

mammalian C2 domain also has an inhibitory effect. C2 domain of PLC- $\beta$ 1, with the type II C2 topology, also exhibited the inhibitory effects in a concentration-dependent manner (Fig. 5D).

Next, to identify the target sites of C2 domains of C2-RGS and PLC- $\beta$ 1, we performed binding experiments. The results demonstrated that both C2 domains bound strongly to G $\alpha$ q subunit (Fig. 7), and that C2 domain of C2-RGS bound strongly to EGL-30, G $\alpha$ q homologue of *C. elegans* (Fig. 8), indicating specific interaction between these C2 domains and G $\alpha$ q subunits. As to PLC- $\beta$ 1, it has been well known that the C-terminal domain (residues ~ 840 to the C-terminal end) is required for activation by G $\alpha$ q and for the GAP activity on G $\alpha$ q subunit (Paulssen et al., 1996; Ilkaeva et al., 2002). However, Wang et al. has revealed that C2 domains of PLC- $\beta$ 1 and PLC- $\beta$ 2 interact specifically with G $\alpha$ q subunit not only at the activated state (in the presence of GTP $\gamma$ S) but also at the inactive state (in the presence of GDP) (Wang et al., 1999). Thus, their report and our results in the present study may add a new member, C2 domain, as a binding module for G $\alpha$ q, in addition to the C-terminal portion of PLC- $\beta$ 1.

Inhibition of Ca<sup>2+</sup> responses by C2 domains of C2-RGS and PLC- $\beta$ 1, and their binding to G $\alpha$ q subunit, strongly suggest some roles of these C2 domains in G $\alpha$ q signaling, but the actual function in each full-length protein is not fully understood yet. Although the Ca<sup>2+</sup> responses were reduced by overexpression of the C2 domain fragment, it remains unsolved whether the inhibition is due to a direct action of C2 domain itself on G $\alpha$ q subunit (such as GAP action), or to C2 domain-induced exclusion of PLC- $\beta$ 1 from the binding interface on G $\alpha$ q. At least, the present results suggest that C2 domain of C2-RGS protein serves as a functional domain with an inhibitory effect, which is in accordance with the effects of C2 domain of PLC- $\beta$ 1 by Wang et al. (Wang et al., 1999). It might also be possible that this C2 domain tunes the character of the whole C2-RGS protein more specific to G $\alpha$ q, than that of the RGS domain, which shows the specificity to G $\alpha$ q and Gai/o.

Furthermore, it is noteworthy that these C2 domains bound to G $\alpha$ q subunits almost equally under all the experimental condition used; the presence of GDP, GDP/AIF<sub>4</sub><sup>-</sup> or GTP $\gamma$ S (Figs. 7 and 8). If C2 domain of PLC- $\beta$ 1 binds with G $\alpha$ q subunits, irrespective of the activation states, the persistent association between the C2 domain and G $\alpha$ q subunits may help the entire PLC- $\beta$ 1 molecule to keep its proper location close to G $\alpha$ q subunit at any stage during GTPase cycle. Thus, the C2 domain may act as a scaffold module (Pawson and Scott, 1997) to organize G $\alpha$ q and PLC- $\beta$ 1 in a stable signaling complex both in the absence and presence of stimulus. It is also possible that the C2 domain of C2-RGS has such a role as a scaffold module, as well as its own inhibitory activity in G $\alpha$ q signaling. The further study is in progress to clarify the detail mechanism and the functional roles of these C2 domains in G $\alpha$ q-mediated intracellular signal transduction.

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## IMAGES IN CARDIOLOGY.....

### Fibromuscular dysplasia in renovascular hypertension demonstrated by multislice CT: comparison with conventional angiogram and intravascular ultrasound

A 36 year old woman with a history of a gradual increase in blood pressure presented at our hospital. A renogram was performed and a decreased blood flow in the first and second phases was observed in the right kidney, with a significantly higher activity of serum renin in the right renal vein compared with the left. Furthermore, a bruit could be heard at the right lateroabdominal site, so right sided renovascular hypertension was suspected.

To evaluate the renal arteries, enhanced multislice computed tomography (CT) (Light Speed Ultra, General Electric, Milwaukee, Wisconsin, USA) was performed with a 1.25 mm slice thickness, helical pitch 7. Following intravenous injection of 100 ml of iodinated contrast material (350 mgI/ml), CT scanning was performed and volume data were transferred to a workstation (M900, Zio, Tokyo, Japan). The curved planar reconstruction image indicated a moniliform irregularity in the mid portion of the right renal arterial lumen (arrowhead in the upper right hand panel), suggesting renovascular hypertension caused by fibromuscular dysplasia.

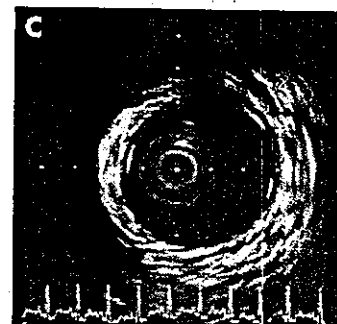
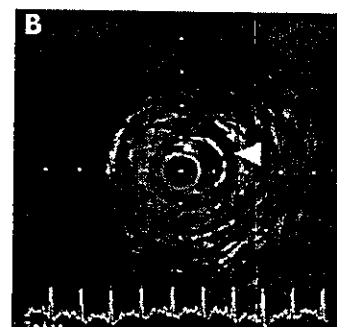
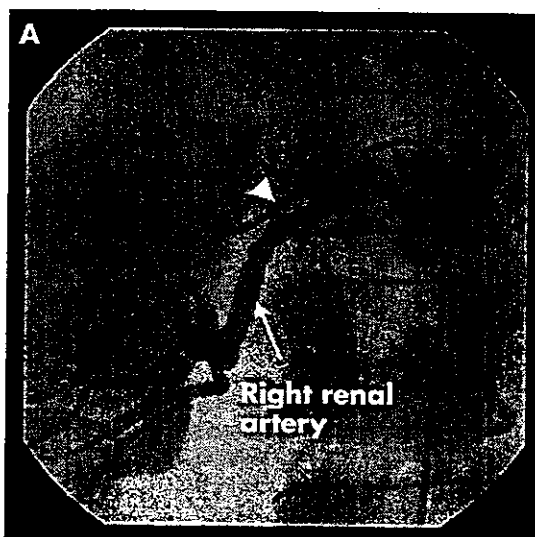
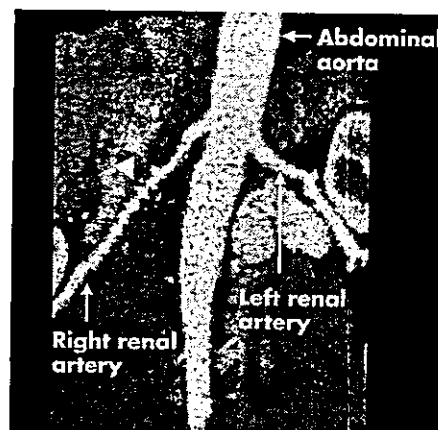
Selective renal arteriography was therefore performed revealing the moniliform lumen of the mid portion of the right renal artery in the same position as the CT image (arrowhead in panel A). Intravascular ultrasound revealed four segmental luminal stenotic sites, approximately 2 mm in diameter, with an echolucent area just outside the intima (arrowhead in panel B), suggesting a thickening of smooth muscle cells. These

thickenings were spotty, located among an average 4.5 mm diameter's normal lumina (panel C), the typical configuration of fibromuscular dysplasia in renovascular hypertension. Percutaneous transluminal angioplasty was therefore performed with good results.

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## Impact of Change in the Price of Percutaneous Coronary Intervention Devices on Medical Expenses

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Percutaneous coronary intervention (PCI) devices are much more expensive in Japan than in the United States, but their prices were reduced in April 2002. This study evaluated the impact of that change in the price of PCI devices on medical expenses. In-hospital costs of 22 consecutive patients who underwent elective single-vessel PCI without a debulking procedure before April 2002 were collected and the in-hospital cost of each patient was recalculated by applying the current prices of the PCI devices and those in the USA. For patients treated with PCI before April 2002, the in-hospital cost was ¥1,456,375±358,781, but when the current price is used, the in-hospital cost is estimated to be ¥1,355,812±313,237 (7% reduction). If the prices of the devices were reduced to those in USA, there would be a 53% reduction (¥689,417±99,139). Although the change in the price of PCI devices in April 2002 has reduced in-hospital costs, the devices are still much more expensive in Japan than in the USA. Further reduction of the price is required to make PCI more cost-effective. (Circ J 2003; 67: 576–578)

**Key Words:** Angioplasty; Cost analysis; Stent

**B**ecause of the prolonged recession, structural reform has been implemented in Japan and the Government sponsored, Japanese health insurance system has not been exempted. Heated debate on reform of the medical insurance system was held in the last Diet session. Because of increasing medical expenses as a result of the growing elderly population, the medical insurance system is mired in red ink, with almost all health insurance associations currently in the red. In this situation, physicians must be more cost-conscious, but little attention has been paid to cost analysis in Japan!

It has been known for a long time that medical devices are much more expensive in Japan than in the United States (USA);<sup>1-7</sup> so in April 2002 the prices of medical devices, such as pacemaker and percutaneous coronary intervention (PCI) devices, were reduced, in an attempt to save medical expenses. This study evaluated the impact of that change in the price of PCI devices on medical expenses.

### Methods

The in-hospital costs of 22 consecutive patients who underwent elective single-vessel PCI without debulking procedure at Sawara Hospital in Chiba, Japan before April 2002 were collected. Coronary stenting was performed in 18 of the 22 patients and procedural success was achieved in all patients except 1 with chronic total occlusion. There were no major in-hospital complications. Non-Q-wave

myocardial infarction occurred in 1 patient. None had a vascular complication that required a longer hospital stay. When patients had other diseases such as diabetes mellitus, the medical cost of those diseases was excluded. Changes in the technique of PCI and modification of the devices affect catheterization laboratory resource utilization, and subsequently in-hospital cost. To evaluate the pure reduction of in-hospital cost by a change in the prices of the PCI devices catheterization laboratory resource utilization, such as the number of balloon catheters and stents used, must be the same. Thus we recalculated the in-hospital cost of each patient by applying the current prices of PCI devices! In addition, to evaluate the difference in the in-hospital cost between Japan and USA because of the different prices of the PCI devices, it was recalculated using the USA prices (\$1=¥120).<sup>2-4</sup>

### Results

In patients treated by PCI before April 2002, the in-hospital cost was ¥1,456,375±358,781. The costs of PCI and PCI devices were ¥1,314,159±357,301 and ¥1,069,819±357,010, respectively, which were 90% and 73% of the total in-hospital cost. Table 1 presents the number of devices used, previous and current prices of PCI devices in Japan and those in the USA. When the current prices of PCI devices are used in the recalculation of the in-hospital cost of those patients, there is a 7% reduction (ie, in-hospital cost=¥1,355,812±313,237). The cost of the PCI device is ¥935,256±311,494 (13% reduction). If the prices of the devices were reduced to those in USA, in-hospital and PCI device costs would be ¥689,417±99,139 (53% reduction) and ¥268,861±96,534 (75% reduction), respectively.

### Discussion

Because of snowballing medical expenses in an aging society, the nation's health insurance system is on the verge

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Table 1 Catheterization Laboratory Resource Utilization, Unit Cost and Procedural Fee for-PCI

|                          | No. of devices used | Previous cost       | Current cost | United States<br>(\$1=¥120) | United Kingdom<br>(£1=¥187) | the Netherlands<br>(1 euro=¥126) | Argentina<br>(\$1=¥120) |
|--------------------------|---------------------|---------------------|--------------|-----------------------------|-----------------------------|----------------------------------|-------------------------|
| Stent                    | 1.0±0.6             | ¥338,000            | ¥324,000     | ¥126,000<br>(\$1,050)*      | ¥108,834<br>(£582)          | ¥102,942<br>(817 euro)           | ¥360,000<br>(\$3,000)   |
| Balloon catheter         | 1.7±0.8             | ¥263,000**          | ¥197,000     | ¥34,800<br>(\$290)          | ¥36,652<br>(£196)           | ¥46,368<br>(368 euro)            | NA                      |
| Intravascular ultrasound | 0.8±0.4             | ¥219,000            | ¥214,000     | ¥55,200<br>(\$460)          | NA                          | ¥68,670<br>(545 euro)            | NA                      |
| Guidewire (0.014")       | 1.7±0.9             | ¥32,700†            | ¥30,800      | ¥12,000<br>(\$100)          | ¥11,220<br>(£60)            | ¥11,970<br>(95 euro)             | NA                      |
| Guiding catheter         | 1.3±0.6             | ¥39,900†            | ¥38,000      | ¥6,240<br>(\$52)            | ¥6,732<br>(£36)             | ¥10,332<br>(82 euro)             | NA                      |
| Procedural fee           | -                   | ¥205,000†/¥199,000* | ¥239,000     | NA                          | NA                          | NA                               | NA                      |

PCI, percutaneous coronary intervention. \*Average price of the Penta, BX Velocity, NIR, and S7 stent. \*\*general I, †standard II, ‡standard I, †conventional balloon angioplasty, and ‡stenting. NA, not available or applicable (the procedural fee in the US was not used to recalculate the in-hospital cost).

of financial crisis. Medical expenses in Japan increased from 20 trillion yen in the fiscal year 1989 to 31 trillion yen in the fiscal year 1999 (the statistical reports of the Health, Labor and Welfare Ministry in Japan). More than 1,700 corporate insurance societies are making losses, the combined total of which is likely to amount to 500 billion yen this fiscal year. Heated discussion was held in the last Diet session, and reforms of the medical insurance system, such as increase in a salaried worker's payment for a medical expense and a decrease in medical insurance reimbursements, were decided. However, it might be difficult to improve the economic situation of the insurance system and thus fundamental reform of the medical insurance system may be inevitable. In that situation, physicians are required to be more cost-conscious and to treat patients efficiently, although there must not be any impairment of quality of care. Although considerable attention has been paid to cost analysis in the USA,<sup>2-7</sup> it has not been fully discussed in Japan!

Increasing demand for PCI is placing a large financial burden on limited healthcare resources. The Japanese Coronary Intervention Study Group conducted a nationwide survey on coronary revascularization procedures during 1997 and reported that a total of 109,788 PCI and 17,667 coronary artery bypass procedures were performed in Japan.<sup>8</sup> The ratio of PCI to coronary artery bypass surgery was 6.2, which is much higher than in the USA.<sup>8</sup> Thus reduction in the cost of PCI in Japan, especially of the PCI device, which constitutes 73% of the total in-hospital cost, is important. Even though some of patients may have multivessel coronary intervention, it can be roughly estimated that the change in the prices of PCI devices in April 2002 saves ¥11 billion/year [¥(1,456,375-1,355,812) × 109,788].

PCI devices are much more expensive in Japan than in USA or Europe (Table 1)!<sup>4,9,10</sup> and although the change in the prices in April 2002 reduces the gap slightly, the devices are still much expensive in Japan, even though most are imported. If the prices were reduced to those in the USA, it would save medical expenses of ¥73 billion/year [¥(1,355,812-689,417) × 109,788]. The numbers of balloon catheters and stents for reimbursement have been less since April 2002, but there are cases with complex lesions that require more devices. If the prices of the devices were reduced to those in the USA, we might be allowed to use the devices as required. Deregulation of the competition in the interventional products is necessary to reduce stent and angioplasty equipment prices.

It has been reported that the difference in overall (in-hospital + follow-up) cost between conventional balloon angioplasty and stenting would be ¥74,016 in the USA (¥1,098,807 vs ¥1,172,823), whereas it is ¥375,655 in Japan (¥1,188,583 vs ¥1,564,238)! Few modern medical advances are truly cost-saving, and many wide-spread practices, including bypass surgery for left main disease,<sup>11</sup>  $\beta$ -blocker therapy after acute myocardial infarction<sup>12</sup> and thrombolytic therapy for acute myocardial infarction<sup>13</sup> prolong life only at the expense of increased healthcare costs. Nevertheless, such treatments are viewed as cost-effective because their benefits to the patient are 'worth the additional cost'. From this viewpoint, stenting is considered to be 'worth the additional cost' (¥74,016) in the USA, but the cost difference in Japan (¥375,655) may be too high for such justification. Recently, a dramatic reduction in the rate of restenosis has been demonstrated with drug-eluting stents!<sup>4</sup> The projected cost of a drug-eluting stent is likely to be approximately \$3,200, which is almost 3-fold more expensive than a bare stent!<sup>5</sup> Thus it might cost one million yen in Japan and all patients might not be able to receive the benefit of drug-eluting stents.

Recent randomized studies demonstrated long-term outcomes in patients with multivessel disease treated with multivessel stenting compared with CABG!<sup>16-18</sup> The long-term mortality was controversial, although target revascularization rates were higher in the stent group than in the surgery group!<sup>16-18</sup> Cost analysis of multivessel stenting and coronary bypass surgery in the USA and Europe showed a lower overall (in-hospital and follow-up) cost in the stent group!<sup>16,19</sup> but a study in Argentina where stenting is much more expensive (\$3,000) than in the USA and Europe, as in Japan, demonstrated no difference in the overall cost between the 2 techniques!<sup>7</sup>

There has been a comparison of the long-term outcome of medical therapy and PCI, particularly in patients with single-vessel disease, which showed that long-term mortality, myocardial infarction, and target lesion revascularization were similar between the 2 therapy groups, but that PCI was superior to medical treatment for the alleviation of angina!<sup>20</sup> Overall cost was less in the medical therapy group than in the PCI group!<sup>9</sup> Thus, particularly in the face of constraints on healthcare spending, it may be recommended that physicians reserve PCI for patients with single-vessel disease whose symptoms of angina are not well controlled by medical treatment!<sup>20</sup>

Because of the Government sponsored, Japanese health

insurance system, all Japanese citizens are able to receive the best treatment equally. However, the nation's health insurance system is on the verge of a financial crisis and so physicians must be more cost-conscious, and furthermore everyone in Japan must consider reforms to the medical insurance system. Otherwise physicians may not be able to provide the best treatment for all patients equally.

#### Study Limitations

The in-hospital cost of patients treated before April 2002 was recalculated by applying the current device prices and those in the USA to estimate those after the change in the prices and in USA! The difference in catheterization laboratory resource utilization affects in-hospital cost. Furthermore, the method of calculating in-hospital cost and the reimbursement system are completely different between Japan and the USA!<sup>7</sup> That is why we used this method to evaluate the pure difference in the in-hospital cost because of the different device prices! Reimbursement prices of the devices were used to calculate the in-hospital cost in Japan. On the other hand, market prices are used in the USA, because there are no reimbursement prices. Thus the difference in the prices of the PCI devices may be less; however, even considering this possible disagreement, PCI devices are much more expensive in Japan!

#### Conclusions

The change in the prices of PCI devices in April 2002 reduces the in-hospital cost by 7%. However, the devices are still much more expensive in Japan than in USA and further reduction of the prices is required to make PCI more cost-effective.

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## THE ROLE OF PPAR $\gamma$ -DEPENDENT PATHWAY IN THE DEVELOPMENT OF CARDIAC HYPERTROPHY

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### Summary

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily. PPARs have three isoforms,  $\alpha$ ,  $\beta$  (or  $\delta$ ) and  $\gamma$ . It has been conceived that PPAR $\gamma$  is expressed predominantly in adipose tissue and promotes adipocyte differentiation and glucose homeostasis. Recently, synthetic antidiabetic thiazolidinediones and natural prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) metabolite, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J<sub>2</sub> (15d-PGJ<sub>2</sub>), have been identified as ligands for PPAR $\gamma$ . Following demonstration that PPAR $\gamma$  is present in a variety of cell types, further study of PPAR $\gamma$  has

been conducted. Although activation of PPAR $\gamma$  appears to have beneficial effects on atherosclerosis and heart failure, it is still largely uncertain whether PPAR $\gamma$  ligands prevent the development of cardiovascular diseases. Recent evidence suggests that some benefit from the antidiabetic agents known as thiazolidinediones may occur through PPAR $\gamma$ -independent mechanisms. In this review, we report on the latest developments concerning the study of PPARs and summarize the roles of the PPAR $\gamma$ -dependent pathway in cardiovascular diseases. © 2003 Prous Science. All rights reserved.

### Introduction

PPARs are transcription factors belonging to the nuclear receptor superfamily that heterodimerize with the retinoid X receptor (RXR) and bind to specific response elements termed PPAR responsive elements in target gene promoters. The PPAR responsive elements are direct repeats of hexam-

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eric consensus sequence AGGTCA, separated by one nucleotide. These nuclear receptors are ligand-dependent transcription factors and activation of target gene transcription depends on the binding of the ligand to the receptor. PPARs have three isoforms,  $\alpha$ ,  $\beta$  (or  $\delta$ ) and  $\gamma$ . PPAR $\alpha$  regulates genes involved in the  $\beta$ -oxidative degradation of fatty acids, whereas PPAR $\gamma$  promotes adipocyte differentiation and glucose homeostasis. The main function of PPAR $\delta$  has yet to be ascertained, but it seems to be implicated in epidermal maturation and skin wound repair. PPAR $\alpha$  is present in the liver, kidney and muscle, whereas PPAR $\gamma$  is expressed predominantly in adipose tissue. PPAR $\delta$  is expressed almost ubiquitously. Recently, it has been demonstrated that PPAR $\alpha$  and PPAR $\gamma$  are also expressed in a variety of cell types. Following reports that the activation of PPAR $\gamma$  suppresses the production of inflammatory cytokines in activated macrophages (1, 2), medical interest in PPARs have grown and a huge effort has been concentrated on related research. The thiazolidinediones, novel insulin-sensitizing agents and high-affinity ligands for PPAR $\gamma$ , have been reported to prevent growth factor-induced proliferation and migration of vascular smooth muscle cells (3, 4). The thiazolidinediones have also been reported to inhibit cytokine-mediated proliferation of endothelial cells (5) and to suppress endothelin-1 secretion from vascular endothelial cells (6). These observations suggest that PPAR $\gamma$  ligands may influence the growth of vascular cells (7). Furthermore, we recently reported that PPAR $\gamma$  plays an important role in the inhibition of cardiac hypertrophy. Here, we present current trends in PPAR $\gamma$  research and discuss the roles of PPAR $\gamma$  in cardiovascular diseases.

### PPAR $\gamma$

The peroxisome is a subcellular organelle which plays a crucial role in cellular metabolism. Peroxisome enzymes are implicated in a broad range of catabolic and anabolic enzymatic pathways such as fatty acid oxidation, biosynthesis of both glycerolipids and cholesterol, and metabolism of reactive oxygen species. The induction of peroxisome proliferation in rodents is associated with cellular responses to a range of chemical compounds. In 1990, Issemann and Green (8) reported that peroxisome proliferators activate a member of the steroid hormone receptor superfamily in mouse liver. This nuclear receptor was named PPAR, and shortly thereafter three major types of PPAR ( $\alpha$ ,  $\beta/\delta$  and  $\gamma$ ) were recognized. While PPAR $\gamma$ 1 is abundant-

ly expressed in various tissues (*e.g.*, liver, kidney, spleen, intestine, muscle, brain and lung), PPAR $\gamma$ 2 is predominantly expressed in adipose tissue (9–13). Several lines of evidence have demonstrated the functional significance of PPAR $\gamma$  in atherosclerotic lesions (14, 15). Recently, PPAR $\gamma$  has also been shown to be expressed in macrophages, vascular smooth muscle cells, endothelial cells and cardiac myocytes (2, 3, 16–18).

PPAR $\alpha$  regulates genes involved in the  $\beta$ -oxidative degradation of fatty acids (19, 20), whereas PPAR $\gamma$  promotes adipocyte differentiation and glucose homeostasis (21–25). In addition, it was recently reported that PPAR $\alpha$  and PPAR $\gamma$  are important immunoinflammatory mediators (1, 2, 26). The function of PPAR $\beta/\delta$  is still relatively unknown. Two different PPAR $\gamma$  splice variants, PPAR $\gamma$ 1 and PPAR $\gamma$ 2, have been cloned. These two forms differ only in their N-terminal 30 amino acids. Both PPAR $\gamma$  isoforms are derived from the same gene by alternative promoter usage and differential splicing. Like other members of the nuclear receptor superfamily, PPARs have several modular domains. The N-terminal A/B domain, which is the least conserved region, contains a ligand-independent activating function-1. The C domain, which is the best conserved domain, is DNA-binding. The D domain allows for bending or conformational alteration of PPAR. The E/F domain is ligand-binding and ligand-dependent transcription requires an activating function-2, located at the C-terminus of the ligand-binding domain. Ligand binding by PPAR $\gamma$  is regulated by intracellular communication between its N-terminal A/B domain and its C-terminal ligand-binding domain. Activity of PPAR $\gamma$  is depressed by phosphorylation of a serine residue (Ser<sup>112</sup>) in the A/B domain, mediated by a member of the mitogen-activated protein kinase family, extracellular signal-regulated protein kinase (27, 28). In addition, another member of the mitogen-activated protein kinase family, c-Jun N-terminal kinase (JNK), also phosphorylates PPAR $\gamma$  at Ser<sup>82</sup> and reduces the transcriptional activity of PPAR $\gamma$  (29). These modifications may control interactions between PPAR $\gamma$  and coactivators or corepressors that have been described to interact with many members of the nuclear receptor family. Recently, several lines of evidence have implicated the functional significance of interaction between nuclear receptors and coactivators in transcriptional activation. cAMP response element binding protein (CREB)-binding protein (CBP)/p300 is a transcriptional coactivator of PPAR $\alpha$ , PPAR $\gamma$  and nu-

clear factor- $\kappa$ B (NF- $\kappa$ B) (30–32). Steroid receptor coactivator-1 (SRC-1) also functions as a coactivator for PPAR and NF- $\kappa$ B (33–35). It has been reported that both CBP and SRC-1 interact with the PPAR $\gamma$ : RXR heterodimer and that the interaction is mediated by initial binding of PPAR coactivator-1 (PGC-1) (36). PGC-1 interacts with several other nuclear hormone receptors including the glucocorticoid receptor, the mineralocorticoid receptor, the estrogen receptor and PPAR $\alpha$ . These observations raise the possibility that nuclear competition for limiting amounts of CBP/p300, SRC-1 or PGC-1 may occur between PPARs and other transcription factors.

#### PPAR $\gamma$ ligands

The PGD<sub>2</sub> metabolite, 15d-PGJ<sub>2</sub>, is the first endogenous ligand for PPAR $\gamma$  to be discovered (37, 38). Although 15d-PGJ<sub>2</sub> is the most potent natural ligand of PPAR $\gamma$ , the extent to which its effects are mediated through PPAR $\gamma$  *in vivo* remains to be determined. Two components of oxidized low-density lipoprotein (LDL), 9-hydroxy and 13-hydroxy octadecadienoic acids (HODE), are also potent endogenous activators of PPAR $\gamma$  (39, 40). Activation of 12/15-lipoxygenase induced by interleukin-4 (IL-4) also produces the endogenous ligands for PPAR $\gamma$  ligands (41). The antidiabetic thiazolidinediones (e.g., troglitazone, pioglitazone, ciglitazone and rosiglitazone) are pharmacological ligands of PPAR $\gamma$  (22, 42, 43). They bind PPAR $\gamma$  with various affinities and it is conceivable that their insulin-sensitizing and hypoglycemic effects are exerted by activating PPAR $\gamma$ . The mechanism by which thiazolidinediones improve insulin resistance has been not fully understood. Okuno *et al.* (44) demonstrated that troglitazone increases the number of small adipocytes and decreases the number of large adipocytes in obese Zucker rats. This action of troglitazone appears to be an important mechanism by which increased expression levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and plasma lipids are normalized, leading to alleviation of insulin resistance. The principal insulin-sensitive glucose transporter in muscle is GLUT4, which is recruited to the sarcolemma in response to elevated levels of insulin. It was reported that PPAR $\gamma$  ligands induce expression of GLUT4 during conversion of fibroblasts into adipocytes (45). Michael *et al.* (46) have demonstrated that PGC-1 increases expression of GLUT4 in part by binding to and coactivating myocyte enhancer factor-2C. In contrast with these results,

heterozygous PPAR $\gamma$ -deficient mice show more insulin-sensitive phenotype compared with wild-type mice on a high-fat diet (47, 48). The function of PPAR $\gamma$  in improving insulin sensitivity is controversial. Several nonsteroidal anti-inflammatory drugs (NSAIDs), including ibuprofen, indomethacin and fenoprofen, also bind to PPAR $\gamma$  and have PPAR $\gamma$  activity at high drug concentrations (49). Their anti-inflammatory effects stem from their ability to inhibit cyclooxygenase-1 (COX-1) and COX-2. Because activation of both PPAR $\alpha$  and PPAR $\gamma$  suppress induction of COX-2 (50, 51), PPARs may contribute to the anti-inflammatory responses produced by NSAIDs. PPAR $\gamma$  is master regulator of adipocyte differentiation and thiazolidinediones enhance adipocyte differentiation through activation of PPAR $\gamma$  (21, 24, 37, 38). As enhanced PPAR $\gamma$  expression induces the differentiation of many cell types, thiazolidinedione-mediated PPAR $\gamma$  activation suppresses the growth of colon cancer cells *in vitro*. However, the effects of thiazolidinediones on colon cancer are controversial and further investigations are needed to clarify the roles of PPAR $\gamma$  in colon carcinogenesis (52–55). RXR, which interacts with PPARs, is activated by 9-*cis* retinoic acid. When combined as a PPAR: RXR heterodimer, PPAR ligands and 9-*cis* retinoic acid can act synergistically on PPAR responses (56–58).

#### PPAR $\gamma$ in cardiovascular diseases

The formation of atherosclerosis is a complex process to which many different factors contribute. The injury of endothelium, the proliferation of smooth muscle cells, the migration of monocytes/macrophages, and the regulatory network of growth factors and cytokines are important in the development of atherosclerosis. In addition, chronic inflammation of the vascular wall is also involved in the process. As mentioned above, PPAR $\gamma$  is implicated in inflammation. PPAR $\gamma$  ligands have been shown to reduce production of inflammatory cytokines such as IL-1 $\beta$ , IL-6, inducible nitric oxide synthase (iNOS) and TNF- $\alpha$  by inhibiting the activity of transcription factors such as activator protein-1 (AP-1), signal transducers and activators of transcription (STATs) and NF- $\kappa$ B in monocytes/macrophages (1, 2). These results suggest that PPAR $\gamma$  activation may have beneficial effects in modulating inflammatory responses in atherosclerosis. It has been reported that PPAR $\gamma$  ligands ameliorate other inflammatory diseases such as arthritis and colitis in animal models (59, 60). On the other hand, the in-

flammatory activation of aortic smooth muscle cells, which is a hallmark of atherosclerosis, is inhibited by PPAR $\alpha$  ligands but not by PPAR $\gamma$  ligands (26). Macrophages affect the vulnerability of plaques to undergo rupture in atherosclerotic lesions. The role of macrophages in plaque rupture is implicated in the secretion of matrix metalloproteinases (MMPs), enzymes that are important in the degradation of extracellular matrix. In macrophages and smooth muscle cells, PPAR $\gamma$  ligands have been shown to reduce expression of MMP-9, which is implicated in both migration of vascular smooth muscle cells and plaque destabilization (2, 3).

Vascular smooth muscle cell proliferation and migration are also critical events in atherosclerosis and restenosis. Thiazolidinediones inhibit these changes of vascular smooth muscle cells and neointimal thickening after vascular injury (4, 61–63). Furthermore, thiazolidinediones induce apoptosis of smooth muscle cells via p53 and Gadd45 (64). Angiotensin II plays an important role in vascular remodeling via angiotensin II type 1 receptor (AT $_1$ R). As activation of PPAR $\gamma$  inhibits AT $_1$ R gene expression at a transcriptional level in vascular smooth muscle cells, PPAR $\gamma$  ligands may prevent angiotensin II-induced vascular remodeling by reduced expression of AT $_1$ R (65). Expression of adhesion molecule by endothelial cells, leading to adhesion of leukocytes to endothelial cells, is a critical early step in atherosclerosis. PPAR $\gamma$  ligands inhibit expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in HUVECs (66, 67) and decrease production of chemokines such as IL-8, and monocyte chemoattractant protein-1 (MCP-1) in human aortic endothelial cells (68). PPAR $\gamma$  ligands also inhibit MCP-1-induced monocyte migration (69). Endothelin-1 is involved in the regulation of vascular tone and endothelial functions. In addition, endothelin-1 is expressed in atherosclerotic lesions and induces proliferation of smooth muscle cells. PPAR $\gamma$  ligands suppress the transcription of the endothelin-1 promoter by interfering with AP-1 in bovine aortic endothelial cells (BAECs) (6).

PPAR $\gamma$  activation by 9-HODE and 13-HODE, the major oxidized lipid components of oxLDL, plays an important role in the development of lipid-accumulating macrophages through transcriptional induction of CD36, a scavenger receptor (39, 40, 70). It has been reported that CD36-deficient mice are protected from atherosclerosis (71). These data suggest that atherogenic oxLDL particles could induce their own uptake through activation of PPAR $\gamma$

and expression of CD36, leading to atherosclerosis. On the other hand, PPAR $\gamma$  ligands could suppress induction of the class A scavenger receptor by TPA (2). Although it is uncertain whether increased expression of CD36 enhances foam cell formation; there is the possibility that PPAR $\gamma$  ligands may promote foam cell formation and have proatherogenic effects. However, Li *et al.* (72) reported that PPAR $\gamma$  ligands (*i.e.*, rosiglitazone and GW7845, a nonthiazolidinedione tyrosine analog) significantly inhibit the development of atherosclerosis in spite of increased expression of CD36 in the arterial wall in LDL receptor-deficient male, but not female, mice. Chen *et al.* (73) demonstrated that thiazolidinediones significantly inhibit the development of atherosclerotic foam cell lesions in apolipoprotein E-knockout mice and suggested that the anti-inflammatory effects of thiazolidinediones may predominate over the induced expression of CD36 in macrophage foam cells. In addition, PPAR $\gamma$  has been shown to induce expression of ABCA1, a transporter that controls apoA1-mediated cholesterol efflux, and cholesterol removal from macrophages through a transcriptional cascade mediated by the nuclear receptor  $\alpha$  LXR (74, 75). Very recently, Jiang *et al.* (76) reported that PPAR $\gamma$  ligands induce hepatocyte growth factor (HGF) mRNA and protein expression in fibroblasts. Because hepatocyte growth factor is among the pleiotropic polypeptides, which have mitogenic and antiapoptotic effects in endothelial cells (77), PPAR $\gamma$  ligands may play beneficial roles in vascular disease via hepatocyte growth factor expression.

The effects of PPARs on heart tissue have not been fully understood. PPAR $\alpha$  has been demonstrated to play an important role in mitochondrial fatty acid  $\beta$ -oxidation (FAO) in the heart. Pressure overload-induced cardiac hypertrophy results in deactivation of PPAR $\alpha$  and subsequent reduction of FAO enzymes (*e.g.*, carnitine palmitoyltransferase I, medium-chain acyl-CoA dehydrogenase, acyl-CoA oxidase) gene expression (78). These results suggest that cardiac hypertrophy induces abnormalities in cardiac lipid homeostasis and energy production via reduction of PPAR $\alpha$  activity. Cardiac-specific PGC-1 transgenic mice display uncontrolled mitochondrial biogenesis, abnormalities of sarcomeric structure and dilated cardiomyopathy (79). PGC-1 appears to control the number and function of cardiac mitochondria. Recently, we demonstrated that PPAR $\gamma$  ligands inhibit cardiac expression of TNF- $\alpha$  at the transcriptional level in

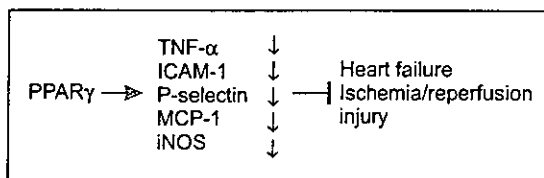


Fig. 1. PPAR $\gamma$  inhibits heart failure and ischemia/reperfusion injury. The effects of PPAR $\gamma$  are associated with decreases in TNF- $\alpha$ , ICAM-1, P-selectin, MCP-1 and iNOS.

part by antagonizing NF- $\kappa$ B activity (80). Because TNF- $\alpha$  expression is elevated in the failing heart and has a negative inotropic effect on cardiac myocytes, treatment with PPAR $\gamma$  ligands may prevent the development of congestive heart failure. Diabetic cardiomyopathy, which is characterized by systolic and diastolic dysfunctions, is a major complication of diabetes; however, the antidiabetic agents known as the thiazolidinediones appear to be beneficial to cardiac function impairment in patients with diabetes mellitus.

Following our study, the role of PPAR $\gamma$  in myocardial ischemia/reperfusion injury has also been elucidated (80–82). PPAR $\gamma$  ligands reduced myocardial infarction size and improved contractile dysfunction after ischemia/reperfusion injury in rats. The beneficial effects of PPAR $\gamma$  ligands were associated with reduction in the expression of ICAM-1, P-selectin, MCP-1 and iNOS (Fig. 1). Moreover, the activation of JNK, AP-1 and NF- $\kappa$ B was inhibited by PPAR $\gamma$  ligands in the animal model.

### PPAR $\gamma$ in cardiac hypertrophy

Cardiac hypertrophy is recognized as an adaptive increase in heart size characterized by a growth of each cardiomyocyte rather than an increase in cell number (83). Cardiac hypertrophy is induced by a variety of diseases such as hypertension, valvular diseases, myocardial infarction and endocrine disorders. Although cardiac hypertrophy may initially be a beneficial response that normalizes wall stress and maintains normal cardiac function, prolonged hypertrophy becomes a leading cause of heart failure and sudden death (84). Therefore, it is very important to understand the precise mechanisms and mediators of cardiac hypertrophy in order to prevent its development. Although numerous signal transduction pathways that promote cardiac hypertrophy have been characterized, only a few studies have focused on inhibitory regulators of hypertrophic response. The modification of anti-

hypertrophic regulators is expected to be a novel therapeutic strategy for cardiac hypertrophy.

Recently, we examined the effects of the thiazolidinediones such as troglitazone, pioglitazone and rosiglitazone on angiotensin II-induced hypertrophy in neonatal rat cardiac myocytes and on pressure overload-induced cardiac hypertrophy in mice (85, 86). To further elucidate the role of PPAR $\gamma$  in the development of cardiac hypertrophy, we examined pressure overload-induced cardiac hypertrophy using heterozygous PPAR $\gamma$ -deficient (PPAR $\gamma^{+/-}$ ) mice. In the study, we demonstrated that the PPAR $\gamma$  ligands troglitazone, pioglitazone and rosiglitazone inhibit angiotensin II-induced hypertrophy of neonatal rat cardiac myocytes. The pressure overload-induced cardiac hypertrophy was more prominent in PPAR $\gamma^{+/-}$  mice than in WT mice. Treatment with the PPAR $\gamma$  ligand pioglitazone inhibited the pressure overload-induced cardiac hypertrophy strongly in WT mice and moderately in PPAR $\gamma^{+/-}$  mice. These results suggest that the PPAR $\gamma$ -dependent pathway inhibits the development of cardiac hypertrophy. Yamamoto *et al.* (87) have also reported that PPAR $\gamma$  ligands such as troglitazone and 15d-PGJ<sub>2</sub> inhibit angiotensin II-, phenylephrine- or mechanical strain-induced cardiac hypertrophy.

However, the molecular mechanism by which PPAR $\gamma$  suppresses cardiac hypertrophy remains to be determined. A variety of transcription factors may be implicated in the development of cardiac hypertrophy. c-Fos and c-jun make the heterodimer complex called AP-1, which transactivates many genes that have a TPA responsible element in their promoter such as atrial natriuretic peptide and endothelin-1 genes (88, 89). STATs are known to play important roles in cytokine signaling pathways (90). Recently, it was reported that STAT3 is activated in cardiac myocytes by the IL-6 family of cytokines and plays a crucial role in generating cardiac hypertrophy through gp130 (91). The cardiac-restricted zinc finger transcription factor GATA4 has also been shown to be required for transcriptional activation of the genes for AT<sub>1</sub>R,  $\beta$ -myosin heavy chain and endothelin-1 during cardiac hypertrophy (92–94). The calcium-dependent phosphatase calcineurin dephosphorylates the transcription factor NF-AT3, and NF-AT3 translocates to the nucleus and interacts with GATA4, resulting in the development of cardiac hypertrophy (95). It was recently reported that PPAR $\gamma$  ligands could downregulate inflammatory responses in monocytes by interfering with AP-1, STAT and NF- $\kappa$ B signaling pathways

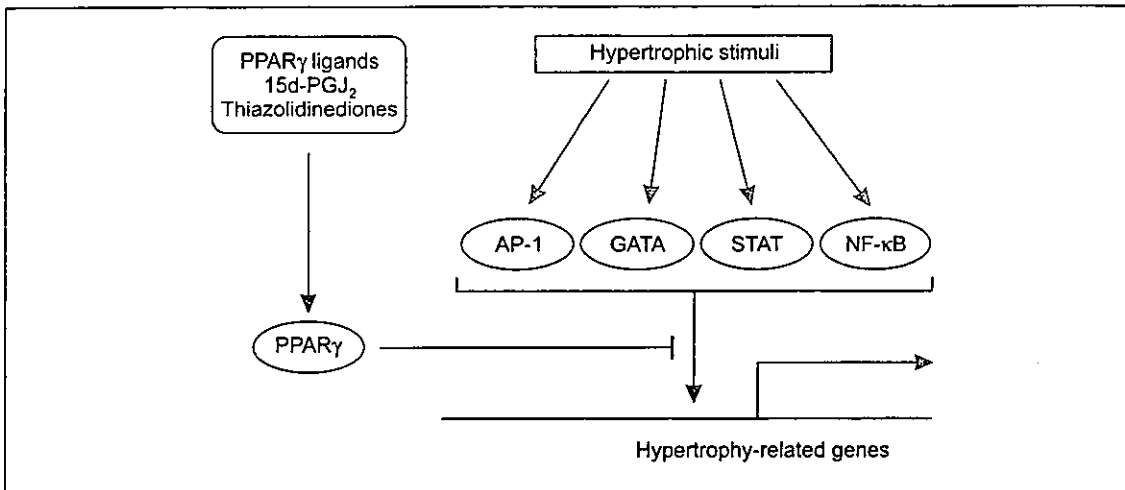


Fig. 2. PPAR $\gamma$  negatively regulates hypertrophic signaling pathways in cardiac myocytes. PPAR $\gamma$  suppresses cardiac hypertrophy by antagonizing the activities of transcription factors such as AP-1, GATA, STAT and NF- $\kappa$ B.

via competition for essential cofactors (2). Furthermore, our laboratory and others have demonstrated that PPAR $\gamma$  ligands inhibit NF- $\kappa$ B activity in cardiac myocytes (18, 87). NF- $\kappa$ B activation is involved in G-protein-coupled receptor agonist- and mechanical strain-induced cardiac hypertrophy (96, 97). Therefore, PPAR $\gamma$  may suppress the development of cardiac hypertrophy by antagonizing the activities of transcription factors such as AP-1, STAT3, GATA4 and NF- $\kappa$ B (Fig. 2).

### Conclusions

Unfortunately, troglitazone was shown to have hepatotoxic effects in some diabetic patients, leading to the withdrawal of this agent from the market. It is hoped that new generations of nonthiazolidinedione PPAR $\gamma$  ligands, which exert antiatherogenic effects and have neither hepatotoxic nor proatherogenic effects, will be explored. Further studies using tissue-specific gene targeting mice are necessary to address the effects of PPAR $\gamma$  on atherosclerosis *in vivo*. Once the beneficial activities of the PPARs have been determined, their modulation may become a promising therapeutic strategy for cardiovascular diseases. Recently, many reports have suggested that insulin resistance and hyperinsulinemia are involved in cardiac hypertrophy (98, 99). Because cardiac hypertrophy can be seen even in normotensive diabetic patients and given the fact that diabetic cardiomyopathy is a major complication of diabetes, antidiabetic agents such as the thiazolidinediones would appear to be ben-

eficial for the treatment of cardiac hypertrophy and dysfunction in patients with diabetes mellitus. Our study suggests the potential clinical efficacy of the thiazolidinediones for the prevention of cardiac hypertrophy, but further studies are necessary to elucidate whether or not the inhibition of cardiac hypertrophy by the PPAR $\gamma$  ligand improves prognosis.

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# Leukemia Inhibitory Factor Enhances Survival of Cardiomyocytes and Induces Regeneration of Myocardium After Myocardial Infarction

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**Background**—Myocardial infarction (MI) is a leading cause of cardiac morbidity and mortality in many countries; however, the treatment of MI is still limited.

**Methods and Results**—We demonstrate a novel gene therapy for MI using leukemia inhibitory factor (LIF) cDNA. We injected LIF plasmid DNA into the thigh muscle of mice immediately after inducing MI. Intramuscular injection of LIF cDNA resulted in a marked increase in circulating LIF protein concentrations. Two weeks later, left ventricular remodeling, such as infarct extent and myocardial fibrosis, was markedly attenuated in the LIF cDNA-injected mice compared with vehicle-injected mice. More myocardium was preserved and cardiac function was better in the LIF-treated mice than in the vehicle-injected mice. Injection of LIF cDNA not only prevented the death of cardiomyocytes in the ischemic area but also induced neovascularization in the myocardium. Furthermore, LIF cDNA injection increased the number of cardiomyocytes in cell cycle and enhanced mobilization of bone marrow cells to the heart and their differentiation into cardiomyocytes.

**Conclusions**—The intramuscular injection of LIF cDNA may induce regeneration of myocardium and provide a novel treatment for MI. (*Circulation*. 2003;108:748-753.)

**Key Words:** gene therapy ■ muscles ■ leukemia inhibitory factor ■ myocardial infarction ■ regeneration

The loss of cardiomyocytes in myocardial infarction (MI) is a major cause of heart failure. Many cardiomyocytes are dead by apoptosis and necrosis in MI,<sup>1</sup> and the myocardium in the infarcted area is gradually replaced by collagen tissue. It has long been believed that adult cardiomyocytes do not proliferate; however, it was recently reported that adult cardiomyocytes could enter the cell cycle and increase the cell number.<sup>2,3</sup> Moreover, it has been reported that cardiomyocytes can be generated from marrow stromal cells from mice *in vitro*<sup>4</sup> and that undifferentiated stem cells in the bone marrow may be transported to the heart and differentiate into cardiomyocytes and vascular endothelial cells *in vivo*.<sup>5-8</sup> Given these possibilities, MI could be treated by enhancing the ability of cardiomyocytes to divide or of bone marrow cells (BMCs) to differentiate into cardiomyocytes.

The interleukin 6 (IL-6) family of cytokines, including IL-6, leukemia inhibitory factor (LIF), ciliary neurotrophic factor, and cardiotrophin-1, have a variety of biological functions not only in the hematopoietic and immune systems but also in other organs, including the nervous and cardiovascular systems.<sup>9,10</sup> The IL-6 family regulates growth and differentiation of many types of cells. Furthermore, these

cytokines contribute to the regeneration of many tissues, such as nerves, skeletal muscle, liver, and bone.<sup>11-14</sup> LIF has been reported to ameliorate denervation-induced muscle atrophy and improve regeneration of muscle and nerves.<sup>11</sup> Locally administered LIF cDNA plasmid in a gelatin carrier can increase bone density and subsequent bone formation.<sup>14</sup> In the heart, gp130, the common receptor of the IL-6 family, is expressed abundantly and has been reported to be critically involved in the growth and survival of cardiomyocytes.<sup>15-18</sup> It has also been reported that LIF receptor is expressed abundantly in cardiomyocytes and that LIF induces marked cardiomyocyte hypertrophy.<sup>19,20</sup> All these findings suggest that LIF may promote survival of cardiomyocytes and regeneration of myocardium. We thus examined the potential usefulness of LIF to treat MI.

## Methods

### Murine MI Model

MI was produced in 12-week-old male C57BL/6 mice by permanent ligation of the left coronary artery.<sup>21</sup> A total of 40 mice were operated on to induce MI and randomly divided into 2 groups, LIF cDNA-injected and vehicle-injected groups (20 mice each). At 2

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days or 1 or 2 weeks after MI, 3 to 6 mice from each group were killed for analysis. The experiments were performed in accordance with the guidelines established by Chiba University for experiments in animals.

### Injection of Plasmids Carrying LIF cDNA

The LIF expression vector was constructed by inserting a chimera of human and mouse LIF cDNA into the pCAGGS plasmid, which is driven by both a cytomegalovirus enhancer and a chicken  $\beta$ -actin promoter.<sup>22</sup> The plasmid was prepared by use of the Concert High Purity Plasmid Purification Systems (Gibco BRL) and injected at a dose of 100  $\mu$ g plasmids per 100  $\mu$ L PBS per 20 g body wt into the thigh muscle of mice immediately after MI. The same volume of PBS alone was injected in littermate mice as controls.

### Assay of LIF Concentration

Plasma LIF concentration was measured with a ELISA kit (Quantikine M) according to the manufacturer's instructions. The LIF ELISA kit has high sensitivity by which the minimum 2 pg/mL of LIF can be detectable. The assay of this kit is specific for natural and recombinant mouse LIF.

### Echocardiography

Transthoracic echocardiography was performed with an Agilent Sonos 4500 (Agilent Technologies Co) provided with an 11-MHz imaging transducer. When the mice had partially recovered from the light anesthesia, M-mode images of the left ventricle (LV) were recorded.<sup>21</sup>

### Histology

Hearts fixed in 10% formalin were embedded in paraffin, sectioned at 4- $\mu$ m thickness, and stained with hematoxylin-eosin and Azan. The extent of fibrosis was measured in 10 fields randomly selected from the LV free wall (LVFW) of each section. Five sections from each heart were measured, and the value was expressed as the ratio of Azan-stained fibrosis area to total myocardium area. The "remained myocardium" in the ischemic area was determined from 5 sections for each heart by calculating the ratio of (LVFW area - fibrotic area) divided by LVFW area.

### TUNEL Analysis

Cardiomyocyte death was assessed in situ by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) of paraffin-embedded heart sections using Cardio TACS (Trevigen Inc).

### Isolation and Transplantation of BMCs

BMCs were isolated from 8-week-old male transgenic mice (C57BL/6) systemically overexpressing green fluorescent protein (GFP), a kind gift from M. Okabe.<sup>23,24</sup> BMCs ( $5 \times 10^7$ ) suspended in 300  $\mu$ L of RPMI medium were injected intravenously into each 8-week-old female C57BL/6 mouse whose total body had been irradiated (9 Gy) for 6 hours. Six weeks after BMC transplantation, the mice were operated on to induce MI. A flow cytometric analysis using an EPICS ALTRA (Beckman Coulter) equipped with a 525-nm filter at a bandwidth of  $\pm 15$  nm revealed that >70% of BMCs were derived from donor cells.

### Immunohistochemistry and Western Blotting

Paraffin-embedded cardiac sections were immunohistochemically stained by use of antibodies to Ki-67 (Dako), GFP (MBL, Japan), platelet and endothelial cell adhesion molecule (PECAM)-1, and cardiac troponin T (cTnT) (Santa Cruz) and MF20, and then with secondary antibodies conjugated with biotin, FITC, or rhodamine. Western blot analysis was performed with antibodies to vascular endothelial growth factor (VEGF) (Santa Cruz). Hybridizing bands were visualized by use of an enzyme-linked chemiluminescence detecting kit (Amersham Pharmacia Biotech).

### Fluorescence In Situ Hybridization for Y Chromosome

Fluorescence in situ hybridization (FISH) for the Y chromosome was performed by use of the Y chromosome probe pUC18-Y351B, a kind gift from C.E. Bishop (Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Tex).<sup>25</sup> Engraftment of cardiomyocytes was assessed by FISH on paraffin-embedded cardiac sections after immunostaining for cTnT. Cardiac sections from normal male and female mice served as positive and negative controls, respectively.

### Statistical Analysis

All values are expressed as mean  $\pm$  SD of 3 experiments in each instance. Comparisons were made by 1-way ANOVA followed by the Dunnett modified *t* test. Differences with a value of  $P < 0.05$  were considered statistically significant.

## Results

### Increases in LIF Proteins After Injection of LIF cDNA

Some cytokines, including the IL-6 family, have been reported to be increased in MI.<sup>26,27</sup> We first examined LIF protein concentrations in blood of mice after inducing MI. Endogenous plasma LIF concentrations were increased transiently after MI (before, 0.06 ng/mL; 2 days, 2.1 ng/mL; 1 week, 0.8 ng/mL; 2 weeks, 0.4 ng/mL; 4 weeks, 0.13 ng/mL). In mice that were injected with LIF plasmid DNA into the thigh muscle immediately after MI had been induced, LIF protein was markedly increased. The concentrations of LIF peaked at 1 week (5.3 ng/mL) after the injection of LIF cDNA and remained persistently high for >4 weeks (1.4 ng/mL).

### Attenuation of Infarct Extent and LV Remodeling and Improvement of Cardiac Function by LIF

To elucidate whether an increase in LIF protein has beneficial effects on MI, we assessed cardiac function and morphology by echocardiography at 2 weeks after MI. In vehicle-injected MI mice, the posterior wall thickness of the LV was decreased significantly compared with sham-operated mice, and the thickness of the interventricular septum was conversely increased (Table). Moreover, LV internal dimensions at both end-diastole and end-systole were increased, whereas the fractional shortening and ejection fraction were decreased (Table), suggesting that LV remodeling and cardiac dysfunction occurred after MI. In LIF cDNA-injected MI mice, all these changes were less prominent and cardiac functions were much better than in vehicle-injected mice (Table). In vehicle-injected MI mice, the infarction area was quite large and the LVFW was very thin (Figure 1A). Fibrosis was so prominent that there were only a few cardiomyocytes in the infarct zone (Figure 1, A and B). In contrast, in LIF cDNA-injected mice, the cardiac muscle was preserved and the LVFW was much thicker (Figure 1A). Fibrosis was less remarkable in the ischemic zone (Figure 1, A and B). All these findings suggest that LIF does exert beneficial effects on MI from the viewpoints of cardiac morphology and function.

### Protection Against Cardiomyocyte Death and Induction of Neovascularization by LIF

Because LIF has been reported to protect cardiomyocytes from injury,<sup>16,18</sup> we examined, by use of the TUNEL method,