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Expression of cytokine genes and presence of enteroviral genomic RNA in endomyocardial biopsy tissues of myocarditis and dilated cardiomyopathy

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Abstract Viral infection, especially by enteroviruses, has been considered to be the most common cause of myocarditis, which may progress to dilated cardiomyopathy (DCM). Although the mechanism of progression remains uncertain, a cytokine-associated injury of myocytes has been proposed. Using reverse transcriptase polymerase chain reaction (RT-PCR), we examined the expression of interleukin 1β (IL-1β), IL-6, IL-8 and tumour necrosis factor alpha (TNF-α) and the presence of enteroviral genomic RNA in endomyocardial biopsy tissues obtained from patients with myocarditis and DCM. We examined endomyocardial biopsy tissues obtained from 6 patients with myocarditis, 21 with DCM and 15 with non-infectious cardiac diseases as controls. In patients with myocarditis, endomyocardial biopsy was performed twice at an interval of 1 month to 8 years after the onset of myocarditis. We used RT-PCR to detect IL-1β, IL-6, IL-8 and TNF-α genes expression and nested RT-PCR (nRT-PCR) to detect enteroviral genomic RNA. IL-1β, IL-6, IL-8 and TNF-α genes were expressed in 100% (6/6) and enteroviral genomic RNA in 67% (4/6) of myocarditis patients at the first biopsy. At the second biopsy, IL-1β, IL-6, IL-8 and TNF-α genes were expressed in none, 50% (3/6), 67% (4/6) and 67% (4/6), respectively, and enteroviral genomic RNA in 67% (4/6). Four patients with myocarditis, in whom IL-8 and TNF-α genes and enteroviral genomic RNA were detected, progressed to DCM at the second biopsy. IL-1β, IL-6, IL-8 and TNF-α genes were expressed in none, 24% (5/21), 38% (8/21), 57% (12/21) of DCM patients, respectively. Enteroviral genomic RNA was detected in 43% (9/21) of DCM. Neither cytokine expression nor enteroviral genomic RNA were detected in the controls. The high incidence of cytokines, especially IL-6, IL-8 and TNF-α, expression in myocarditis and DCM, which might be induced by enteroviral infection, suggests that cytokines play an important role in myocytic damage leading to DCM.

Key words Enterovirus · Myocarditis · Dilated cardiomyopathy · Cytokine · Reverse transcriptase polymerase chain reaction

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Introduction

Coxsackievirus (CV), especially group B, are the most commonly identified agents in the aetiology of acute myocarditis in humans [29]. Dilated cardiomyopathy (DCM) is a myocardial disorder of unknown aetiology, defined by dilatation of the left ventricle, either alone or with right ventricular dilatation, in the absence of coronary, valvular, pericardial or congenital diseases [20], which has been reported to be the probable sequela of long-term or recurrent viral myocarditis [1, 2, 6, 29]. DCM is accompanied by impairment of ventricular function [20]. We have previously demonstrated the persistence of enteroviral genomic RNA in endomyocardial tissues from patients with myocarditis and DCM by polmyerase chain reaction (PCR) with slot blot hybridization [22, 23] and examined the progression from myocarditis to DCM in patients with persistent enteroviral genomic RNA [22]. Recently, other investigators have proposed that cell-mediated immunity plays an important role in the pathogenesis of myocardial cell damage during this process [1, 2, 6, 24]. In a murine model of CV B3-induced myocarditis, lipopolysaccharide increases the cytokine concentration as well as MHC class I and II expression in myocardial tissues [15]. Sato et al. [21] reported that myocardial cell damage in athymic C3H/HeN mice of CVB3-induced myocarditis was associated with the expression of tumour necrosis factor-α (TNF-α) and other cytokines. In addition, Henke et al. reported that CVB 3 can induce interleukin (IL)-1, IL-6 and TNF-α

from human monocytes without affecting their viability, and suggested the involvement of cytokines in the myocardial cell damage induced by persistent CVB3 infection could lead to cardiomyopathy [9]. However, the mechanism of the progression of myocarditis to DCM remains uncertain. It is not clear whether human DCM is due to a direct or indirect cytopathic effect of the viruses upon myocardial cells, or due to an infection-related immune or other response to myocardial cells.

In this study, we investigated cytokine (IL-1β, IL-6, IL-8 and TNF-α) genes expression and the presence of enteroviral genomic RNA in endomyocardial biopsy tissues obtained from patients with myocarditis and DCM using the reverse transcriptase polymerase chain reaction (RT-PCR) and nested RT-PCR (nRT-PCR). The correlation between cytokine expression and clinicopathological findings was studied with a long-term follow-up including repeated endomyocardial biopsies in patients with myocarditis.

Materials and methods

We examined 48 endomyocardial biopsy tissues obtained from 6 patients with myocarditis, 21 patients with DCM and 15 control patients with non-infectious cardiac diseases. Patients with myocarditis included 3 males and 3 females with a mean age of 45 years (range, 20 to 70 years), and endomyocardial biopsy was performed twice at an interval of 2 months to 8 years from the onset of myocarditis. Patients with DCM included 14 males and 7 females with a mean age of 60 years (range, 28 to 76 years). Myocarditis was diagnosed according to the Dallas criteria [3]. DCM was diagnosed according to the criteria of the WHO/ISFC task force, [20] and on the basis of the following two-dimensional echocardiographic findings: a left ventricular ejection fraction of less than 50% and a left ventricular end-systolic diameter of more than 50 mm. Patients were excluded if there was any angiographic evidence of coronary heart disease, ischaemic changes during exercise testing, systemic hypertension, concomitant systemic or endocrine diseases that might cause impairment of left ventricular function, or excessive alcohol consumption. Controls (8 males and 7 females with a mean age of 49 years; range, 20 to 80 years) consisted of 6 patients with hypertensive heart diseases, 6 with a history of ventricular tachycardia, and 3 with sick sinus syndrome.

Right and left cardiac catheterization, selective coronary angiography, left ventriculography, and two dimensional echocardiography were performed in all patients. Right ventricular endomyocardial tissues were obtained from all patients with a bioptome (Type A, Yufu Corp., Tokyo, Japan) through the femoral venous approach. Four tissue samples were obtained from each patient. Three of these were fixed in 10% formalin, embedded in paraffin after dehydration in a graded alcohol series, and cut into 4 μm-thick sections. Sections were stained with haematoxylin and eosin. In DCM, each section was examined independently by three investigators who did not know the clinical features of the patient. The histological changes were graded on a scale from 0 (not present) to 3 (severe); hypertrophy, disarrangement and degeneration of myocytes, myocardial fibrosis and cellular infiltration, as previously reported [23]. The remaining tissue sample was frozen immediately after biopsy at −80° C and used for PCR.

CVB-specific neutralizing antibody titer and the titre of CVB-specific serum IgM were determined using a microtitre method and an IgM capture enzyme linked immunosorbent assay (ELISA), respectively [8, 30].

Peripheral blood monocytes were prepared from normal blood donors. After centrifugation, monocytes were washed and resuspended in AIM-R medium (GIBCO Lab., N.Y., USA) supplemented with 2 μg of anti-human Leu-4 [CD3] (Becton Dickinson, Calif., USA). Those monocytes were allowed to adhere to tissue culture flasks for 24 h at 37° C in a humidified 5% CO_2-95% air atmosphere. Subsequently, these cells were used as positive controls for the detection of cytokines expression.

CVB3-positive HeLa cells were used as a positive control for the presence of enteroviral genomic RNA.

Total RNA was extracted by the acid guanidinium thiocyanate-phenol-chloroform method from HeLa cells, cultured monocytes and endomyocardial biopsy tissues [7]. After the biopsy tissues, cultured cells and cultured monocytes were homogenized in guanidine thiocyanate, the total RNA was extracted with phenol-chloroform, then recovered with isopropanol. The purity of extracted total RNA was confirmed by determining the ratio of absorbance at 260 nm to that at 280 nm. The extracted RNA was diluted to 200 ng/μl with double-distilled water (DDW).

The expression of cytokine genes and the presence of enteroviral genomic RNA were analysed using the RT-PCR method followed by Southern blot hybridization. Table 1 shows the sequences of primers and probes. Sets of primers and a probe for enterovi-

Table 1 Sequences of primers and probes

Cytokines		Sequence (5'→3')	Fragment [Reference]
IL-1β	sense	GACCTGGACCTCTGCCCTCTG	408 bp [17]
	anti-sense	AGGTATTTTGTCATTACTTTC	
	probe	TATTTTGTCATTACTTTCTTCTCCTTGTACAAA	
		GGACATGGAGAACACCACTTGTTGCTC	
IL-6	sense	GTACCCCCAGGAGAAGAT	388 bp [31]
	anti-sense	CATTTGCCGAAGAGCCCTCA	
	probe	TTTTGTACTCATCTGCACAGCTCTGGCTTGTTC	
		CTCACTCTCAAATCTGTTCTGGAG	
IL-8	sense	GCTTTCTGATGGAAGAGAGC	585 bp [18]
	anti-sense	GGCACAGTGGAACAAGGACT	
	probe	CTTCAAAAACTTCTCCACAACCCTCTGCACCCA	
		GTTTTCCTTGGGGTCCAGACA	
TNF-α	sense	GCCTGTAGCCCATGTTGTAG	438 bp [25]
	anti-sense	AATGATCCCAAAGTAGACCTGCCC	
	probe	AAAGTCGAGATAGTCGGGCCGATTGATCTCAGC	
		GCTGAGTCGGTCATTCTTCTCCAGCTG	
β actin	sense	CCTGGCACCCAGCACAATGA	629 bp [19]
	anti-sense	TTGGGAAGGTTGGATGTTCG	
	probe	TTTGCGGTGGACGATGGAGGGGCCGGAATAGTA	
		ATACTCCTGCTTGCTGATCCACATCTG	

ral gemonic RNA, located in the VP4 region, were used as previously described [22, 23]. The primers and probes were synthesized by the phosphoramidite method using a DNA synthesizer (Milli-Gen/Biosearch, Bedford, Mass., USA).

RT-PCR was performed with an RNA-PCR kit (GeneAmp, Perkin Elmer-Cetus Corp., Norland, Conn., USA). The cDNA was synthesized from 200 ng of total RNA by reverse transcription at 42° C for 15 min using the anti-sense primer. The products were amplified with 45 cycles of PCR using a temperature cycler (Hybaid, Middlessex, UK). Each cycle consisted of denaturation at 94° C for 30 s, annealing at 55° C for 60 s, and extension at 72° C for 60 s. For detection of enteroviral genomic RNA, a second PCR was performed. Two microlitres of the first PCR product was transferred to a reaction buffer (50 mM KCl, 0.01% gelatin and 10 mM TRIS buffer, pH 8.3) containing 20 pmol of internal primers, 1 mM $MgCl_2$, 0.2 mM of deoxynucleotide triphosphate and 0.5 units of AmpliTaq DNA polymerase (Perkin Elmer-Cetus), then amplified with 45 cycles as in the first PCR.

The PCR products (10 µl) were electrophoresed on a 3% NuSieve 1% Seakem agarose gel (FMC Bioproducts, Rockland, Me., USA). DNA bands were visualized after ethidium bromide staining by exposure to ultraviolet light. Hae III-digested ϕx174 DNA (Toyobo, Osaka, Japan) was used as a size marker for electrophoresis. The DNA bands were denatured and transferred onto a nylon membrane (Hybond-N, Amersham, UK) using 0.4 M of NaOH as denaturant buffer and 20×SSC (0.3 M NaCl and 0.03 M sodium citrate at pH 7.0) as transferring buffer. The DNA bands were fixed on the membrane by a UV linker (Spectromics, Westbury, New York, USA). The membrane was then prehybridized in a solution of 50 mM TRIS-HCl, 1 M NaCl, 10% dextran sulphate, 1% sodium dodecyl sulphate (SDS) and sheared salmon sperm DNA at 65° C for 2 h. Hybridization was carried out in the same solution with a [γ^{32}P]-5'-end-labelled probe, prepared using a kit (MEGALABEL, Takara, Kyoto, Japan). After incubation for 16 h at 65° C, the membrane was washed three times at room temperature for 10 min, and twice at 65° C for 10 min in 2×SSC containing 0.1% SDS. The membrane was then autoradiographed at −80° C for 24 h.

All clinical results of DCM were expressed as mean values ±SE (standard error). The relationship of PCR results to histological grade was analysed using the Mann-Whitney rank-sum test.

Results

The total RNAs which were extracted from cultured monocytes and HeLa cells were used for RT- and nRT-PCR as positive controls. Expression of cytokines and β actin in cultured lymphocytes and enteroviral genomic RNA in HeLa cells were confirmed by Southern blot hybridization after PCR (Fig. 1).

In 15 control patients with various non-infectious cardiac diseases, neither expression of cytokine genes nor enteroviral genomic RNA were detected (Fig. 2). The expression of β actin was confirmed in all controls.

The expression of cytokines and enteroviral genomic RNA were detected in all patients with myocarditis at first biopsy (Fig. 3). At the second biopsy, IL-1β, IL-6, IL-8 and TNF-α genes were expressed in 16% (1/6), 50% (3/6), 67% (4/6) and 67% (4/6), respectively and enteroviral genomic RNA in 67% (4/6) (Fig. 3). Table 2 shows correlation between PCR results and clinicopathological findings. In four patients (patient 3, 4, 5 and 6) with active myocarditis at first biopsy (Fig. 4), IL-8, TNF-α and enteroviral genomic RNA were consistently detected at the first and second biopsies, although active myocarditis had resolved at the second biopsy (Fig. 5). The clinical course of those four patients were of DCM. In the remaining two patients, cytokine genes expression and enteroviral genomic RNA were disappeared at the second biopsy and clinico-

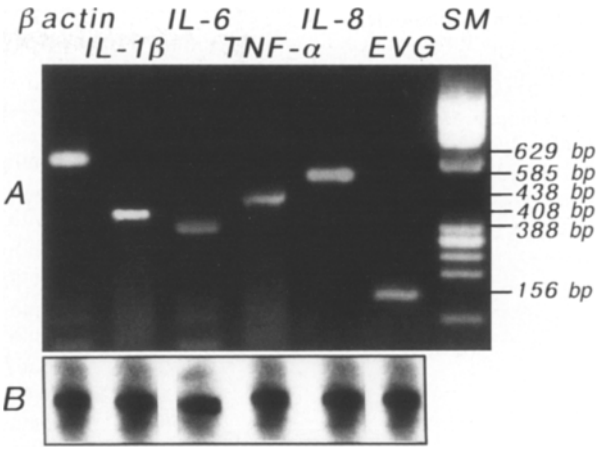

Fig. 1A, B Results of RT-PCR of β actin, IL-1β, IL-6, IL-8 and TNF-α genes and enteroviral genomic RNA of positive control. **A** Agarose gel electrophoresis; **B** Southern blot hybridization; *EVG*, enteroviral genomic RNA, *SM* size marker

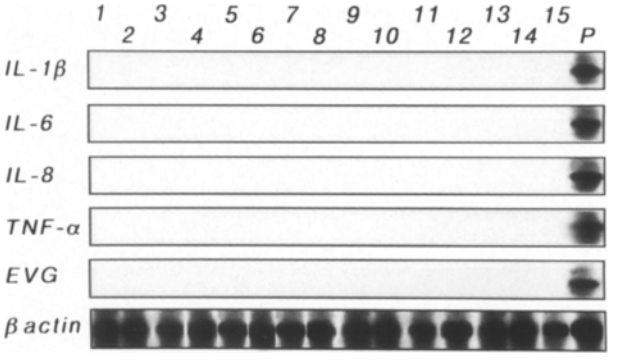

Fig. 2 Results of RT-PCR in biopsy tissues from 15 patients with controls. *EVG* Enteroviral genomic RNA, *P* positive control

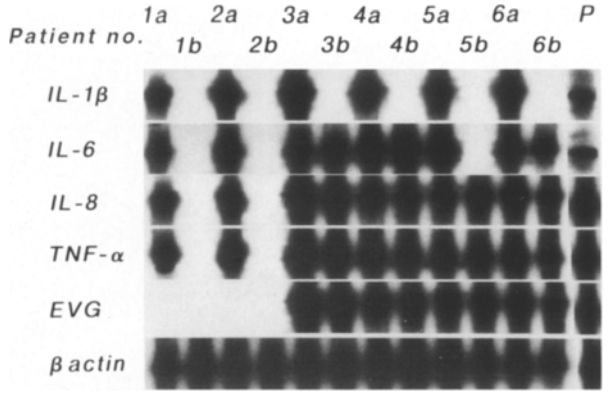

Fig. 3 Results of RT-PCR in 6 patients with myocarditis. *EVG* Enteroviral genomic RNA

Table 2 Clinicopathological findings and PCR results in myocarditis (*EVG* enteroviral genomic RNA, *a* first biopsy, *b* second biopsy, *M* male, *F* female, *MC* myocarditis, *DCM* dilated cardiomyopathy)

Patient	Age/sex	Time after onset	Clinical diagnosis	Diagnosis (Dallas criteria)	PCR results				
					IL-1β	IL-6	IL-8	TNF-α	EVG
1a	25/F	1 month	MC	Active MC	+	+	+	+	−
b		6 months	MC	Resolved MC	−	−	−	−	−
2a	43/F	3 days	Acute MC	Active MC	+	+	+	+	−
b		1 month	MC	Resolving MC	−	−	−	−	−
3a	69/M	2 months	Acute MC	Active MC	+	+	+	+	+
b		7 months	DCM	Resolved MC	−	+	+	+	+
4a	43/M	1 month	Acute MC	Active MC	+	+	+	+	+
b		3 months	DCM	Resolving MC	−	+	+	+	+
5a	20/M	1 month	MC	Active MC	+	+	+	+	+
b		6 months	DCM	Resolved MC	−	−	+	+	+
6a	70/F	2 months	MC	Active MC	+	+	+	+	+
b		8 years	DCM	Resolved MC	−	+	+	+	+

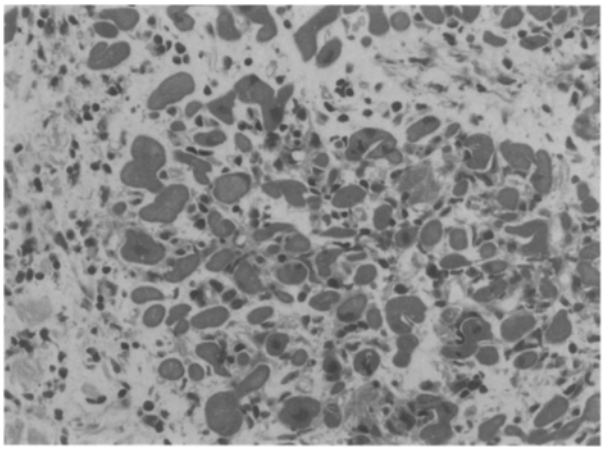

Fig. 4 Patient 5a. There is severe inflammatory infiltration with myocytic necrosis (HE, ×200)

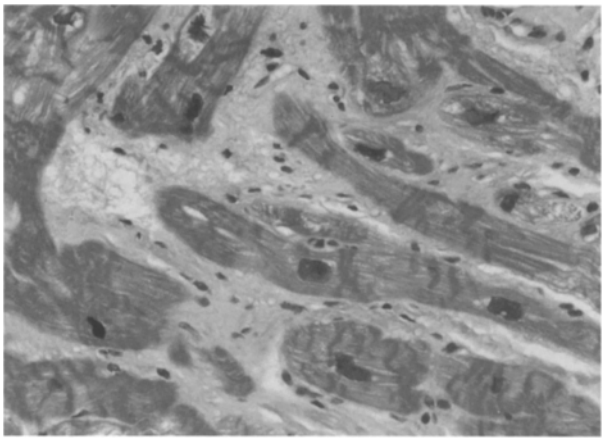

Fig. 5 Patient 5b. There is a mild interstitial fibrosis. Inflammatory cells are not evident (HE, ×400)

pathological findings revealed marked improvement of myocarditis.

Table 3 shows the PCR results. IL-1β, IL-6, IL-8 and TNF-α genes were expressed in none, 24% (5/21), 38% (8/21) and 57% (12/21) of DCM, respectively (Fig. 6).

Table 3 Clinical findings and PCR results in DCM (*EVG* enteroviral genomic RNA, *M* male, *F* female, *DCM* dilated cardiomyopathy)

Patient no.	Age/sex	PCR results				
		IL-1β	IL-6	IL-8	TNF-α	EVG
1	69/M	−	+	+	+	+
2	75/M	−	+	−	+	+
3	76/F	−	−	−	−	−
4	57/M	−	−	−	−	−
5	57/M	−	−	−	−	−
6	46/F	−	−	−	−	−
7	64/F	−	+	+	+	−
8	60/M	−	+	+	+	+
9	69/M	−	−	+	+	+
10	65/M	−	−	−	−	−
11	68/F	−	−	−	−	−
12	54/F	−	−	−	+	+
13	61/M	−	−	+	+	−
14	59/M	−	−	+	+	+
15	61/M	−	−	−	−	−
16	66/M	−	−	−	+	+
17	41/M	−	−	+	+	+
18	38/F	−	−	−	−	−
19	57/M	−	+	−	+	−
20	73/F	−	−	+	+	+
21	28/M	−	−	−	−	−

Enteroviral genomic RNA was detected in 43% (9/21) (Fig. 6). The expression of IL-8 and TNF-α and the presence of enteroviral genomic RNA were associated with myocardial fibrosis (Table 4). The mean histological grades in endomyocardial tissues, with and without IL-8, TNF-α genes expression and enteroviral genomic RNA were 1.4 versus 0.8 (P=0.04), 1.5 versus 0.5 (P=0.003) and 1.4 versus 0.9 (P=0.04) on myocardial fibrosis, respectively. Myocytic hypertrophy was more prominent in TNF-α-positive than TNF-α-negative tissues (Table 4). The mean histological grade with and without TNF-α gene expression was 1.4 versus 0.7 (P=0.03). By contrast, myocytic degeneration was more prominent in TNF-α and enteroviral genomic RNA-negative than RNA-positive findings (Table 4). The mean histological grades with and without TNF-α was 0.7 and 1.7, respec-

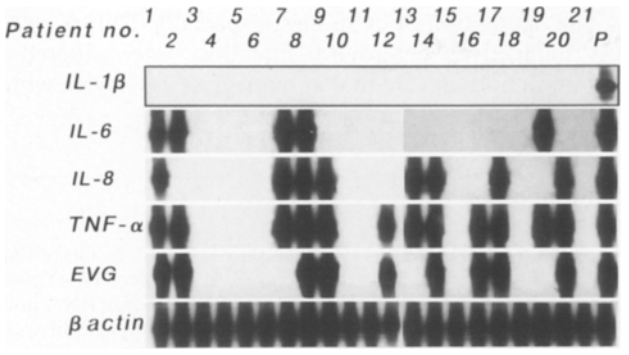

Fig. 6 Results of RT-PCR in 21 patients with DCM. *EVG* Enteroviral genomic RNA

Table 4 Histology of endomyocardial biopsy tissues in DCM (Grading scale: 0, not present; 1, mild; 2, moderate; 3, severe. *EVG* Enteroviral genomic RNA)

	IL-6	IL-8	TNF-α	EVG
Histological grade				
Myocytic hypertrophy				
Positive	1.5	1.3	1.4	1.4
Negative	0.9	1.0	0.7	0.9
p value	0.1	0.2	0.03	0.09
Degeneration				
Positive	1.2	1.2	0.7	0.7
Negative	1.1	1.1	1.7	1.3
p value	0.6	0.7	0.002	0.002
Disarrangement				
Positive	1.2	1.2	1.2	1.3
Negative	1.1	1.1	1.0	0.9
p value	0.6	0.7	0.4	0.1
Cellular infiltration				
Positive	1.0	1.1	1.1	0.9
Negative	1.1	1.0	1.0	1.2
p value	0.9	0.9	0.9	0.4
Extent of fibrosis				
Positive	1.5	1.4	1.5	1.4
Negative	1.0	0.8	0.5	0.9
p value	0.08	0.04	0.003	0.04

tively (P=0.002) and that with and without enteroviral genomic RNA was 0.7 versus 1.3 (P=0.002). In echocardiographic findings, there was no significant difference between positive and negative PCR results (Table 5).

All myocarditis, DCM and control patients were negative for CVB-specific neutralization and IgM antibodies in serum.

Discussion

Although a post-viral aetiology for DCM has been proposed and widely accepted, the actual role of the viral infection in DCM has not been well defined because detection of virus is difficult. Attempts to identify viruses by electron microscopy, to isolate viruses from myocardial tissues, and to detect enteroviral-specific antigens in myocardium have generally been unsuccessful [4, 29]. A

Table 5 Clinical characteristics in DCM (*EVG* enteroviral genomic RNA, *LVEF* left ventricular ejection fraction, *LVEDD* left ventricular endo-diastolic diameter, *LVESD* left ventricular endo-systolic diameter)

		IL-6	IL-8	TNF-α	EVG
LVEF (%)	Positive	30.0±3.8	28.8±3.5	25.8±3.1	26.4±3.5
	Negative	28.9±3.2	29.4±3.4	33.7±3.5	32.0±3.4
	p value	0.8	0.9	0.1	0.3
LVEDD (mm)	Positive	65.0±2.2	65.0±3.1	64.4±2.1	64.4±6.6
	Negative	64.8±2.5	64.8±2.1	65.5±2.9	65.3±2.5
	p value	0.9	0.9	0.8	0.8
LVESD (mm)	Positive	55.5±2.7	55.6±3.3	56.2±2.0	56.0±2.4
	Negative	55.2±2.2	55.1±2.0	54.1±2.9	54.6±2.5
	p value	0.9	0.9	0.6	0.7

promising molecular biological approach has recently been developed for detection of viral genomes; development of PCR has made it possible to detect viral nucleic acids in low amounts. In our previous studies and those by other groups, enteroviral genomic RNA or CVB3 RNA were detected at various rates in endomyocardial biopsy tissues from patients with DCM and myocarditis by PCR and hybridization assay [10, 11, 22, 23, 26]. However, it is not clear whether DCM is the result of a direct cytopathic effect of viruses upon myocardial cells or of infection- or host-induced immune responses against myocardial cells. In human myocarditis, myocytic damage is related to an impairment of cell-mediated and humoral immune responses during the chronic stage of myocarditis [1, 2, 6, 29]. In another model of CVB3-induced myocarditis, the persistent expression of intercellular adhesion molecule (ICAM)-1 and MHC antigens induced by cytokines (INF-γ and TNF-α) may cause further myocardial cell damages in a later phase of myocarditis [24]. Recently, Henke and his colleagues [9] have suggested that cytokines (TNF-α, IL-1β and IL-6) which cause myocytic damage leading to DCM, were released from monocytes and fibroblasts in response to CVB infection. Thus, the immune response to the infection would be an important factor in the development of DCM. However, to our knowledge, there have been no reports in which the expression of cytokines was studied in endomyocardial biopsy specimens from patients with myocarditis and DCM.

In the present study, although both cytokines (IL-1β, IL-6, IL-8 and TNF-α) and enteroviral genomic RNA were detected in four patients with myocarditis at the first biopsy (patient no. 3, 4, 5 and 6), the expression of cytokines (IL-6, IL-8 and TNF-α) and the presence of enteroviral genomic RNA was prolonged in the myocardium in three patients with myocarditis (patient no. 3, 4 and 6). In one patient (patient no. 5), IL-8, TNF-α and enteroviral genomic RNA were detected, but IL-6 disappeared at 6 months from onset. These patients showed progression from myocarditis to DCM after the active myocarditis resolved. In the remaining patients with myocarditis (patient no. 1 and 2), the expression of cytokine genes had disappeared at the second biopsy, and

clinicopathological findings improved, although cytokines (IL-1β, IL-6, IL-8 and TNF-α) were expressed at the first biopsy. This suggests that cytokines, especially IL-6, IL-8 and TNF-α are induced as parts of the immune response to enteroviral infection and play an important role in the progression from viral-induced myocarditis to DCM.

TNF-α was detected in twelve patients with DCM. Enteroviral genomic RNA, IL-8 and IL-6 were simultaneously detected in 9, 8 and 5 DCM patients with TNF-α expression, respectively. The high incidences of cytokine genes expressions, especially the TNF-α gene with the presence of enteroviral genomic RNA suggest that the persistent expression of viral-induced cytokines is the pathogenesis of DCM, progressed from myocarditis. Wee et al. [28] reported that viral RNA persists until 6 weeks after inoculation, with ventricular enlargement or dilatation. The pathology revealed only a few foci of cellular infiltration and was dominated by fibrosis and dystrophic calcification in this mouse model of virus-induced myocarditis. In another model, Kyu et al. [14] reported that viral genome was detected by PCR until 90 days after inoculation, and microscopic examination revealed diffuse fibrosis, but myocardial necrosis or cellular infiltration disappeared in the chronic stage. In our study of DCM, the major histological changes with the expression of cytokines and the presence of enteroviral genomic RNA revealed myocardial fibrosis and hypertrophy of myocytes similar to those in the chronic stage of murine viral-induced myocarditis. The histological changes in the endomyocardial tissue showing cytokine expression with enteroviral genomic RNA resembled the sequela of viral myocarditis, in which myocardial fibrosis and myocytic hypertrophy develop [12]. These findings strongly suggested that the histological findings of DCM with cytokine expression and demonstrable viral genome were similar to that in the myocarditis patient showing progression from myocarditis to DCM.

Although we did not examine the localization of cytokines and enteroviral genomic RNA in the present study, Kandolf and Hofschneider [11] reported that the enteroviral RNA and CVB3 cloned cDNA probe were localized in myocytes of human DCM using in situ hybridization. Proinflammatory mediators such as IL-1, IL-6 and TNF-α are produced by a number of cell types. In addition, IL-8 is produced by non-circulating extravascular cells such as fibroblasts in response to proinflammatory mediators such as IL-1 and TNF [16]. TNF-α has especially been reported to be a representive cytokine inducing cachaxia, cell damage, high fever and shock [5, 13, 27]. Therefore, we assume that the mechanisms of myocytic damage in DCM are as follows; at first, enteroviruses, such as CVB3, infect myocardial cells and acute myocarditis develops. Secondly, myocytes and other cells such as fibroblasts and inflammatory cells express cytokines during the acute stage of myocarditis and chronic myocarditis progresses to DCM as a result of long-term myocytic damage by persistent cytokine expression in an autocrine and/or paracrine effect.

The expression of cytokines, especially TNF-α, probably induced by enteroviral infection, is considered to play an important role in that damage of myocytes which leads to DCM.

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