Differential expression of \( \alpha_1 \), \( \alpha_3 \) and \( \alpha_5 \) integrin subunits in acute and chronic stages of myocardial infarction in rats

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Abstract

Objective: Anchoring cardiac myocytes to extracellular matrix, which is mediated mainly by integrins on their surfaces, is important for maintaining the architecture of myocardial tissues and transmitting mechanical force. We evaluated the expression of \( \alpha \) integrin subunits on myocytes and the accumulation of interstitial collagen and fibronectin at acute and chronic stages after myocardial infarction.

Methods: Myocardial infarction was induced by ligation of left coronary arteries in rats. The expression of \( \alpha_1 \), \( \alpha_3 \) and \( \alpha_5 \) integrin subunits, and accumulation of collagen and fibronectin were analyzed with immunohistochemistry or sirius-red staining.

Results: In hearts without infarction, moderate expression of the \( \alpha_3 \) subunit and only slight expression of the \( \alpha_5 \) subunit were observed on myocytes. In the first week after infarction, the \( \alpha_1 \) subunit, collagen and fibronectin were increased only in the peri-infarcted area, while the \( \alpha_5 \) subunit was increased both in peri-infarcted and non-infarcted areas. At day 42, the expression of the \( \alpha_1 \) subunit and collagen were still increased, although the \( \alpha_5 \) subunit and fibronectin were decreased. The expression of the \( \alpha_3 \) subunit was not altered throughout the experimental period. Conclusion: These data suggest that integrin subunits play an important role in healing and remodeling processes after myocardial infarction. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Connective tissue; Infarction; Interstitial spaces

1. Introduction

Myocardial tissues are composed of cardiac myocytes, fibroblasts, the coronary vascular system, and extracellular matrix proteins. At acute (healing process) and chronic stages (ventricular remodeling process) of myocardial infarction, qualitative and quantitative alterations of these components are observed in infarcted and non-infarcted tissues. In experimental myocardial infarction, the remaining cardiac myocytes become hypertrophied [1]. An increased synthesis of DNA, probably mediated by cardiac fibroblasts, is also observed even in non-infarcted tissues [2]. Fibronectin in infarcted tissues is increased within the first 2–4 days and declines thereafter [3,4]. For the first week, the accumulation of interstitial collagen in infarcted tissues begins to increase, and the accumulation continues to increase until the chronic stage at which congestive heart failure is often observed [5]. The remodeling of myocardial tissues after the onset of infarction occurs as a result of these cellular and molecular events.

On the other hand, anchoring of cells to cells or extracellular matrix proteins, which is mediated mainly by integrins expressed on cell surface, is important for maintaining the architecture of tissues [6,7]. In the myocardial tissue, integrins play a role in transmitting and distributing the mechanical force generated by the contraction of each myocyte to the extracellular matrix, and preventing myocytes from overstretching by elevated tension [8,9]. Integrins form a heterodimer of \( \alpha \) and \( \beta \) subunits [6,7]. Eight kinds of \( \beta \) subunits are known today, and adhesion to the extracellular matrix is mainly mediated by the \( \beta 1 \) integrin subfamily. It is currently thought that this subfamily contains ten kinds of \( \alpha \) integrin subunits which determine the types of the corresponding extracellular matrix. For example, \( \alpha_1 \beta 1 \) and \( \alpha_2 \beta 1 \) integrin complexes can bind to collagen and laminin, \( \alpha 4 \beta 1 \) and \( \alpha 5 \beta 1 \)
integrin complexes only to fibronectin, and α3β1 integrin complex to collagen, laminin and fibronectin [6,7]. Therefore, it is hypothesized that the expression of integrins in cardiac tissues is changed in accordance with the alteration of the components of the extracellular matrix as mentioned above, contributing to the maintenance of the tissue structure and function of diseased hearts. However, there are few reports concerning the relationship between alterations in the expression of integrins and in extracellular matrix proteins in diseased hearts including myocardial infarction. There is only one report describing the expression of α1β1 and α3β1 integrins in myocytes hypertrophied by aortic coarctation [10]. To address this hypothesis, we examined the expression of α1, α3 and α5 integrin subunits using a rat model of myocardial infarction, as well as the accumulation of collagen and fibronectin which are major components of extracellular matrix proteins in myocardial tissues.

2. Methods

2.1. Induction of myocardial infarction

Myocardial infarction was produced in 8-week-old male Wistar rats (Funabashi Farm, Shizuoka, Japan) as previously described [11]. Briefly, rats were anesthetized with methohexitol sodium (50 mg/kg i.p.), intubated endotracheally and ventilated mechanically. The left anterior descending coronary artery, via a left-sided thoracotomy, was ligated with a 6-0 nylon suture (MI group). The chest was closed, and the rats were maintained in cages. The same procedures except for the coronary ligation were repeated in a sham-operated (SO) group of rats. Hemodynamic measurements of which the technique was described elsewhere were performed at 2, 4, 7, 14 and 42 days after the induction of infarction in the MI group, and at 7 and 42 days after the sham-operation in the SO group as well in pre-operated rats (PO group) [12]. After arterial blood was obtained for determination of plasma renin activity, the hearts were excised, and the myocardial tissue samples were processed for histological examination. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985) and was approved by the ethical committee on Animal Experiments of Tohoku University School of Medicine.

2.2. Tissue preparation

Immediately after opening the thorax, hearts were isolated and rinsed with 10 mM phosphate-buffered saline (pH 7.4) at 4°C. After vessels and connective tissues were carefully removed, the heart was weighed. Then, the hearts were cut along the short axis at the level of the coronary ligation. Tissue samples were frozen in OCT compound (Miles Inc., Elkhart, IN, USA) using liquid nitrogen and stored at −80°C until use for the following histological study.

2.3. Infarct size and cell width

The infarct size of hearts was evaluated by planimetry analysis as previously described [13]. Four coronal sections of approximately 2-mm thickness were cut out from the level of the coronary ligation to the apex. Each section was fixed in 4% phosphate-buffered formaldehyde for 20 min at room temperature followed by staining with hematoxylin–eosin, and then observed with an imaging analyzer (Micro Computer Imaging Device, Maging Research Inc., St. Catharines, Canada) at a magnification of ×40. The lengths of the epicardium and the endocardium in the infarcted walls were measured and expressed as the percentage of epicardial and endocardial circumferences of left ventricle in each section. The mean value of four sections was considered as the infarct size of each heart.

Cell widths were measured on the coronal sections obtained at the papillary muscle level. Fields for the measurement of myocyte diameter were selected in non-infarcted left ventricular area, which was defined as the left ventricular wall opposite to the infarcted area. In each field, myocytes containing their nuclei in the center were selected, and the shortest length across the nuclei was measured as the diameter. The mean value of 50 myocytes was considered as the myocytes width of each heart [1].

2.4. Sirius red staining

Sirius red staining was performed according to the method previously described [5]. Cryostat sections 5 μm thick were mounted on poly-L-lysine-coated slides, and fixed with 4% phosphate-buffered formaldehyde. After washing in distilled water, sections were incubated with 0.2% phosphomolybdic acid followed by incubation with 0.1% sirius red (Polysciences Inc., Warrington, PA, USA). Then they were washed well in 0.01 N HCl before being dehydrated and mounted. In each of peri-infarcted area, non-infarcted left ventricular area and non-infarcted right ventricular area of each section, the pictures of three fields, except for vessels, were taken into the imaging analyzer. The sirius red positive area was expressed as the percentage of sirius red-positive pixels in total pixels as previously described [5]. Peri-infarcted area was defined as the left ventricular wall adjacent the infarct tissue where some myocytes were confirmed to remain by the observation of parallel sections stained with hematoxylin–eosin. Non-infarcted left ventricular area was defined as mentioned above. Non-infarcted right ventricular area was
defined as the right ventricular wall opposite to the infarcted tissue.

2.5. Immunohistochemistry

Immunohistochemistry was performed on cryostat sections 5 μm thick. The sections were fixed with acetone for 10 min at room temperature. Immunoreactive α1, α3, and α5 integrin subunits were detected with rabbit antisera for the α1 subunit (Chemicon International Inc. Temecula, CA, USA), and with rabbit polyclonal purified antibody for the α3 and α5 subunits (Chemicon) using the peroxidase anti-peroxidase (PAP) method previously described [14]. Sections were developed with diaminobenzidine (Dako Corporation, Carpinteria, CA, USA) according to the manufacturer’s protocol. Normal rabbit serum and IgG were used as the control antibodies, respectively. Since synthetic peptide derived from the COOH terminal sequence of each α integrin subunit was used as the immunogen, the intracellular domain of these integrins was stained in the present study.

After immunostaining, pictures of three fields in each peri-infarcted and non-infarcted left ventricular, and non-infarcted right ventricular areas which were defined as described above were taken in black and white at a magnification of ×100 using a light microscope (Carl Zeiss, Jena, Germany). Then, the intensity of the staining on myocytes was estimated with NIH image 1.61, and expressed as a gray scale from 0 (=white) to 255 (=black). Myocytes were differentiated from fibroblasts or myofibroblasts by morphological characteristics in parallel sections stained with hematoxylin–eosin. In each field, 30 myocytes were randomly selected, and the mean intensity of 90 myocytes was considered the value of each area.

With this analysis, the error of intensity between staining with anti-integrin antibodies and with control antibodies was defined as the amount of the integrin.

Integrins localized in vascular smooth muscle cells were also observed. The coronary arteries which were confirmed using parallel sections stained with hematoxylin–eosin were selected in each area.

Immunolocalization of fibronectin was examined with rabbit anti-rat plasma fibronectin antibody (Chemicon) diluted to 1:800 using the ABC method as previously described [15]. In this study, normal goat serum for blocking non-specific staining was not used, because it cannot be excluded that the anti-fibronectin antibody cross-reacts with fibronectin in goat serum [3]. Fibronectin-positive areas were estimated by the same method used for sirius red-positive areas as described above.

2.6. Measurements of plasma renin activity

Blood was mixed with sodium–EDTA and centrifuged at 1200×g for 10 min to collect plasma, which was stocked at −80°C until use for the measurement of plasma renin activity. Plasma renin activity was measured by radioimmunoassay.

2.7. Statistical analysis

Values are presented as mean±S.E.M. Unpaired two tailed t-test was used to compare the differences between the values in the MI and SO groups at days 7 and 42. The differences among the values at multiple time-points in each group were estimated with one way analysis of variance, followed by Scheffe’s test. The level of significance was taken at *P*<0.05.

3. Results

3.1. Characteristics of experimental myocardial infarction

As summarized in Table 1, infarct size tended to decrease with time after the induction of myocardial infarction, but the changes were not statistically significant. At day 7, the heart weight/body weight ratio and the width of myocytes in the non-infarcted area of hearts were significantly increased in the MI group compared with the SO group. Pressure measurement revealed decreases in systolic and diastolic aortic pressures and an elevation of left ventricular end-diastolic pressure. Thereafter, the heart weight/body weight ratio was slightly decreased, but the width continued to increase in the MI group. Aortic pressures gradually returned to the level of the SO group and end-diastolic pressure continued to elevate. At day 42, the increases in the heart weight/body weight ratio and the width of myocytes were still significant compared with their respective values at day 42 in the SO group. Furthermore, end-diastolic pressure was significantly higher than not only that at day 42 in the SO group (*P*<0.05) but also that at any time point in the MI group (*P*<0.05). Plasma renin activity was gradually elevated from day 2 in the MI group, and the elevation was significantly higher than that at day 42 in the SO group.

3.2. Expression of α1, α3 and α5 integrin subunits on myocytes

In hearts of the PO and SO groups, the α1 integrin subunit was not detected on any cells (Fig. 1, panel A). At day 7 (Fig. 1, panel B, D and E) and 42 (Fig. 1, panel C) in the MI group, the α1 integrin subunit was detected on normal myocytes in the peri-infarcted area (Fig. 1, panel D). In addition, a focused and strong expression of the α1 integrin subunit was observed on the apparently dead myocytes (Fig. 1, panel E). The expression was observed neither in non-infarcted areas of the left ventricle nor in the...
right ventricle at any time points. As illustrated in Fig. 2, semi-quantitative analysis revealed that the expression of the α1 integrin subunit on myocytes was significantly elevated from day 4 in the peri-infarcted area compared with that in the PO group (P<0.05). Expression of the α3 integrin subunit was observed on all myocytes in both the left (Fig. 3, panel A) and right ventricle in the PO and SO groups. The expression in the MI group (Fig. 3, panels B and C) was not significantly altered, as illustrated in Fig. 4. The α5 integrin subunit was only slightly expressed on myocytes in the PO (Fig. 5, panel A) and SO groups (data not shown). As illustrated in Fig. 6,

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Table 1
Characteristics of rats in each group

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<th>Days after surgery</th>
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<th>MI group</th>
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<td>273±13</td>
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<tr>
<td>(g)</td>
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<td>5</td>
<td>8</td>
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<tr>
<td>HW</td>
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<td>628±5</td>
<td>736±28</td>
</tr>
<tr>
<td>(mg)</td>
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</tr>
<tr>
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<td>2.70±0.04</td>
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<td>Width of myocytes</td>
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<td>20.2±0.3</td>
<td>21.8±0.1</td>
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<td>(μm)</td>
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<td>4</td>
<td>7</td>
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<td>Systolic AoP (mmHg)</td>
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<td>Diastolic AoP (mmHg)</td>
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<td>PRA (ng/ml/h)</td>
<td>13.8±1.1</td>
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<tr>
<td>Infarct size (%)</td>
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BW, body weight; HW, heart weight; AoP, aortic pressure; LVEDP, left ventricular end-diastolic pressure; PRA, plasma renin activity.

* Values are mean±S.E.M.

* P<0.05 as compared with the values at the same day after the surgery in SO group.

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Fig. 1. The expression of α1 integrin subunit in cardiac tissues. The expression of α1 integrin subunit was not observed in hearts without infarction (panel A). After infarction, α1 integrin subunit was detected in the infarcted and peri-infarcted areas at day 7 (panel B). Panel C represents the expression of the α1 integrin subunit on myocytes in the peri-infarcted area at day 42. Panel D and E, the magnified pictures of the squared areas in panel B, represents normal myocytes in peri-infarcted area and dead myocytes in infarcted area, respectively.
Fig. 2. Semi-quantitative evaluation of $\alpha_1$ integrin subunit on myocytes. The expression of $\alpha_1$ integrin on myocytes was significantly increased in peri-infarcted areas of the left ventricle from day 4, and the expression continued to day 42. Values are mean±S.E.M ($n=5$). LV, left ventricle; RV, right ventricle. * $P<0.05$, compared with the value in PO group.

3.3. Expression of $\alpha_1$, $\alpha_3$, and $\alpha_5$ integrin subunits on coronary vessels

The $\alpha_1$ integrin subunit was not detected on coronary vessels in the PO group (Fig. 7, panel A) as previously reported [16]. In the MI group, however, the $\alpha_1$ integrin subunit was observed on the media of vessels in both the infarcted (Fig. 7, panel D) and non-infarcted left and right ventricular areas (data not shown) throughout the experimental period. In contrast, the $\alpha_3$ integrin subunit was detected on the media of all coronary vessels in the PO group (Fig. 7, panel B). This expression was not altered after the induction of infarction (Fig. 7, panel E) throughout the experimental period. The $\alpha_5$ integrin subunit was detected only at the subendothelial space in all coronary vessels in the PO group (Fig. 7, panel C). The expression of the $\alpha_5$ integrin subunit was observed on the media, in addition to the subendothelial space, of vessels only in the infarcted area at the acute stage of infarction (day 2, 4, and 7) (Fig. 7, panel F), and decreased thereafter. At day 42, the staining on the media was detected only on a few vessels (data not shown).

Fig. 3. The expression of $\alpha_3$ integrin subunits in cardiac tissues. $\alpha_3$ Integrin subunit was moderately expressed on myocytes in hearts without infarction (panel A). Panel B and C represent the expression of the $\alpha_3$ integrin subunit in peri-infarcted area after infarction at day 7 and 42, respectively.
3.4. Extracellular matrix after myocardial infarction

The accumulation of interstitial collagen was significantly increased at day 7 in the peri-infarcted area ($P<0.05$) compared with that in hearts of the PO group. The accumulation was increased further at days 14 and 42 (Fig. 8). The increase of collagen deposition was accompanied with the increased expression of the $\alpha_1$ integrin on myocytes. The accumulation in the non-infarcted left and right ventricles of the MI group was slightly increased, but not significantly.

Fibronectin was accumulated around myocytes and the perivascular space in hearts of the PO group. In hearts with infarction, the accumulation was significantly increased in
Fig. 6. Semi-quantitative evaluation of α5 integrin subunit on myocytes. The expression of the α5 integrin subunit was significantly increased in both peri-infarcted and non-infarcted areas. The expression peaked at day 4 and 7, and declined to the level in hearts without infarction at day 42. Values are mean ± S.E.M (n=5). LV, left ventricle; RV, right ventricle. * P<0.05, compared with the value in PO group.

4. Discussion

In the present study using experimental myocardial infarction, we demonstrated for the first time that α1, α3

Fig. 7. The expression of α1, α3 and α5 integrin subunits in coronary vessels. Panel A–C represent immunohistochemical staining for α1, α3 and α5 integrin subunit in hearts without infarction, respectively. The expression of the α3 integrin subunit was observed in vascular media, while that of the α5 integrin subunit was observed in subendothelial spaces. α1 Integrin subunit was not detected on vascular cells. Panel D–F represent immunohistochemical staining for α1, α3 and α5 integrin subunits in infarcted areas at day 7 after infarction, respectively. Each integrin subunit was expressed in the vascular media of infarcted area.
and α5 integrin subunits on myocytes were distinctively expressed depending on the time after infarction and the sites of the myocardial tissues, and that the alteration of integrin expression on myocytes was accompanied by the accumulation of extracellular matrix components which are counter-ligands of each integrin. We observed also the alteration of integrin expression on coronary vessels, which was similar to that on cardiac myocytes.

In regard to the expression of α integrin subunits, Terracio et al. [10] previously reported that all of the α1, α3 and α5 integrin subunits were expressed on cardiac myocytes in fetal rats, while only the α3 integrin subunit was observed on those in adult rats. They further demonstrated that, after the establishment of myocardial hypertrophy by aortic coarctation, α1 and α5 integrin subunits were reexpressed on cardiac myocytes. In the present study, the α1 integrin subunit was expressed on the remaining myocytes of the peri-infarcted area, but not on the myocytes of the non-infarcted area in spite of the presence of myocyte hypertrophy throughout the experimental period. In contrast, the α5 integrin subunit on myocytes was transiently expressed at days 4 and 7 in both peri-infarcted and non-infarcted area. Therefore, the expression of α1 and α5 integrin subunits after myocardial
infarction was not simply related to the presence or absence of myocardial hypertrophy.

It has been reported that various types of myocytes such as necrotic, apoptotic and stunned, as well normal, myocytes are existed in infarcted tissues [17–19]. In the present study, although not divided into each type, myocytes were classified as normal or necrotic based on the observation in parallel sections stained with hematoxylin–eosin. Necrotic myocytes which were characterized by disappearance of nuclei and hyperesinophilic staining of cytoplasm in the parallel sections were observed at 2, 4 and 7 days in the infarcted area. At any time point, these myocytes were more strongly stained in immunohistochemistry with either anti α1, α3 or α5 integrin antibody than normal myocytes, the staining of which was as mentioned above. The reason of the different intensity of the immunohistochemical staining between necrotic and normal myocytes was unclear.

It has been suggested in other types of cells that the expression of individual integrins is regulated by a variety of factors that affect cell growth and differentiation. In vascular smooth muscle cells, PDGF-BB and TGF-β1 distinctively regulate the expression of α integrin subunits [20]. PDGF-BB upregulates only the expression of the α5 integrin subunit not followed by an increase of the β1 integrin subunit. In contrast, TGF-β1 enhances the expression of both α1 and α5 integrin subunits. In human dermal fibroblasts, PDGF increases the expression of the α5 integrin subunit and decreases that of the α1 integrin subunit. Furthermore, inflammatory cytokines such as IL-1β, TNF-α or IFN-γ enhance the expression of both α1 and α5 integrin subunits [21]. The expression of these cytokines has been observed in cardiac tissues after myocardial infarction in animal models [22,23]. Taken together, the expression of α integrin subunits on myocytes after myocardial infarction is likely to be regulated by these cytokines and growth factors.

On the other hand, there are some evidences suggesting that integrin–matrix interaction is one of the candidates regulating the expression of α integrin subunits. PDGF-BB increases the expression of α3 and α5 integrin subunits in human dermal fibroblasts cultured on fibronectin-rich or fibrin gel, whereas it increases the expression of the α2 integrin subunit in those cultured on collagen gel [24]. Culture on fibronectin or type IV collagen was shown to maintain α1 and α5 integrin subunit expression on the surface of NIH-3T3 fibroblasts [25]. In the present study, the accumulation of interstitial collagen which can bind to both α1 and α3 integrin subunits was related to the expression of the α1 integrin subunit in terms of time and location. The accumulation of fibronectin which can bind to both α3 and α5 integrin subunits was increased in the peri-infarcted area but not in the non-infarcted area, while in both areas, the α5 integrin subunit was expressed on myocytes. Therefore, the expression and maintenance of a part of the α integrin subunits observed on myocytes in this model may be influenced at least in part by integrin–matrix interaction.

The renin–angiotensin system is known to play an important role in cardiovascular remodeling including that after myocardial infarction [26–28]. The contribution of the renin–angiotensin system to the expression of α integrin subunits has not been determined. However, Burgess et al. [29] showed that incubation with angiotensin II enhanced the expression of β1 integrin subunits, which form heterodimers with α subunits, on cultured cardiac fibroblasts. In experimental myocardial infarction, the angiotensin II contents in infarcted tissues were 4.2-fold higher than those in hearts without infarction, while those in non-infarcted tissues were not increased when examined 3 weeks after the induction of infarction [27]. Angiotensin converting enzyme mRNA and protein were shown to be increased in the border zone of the infarcted area at 4 days after infarction [28]. These previous observations suggest that the renin–angiotensin system may be involved also in the regulation of α integrin expression on myocytes after myocardial infarction.

There are several pathological changes after myocardial infarction. Acute myocardial expansion, i.e. regional dilatation and thinning of the infarcted wall occurs within the first 24 hours after infarction [30]. In the next 2–4 days, thinning occurs also in non-infarcted left ventricular and septal walls [31,32]. It has been noted that side-to-side slippage of myocytes contributes to these structural changes [31,32]. Although the mechanisms of side-to-side slippage have not been elucidated, rearrangement of the structural framework provided by extracellular matrix is thought to be needed, as Whittaker et al. [32] reported that damage to collagen, which is one of the representative extracellular matrix components, was observed after myocardial infarction in rats. In the present study, the expression of the α5 integrin subunit on myocytes was increased in association with an alteration of the extracellular matrix components at day 4 and 7 after myocardial infarction. Therefore, the α5 integrin subunit on myocytes might be involved in the side-to-side slippage by binding to the extracellular matrix rearranged after the myocardial infarction.

Another important pathological change is scar formation in infarcted walls. Scar formation has been thought to play a role in preventing further dilatation of ventricular walls by infarction [33]. A representative event in this process is collagen deposition, which occurs approximately 1 week after infarction [5]. In the present study, the expression of the α1 integrin subunit on myocytes was observed in peri-infarcted areas at day 4 as interstitial collagen was increased. α1β1 integrin was shown to induce the collagen matrix reorganization as estimated by collagen gel contraction in vascular smooth muscle cells [34]. Simpson et al. [35] reported that neonatal myocytes assumed a tissue-like pattern of organization only through α1β1 integrin–collagen binding. These findings support the contribution of
the α1 integrin subunit expressed on remaining myocytes in peri-infarcted areas to the scar formation after myocardial infarction.

To our knowledge, there have been only a few reports about the cellular and molecular changes of vascular vessels in infarcted areas after myocardial infarction, such as perivascular deposition of collagen and fibronectin [3–5], and the increase of the vascular wall thickness [36]. In the present study, we demonstrated the expression of integrin subunits on coronary vessels in hearts with and without infarction. In hearts without infarction, the expression of the α3 integrin subunit, but not the α1 or α5 subunit, was observed in the vascular media. In hearts with infarction, of interest, the expression of α1 integrin subunit in vascular vessels was observed not only in infarcted area but also in non-infarcted area, while the expression on cardiac myocytes was observed only in the peri-infarcted area. In contrast, α5 integrin subunit expression in vascular vessels was observed only in the infarcted area, while the expression on cardiac myocytes in the peri-infarcted and non-infarcted areas. Thus, depending on the integrins, the expression patterns of the integrin were different between cardiac myocytes and smooth muscle cells. From the present study, it is not clear whether the difference resulted from the difference in cell types or from the difference in stimuli given to each type of cells.

There have been several reports concerning the functional roles of integrins on vascular smooth muscle cells, of which one is neointima formation after vascular injury [34,37]. α1β1 Integrin has been shown to be expressed in vascular walls after balloon injury and to induce collagen gel contraction which is accepted as an in vitro model of collagen matrix remodeling [34]. Furthermore, RGD peptide, which is an antagonist of several integrins including the α5 subunit, suppresses the neointima formation induced by balloon injury in a rat model [37]. These findings and ours suggest that coronary occlusion has injurious effects on vascular walls, and that the α1 and α5 integrin subunits are involved in the repair processes of injured vessels. Taken together, vascular remodeling including the expression of α1 and α5 integrin subunits on vascular vessels should be further investigated as one of the important events in ventricular remodeling after myocardial infarction.

While α1, α3 and α5 integrin subunits on fibroblast-like cells were undetectable in PO and SO groups, the expression of α1 and α3, but not α5, integrin subunit were observed on fibroblast-like cells in infarcted, but not in non-infarcted, area at day 4, 7 and 14 in MI group. However, the cells expressing these integrins were very few, and, furthermore, the intensity of the staining in those cells was faint.

We evaluated the expression of α integrin subunits in β1 family and the accumulation of collagen and fibronectin in the myocardial tissue at the acute and chronic stages of infarction. We demonstrated that the expressions of α1, α3 and α5 integrin subunits not only on myocytes but also on coronary vascular cells were independently regulated in peri-infarcted and non-infarcted areas at acute and chronic stages. The region- and stage-dependent expression of integrin subunits may be important for the healing and remodeling of myocardial tissues after infarction. Regulatory mechanisms and the contribution of integrins to cardiac function need to be further investigated.

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