Two cases of dermatomyositis associated with interstitial pneumonia: a comprehensive study of gene expression

Sirs,

We recently encountered two cases of dermatomyositis (DM) associated with interstitial pneumonia (IP). To investigate the expression of genes related to the prognosis and/or disease activity of DM associated with IP, we performed a comprehensive study of gene expression using the DNA microarray method. Patient 1 (a 40-year-old woman) had severe muscle weakness and myalgia in the upper and lower limbs, while typical DM-related skin changes (Gottron signs) were observed on her hands. Elevation of muscle enzymes was also noted, with creatine phosphokinase (CK) at 8,521 IU/ml (normal (n) < 250 IU/ml), aldolase at 22.6 IU/l (n=2.1 – 6.1 IU/l), glutamate-oxaloacetate transaminase (GOT) at 421 IU/ml (n =13 -33 IU/l), glutamate-pyruvate transaminase (GPT) at 421 IU/ml (n=8 – 42 IU/l). Anti-Jo1 antibody was also positive. Patient 2 (a 57-year-old woman) had typical dermatitis of DM (Gottron signs and heliotrope rash) and the levels of muscle enzymes were increased (CPK was 1,280 IU/ml, aldolase was 21.2 IU/l, GOT was 315 IU/l, and GPT 129 IU/l, respectively). In both patients, electromyography (EMG) indicated a myogenic pattern and muscle biopsy revealed infiltration of lymphocytes. Both patients satisfied diagnostic criteria of DM by Peter and Bohan (1). Paraneoplastic screening tests revealed no evidences of malignant tumors in both patients. The chest x-ray and computed tomography (CT) features indicated the presence of IP in both patients. Based on these findings, they were diagnosed as having with DM associated with IP. In patient 2, the main symptoms were dermatitis and muscle-related manifestations and the laboratory changes were less severe than in patient 1.

Both patients were treated with steroid therapy (60 mg/day), including pulse therapy (1000 mg/day for 3 days). Patient 1 showed a good response to treatment, with her clinical (myopathy and IP) and laboratory findings improving gradually. In contrast, although the pulse therapy was repeatedly performed in patient 2, she showed steroid-resistance, and her IP became dramatically worse. She died of severe respiratory failure due to exacerbation of IP despite intensive treatment including cyclosporine and cyclophosphamide.

In order to investigate changes of gene expression by the peripheral blood mononuclear cells (PBMC) of these patients, we performed comprehensive gene analyses using the DNA microarray method, as described previously (2). After receiving the approval of our ethics committee, samples were obtained with the written consent of the patients. Briefly, RNA was purified from PBMC of patients 1 and 2, and also from PBMC of a healthy control (a 30-year-old woman) using an RNeasy Mini Kit (Qiagen Inc., Valencia, CA). RNA quality was checked by an Agilent 2100 Bioanalyzer and an RNA 6000 Nano LabChip Kit (Agilent Technologies, Palo Alto, CA). Then, fluorescence-labeled RNA targets were synthesized using an amino-allyl RNA amplification Kit (Sigma-Aldrich Co., St. Louis, MO). These labeled targets were hybridized to an AceGene Human 30K 1 Oligo Chip Version (DNA Chip Research Inc., Kanagawa, Japan), and hybridized images were scanned by using a ScanArrray Lite (PerkinElmer, Inc., Wellesley, MA). The signal intensity data were quantified with ScanArray Express Software (PerkinElmer, Inc.). The analyses were done with Microsoft Excel (Microsoft, Redmond, WA) and Avadis (Strand Life Sciences, Bangalore, India). The results of gene analysis are summarized in Table I. Several previous studies have found a marked increase of ICAM-1 expression in the muscle vessels of DM patients, suggesting a possible important role of ICAM-1 in the pathophysiology of inflammatory myopathy (3). As shown in Table IA, an ICAM-1 gene expression was dramatically decreased by treatment in patient 1 (Table IA). This finding seemed to suggest that ICAM-1-mediated processes may cause muscle lesions, although our data were obtained from PBMC rather than muscle tissue. Some cytokine genes, such as IL-4 and IL-1 (F5 and F6) (which are classified as T helper 2 cytokines; Th-2), were decreased after treatment, while expression of IL-2 (a Th-1 gene) was increased (Table IA and B). These changes may support the concept that DM is generally considered to be a disease related to Th-2 dominance (4). The genes showing higher expression in patient 2 compared with patient 1 are shown in Table I (C). Expression of gene for chemokine-related ligand and/or receptors (such as CCL21, CCL24, CCL25, and CCR1) was higher in patient 2 than those in patient 1, as well as expression of the TLR-1, IL-1 F5, and TNF receptor-related (TNFRSF17 and TNFRSF5) genes. This may have occurred because several chemokines play an important role in the infiltration of inflammatory cells into the muscles of patients with DM (5, 6). Among these genes, those from the IL-1 family (especially IL-1 F5) may be important for understanding disease activity and/or the prognosis, because expression of this gene decreased after therapy (Table IA) and patient 2 showed higher expression compared with patient 1 (Table IC). Although the precise role of IL-1 in the pathogenesis of DM is unclear, this cytokine seems to contribute to the development of DM in addition to ICAM-1, which was previously reported to have a role (7). Thus, our study suggested that expression of the ICAM-1, IL-1 (F5), and chemokine-related ligand/receptor genes by PBMC may be important for understanding the effect of treatment and/or prognosis of DM associated with IP. It is also interesting that immunohistochemical studies have revealed prominent staining of ICAM-1 and IL-1 in the skeletal muscles of DM patients (3, 7). Furthermore, the phenomenon of higher expressions of TNF receptor-related genes in patient 2 than patient 1 (Table IC)

Table I. Gene profile of 2 patients with dermatomyosistis: influence of treatment **(A, B)** and prognosis **(C)**.

Parient 1[1]			
(A) Decreased expression after therapy symbol (gene name)	E.I.[2]	**(B)** Increased expression after therapies symbol (gene name)	E.I.
IL-4 (interleukin-4)	0.12	CD6 (CD6 antigen)	6.87
CCBP2 chemokine-binding protein 2)	0.13	IL-2 (interleukin-2)	6.55
ICAM-1 (intercellular adhesion molecule-1)	0.15	IL-1R (interleukin-1 receptor)	4.66
IL-1F5 (interleukin-1 family 5)	0.2	CD164 (CD164 antigen)	4.18
IL-1F6 (interleukin-1 family 6)	0.28	CCL24 (chemokine ligand 24)	4.13

(C) Patient 1 < Patient 2[3]		
	symbol (gene name)	E.I.[4]
	TNFRSF17 (tumor necrosis factor receptor superfamily 17)	26.33
	TLR-1 (toll-like receptor-1)	18.11
	IL-1F5 (interleukin-1 family 5)	8.63
	CCL21 (chemokine ligand 21)	9.09
	CCL24 (chemokine ligand 24)	8.32
	TNFRSF5 (tumor necrosis factor receptor super family 5)	6.83
	CCR1 (chemokine receptor-1)	5.14
	CCL25 (chemokine ligand 25)	4.04

[1]Before treatment, the genes listed in **(A)** showed increased expression and those listed in **(B)** were decreased in patient 1 compared with the control.

[2]The expression index (E.I.) was calculated as the level of gene expression after treatment divided by that before treatment in patient 1.

[3]Higher gene expression in patients 2 compared with patient 1.

[4]The E.I. was calculated as the level of gene expression in patient 2 divided by the level in patient 1.

may be concerned with the recent findings of therapeutic efficacy of anti-TNF agents to refractory DM (8).

Although further studies in more DM patients are required to confirm our results, comprehensive gene analysis appears to be useful to elucidate the pathophysiologic mechanisms of autoimmune diseases such as DM as well as to understand disease activity and/or the prognosis.

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