

*Medicine**Cardiovascular Diseases fields*

Okayama University

Year 2008

Expression of monocyte chemoattractant  
protein-1 in idiopathic dilated  
cardiomyopathy

Makoto Kobayashi\*      Kazufumi Nakamura<sup>†</sup>      Kengo Fukushima Kusano<sup>‡</sup>  
Yoichi Nakamura\*\*      Keiko Ohta-Ogo<sup>††</sup>      Satoshi Nagase<sup>‡‡</sup>  
Satoru Sakuragi<sup>§</sup>      Tohru Ohe<sup>¶</sup>

\*Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

<sup>†</sup>Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, [ichibun@cc.okayama-u.ac.jp](mailto:ichibun@cc.okayama-u.ac.jp)

<sup>‡</sup>Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

\*\*aDepartment of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

<sup>††</sup>Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

<sup>‡‡</sup>aDepartment of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

<sup>§</sup>Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

<sup>¶</sup>Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

This paper is posted at eScholarship@OUDIR : Okayama University Digital Information Repository.

<http://escholarship.lib.okayama-u.ac.jp/cardiovascular.diseases/2>

**Expression of Monocyte Chemoattractant Protein-1 in Idiopathic Dilated  
Cardiomyopathy**

Makoto Kobayashi, MD; Kazufumi Nakamura, MD; Kengo Fukushima Kusano, MD;  
Yoichi Nakamura, MD; Keiko Ohta-Ogo, MD; Satoshi Nagase, MD; Satoru Sakuragi,  
MD; Tohru Ohe, MD

Department of Cardiovascular Medicine, Okayama University Graduate School of  
Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama  
700-8558, Japan.

Telephone: +81-86-235-7351, Fax: +81-86-235-7353,

Correspondence to Kazufumi Nakamura, MD

E-mail: [ichibun@cc.okayama-u.ac.jp](mailto:ichibun@cc.okayama-u.ac.jp)

991 words

## **Abstract**

Immunological factors have been involved in the pathogenesis of dilated cardiomyopathy (DCM). The cytotoxic action of macrophages is one of the main factors causing cardiac myocyte damage. Monocyte chemoattractant protein-1 (MCP-1) is a major signal for the accumulation of monocytes/macrophages. We examined whether MCP-1 was expressed in the myocardium of DCM patients and whether the expression level was correlated with the degree of impairment of cardiac function. The expression of MCP-1 in the myocardium was determined by immunohistochemistry in endomyocardial biopsy samples from 13 patients. The expression of MCP-1 was found in all myocardial samples from DCM patients but not in those from control subjects. Positive staining for MCP-1 was distinct in cardiac myocytes, interstitium and infiltrating cells. Semi-quantitative analysis revealed that the expression of MCP-1 was inversely correlated with left ventricular ejection fraction. In conclusions, the expression level of MCP-1 in the myocardium was correlated with the degree of impairment of cardiac function in patients with DCM.

**Key Words :** cardiomyopathy, immunohistochemistry, monocyte

## **1. Introduction**

Immunological factors have been involved in the pathogenesis of dilated cardiomyopathy (DCM). In particular, the cytotoxic action of mononuclear cells is thought to be an important factor causing cardiac myocyte damage. Infiltration of mononuclear cells such as macrophages has been demonstrated in the hearts of patients with DCM [1]. Macrophages release various proinflammatory cytokines and generate reactive oxygen species, which have various effects such as causing cardiac myocyte injury, hypertrophy and apoptosis [2].

Monocyte chemoattractant protein-1 (MCP-1) is a major signal for the accumulation of monocytes/macrophages in several diseases [3]. Aukrust et al. reported that circulating levels of MCP-1 were increased in patients with congestive heart failure and that the levels were inversely correlated with left ventricular ejection fraction (LVEF) [3]. However, the expression of MCP-1 in failing hearts remains uncertain. Therefore, we examined whether MCP-1 was expressed in the myocardium of DCM patients and whether the expression level was correlated with the degree of impairment of cardiac function.

## **2. Methods**

### *2.1. Subjects*

The patient population studied comprised 20 consecutive patients (13 men and 7 women; mean age  $54 \pm 11$  years) admitted to Okayama (Japan) University Hospital between 1998 and 2000.

### *2.2. MCP-1 assay*

Serum levels of MCP-1 were measured using a human MCP-1 ELISA system

(Amersham Pharmacia Biotech, UK). Immunoenzymatic staining in endomyocardial biopsy samples obtained from 13 patients with DCM was performed using a DAKO LSAB System (Dako). Mouse monoclonal anti-human MCP-1 antibody (1:1000 dilution, American Research Products, Inc.) or anti-human CD68 antibody (1:100 dilution, DAKO) was added. The intensity of the immunostaining of MCP-1 was graded semiquantitatively on a four-point scale from 0 to 3 in randomly selected 5 separate high-power fields (x200) from 3 or 4 sections per patient. Grade 0 indicated no staining; grade 1, positive immunostaining in < 25% of the cells; grade 2, positivity of 25% to 50% of the cells; and grade 3, positivity of >50% of the cells.

### **3. Results**

The serum levels of MCP-1 in 20 patients with DCM were significantly elevated compared with those in healthy 33 control subjects ( $226 \pm 54$  versus  $176 \pm 32$  pg/mL,  $p < 0.0001$ ). Expression of MCP-1 was found in all myocardial biopsy samples from DCM patients but not in those from control subjects. Positive immunohistochemical staining for MCP-1 was distinct in cardiac myocytes (Fig. 1B), interstitium and infiltrating cells (Fig. 1C). Semi-quantitative analysis revealed that the expression of MCP-1 in the myocardium was inversely correlated with LVEF ( $r = -0.669$ ,  $p < 0.05$ ) (Fig. 2). To determine the chemotactic effect of MCP-1 in the myocardium, we calculated the numbers of macrophages that were positively stained with anti-human CD68 antibody in the endomyocardial biopsy samples. The mean number of macrophages in the myocardial biopsy samples from DCM patients was 12-fold higher than that in samples from control subjects ( $1.2 \pm 2.1$  versus  $0.1 \pm 0.3/\text{mm}^2$ ,  $p < 0.05$ ).

#### **4. Discussion**

The major new finding of this work is that MCP-1 was expressed in the myocardium of DCM patients and that the expression level was inversely correlated with LVEF.

Seino et al. reported the detection of MCP-1 mRNA expression in human endomyocardial biopsy specimens from DCM patients by polymerase chain reaction analysis [4]. The present study is the first to demonstrate expression of MCP-1 protein in the cardiac interstitium and myocytes of DCM patients. Furthermore, the number of infiltrating macrophages was increased in the myocardium of DCM patients. MCP-1 may be an important factor in the pathogenesis of DCM.

## References

- [1] Holzinger C, Schollhammer A, Imhof M, et al. Phenotypic patterns of mononuclear cells in dilated cardiomyopathy. *Circulation*. 1995;92:2876-2885.
- [2] Nakamura K, Fushimi K, Kouchi H, et al. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-alpha and angiotensin II. *Circulation*. 1998;98:794-799.
- [3] Aukrust P, Ueland T, Muller F, et al. Elevated circulating levels of C-C chemokines in patients with congestive heart failure. *Circulation*. 1998;97:1136-1143.
- [4] Seino Y, Ikeda U, Sekiguchi H, et al. Expression of leukocyte chemotactic cytokines in myocardial tissue. *Cytokine*. 1995;7:301-304.

## FIGURE LEGENDS

Figure 1. Immunohistochemical examination of MCP-1 in myocardial biopsy samples. A, Immunohistochemical staining in a myocardial biopsy sample from a control subject. B, Positive immunohistochemical staining (brown) in a myocardial biopsy sample from a DCM patient. Bar = 100  $\mu$ m. C, Positive staining (brown) in the interstitium and infiltrating cells. Bar = 50  $\mu$ m. D and E, Negative immunohistochemical staining incubated without a primary antibody in a myocardial biopsy sample from a DCM patient.

Figure 2. Correlation between LVEF and the expression levels of MCP-1 in myocardial biopsy samples from 13 DCM patients.



**Figure1**  
[Click here to download high resolution image](#)

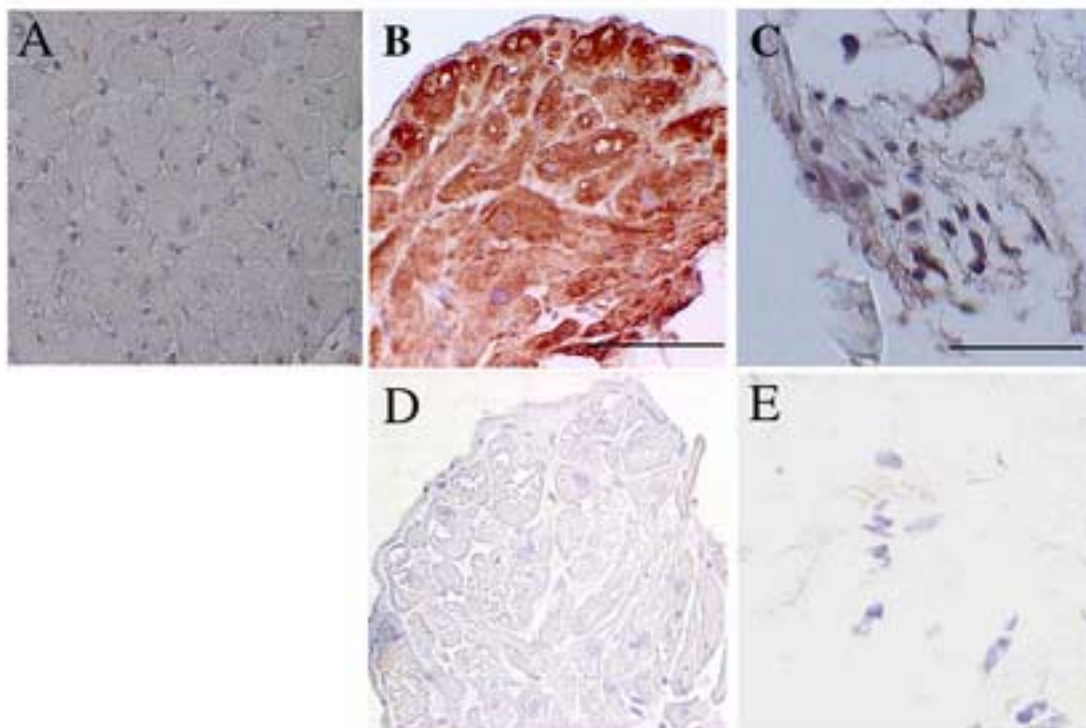


Figure 2

[Click here to download high resolution image](#)

