growth factor-ß (TGF-ß), which is produced by a wide range of cells including endothelial cells and enhances the fibrotic changes in various tissues, is increased in PM/DM with IP. Endothelial damage is likely to exist in PM/DM. We studied the relationship between endothelial damage and IP in PM/DM.

Methods. Blood levels of sialylated carbohydrate antigen KL-6, TGF-ß, endothelin-1 (ET-1), thrombomodulin (TM), and plasminogen activator inhibitor-1 (PAI-1) were determined in 43 patients with PM or DM with or without IP, and the relationship between these measures was analyzed.

Results. Blood levels of KL-6 and TGF-ß were higher in the patients with IP than those without, and these measures were well correlated with each other. Levels of ET-1, TM, and PAI-1, all known to reflect the extent of endothelial damage, were also increased in patients with IP, and these measures correlated well with TGF-ß.

Conclusion. Our data suggest that endothelial damage might play an important role through the production of fibrosis-enhancing factors such as TGF-ß or ET-1 in PM/DM. (J Rheumatol 2006;33:903–6)

Key Indexing Terms:
INTERSTITIAL PNEUMONITIS TRANSFORMING GROWTH FACTOR-ß
ENDOTHELIAL DAMAGE POLYMYOSITIS DERMATOMYOSITIS

Both polymyositis (PM) and dermatomyositis (DM) are forms of autoimmune myositis that systemically involve muscle fibers and, in the latter, also skin. Although the prognosis has been improved by progress in treatment using corticosteroids or various immunosuppressive agents, interstitial pneumonitis (IP) remains an important cause of death in PM/DM.

One of the most common autoimmune diseases often complicated by IP or pulmonary fibrosis is systemic sclerosis (SSc). In SSc, it has been reported that endothelial damage may be a factor in the abnormal regulation of several vasoactive agents such as endothelin (ET) and nitric oxide (NO). ET is both a strong vasoconstrictor and a growth factor for fibroblasts, while NO is simultaneously a vasodilator, and a mediator of free-radical related tissue injury. It has been reported that plasma concentrations of ET and NO are increased in plasma from SSc patients with pulmonary fibrosis[1-3]. Thus it might be important to also investigate the relationship between endothelial damage and IP in PM/DM.

On the other hand, transforming growth factor-ß (TGF-ß), which is produced by a wide range of cells including endothelial cells, is known to be associated with fibrosis of the tissues[4-7] and increased in patients with PM/DM with IP[8]. In this study, in addition to TGF-ß, we investigated blood levels of ET-1 (an isoform of ET), thrombomodulin (TM), and plasminogen activator inhibitor (PAI-1), all of which are known to reflect the extent of endothelial damage[9-11] in patients with PM/DM.

MATERIALS AND METHODS

Patients. This study examined peripheral blood samples from 43 patients who consulted our hospital between January 2000 and May 2005 and met Bohan and Peter's criteria for PM/DM[12]. Patients were divided into 2 groups, those without IP (Group A, n = 22) or with IP (Group B, n = 21), irrespective of whether the diagnosis was PM or DM. IP was diagnosed by the clinical findings as follows: (1) 2 or more of the following items: (i) fine crackles, (ii) exertional dyspnea, and (iii) nonproductive cough; (2) decreased vital capacity (< 80%) or lung diffusing capacity for carbon monoxide (< 80%); and (3) reticulonodular shadow on the chest radiographs. Table 1 shows profiles of the patients in this study. DM was diagnosed in 18.2% of patients in Group A and 61.9% of those in Group B. The mean age of patients was 55.9 years in Group A and 51.1 years in Group B. Disease duration prior to the study was significantly longer in Group A than Group B (12.8 vs 3.2 yrs; $p < 0.05$). All patients were taking 5 to 20 mg per day prednisolone, and 2 in Group A and 8 in Group B received some immunosuppressive agents, including azathio-

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Table 1. Data for PM/DM patients with and without IP.

	Group A, n = 22	Group B, n = 21
DM/PM, n (% DM)	4/18 (18.2)	13/8 (61.9)
Age, yrs, mean ± SD (range)	55.9 ± 11.8 (24–69)	51.1 ± 12.8 (24–71)
Disease duration, yrs, mean ± SD (range)*	12.8 ± 10.9 (2.7–26)	3.2 ± 3.5 (0.2–10)
Dose of prednisolone, mg, mean ± SD	6.0 ± 2.9	15.4 ± 8.7
No. of patients taking immunosuppressants** (%)	2 (9.1)	8 (38.1)
Mortality, n (%)***	0 (0)	6 (28.6)

* $p < 0.05$. ** Azathioprine, cyclosporin A, or cyclophosphamide (monthly bolus venous infusion). *** Mortality rate calculated using data from current study.

prine, cyclosporin A, and cyclophosphamide (monthly bolus venous infusion). There was no evidence of disseminated intravascular coagulopathy, thrombotic thrombocytopenic purpura, renal injury, nephrotic syndrome, malignancy, or other rheumatic diseases in any patient.

Measurements. Blood samples were collected during routine clinic consultations or during hospitalization, and chest radiographs and respiratory function tests were performed within 3 weeks of the day blood was collected. In addition to common blood cell counts, serum levels of creatinine phosphokinase (CK) activity, sialylated carbohydrate antigen KL-6 (one of indices for pulmonary fibrosis; normal < 500 u/ml, electrochemical luminescence immunoassay kit, Sanko-Junyaku, Tokyo, Japan), and plasma levels of TGF-ß (enzyme immunoassay kit, Cosmo Bio Co. Ltd., Tokyo, Japan), TM (enzyme immunoassay kit, Mitsubishi-Kagaku Bio-Clinical Laboratories, Inc., Tokyo, Japan), ET-1 (enzyme immunoassay kit, IBL Co. Ltd., Gunma, Japan), and PAI-1 (latex photometric immunoassay kit, BML Inc., Tokyo, Japan) were determined. Determination of these measures was done using blood samples drawn with or without sodium citrate on the same day and preserved at –80° until measurement.

Statistical analysis. Differences in mean values between the 2 groups were analyzed by Student t test and correlation was analyzed using t-distribution ($\nu = N - 2$).

RESULTS

Myogenic enzymes and blood cell counts. Table 2 shows the mean serum levels of CK, leukocytes, red blood cells, and platelets in the 2 groups. There was no difference in CK levels and red blood cell counts between the 2 groups. The numbers of leukocytes and platelets in Group B were significantly higher than in Group A ($p < 0.05$, $p < 0.05$, respectively).

KL-6 and endothelial damage. Table 3 shows blood levels of KL-6 and measures for endothelial damage. Serum levels of KL-6 in Group B were significantly higher than in group A ($p < 0.001$). Plasma levels of TGF-ß, ET-1, TM, and PAI-1 in Group B were significantly higher than in Group A ($p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.05$, respectively). Figure 1 shows the relationships between KL-6 and TGF-ß, and between TGF-ß and other indicators of endothelial damage. There was a positive correlation between the serum KL-6 level and plasma TGF-ß level ($p < 0.01$). The plasma level of TGF-ß correlated positively with ET-1, TM, and PAI-1 levels ($p < 0.01$, $p < 0.01$, $p < 0.01$, respectively).

Relationship between mediators and corticosteroids or immunosuppressive agents. There were no relationships between the blood levels of TGF-ß, ET-1, TM, and PAI-1 and the dose of corticosteroids or immunosuppressive agents.

Table 2. CK and blood cell counts. Data are mean ± SD.

	Group A, n = 22 Without IP	Group B, n = 21 With IP	p
CK, IU/l	175 ± 184	233 ± 364	> 0.10
White blood cells, /μl	8100 ± 3200	11300 ± 4400	< 0.05
Red blood cells, 10^4/μl	360 ± 33	355 ± 40	> 0.10
Platelets, /μl	276,000 ± 91,000	358,000 ± 105,000	< 0.05

CK: creatinine phosphokinase.

Table 3. KL-6 and endothelial damage. Data are mean ± SD.

	Group A, n = 22 Without IP	Group B, n = 21 With IP	p
KL-6, u/ml	282 ± 128	1969 ± 1594	< 0.001
TGF-ß, ng/ml	26.4 ± 8.7	32.3 ± 7.9	< 0.05
ET-1, pg/ml	2.6 ± 0.8	5.1 ± 1.4	< 0.01
TM, u/ml	9.5 ± 2.7	13.5 ± 4.6	< 0.01
PAI-1, ng/ml	35.3 ± 37.3	83.0 ± 88.5	< 0.05

ET-1: endothelin-1; TM: thrombomodulin. Normal ranges: KL-6: < 500 u/ml; ET-1: < 2.3 pg/ml; TM: < 4.5 u/ml; PAI-1: < 50 ng/ml; TGF-ß: not known.

DISCUSSION

Both PM and DM are characterized by autoimmune myopathy and frequent complication of IP. Although it might be difficult to conclusively diagnose early-stage IP with the criteria used here, there were certain differences between patients with and those without IP. The average duration of disease in patients with IP was shorter than in those without IP, while there was no difference in mean age between the 2 groups. The patient group with IP included more patients with DM than the group without IP, and blood concentrations of TGF-ß, ET-1, TM, and PAI-1 were higher in DM than in PM, although the difference was not significant. Further, IP was frequently seen in cases of amyopathic myositis in which myositic symptoms such as muscle weakness or elevated CK were merely trace or transient (data not shown). Based on these findings, the pathophysiology of IP might not correlate to the muscle disorders but to the pathophysiology, in which certain growth factors for fibroblasts such as TGF-ß or connective tissue growth factor play an important role. This hypothesis seems to be supported by reports indicating that the plasma level of TGF-ß is elevated in cases of glomerulosclerosis, SSc, and PM/DM with

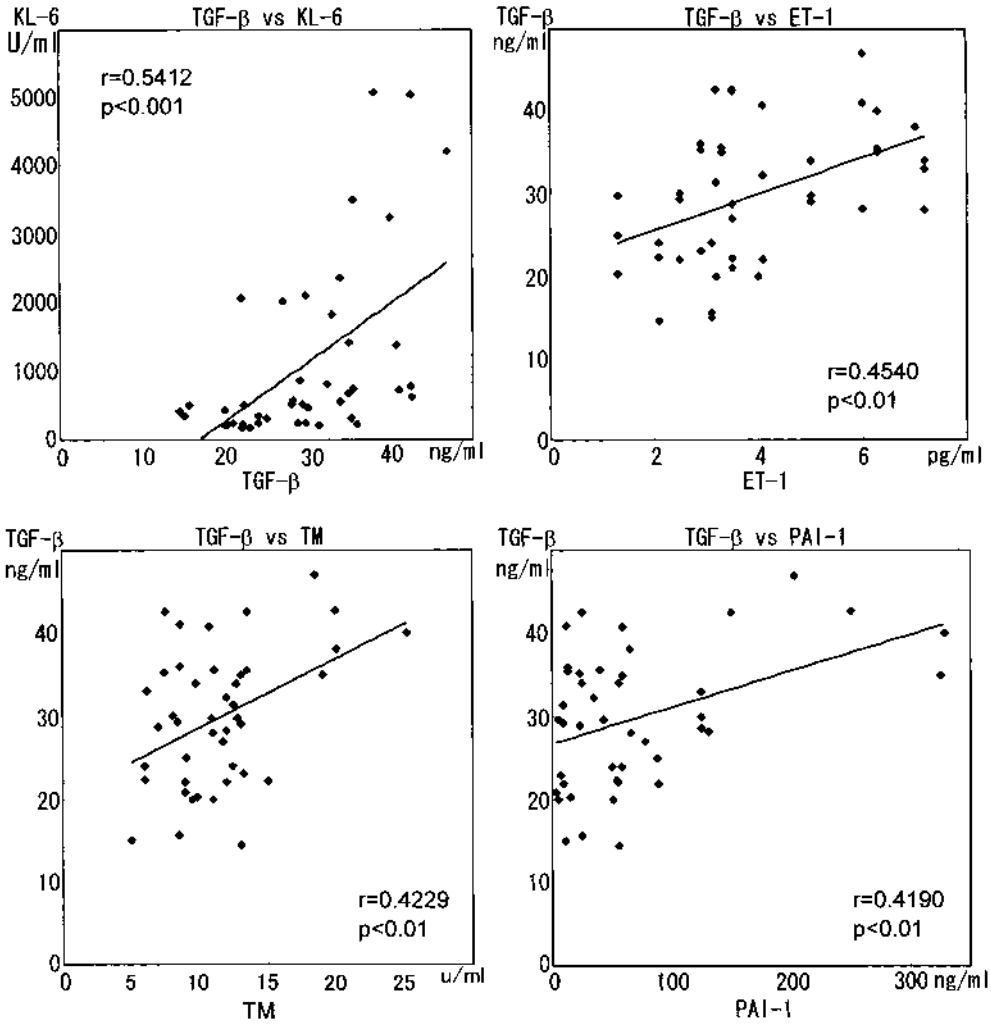

Figure 1. Relationship between endothelial damage and plasma KL-6. Combined samples from the 43 patients were used for determination of TGF-ß, ET-1, TM, and PAI-1.

IP[8,13]. Histological findings from lung biopsies performed in 4 cases in this series indicated nonspecific IP, and histopathologically-proven vasculitis in the lung or other tissues has been reported to be rare[14,15]. However, diffuse endothelial damage could occur in the vessels or microvessels due to various causes. Therefore, investigation of the relationship between IP and endothelial damage seems important.

KL-6 has been reported to be useful for the diagnosis and followup of IP, although this is not always true in all cases[16,17]. In our study, the blood level of KL-6 was remarkably elevated in patients with IP, and there was a positive correlation between KL-6 and TGF-ß. It is known that TGF-ß is produced by endothelial cells or macrophages, and that its production is enhanced by endothelial damage. We investigated whether other substances that are stimulated by endothelial damage parallel the TGF-ß in PM/DM. It has been reported that plasma ET levels reflect the extent of endothelial damage[18], and anti-ET antibodies have been detected in inflammatory muscle diseases[19], although the pathological significance of this is unclear. TM is produced by vascular endothelial cells and inhibits thrombin production. TM production is also enhanced by endothelial damage. PAI-1 is produced by endothelial cells or smooth muscle cells of the vessels, or hepatic cells, and binds to tissue plasmin activator, leading to inhibition of fibrinolysis. Production of PAI-1 is enhanced by vascular damage, or it may act as an acute reactant. We observed that blood levels of TGF-ß, ET-1, PAI-1, and TM were elevated and correlated to one another in cases of PM/DM with IP. It is known that TGF-ß and ET-1 are capable of facilitating fibrosis, while all of these mediators are induced by endothelial damage. On the other hand, TGF-ß is also produced by airway epithelial cells and type II pneumocytes[20,21], and PAI-1 by alveolar macrophages and cuboidal epithelial cells[22], and thus they could be regarded as endogenous repair factors. Therefore, elevated levels of TGF-ß and PAI-1 might be the result of IP. However, we observed the

level of TGF-ß correlated to that of TM, which is relatively specific to endothelial damage. These findings suggest it is possible that endothelial damage induced by some causes might play an important role through the production of fibrosis-enhancing factors such as TGF-ß or ET-1 in PM/DM.

With regard to the influence of corticosteroids and immunosuppressive agents, there is a possibility that these agents might have induced a series of infections leading to IP, although there was no significant relationship between the blood levels of these mediators and the doses of them. At least, production of TGF-ß would not be stimulated directly by corticosteroids[23]. In the blood cell counts, a tendency toward increased numbers of leukocytes was seen in the cases of IP. This might be the result of a wide range of inflammatory activity, such as IP itself, or vascular damage, or alternatively, of the influence of corticosteroids, although there was no relationship between the leukocyte count and the dose of corticosteroids (data not shown). On the other hand, the platelet count was significantly increased in the cases of IP. Because there was no evidence of bleeding or malignancy and no history of splenectomy, there is a possibility that some growth factors for megakaryocytes might have been induced in response to chronic inflammation or endothelial damage.

There has been a report that bosentan, an antagonist of ET-1 receptor, is effective for pulmonary hypertension due to pulmonary fibrosis in SSc[24]. Together with the fact that ET-1 is induced by endothelial damage, this finding might also support the hypothesis that endothelial damage causes upregulation of fibrosis-enhancing factors leading to the fibrosis of various tissues.

The relationship between TGF-ß or endothelial damage and autoantibodies specific to PM/DM such as anti-Jo-1 antibody was not evaluated in our study because of an insufficient number of cases tested for these autoantibodies. However, detection of these autoantibodies might be important to determine the pathogenesis of endothelial damage in PM/DM. It is known that endothelial damage is caused by various disorders such as hyperglycemia, sepsis, thrombosis, and disseminated intravascular coagulation, as well as vasculitis. Therefore, endothelial damage induced by any of these factors might become a trigger for the exacerbation of IP in patients with PM or DM.

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