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Myocardial CD36 expression and fatty acid accumulation in patients with type I and II CD36 deficiency

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Long-chain fatty acids (LCFA) are one of the major cardiac energy substrates, so understanding LCFA metabolism may help in elucidating the mechanisms of various heart diseases. CD36 is a multifunctional membrane glycoprotein that acts not only as a receptor for thrombospondin, collagen and oxidized low density lipoprotein but also as a receptor for LCFA. We investigated the relationship between CD36 expression in myocardial capillary endothelial cells and myocardial LCFA uptake in patients with CD36 deficiency. We analyzed CD36 expression in blood cells from 250 patients with heart diseases by means of a flow cytometer. In 218 patients, myocardial LCFA scintigraphy was performed with ^{123}I-β-methyl-p-iodophenyl pentadecanoic acid (BMIPP). In 5 patients, myocardial capillary endothelial cells were examined immunohistochemically for CD36 expression. Eleven patients (4%) showed signs of type I CD36 deficiency (neither platelets nor monocytes expressed CD36). Twenty patients (8%) had type II CD36 deficiency (monocytes expressed CD36 but platelets did not). In all 11 patients with type I CD36 deficiency, no BMIPP accumulation was observed in the heart, but in 13 patients with type II CD36 deficiency, BMIPP accumulation in the heart was focally reduced, but there were no patients without BMIPP accumulation in the heart. Although the myocardial capillary endothelial cells from two CD36-positive patients expressed CD36, those from two patients with type I CD36 deficiency did not. In a patient with type II CD36 deficiency, some capillary endothelial cells displayed patchy CD36 expression.

CD36 deficiency was documented in 31 (12%) patients with heart diseases. Because CD36 was not expressed in the myocardial capillary endothelial cells in patients with type I CD36 deficiency, type I CD36 deficiency is closely related to lack of myocardial LCFA accumulation and metabolism in the myocardium.

Key words: CD36 deficiency, fatty acid metabolism, ^{123}I-BMIPP, heart disease, cardiomyopathy

INTRODUCTION

BECAUSE LONG-CHAIN FATTY ACIDS (LCFA) are one of the major cardiac energy substrates, understanding LCFA metabolism may help in elucidating the mechanisms of various heart diseases. Reduced myocardial LCFA uptake or a lack of it has been demonstrated in some patients with hypertrophic cardiomyopathy (HCM), dilated

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Table 1 Type I and II CD36 deficiency

Case	Age	Sex	Clinical diagnosis	^{123}I-BMIPP	^{201}TlCl
(A) type I					
1	57	M	Dilated phase HCM	no uptake	uptake (+)
2	66	F	Inferior OMI, DM	no uptake	uptake (+)
3	70	M	Subendocardial infarction	no uptake	uptake (+)
4	52	F	Angina pectoris, CABG	no uptake	uptake (+)
5	61	M	Vasospastic angina	no uptake	uptake (+)
6	65	M	DCM	no uptake	uptake (+)
7	57	M	HCM	no uptake	uptake (+)
8	62	F	Ischemic heart disease	no uptake	uptake (+)
9	36	M	Hypertension	no uptake	uptake (+)
10	30	F	HCM	no uptake	uptake (+)
11	67	M	Vasospastic angina	no uptake	uptake (+)
(B) type II					
12	66	M	DCM	uptake (+)	uptake (+)
13	84	F	Angina pectoris	uptake (+)	uptake (+)
14	71	M	HCM	uptake (+)	uptake (+)
15	49	M	Vasospastic angina	uptake (+)	uptake (+)
16	76	M	Ischemic heart disease	uptake (+)	uptake (+)
17	70	M	Ischemic heart disease, SSS	uptake (+)	uptake (+)
18	72	M	Vasospastic angina	uptake (+)	uptake (+)
19	66	M	Hypertension, Hyperlipidemia	not done	not done
20	71	M	Angina pectoris	uptake (+)	uptake (+)
21	73	M	HCM	uptake (+)	uptake (+)
22	60	M	Vasospastic angina	uptake (+)	uptake (+)
23	83	F	Valvular heart disease	uptake (+)	uptake (+)
24	58	F	HCM	uptake (+)	uptake (+)
25	75	F	OMI	uptake (+)	uptake (+)
26	68	F	Hypertension	not done	not done
27	66	F	Ischemic heart disease	not done	uptake (+)
28	57	M	Valvular heart disease	not done	uptake (+)
29	62	F	Arrhythmia	not done	uptake (+)
30	62	M	Vasospastic angina	not done	uptake (+)
31	77	F	Hypertension	not done	not done

^{123}I-BMIPP = ^{123}I-β-methyl-p-iodophenyl pentadecanoic acid, HCM = hypertrophic cardiomyopathy, OMI = old myocardial infarction, DM = diabetes mellitus, CABG = A-C bypass graft, DCM = dilated cardiomyopathy, SSS = sick sinus syndrome

cardiomyopathy (DCM), old myocardial infarction (OMI) and other heart diseases.[1,2]

CD36 is a multifunctional membrane glycoprotein that acts not only as a receptor for thrombospondin, collagen and oxidized low density lipoprotein but also as a receptor of LCFA.[3–6] CD36 is unique in that it is highly expressed in adipocytes and mammary secretory epithelial cells, both of which are actively involved in fatty acid uptake, and the synthesis, storage and secretion of triacylglycerol. CD36 is also expressed in capillary endothelial cells of cardiac and skeletal muscle.[7] Since cardiac and skeletal muscles, but not brain tissue, are highly oxidative and capable of oxidizing large amounts of LCFA, it has been hypothesized that CD36 expression in capillary endothelial cells is related to parenchymal cell lipid metabolism. CD36 acts as a direct signal transducer and a specific ligand of LCFA.[7]

In the present study, we investigated CD36 expression on blood cells in patients with several heart diseases, as well as the relationship of CD36 expression in myocardial capillary endothelial cells and myocardial LCFA uptake.

MATERIALS AND METHODS

Patients

We studied 250 patients, 142 males and 108 females with a mean age of 62 years (SD = ± 5, range 30 to 84), with various cardiac disorders. All 44 patients with HCM showed signs of myocardial hypertrophy based on the echocardiographic demonstration of an interventricular septum wall thickness greater than 13 mm and asymmetrical septal hypertrophy with a septal/posterior wall ratio > 1.3, along with the absence of other cardiac or systemic diseases capable of producing such hypertrophy. All 26 patients with DCM had impaired left and/or right ventricular systolic function with cardiac enlargement and

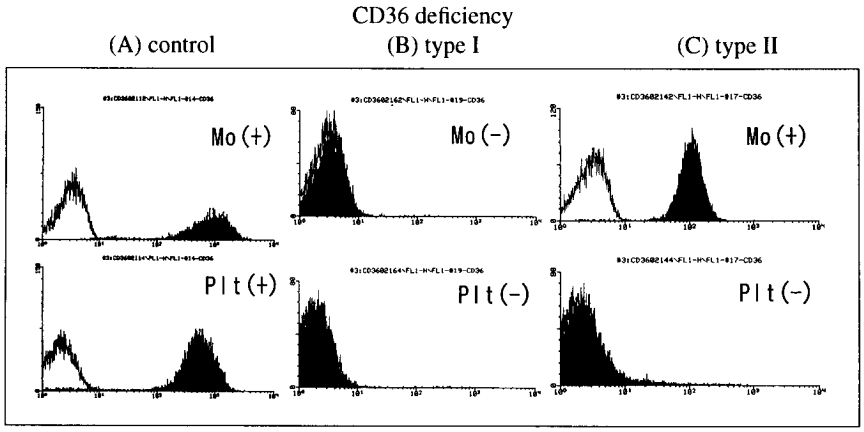


Fig. 1 CD36 expression in blood cells. Control patient: Platelets and monocytes expressed CD36. Patient with type I CD36 deficiency: Platelets and monocytes did not express CD36. Patient with type II CD36 deficiency: Monocytes expressed CD36, while platelets did not.

symptoms of congestive heart failure. Ninety-six subjects, consisting of 36 patients with OMI and 60 with angina pectoris, were studied. The diagnoses were based on a typical history, electrocardiographic changes, coronary arteriography and/or a significant increase in myocardial serum enzymes. The remaining 84 patients with heart diseases included 24 patients with arrhythmia, 19 with hypertension, 28 with valvular heart diseases and 13 with congenital heart diseases.

Written informed consent was obtained from all patients prior to all examinations. This study protocol was in agreement with the guidelines of the ethical committee at our institution.

Flow cytometric analysis of CD36 expression

Peripheral blood from patients drawn into EDTA-2Na tubes was centrifuged at 700 × g for 10 min to obtain platelet-rich plasma (PRP). Aliquots of 100 μl of PRP were washed once with washing buffer (PBS supplemented with 0.1 human serum albumin and 0.4% citrate), incubated with FITC-conjugated anti-CD36 (Immunotech, U.S.A.) antibody or control IgG1-FITC (Becton Dickinson, U.S.A.) for 30 min on ice, washed once with washing buffer, then analyzed with a FACScan™ flow cytometer (Becton Dickinson). The acquisition rate was set on platelet area with a light scattergram. Peripheral blood mononuclear cells were isolated by Lymphoprep (1.077, Nicomed Pharma AS, Norway) density centrifugation. Light density cells were obtained, washed with a buffer, and aliquots (1×10^6 cells/tube) were stained simultaneously with PE-conjugated anti-CD14 (Leu-M3, Becton Dickinson) and FITC-conjugated anti-CD36 or control IgG1-FITC. CD14-positive monocytes were acquired with a gate on a dot plotgram (CD14-PE vs. SSC), and the CD36-FITC fluorescence was analyzed. Patients with CD36 deficiency were divided into 2 groups: in type I there was no CD36 expression either in platelets or monocytes, but in type II monocytes expressed CD36 despite its lack in platelets.[8,9]

Myocardial LCFA uptake

Myocardial LCFA uptake and myocardial perfusion were evaluated with ^{123}I-β-methyl-p-iodophenyl pentadecanoic acid (BMIPP, Nihon Medi-Physics, Japan), a radiolabeled LCFA analog, and ^{201}TlCl, respectively.[8]

After an overnight fast, BMIPP was administered intravenously. Dynamic scan was carried out every second from 1 to 12 seconds after BMIPP injection. Planar and single photon emission computed tomography (SPECT) imagings were carried out at 15 min (early imaging) and at 4 hours (delayed imaging) after BMIPP injection. If myocardial BMIPP accumulation was severely decreased and no SPECT image could be obtained, we decided that there was a lack of myocardial BMIPP accumulation. ^{201}TlCl SPECT imaging was performed on another day.

The SPECT system used in this study consisted of a single head, large field digital γ-camera equipped with a general purpose, low energy, parallel hole collimator (ZLC-D-ORBITER, Siemens, German) connected to a microcomputer (SCINTIPAK 24000, Shimadzu Co., Japan). Both image sequences consisted of 32 projections with a 64 × 64 matrix aquired for 40 seconds (BMIPP) and 30 seconds (^{201}TlCl) over a 180° circular orbit, from 30° right anterior oblique to 60° left posterior oblique.

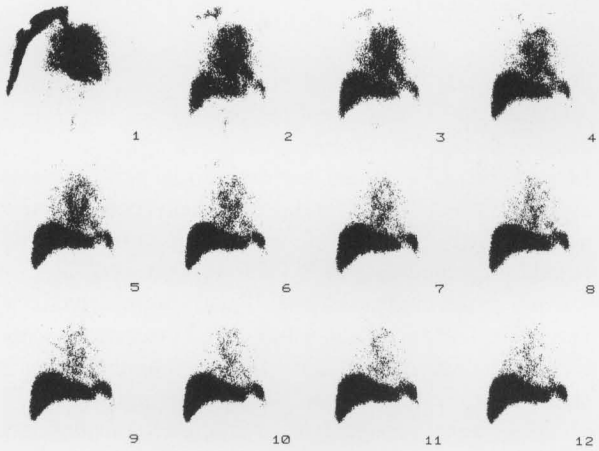
Cardiac catheterization and endomyocardial biopsy

On cardiac catheter examinations, endocardial biopsy specimens were obtained from the left ventricular lateral wall after left ventriculography and coronary angiography from 62 patients with HCM and DCM. Five biopsy specimens were stained for histological and immunohistochemical examination.

Immunohistochemistry

Endomyocardial tissues were embedded in Tissue-Tek^R O.C.T. compound (Sakura, Japan), frozen in dry ice-

acetone, and cut into sections 6 μm-thick. Sections on slide glasses were fixed in 2% paraformaldehyde for 10 min. After inhibition of endogenous peroxidase activity with methanol supplemented with 10% hydrogen peroxide for 60 min, we performed immunohistochemical analysis with anti-CD36 or anti-CD34 (for capillary endothelial cells) mouse monoclonal antibodies (Immunotech) at 4°C overnight. After washing with PBS, the tissues were incubated with biotinylated rabbit anti-mouse immunoglobulin and then streptavidin-conjugated peroxidase (Nichirei, Japan) for 1 to 2 hours at room temperature. After visualization with 3,3′-diaminobenzidine (Dojin Chemical Co., Japan), the tissues were counter stained with Mayer-Hematoxylin.



^{123}I-BMIPP

Fig. 2 Long-chain fatty acid myocardial scintigrams (^{123}I-BMIPP, dynamic images, Case 3). There was no myocardial ^{123}I-BMIPP accumulation in the patient of type I CD36 deficiency at 1–12 seconds after ^{123}I-BMIPP injection.

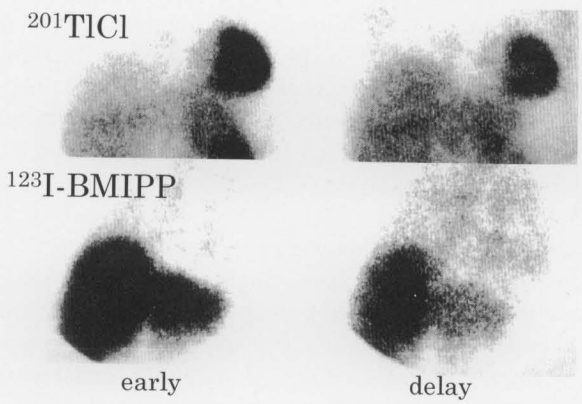


Fig. 3 Planar anterior view images of ^{201}TlCl and long-chain fatty acid (^{123}I-BMIPP) myocardial scintigrams (early and delayed images, Case 2). Although there was ^{201}TlCl myocardial accumulation, there was no myocardial ^{123}I-BMIPP accumulation in the patient of type I CD36 deficiency.

RESULTS

Flow cytometric analysis (Table 1 and Fig. 1)
Flow cytometric analysis revealed that 11 patients (4%) (3 with HCM, 1 with DCM, 2 with OMI, 4 with angina pectoris, and 1 with hypertension) showed signs of type I CD36 deficiency. Twenty patients (8%) (3 with HCM, 1 with DCM, 1 with OMI, 9 with angina pectoris and 6 with

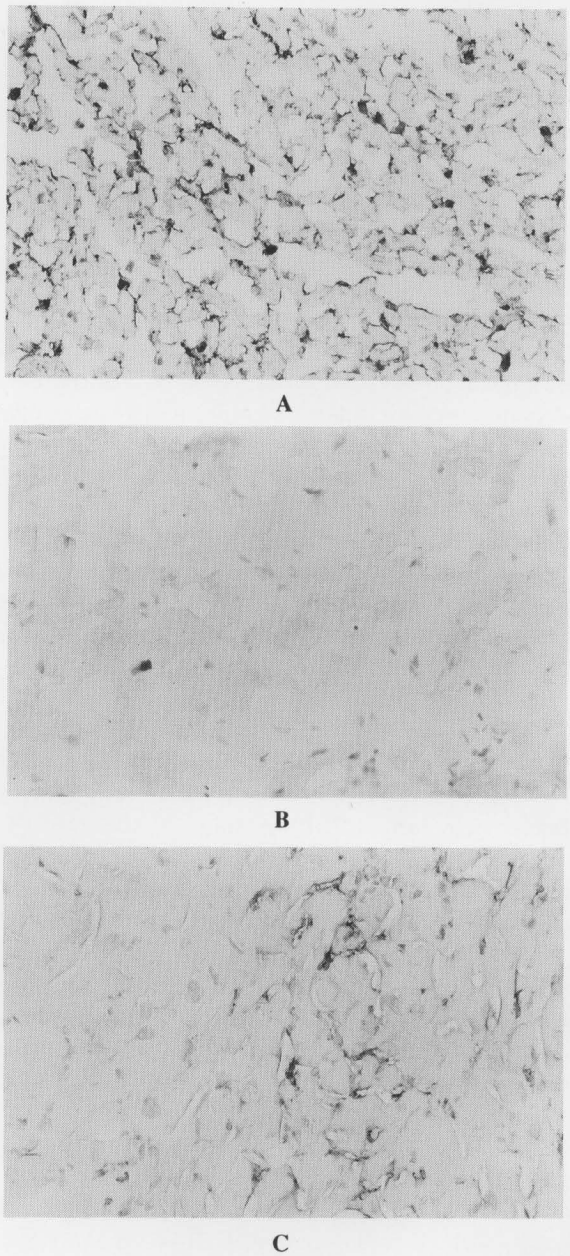


Fig. 4 CD36 expression in myocardial capillary endothelial cells. (A) Control patient: Myocardial capillary endothelial cells expressed CD36. (B) Patient with type I CD36 deficiency (Case 7): Myocardial capillary endothelial cells did not express CD36. (C) Patient with type II CD36 deficiency (Case 12): The myocardium showed a mosaic pattern (chimerism) formed by CD36-positive and negative capillary endothelial cells. (× 1000).

other heart diseases) showed signs of type II CD36 deficiency, so that overall 31 patients (12%) with heart diseases had type I or II CD36 deficiency.

Myocardial BMIPP and ^{201}TlCl scintigraphies (Figs. 2 and 3)

All 11 patients with type I CD36 deficiency showed no sign of BMIPP accumulation in the heart. Myocardial BMIPP scintigraphy was performed in 13 of 20 patients with type II CD36 deficiency. Among the cases with type II CD36 deficiency, all patients had BMIPP accumulation in the heart. In some patients with type II CD36 deficiency, BMIPP accumulation in the whole heart was mildly reduced. The reduced regions correlated with the hypertrophic regions in HCM and the ischemic regions in angina pectoris or OMI. All 24 patients with type I (11 patients) and type II (13) CD36 deficiency had ^{201}TlCl accumulation in the heart.

Immunohistochemistry (Fig. 4)

CD34 and CD36 expressions were examined in capillary endothelial cells from myocardial biopsy specimens from 2 patients without CD36 deficiency, two patients with type I and one patient with type II CD36 deficiency.

CD34 was expressed in the myocardial capillary endothelial cells in all patients with or without CD36 deficiency. Although CD36 was expressed in the myocardial capillary endothelial cells from the CD36-positive patients, it was not expressed in those from the two patients with type I CD36 deficiency. In a patient with type II CD36 deficiency, some capillary endothelial cells were CD36-negative, displaying patchy expression of CD36.

DISCUSSION

BMIPP is one of the analogs of LCFA that is retained in the cardiac muscle for a relatively prolonged period, and is an optimal radiopharmaceutical agent for SPECT.[10] Some studies have indicated that fatty acid metabolic disorders develop prior to myocardial blood flow disorder in patients with HCM and other heart diseases, and that fatty acid utilization is decreased in these patients.[10] Other studies have shown that BMIPP accumulation is reduced at hypertrophic sites, although few patients without BMIPP accumulation in the myocardium have been reported.[1,2,9]

Myocardium is highly oxidative and catabolizes LCFA as a source of energy. Glucose is the major energy substrate in the fetal heart, but LCFA utilization is limited. During the early postnatal period, a marked increase in LCFA utilization occurs, and ultimately LCFA becomes the chief myocardial energy substrate.[11,12] If myocardial LCFA uptake does not function normally, serious sequelae such as sudden death may occur as a consequence of stress. Although subjects with CD36 deficiency have been reported to be apparently healthy, in the present study 3 patients (cases 1, 6 and 7) had a family history of sudden death in younger relatives.

Although the physiological function of CD36 is not known, CD36 has been suggested to be one of the receptors for oxidized low density lipoprotein and a LCFA transporter.[3–6] Nozaki et al.[6] demonstrated a marked reduction in the uptake of oxidized low density lipoprotein by CD36-deficient macrophages, which suggested that there might be some differences between CD36-deficient and CD36-positive subjects in atherogenesis. CD36 deficiency may play an important role in the pathogenesis of arteriosclerosis.

There is clinical and experimental evidence of a possible relationship between a shift in myocardial substrate utilization and cardiac hypertrophy.[13,14] Kusaka et al.[15] showed that inhibition of myocardial LCFA oxidation induced cardiac hypertrophy in rats. Altered myocardial LCFA uptake may play an important role in the pathogenesis of HCM and DCM, and therefore the association of CD36 deficiency with HCM and DCM is reasonable.[2,16,17] Some studies have indicated that a high proportion (39%) of patients with HCM showed signs of type I or type II CD36 deficiency.[2] CD36 deficiency and fatty acid metabolic disorders may be involved in cardiac hypertrophy. We hypothesize that a defect in the first step of LCFA metabolism, i.e. the membrane LCFA transporter, may be related to impared myocardial LCFA uptake in HCM and DCM, and to unbalanced arterial LCFA uptake in arteriosclerosis. This transporter abnormality may be linked to the etiology of HCM, DCM and arteriosclerosis.

CD36 functions in various cells. Fat cells have a membrane transport system for LCFA by means of fatty acid-transporting protein. Abumrad et al.[18] purified an 88 kD fat cellular-membrane protein similar to CD36, and reported that inhibition of this protein suppressed fatty acid intake by 75%. The incidence of type II CD36 deficiency is relatively high at about 3% in the Japanese population and 0.3% in that of the U.S.A., but the incidence of type I CD36 deficiency has been estimated to be much lower than that of type II.[19,20] In the present study, 11 patients (4%) had type I and 20 patients (8%) had type II CD36 deficiency. CD36 deficiency was observed in a higher proportion (31 patients, 12%) of patients with heart diseases in this study than in a previous study.

CD34 is known to be expressed in capillary endothelial cells in humans, so we stained for CD34 to identify the cells in the specimens. Although CD34 was expressed in the myocardial capillary endothelial cells in patients with or without CD36 deficiency, this molecule was not expressed in the myocardial capillary endothelial cells from patients with type I CD36 deficiency. In type II CD36 deficiency, the myocardium had a mosaic pattern (chimerism) formed by CD36-positive and negative capillary endothelial cells. In contrast, CD36 expression on monocytes from patients with type II CD36 deficiency showed a single peak in flow cytometer. The reason for the difference between the patterns of expression of CD36 on

monocytes and endothelial cells is not known. The amount of CD36 expressed on monocytes from patients with type II CD36 deficiency measured by flow cytometry was only a half of that expressed in normal subjects.[4] If one of the two CD36 alleles is inactivated in a differentiation stage before CD36 expression in endothelial cells, CD36-positive and -negative cells may be observed in patients with type II CD36 deficiency.

There were 11 patients with type I CD36 deficiency treated for various heart diseases in our clinic. All 11 patients with type I deficiency underwent BMIPP scintigraphy, and none had BMIPP accumulation in the heart, but in patients with type II CD36 deficiency BMIPP accumulation was focally reduced, but there were no patients without BMIPP accumulation. Therefore, the absence of BMIPP accumulation in the heart is associated with type I CD36 deficiency and absence of CD36 in the myocardial capillary endothelial cells.

Although further investigations are required, our results suggest that CD36 might be closely related to myocardial LCFA metabolism.

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