Therapeutic Activation of Signal Transducer and Activator of Transcription 3 by Interleukin-11 Ameliorates Cardiac Fibrosis After Myocardial Infarction

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Background—Glycoprotein 130 is the common receptor subunit for the interleukin (IL)-6 cytokine family. Previously, we reported that pretreatment of IL-11, an IL-6 family cytokine, activates the glycoprotein 130 signaling pathway in cardiomyocytes and prevents ischemia/reperfusion injury in vivo; however, its long-term effects on cardiac remodeling after myocardial infarction (MI) remain to be elucidated.

Methods and Results—MI was generated by ligation of the left coronary artery in C57BL/6 mice. Real-time reverse transcription polymerase chain reaction analyses showed that IL-11 mRNA was remarkably upregulated in the hearts exposed to MI. Intravenous injection of IL-11 activated signal transducer and activator of transcription 3 (STAT3), a downstream signaling molecule of glycoprotein 130, in cardiomyocytes in vivo, suggesting that cardiac myocytes are target cells of IL-11 in the hearts. Twenty-four hours after coronary ligation, IL-11 was administered intravenously, followed by consecutive administration every 24 hours for 4 days. IL-11 treatment reduced fibrosis area 14 days after MI, attenuating cardiac dysfunction. Consistent with a previous report that STAT3 exhibits antiapoptotic and angiogenic activity in the heart, IL-11 treatment prevented apoptotic cell death of the bordering myocardium adjacent to the infarct zone and increased capillary density at the border zone. Importantly, cardiac-specific ablation of STAT3 abrogated IL-11–mediated attenuation of fibrosis and was associated with left ventricular enlargement. Moreover, with the use of cardiac-specific transgenic mice expressing constitutively active STAT3, cardiac STAT3 activation was shown to be sufficient to prevent adverse cardiac remodeling.

Conclusions—IL-11 attenuated cardiac fibrosis after MI through STAT3. Activation of the IL-11/glycoprotein 130/STAT3 axis may be a novel therapeutic strategy against cardiovascular diseases. (Circulation. 2010;121:684-691.)

Key Words: interleukins ▪ myocardial infarction ▪ remodeling ▪ signal transduction

After myocardial injury, various kinds of neurohumoral factors and cytokines modulate cardiac remodeling. Among them, leukemia inhibitory factor (LIF) and cardiotrophin-1, which belong to the interleukin (IL)-6 family, play important roles in cardioprotection.1,2 LIF and cardiotrophin-1 are secreted from cardiomyocytes in response to pathological stress.3,5 These cytokines bind and activate LIF receptor in cardiomyocytes.6 Activated LIF receptor makes a dimer with glycoprotein 130 (gp130), followed by activation of signal transducer and activator of transcription 3 (STAT3).7 STAT3 activation promotes cardiomyocyte survival and vascular formation in the heart.8–10 Thus, cardiac activation of the gp130/STAT3 system may be a potential therapeutic strategy against cardiovascular diseases; however, therapies targeting gp130 have not been proposed.

Clinical Perspective on p 691

The difficulty in therapeutic activation of gp130 is derived from its receptor system. Gp130 is expressed ubiquitously as the common receptor subunit of IL-6 family cytokines.11 IL-6 family cytokines bind their specific receptor α subunits, followed by activation of a common gp130 receptor. Pleiotropic effects of IL-6 family cytokines are explained by the differential expression of receptor α subunits. Most members of the IL-6 family, whose receptor α subunits are expressed abundantly in inflammatory cells, would evoke severe in-

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flammation\(^2\) as a serious adverse event if administered systemically. Therefore, to achieve clinical use of IL-6 family cytokines, the cytokine that induces only a tolerable level of inflammation should be selected.

IL-11 is a hematopoietic IL-6 family cytokine with pleiotropic effects. IL-11 exhibits thrombopoietic activity, and recombinant human IL-11 is used clinically for thrombocytopenia.\(^13\) In contrast to other IL-6 family members, IL-11 exhibits anti-inflammatory activity against chronic inflammatory diseases, such as Crohn disease.\(^14\) Moreover, recombinant human IL-11 protects epithelial cells of the intestine from tissue damage, suggesting its cytoprotective property.\(^15\) Recently, we demonstrated that the IL-11 receptor is expressed in cardiomyocytes and that pretreatment of IL-11 confers resistance to ischemia/reperfusion injury in a murine model as a preconditioning effect.\(^16\) When the limited level of clinical adverse effects of recombinant human IL-11 is considered,\(^13\) IL-11 may be a candidate to be available clinically as cardiac gp130-targeting therapy against heart diseases.

In this study, we investigated the long-term effects of IL-11 treatment after myocardial infarction (MI). In addition, we report that IL-11 treatment prevents adverse cardiac remodeling through the STAT3 pathway.

**Methods**

**Animal Care**

The care of all animals was in compliance with the Osaka University animal care guidelines. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (National Institutes of Health publication No. 85-23, revised 1996).

**Coronary Artery Ligation and IL-11 Treatment**

MI was generated by coronary artery ligation according to the previous report\(^1\) with minor modifications. Briefly, C57BL/6 mice (8 to 10 weeks old; Japan SLC) were anesthetized and ventilated with 80% oxygen containing 1.5% isoflurane (Merek). After left-side thoracotomy, the left coronary artery was ligated with 7-0 silk sutures. Infarction was confirmed by discoloration of the ventricle and ST-T changes on ECG. The chest and skin were closed with 5-0 silk sutures. Infarct area was separated from the infarcted wall at 3 separate regions and averaged.

**Histological Estimation of Cardiac Fibrosis**

The frozen sections (5-μm thick) were prepared from the portion 300 μm distal to the ligation point and stained with Masson’s trichrome. Photomicrographs were taken, and fibrotic circumference and area were measured with the use of Scion Image (Scion Corporation) by a researcher who was blinded to the treatment. Fibrotic circumference and area were calculated as a percentage of LV circumference and area, respectively. Infarct wall thickness was measured perpendicular to the infarcted wall at 3 separate regions and averaged.

**Immunofluorescent Microscopic Analyses**

The hearts were harvested 15 minutes after intravenous injection of IL-11, and the frozen sections were prepared. The sections were stained with anti-p-STAT3 and anti-sarcomeric α-actinin antibodies. Alexa Fluor 488–conjugated goat anti-rabbit IgG (Molecular Probes) and Alexa Fluor 546–conjugated goat anti-mouse IgG (Molecular Probes) were used as secondary antibodies. Nuclei were also stained with Hoechst 33258.

**Immunohistochemical Analyses**

The frozen sections were prepared as described above. Capillary density was examined by immunohistochemical staining with the use of the Vectastain ABC kit (Vector Laboratories) with anti-CD31 antibody (BD Biosciences, San Jose, Calif). To estimate capillary density, the expression of GAPDH mRNA was estimated with the SYBR green system. The primers for IL-11 or GAPDH are as follows: IL-11 forward: 5′-CTGGCC-ACCTTGGCCCATGAG-3′; IL-11 reverse: 5′-CCAGGGACACA-TCAGGAAGA-3′; GAPDH forward: 5′-GGCTGTTGCTGATAGT-GTCGT-3′; GAPDH reverse: 5′-CCCTTTGTCCTACCCCT-3′.

**Immunoblot Analyses**

Immunoblot analyses were performed as described previously.\(^17\) Heart homogenates were prepared in buffer containing 150 mmol/L NaCl, 10 mmol/L Tris-HCl (pH 7.5), 1 mmol/L EDTA, 1% Triton X-100, 1% deoxycholic acid, and 1 mmol/L dithiothreitol. Proteins were separated by SDS-PAGE and transferred onto the polyvinylidene difluoride membrane (Millipore). The membrane was immunoblotted with anti-phospho-STAT3 (p-STAT3) (Cell Signaling Technology, Danvers, Mass), anti-Bcl-2 (BD Transduction Laboratories), anti-survivin (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), or anti-cleaved caspase 3 (Cell Signaling Technology) antibody. The membrane was reprobed with anti-STAT3 (Santa Cruz Biotechnology) or anti-GAPDH (Chemicon, Temecula, Calif) antibody to show equal amount loading.

**Real-Time Reverse Transcription Polymerase Chain Reaction**

Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed according to the manufacturer’s protocol. Total RNA was prepared from hearts at various time points after operation. In some experiments, the hearts were cut into 2 pieces: infarct area and remote area. The infarct area is the damaged or fibrotic region with its surrounding border zone, and the remote area is the portion separated from the infarct area by >1 mm.

Total RNA (1 μg) was subjected to first-strand cDNA synthesis with oligo(dT) primer. IL-11 was quantified by real-time RT-PCR with the use of the ABI-PRISM 7700 sequence detection system (Applied Biosystems Inc) with the SYBR green system (Applied Biosystems). As an internal control, the expression of GAPDH mRNA was estimated with the SYBR green system. The primers for IL-11 or GAPDH are as follows: IL-11 forward: 5′-CTGCCC-ACCTTGGCCCATGAG-3′; IL-11 reverse: 5′-CCAGGGACACA-TCAGGAAGA-3′; GAPDH forward: 5′-GGCTGTTGCTGATAGTGTCGT-3′; GAPDH reverse: 5′-CCCTTTGTCCTACCCCT-3′.
Conditional Ablation of the STAT3 Gene in Cardiomyocytes of Adult Murine Heart

The transgenic mice in which Cre recombinase fused to the mutated estrogen receptor domains (MerCreMer) were driven by the cardiomyocyte-specific α-myosin heavy chain (α-MHC) promoter, designated as α-MHC-MerCreMer mice, were a gift from Dr. Molkentin.18 We crossed the α-MHC-MerCreMer mice with mice that carried floxed STAT3 alleles (STAT3 flox/flox)19 and produced α-MHC-MerCreMer/STAT3flox/flox mutant mice. To induce Cre-mediated recombination, mice were treated with 20 mg/kg tamoxifen (Sigma, St. Louis, Mo) by intraperitoneal injection once per day for 5 consecutive days. Five days after the last treatment, the level of STAT3 expression decreased dramatically, and the mutant mice underwent MI as described above.

Cardiac-Specific Transgenic Mice Expressing Constitutively Active STAT3

Generation of cardiac-specific transgenic mice expressing constitutively active STAT3 was described previously.9

Statistical Analysis

Data are presented as mean±SD. Comparisons between 2 groups were performed with the use of the unpaired t test. One-way ANOVA with the Bonferroni test was used for multiple comparisons. Differences were considered statistically significant when the calculated (2-tailed) P value was <0.05.

Results

IL-11 Is a Cardiac Cytokine That Activates STAT3 in Cardiomyocytes In Vivo

We analyzed the expression of IL-11 mRNA in hearts at various time points after MI. Hearts were separated into infarct area and remote area, and the expression of IL-11 mRNA was measured by real-time RT-PCR (Figure 1A). The expression of IL-11 transcript was elevated, with its peak at 1 day after MI, and was gradually reduced at both infarct and remote areas. In the infarct area, the enhanced expression of IL-11 was sustained for 14 days. These data indicate that IL-11 is produced in the heart during cardiac remodeling after MI.

Next, we examined whether intravenous administration of IL-11 stimulates STAT3 in hearts by immunoblot analysis with anti-p-STAT3 antibody (Figure 1B). STAT3 phosphorylation was induced rapidly and reduced to the basal level within 180 minutes after IL-11 injection. IL-11 activated STAT3 in the heart in a dose-dependent manner (Figure 1 in the online-only Data Supplement).

To confirm that STAT3 activation occurred in cardiomyocytes, we performed immunohistological analyses to detect the nuclear localization of activated p-STAT3 (Figure 1C). Nuclear staining of p-STAT3 was detected in the IL-11–treated hearts but not in untreated hearts. Notably, >90% of nuclei of sarcomeric α-actinin–positive cells were also positively stained with anti-p-STAT3 antibody, indicating that IL-11 treatment results in STAT3 activation in cardiomyocytes in vivo. As is the case with noninfarcted mice, p-STAT3 was localized mainly in cardiomyocyte nuclei of postinfarct hearts, and IL-11 treatment increased the frequency of p-STAT3–positive cardiac myocytes (Figure II in the online-only Data Supplement).

IL-11 Administration Attenuates Cardiac Remodeling After MI

To examine the effects of IL-11 on adverse cardiac remodeling, IL-11 was administered to the mice after MI operation, and cardiac fibrosis was histologically estimated at day 14 after MI (Figure 2). Both fibrotic circumference and fibrotic area were reduced by IL-11 in a dose-dependent manner (Figures 2B and C). Treatment of IL-11 at 8 μg/kg achieved a submaximal reduction in fibrotic circumference by 28.9% (PBS, 58.6±9.6%; IL-11, 41.7±10.0%) and fibrotic area by 33.1% (PBS, 39.8±9.3%; IL-11, 26.6±7.4%). Interestingly, IL-11–treated hearts showed an increase in infarct wall thickness compared with PBS-treated hearts (Figure 2D).
examine the effects of IL-11 on LV hypertrophy, we analyzed expression of α-skeletal muscle actin mRNA, a well-known marker of LV hypertrophy (Figure II in the online-only Data Supplement). IL-11 treatment showed a tendency to reduce α-skeletal muscle actin expression, although its reduction was not statistically significant.

To clarify whether IL-11 prevents cardiac dysfunction after MI, we measured LV developed pressure and ±dP/dt at day 1 (Table I in the online-only Data Supplement) before IL-11 administration was started. Because IL-11 treatment attenuated cardiac fibrosis that occurred during the following 2 weeks, we examined the effects of IL-11 on cardiac function 2 weeks after MI (Table). IL-11 treatment ameliorated chronic cardiac dysfunction in a dose-dependent manner compared with the PBS-treated group. Consistent with the attenuation of fibrosis, treatment of IL-11 at a dose of 8 μg/kg submaximally prevented cardiac dysfunction. Thus, further experiments were performed with the use of IL-11 at a dose of 8 μg/kg.

We also confirmed the inhibitory effects of IL-11 on adverse cardiac remodeling 28 days after MI. IL-11 treatment prevented cardiac fibrosis and preserved cardiac function (Figure IV in the online-only Data Supplement).

To examine whether IL-11 reduces infarct size, infarct size was measured by Evans blue exclusion 2 days after MI. There was no significant difference in infarct size (PBS, 23.0±7.1%; IL-11 [8 μg/kg], 22.0±4.3%; n=4 mice for each group).

**Table. Effects of IL-11 on Cardiac Function at Day 14 After MI**

<table>
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<th>Parameters</th>
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<th>20</th>
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<td>LVDP, mm Hg</td>
<td>32.4±4.4</td>
<td>34.9±6.3</td>
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<td>44.3±13.5*</td>
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<td>±dP/dt, mm Hg/s</td>
<td>884±113</td>
<td>958±162</td>
<td>1210±148*</td>
<td>1243±324*</td>
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<td>−dP/dt, mm Hg/s</td>
<td>−713±93</td>
<td>−737±176</td>
<td>−1022±210*</td>
<td>−1043±326*</td>
</tr>
</tbody>
</table>

Data are mean±SD (n=8 mice for PBS; n=9 mice for 3 μg/kg; n=9 mice for 8 μg/kg; n=8 mice for 20 μg/kg). LVDP indicates LV developed pressure.

*P<0.05 vs PBS by 1-way ANOVA followed by Bonferroni test.
To assess the importance of cardiac STAT3 activation in IL-11–mediated prevention against adverse cardiac remodeling was abrogated in STAT3 CKO mice (Figure 5C and 5D). Interestingly, enlargement of LVs was observed in STAT3 CKO mice compared with wild-type mice exposed to MI without IL-11 treatment (18.02 ± 3.20 mm [n = 6] versus 15.08 ± 1.81 mm [n = 8]; P < 0.05), probably because STAT3 is endogenously activated after MI, even without IL-11 treatment (Figures II and VI in the online-only Data Supplement), and contributes to the prevention of adverse cardiac remodeling. Consistently, we also confirmed that IL-11–mediated attenuation of cardiac dysfunction was canceled in STAT3 CKO mice (Figure VII in the online-only Data Supplement). Moreover, the increase in capillary density, which was observed in response to IL-11 in the mice with STAT3 wild/wild background, was abrogated by STAT3 gene ablation (Figure VIII in the online-only Data Supplement). These data indicate that STAT3 is required for IL-11–mediated amelioration of adverse cardiac remodeling after MI.

To reinforce the importance of STAT3 in cardioprotection, transgenic hearts expressing constitutively active STAT3 (caSTAT3) were exposed to MI, and cardiac fibrosis was estimated. In caSTAT3 transgenic hearts, cardiac fibrosis was reduced by 47% compared with nontransgenic littermates (wild-type) (Figure 6). Moreover, similar to IL-11–treated hearts, caSTAT3 transgenic hearts showed an increase in fibrotic wall thickness. Importantly, cardiac dysfunction was ameliorated in caSTAT3 transgenic mice compared with wild-type mice (Figure IX in the online-only Data Supplement). Therefore, activation of STAT3 in cardiomyocytes was sufficient to suppress cardiac remodeling after MI.

**Activation of STAT3 Is Necessary and Sufficient for IL-11–Mediated Prevention of Cardiac Remodeling**

To assess the importance of cardiac STAT3 activation in IL-11–mediated attenuation of adverse remodeling, we generated cardiac-specific conditional STAT3-deficient mice (STAT3 CKO mice) by establishing α-MHC-MerCreMer mice on STAT3<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> background (Figure 5A). The ablation of the STAT3 gene did not induce notable histological alterations at day 14 after sham operation. IL-11 treatment ameliorated postinfarct fibrosis in α-MHC-MerCreMer mice on STAT3<sup>wild/wild</sup> background after MI (Figure V in the online-only Data Supplement), as in the case with nontransgenic mice (Figure 2). Importantly, IL-11–mediated prevention against adverse cardiac remodeling was abrogated in STAT3 CKO mice (Figure 5C and 5D). Interestingly, enlargement of LVs was observed in STAT3 CKO mice compared with wild-type mice exposed to MI without IL-11 treatment (18.02 ± 3.20 mm [n = 6] versus 15.08 ± 1.81 mm [n = 8]; P < 0.05), probably because STAT3 is endogenously activated after MI, even without IL-11 treatment (Figures II and VI in the online-only Data Supplement), and contributes to the prevention of adverse cardiac remodeling. Consistently, we also confirmed that IL-11–mediated attenuation of cardiac dysfunction was canceled in STAT3 CKO mice (Figure VII in the online-only Data Supplement). Moreover, the increase in capillary density, which was observed in response to IL-11 in the mice with STAT3 wild/wild background, was abrogated by STAT3 gene ablation (Figure VIII in the online-only Data Supplement). These data indicate that STAT3 is required for IL-11–mediated amelioration of adverse cardiac remodeling after MI.

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In this study, we examined the effects of IL-11 treatment on adverse cardiac remodeling after MI. IL-11 activated STAT3 in cardiomyocytes in vivo. IL-11 ameliorated cardiac fibrosis and attenuated cardiac dysfunction after MI. IL-11 reduced the number of apoptotic myocytes, and IL-11–treated hearts showed an increase in capillary density. Importantly, the preventive effects of IL-11 against adverse remodeling were suppressed in cardiac-specific STAT3-deficient mice. Moreover, cardiac STAT3 activation was sufficient to suppress adverse remodeling. These findings suggest that IL-11 treatment is a promising strategy against adverse cardiac remodeling after MI.

Previously, G-CSF and erythropoietin were reported to exhibit preventive effects against postinfarct fibrosis, however, a large difference in signaling pathways exists among these cytokines. IL-11 rapidly activates STAT3 in cardiomyocytes at a lower concentration than G-CSF, suggesting that IL-11 is a more potent activator of STAT3. Akt, another cytoprotective signal transducer, is activated by G-CSF and erythropoietin in cardiomyocytes and to a lesser extent by IL-11 (data not shown). Moreover, G-CSF confers resistance to ischemia/reperfusion through endothelial Akt activation and IL-11 through cardiomyocyte activation of STAT3. Thus, IL-11 transduces cardioprotective signals via pathways that are distinct from G-CSF or erythropoietin.

IL-11 belongs to the IL-6 cytokine family, which uses gp130 as its common receptor. Interestingly, LIF, a member of the IL-6 cytokine family, has been reported to enhance survival of cardiomyocytes, with mobilization of bone marrow cells to the heart. Similarly, G-CSF stimulates the homing of bone marrow cells to the heart, leading to regeneration of the injured heart. These studies propose that bone marrow cells may contribute to cardiac repair in response to cytokine stimuli. In our experiments, IL-11 failed to show cardioprotective effects in cardiomyocyte-specific STAT3-deficient mice. Therefore, cardiomyocytes are important components in the action of IL-11, and STAT3 activation in cardiomyocytes is a critical factor for IL-11–mediated prevention of adverse cardiac remodeling, although we cannot exclude the possibility that bone marrow cell
mobilization is regulated by cardiac STAT3 through some paracrine systems.

Previously, we reported that IL-11 shows cytoprotective effects through STAT3 and prevents ischemia/reperfusion injury in the heart. IL-11 reduced cardiomyocyte apoptosis after MI consistently in this study. In addition to cell-autonomous cytoprotection, we have demonstrated that IL-11 promoted vessel growth in the heart. Importantly, the enhancement of capillary density by IL-11 was not observed in cardiac-specific STAT3-deficient mice. Taken together with previous reports that cardiac activation of STAT3 promotes vascular formation in the heart, STAT3 is a critical regulator of the interaction between myocardium and endothelium. Interestingly, IL-11-mediated enhancement of vascular formation was closely associated with improvement of cardiomyocyte viability. Similarly, it was reported recently that cardiac production of angiogenic growth factors was impaired under decompensatory conditions during cardiac remodeling and that angiogenesis was critical to prevent onset of heart failure in response to pathological stress. Collectively, this suggests that IL-11 mediates cardiomyocyte survival and capillary growth in an interdependent manner and that these signals are coordinated by STAT3.

IL-11 exerted preventive effects against adverse cardiac remodeling after MI at almost the same level as G-CSF, whose clinical use was reported to be at least partially beneficial in cardiovascular diseases. Thus, IL-11 treatment may be proposed as a novel cytokine therapy. Although one of the most serious adverse effects of cytokine therapy is inflammation, IL-11 is known to be an anti-inflammatory cytokine. Indeed, IL-11 significantly suppressed expression of proinflammatory cytokines, such as IL-6 and tumor necrosis factor-α, in postinfarct myocardium (Figure X in the online-only Data Supplement), which provides evidence supporting its safety.

In summary, IL-11 transduced cardioprotective signals through STAT3 and suppressed adverse cardiac remodeling after MI. The IL-11/gp130/STAT3 axis may be a novel therapeutic target against cardiovascular diseases.

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Disclosures
None.

References
IL-11 as a Novel Cardioprotective Cytokine

In ischemic heart diseases, myocardial damage is initially induced by reduction of blood supply and is subsequently expanded by cardiac remodeling, leading to heart failure. A therapeutic strategy to limit myocardial remodeling, such as angiotensin-converting enzyme inhibitors and β-blockers, improves the survival rate; however, the prognosis of heart failure is still not satisfactory. Cardiac remodeling is positively or negatively regulated by a number of neurohumoral factors and cytokines. Here, we examined whether interleukin (IL)-11, a member of the IL-6 family of cytokines, ameliorates postinfarct remodeling, using a model of myocardial infarction by coronary ligation. Treatment with IL-11 reduced fibrosis after myocardial infarction, with attenuation of myocardial dysfunction. IL-11 decreased the frequency of cardiomyocyte death and increased capillary density. IL-11 treatment resulted in STAT3 activation in cardiomyocytes in vivo. Using conditional knockout mice and cardiac-specific transgenic mice, we demonstrated that activation of STAT3 in cardiomyocytes was necessary and sufficient for IL-11-mediated prevention of cardiac adverse remodeling. These findings suggest that IL-11 treatment may be useful as a therapeutic strategy against the onset of heart failure after myocardial infarction. Because human recombinant IL-11, oprelvekin, is clinically used for thrombocytopenia, with a tolerable level of adverse drug effects providing some proof of its safety, our results suggest that IL-11 treatment is a promising novel cytokine therapy for prevention against heart failure.
Therapeutic Activation of Signal Transducer and Activator of Transcription 3 by Interleukin-11 Ameliorates Cardiac Fibrosis After Myocardial Infarction
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SUPPLEMENTAL MATERIAL
Supplementary Materials and Methods

Real time RT-PCR

The expression of α-skeletal muscle actin mRNA was quantified by real time RT-PCR using the ABI-PRISM® 7700 sequence detection system with SYBR green system. The primers for α-skeletal muscle actin are as follows;

α-skeletal muscle actin forward: 5’-AGGGCCAGAGTCAGAGCAG -3’,
α-skeletal muscle actin reverse: 5’-CCGTTGTCACACACAAGAGC -3’.

The expression of IL-6 or TNF-α mRNA was quantified by real time RT-PCR using the ABI-PRISM® 7700 sequence detection system with TaqMan Assay on Demand Reagents (PE Applied Biosystems Inc.).

Supplementary Figure Legends

Supplementary Figure 1
IL-11 activated STAT3 in murine hearts in a dose-dependent manner.
The various concentrations of IL-11 were intravenously administered to mice. Fifteen minutes after injection, mice were sacrificed. The lysates were prepared from hearts and immunoblotted with anti-phospho-specific STAT3 (p-STAT3) antibody. Blots were reprobed with anti-STAT3 antibody. Experiments were repeated three times with similar results.

Supplementary Figure 2
IL-11 activated STAT3 in the cardiomyocytes at day 1 after MI.
IL-11 (8 µg/kg) or PBS was intravenously administered in mice at day 1 after MI. Fifteen minutes after injection, the hearts were harvested and frozen sections were prepared. The sections were co-stained with anti-phospho-specific STAT3 (p-STAT3) and anti-sarcomeric α-actinin antibodies. Hoechst 33258 staining was also performed to identify the nuclei. The images shown are representative of 15 obtained from 3 mice (5 fields from each mouse). Arrowheads show the p-STAT3-positive nuclei. Bar, 50 µm.

Supplementary Figure 3
The effect of IL-11 on the expression of α-skeletal muscle actin after MI.
Total RNA was prepared from infarct (I) or remote (R) area of hearts after MI and real time RT-PCR was performed for α-skeletal muscle actin. The expression of α-skeletal muscle actin
was normalized with that of GAPDH. Data are shown as mean ± S.D. (n=5 mice for each group).

Supplementary Figure 4

**IL-11 attenuated the cardiac remodeling at day 28 after MI.**

Mice were exposed to MI operation, followed by the treatment of IL-11 for 5 days. PBS was used as control. Heart sections were prepared 28 days after MI and stained with Masson’s Trichrome method to determine fibrosis. The ratio of fibrotic circumference to LV circumference was quantitatively estimated. LVDP and ±dp/dt were measured by a Langendorff apparatus at day 28 after MI. Data are shown as mean ± S.D. (n=4 mice, for PBS; n=7 mice, for IL-11). *P<0.05 vs. PBS, by unpaired t test.

Supplementary Figure 5

**IL-11 treatment reduced the cardiac fibrosis in α-MHC-MerCreMer mice on STAT3\(^{\text{wild/wild}}\) background after MI.**

MI was generated in α-MHC-MerCreMer mice on STAT3\(^{\text{wild/wild}}\) background, followed by IL-11 treatment for 5 days. Heart sections (3 sections from each mouse) were prepared 14 days after MI and stained with Masson’s Trichrome method to determine fibrosis. (A) The images are representative of 18 obtained from 6 mice. Bar, 1 mm. (B) The ratio of fibrotic circumference to LV circumference was quantitatively estimated. Data are shown as mean ± S.D. (n=6 mice for each group). *P<0.01 vs. PBS, by unpaired t test.

Supplementary Figure 6

**Cardiac activation of STAT3 was sustained during cardiac remodeling after MI in mice.**

Mice were exposed to MI operation and the hearts were harvested at the indicated time point. The lysates were prepared from hearts and immunoblotted with anti-phospho-specific STAT3 (p-STAT3) antibody. Blots were reprobed with anti-STAT3 antibody.

Supplementary Figure 7

**STAT3 was required for IL-11-mediated amelioration of cardiac dysfunction after MI.**

After tamoxifen treatment, MI was generated in α-MHC-MerCreMer/STAT3\(^{\text{flox/flox}}\) (flox/folx, n=4) or α-MHC-MerCreMer/STAT3\(^{\text{wild/wild}}\) (wild/wild, n=4) mice, followed by the administration of IL-11. At day 14 after MI, LVDP and ±dp/dt were measured by a Langendorff apparatus. Data are shown as mean ± S.D. *P<0.05 vs. α-MHC-MerCreMer/STAT3\(^{\text{wild/wild}}\), by unpaired t test.
Supplementary Figure 8

The enhancement of capillary density by IL-11 was not observed in cardiac-specific STAT3-deficient mice.

After tamoxifen treatment, MI was generated in α-MHC-MerCreMer/STAT3\textsuperscript{floxflo} or α-MHC-MerCreMer/STAT3\textsuperscript{wild/wild} mice, followed by the administration of IL-11. Heart sections were prepared 14 days after MI and stained with anti-CD31 antibody, to detect capillary endothelial cells. The CD31-positive capillary density was quantitatively estimated. Ten visual fields were randomly selected. Data are shown as mean ± S.D. (n=6 mice for each group). *\( P < 0.01 \) vs. STAT3\textsuperscript{wild/wild} without IL-11 treatment. #\( P < 0.01 \) vs. STAT3\textsuperscript{floxflo} with IL-11 treatment, by one-way ANOVA followed by Bonferroni test.

Supplementary Figure 9

Activation of STAT3 in cardiomyocytes was sufficient for amelioration of cardiac dysfunction.

Cardiac-specific transgenic mice expressing constitutively active STAT3 (caSTAT3) or wild-type mice were exposed to MI. At day 14 after MI, LVDP and ±dp/dt were measured by a Langendorff apparatus. Data are shown as mean ± S.D. (n=5 mice for each group). *\( P < 0.05 \) vs. wild-type, by unpaired \( t \) test.

Supplementary Figure 10

IL-11 suppressed the inflammatory reaction in post-infarct myocardium.

Total RNA was prepared from infarct hearts at day 4 after MI or sham operation. The expression level of IL-6 or TNF-α mRNA was measured by real time RT-PCR methods and normalized with that of GAPDH. The cytokine level was expressed as fold induction of that in non-infarct hearts. Data are shown as mean ± S.D. (n=4 mice for each group). *\( P < 0.05 \) vs. sham, #\( P < 0.05 \) vs. PBS, by one-way ANOVA followed by Bonferroni test.
### Supplementary Table 1. Cardiac function at day 1 after MI before IL-11 treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>One day post MI</th>
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</thead>
<tbody>
<tr>
<td>LVDP (mmHg)</td>
<td>83.0±8.8</td>
<td>53.9±12.6*</td>
</tr>
<tr>
<td>+dp/dt (mmHg/s)</td>
<td>2321±390</td>
<td>1485±316*</td>
</tr>
<tr>
<td>-dp/dt (mmHg/s)</td>
<td>-2030±353</td>
<td>-1242±290*</td>
</tr>
</tbody>
</table>

Data were shown as mean ± S.D. (n=7 mice, for baseline; n=7 mice, one day post MI). *P<0.01 vs. Baseline, by unpaired t test.

### Supplementary Table 2. The dependency of IL-11 therapy on the treatment timing.

<table>
<thead>
<tr>
<th></th>
<th>Fibrotic area / LV area (%)</th>
<th>Capillaries (/mm²)</th>
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</thead>
<tbody>
<tr>
<td>PBS</td>
<td>42.6±13.1</td>
<td>2052±118</td>
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<tr>
<td>IL-11</td>
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<tr>
<td>d1-d3</td>
<td>26.4±8.0*</td>
<td>2304±179*</td>
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<tr>
<td>d3-d5</td>
<td>34.8±9.0</td>
<td>2153±232</td>
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</table>

Data were shown as mean ± S.D. (n=8 mice, for PBS; n=9 mice, for d1-d3; n=9 mice, for d3-d5). *P<0.05 vs. PBS, by one-way ANOVA followed by Bonferroni test.
Supplementary Figure 1
Supplementary Figure 3
Supplementary Figure 5
<table>
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<th>non-MI</th>
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<tr>
<td><strong>p-STAT3</strong></td>
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<td></td>
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<tr>
<td><strong>total STAT3</strong></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Supplementary Figure 6
Supplementary Figure 7
Supplementary Figure 8

![Bar chart showing capillaries/mm² for different conditions.](image-url)

- IL-11: 
  - wild/wild: 2000 capillaries/mm²
  - flox/flox: 2500 capillaries/mm²
- * indicates significant difference
- # indicates another significant difference
Supplementary Figure 9