Inflammatory Response to Acute Myocardial Infarction Augments Neointimal Hyperplasia After Vascular Injury in a Remote Artery

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Objective—Percutaneous coronary intervention (PCI) is currently the most widely accepted treatment for acute myocardial infarction (AMI). It remains unclear, however, whether post-AMI conditions might exacerbate neointimal hyperplasia and restenosis following PCI. Given that both a medial smooth muscle cell lineage and a bone marrow (BM)-derived hematopoietic stem cell lineage are now thought to contribute to neointima formation, the primary aims of the present study were to determine whether AMI augments neointimal hyperplasia at sites of arterial injury, and whether BM-derived cells contribute to that process.

Methods and Results—We simultaneously generated models of AMI and arterial injury in the same mice, some of which had received BM transplantation. We found that AMI augments neointimal hyperplasia at sites of femoral artery injury by ≈35% (P<0.05), but that while BM-derived cells contributed to neointimal hyperplasia, they did not contribute to the AMI-related augmentation. Expression of interleukin (IL)-6 mRNA was ≈7-fold higher in the neointimas of mice subjected to both AMI and arterial injury than in those of mice subjected to arterial injury alone. In addition, we observed increased synthesis of tumor necrosis factor (TNF)-α within infarcted hearts and TNF-α receptor type 1 (TNFR1) within injured arteries. Chronic treatment with pentoxifylline, which mainly inhibits TNF-α synthesis, reduced levels of circulating TNF-α and attenuated neointimal hyperplasia after AMI.

Conclusions—Conditions after AMI could exacerbate postangioplasty restenosis, not by increasing mobilization of BM-derived cells, but by stimulating signaling via TNF-α, TNFR1 and IL-6. (Arterioscler Thromb Vasc Biol. 2006; 26:000-000.)

Key Words: Bone marrow ■ Inflammation ■ Myocardial infarction ■ Restenosis ■ Smooth muscle cell

Both the occurrence and eventual healing of acute myocardial infarction (AMI) evoke inflammatory processes that lead to clinical components of instability, as evidenced by the high rate of subsequent coronary artery events, including recurrent MI and in-stent restenosis after percutaneous coronary intervention (PCI). It is well known from both experimental and clinical observations that local upregulation of the expression of proinflammatory cytokines in activated smooth muscle cells (SMCs) contributes significantly to restenosis after balloon angioplasty and stent implantation. In the setting of AMI, moreover, various proinflammatory cytokines and growth factors, including TNF-α, IL-1β, IL-6 and vascular endothelial growth factor (VEGF), are expressed in both infarcted and noninfarcted regions of the heart, and their plasma levels are elevated for ≈2 weeks after AMI, raising the possibility that they, too, contribute to neointimal hyperplasia after PCI.

Recent findings suggest that 2 lineages of neointimal SMCs are involved in vascular remodeling after injury: a medial SMC lineage whose activation is triggered by various proinflammatory cytokines (the classical scenario), and a newly identified bone marrow (BM)-derived hematopoietic stem cell lineage. It now appears that hematopoietic stem cells and endothelial progenitor cells are released from BM into the peripheral circulation during the early phase of AMI. Thus, the mechanism for AMI-related vascular remodeling is apparently more complex than was recognized before the emergence of these new findings.

Within that context, the first aim of the present study was to determine whether AMI is, itself, capable of promoting neointimal hyperplasia at distant sites of arterial injury, such as would be caused by PCI. If so, the second aim of this study was to determine whether BM-derived cells contribute to that process. To accomplish these aims, we simultaneously gen-
ered experimental models of AMI and femoral arterial injury in the same mice, some of which had previously received BM transplantation (BMT) from green fluorescence protein (GFP) mice. Here we show that the inflammatory response to AMI augments neointimal hyperplasia in the injured femoral artery, but whereas BM-derived cells contribute to that neointima formation, they do not significantly contribute to the AMI-related augmentation of the response. Moreover, we demonstrate that the TNF-α synthesis inhibitor pentoxifylline (PTX) reduces levels of circulating TNF-α and attenuates neointimal hyperplasia after AMI. Apparently, cross-talk between the heart and injured artery via signaling pathways mediated by inflammatory cytokines, especially TNF-α, TNF receptor type 1 (TNFR1) and IL-6, are involved in this process.

Materials and Methods

Animals

C57BL/6 mice were purchased from SLC (Shizuoka, Japan). Transgenic mice (C57BL/6 background) that ubiquitously express enhanced GFP (GFP mice) were a generous gift from Dr Masaru Okabe (Osaka University, Osaka, Japan). All experimental procedures were performed in accordance with protocols approved by the Ethics Review Committee for Animal Experimentation of Nara Medical University.

Bone Marrow Reconstitution

Bone marrow reconstitution (BMT) was performed as described previously. One day after exposing 8-week-old male wild-type mice to a lethal dose (9.0 Gy) of X-irradiation, they received a tail vein injection of unfraccionated BM cells (1×10⁶) that had been harvested from the femora and tibias of GFP mice and suspended in 0.2 mL of phosphate-buffered saline. Eight weeks after BMT, peripheral leukocytes had been reconstituted to >90% of control, as determined by flow cytometry.

AMI

AMI was induced in mice as described previously. The precise methods are described online only. Please see http://atvb.ahajournals.org.

Vascular Injury

Vascular injury (VI) was induced as described previously. Mice were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg), and the femoral artery was exposed. A straight spring wire (0.38 mm in diameter, No. C-SF-15 to 15; COOK, Bloomington, Ind) was then inserted into the femoral artery, left in place for 1 minute to denude and dilate the artery, then removed.

Measurement of Neointimal Hyperplasia

For morphometric studies, femoral arteries were harvested 4 weeks after injury, and digitalized images of these vessels were obtained and analyzed using image analysis software (Version 3.2; Soft Imaging System, Munster, Germany). The lumen, internal elastic lamina (IEL), and external elastic lamina (EEL) were defined, and the intimal (tissue between lumen and IEL) and medial (tissue between IEL and EEL) areas were recorded. Neointima/media area (NI/M) ratios were also calculated.

Immunohistochemistry and Immunofluorescent Staining

Methods for immunohistochemistry and immunofluorescent staining were performed standard methods, and their details were described online only. Please see http://atvb.ahajournals.org.

cDNA Array Analysis

Total RNA was isolated from pooled arteries (n=6 for each group) using a QIAGEN RNeasy Minikit (QIAGEN Inc, Valencia, Calif). Murine U74A version 2 GeneChips were purchased from Affymetrix (Santa Clara, Calif) and hybridization was carried out according to the manufacturer’s instructions.

Measurement of Proinflammatory Cytokine mRNA

RNA was isolated from pooled arteries (n=6 to 8 for each group) using a QIAGEN RNeasy Minikit (QIAGEN Inc, Valencia, Calif) and then amplified using a MessageAmp Kit (Ambion, Austin, Tex), which ensures amplification of very small amounts of RNA. RNA also was isolated from hearts using TRizol Reagent (Invitrogen, Carlsbad, Calif), after which cDNA was generated using both RNA samples and an Invitrogen SuperScript II Reverse Transcriptase Kit (Invitrogen, Carlsbad, Calif). Real-time polymerase chain reaction (PCR) was then performed in an ABI-Prism 7700 (Applied Biosystems, Foster City, Calif) using Taqman Universal PCR MasterMix (Applied Biosystems). The oligonucleotide probes and primers for IL-6, MCP-1, VEGF, transforming growth factor (TGF)-β, stromal cell-derived factor (SDF)-1α, IL-1β, and TNF-α were purchased from Applied Biosystems.

Measurements of Plasma TNF-α Levels

Plasma TNF-α levels were measured using a mouse TNF-α enzyme-linked immunosorbent assay kit (eBioscience, San Diego, Calif) according to the manufacturer instructions. The minimum detectable concentration of TNF-α was 8 pg/mL.

Experimental Protocols

Depending on the experiment, mice were placed into one of four groups: the AMI+VI group were subjected to both AMI and femoral arterial injury; the VI group was subjected to a sham operation and femoral arterial injury; and the AMI and sham-operated groups received only AMI or the sham operation, respectively. Mice that did not receive BM cells were used to compare neointimal hyperplasia and mRNA expression among the groups. Two weeks after AMI, femoral arteries were carefully excised from 6 to 8 mice in each group and pooled for analysis of mRNA expression. Data from 2 independent experiments were averaged. Four weeks after AMI, femoral arteries were excised from 10 mice in each group to measure neointimal hyperplasia. Again, 2 series of these experiments were performed. In addition, to detect BM-derived cells within the neointima, we performed similar experiments using 6 mice that had received BM cells in each group.

In some mice in AMI+VI and VI groups, PTX (30 mg/kg per day) or vehicle (Veh) (phosphate-buffered saline) was infused intra-arterially using an osmotic minipump (Alzet, Cupertino, Calif) for 4 weeks after AMI or sham operation. At the end of the 4-week treatment period, the mice were euthanized and peripheral blood was collected to measure circulating TNF-α levels, and the injured and sham-operated femoral arteries were collected to assess the neointimal hyperplasia.

Statistical Analysis

All results are expressed as means±SEM. Differences between groups were evaluated for statistical significance using Student t test. Values of P<0.05 were considered significant.

Results

Myocardial Infarction Augments Neointimal Hyperplasia in Injured Arteries

Four weeks after the surgery, neointima formation was observed in mice in both the AMI+VI and VI groups (Figure 1A and 1B). As can be seen in Figure 1, however, the neointimal hyperplasia was substantially more prominent in
the AMI+VI group than in the VI group. Immunohistochemical staining revealed that the neointimas in both groups were mainly composed of α-SMA-positive SMCs (Figure 1C and 1D), suggesting that the inflammatory response to AMI increases SMC numbers within the neointimas of distant injured arteries. When we measured the neointimal and medial areas using computerized morphometry, we found that the NI/M ratios and neointimal areas were significantly greater in the AMI+VI group than in the VI group (Figure 1E and 1F).

BM-Derived Cells Contribute to Neointima Formation but not to the AMI-Related Augmentation

To determine the extent to which BM-derived cells contribute to the AMI-related augmentation of neointimal hyperplasia in injured arteries, we next performed a set of experiments using mice that had received BM cells from GFP mice. Four weeks after the vascular injury, we observed that GFP-positive cells had accumulated in the neointimas and medias of the injured arteries (Figure 2A and 2B) in both AMI+VI and VI mice. Moreover, immunofluorescent staining showed that some of the GFP-positive cells expressed α-SMA (Figure 2C), suggesting they had differentiated into cells similar to SMCs. The numbers of GFP-positive cells did not significantly differ in the neointimas or medias of mice in the AMI+VI and VI groups (Figure 2D), though they tended to be larger in AMI+VI mice than in VI mice.

It thus appears that BM-derived cells do indeed contribute to vascular remodeling after injury, but they are not responsible for the AMI-related augmentation of the response. It also appears that the inflammatory response to AMI did not promote significant mobilization of progenitor cells with the potential to differentiate into SMCs.

Expression of Proinflammatory Cytokines in Injured Femoral Arteries

Given the absence of a significant contribution by BM-derived cells to the augmented neointimal hyperplasia seen in injured arteries after AMI, we next sought to identify any molecules that might trigger migration and proliferation of medial SMCs by analyzing the expression profiles of various mRNAs using cDNA arrays. Among a number of upregulated molecules, levels of IL-6, MCP-1, VEGF, TGF-β, SDF-1α, and IL-1β mRNA were markedly higher in the injured arteries of AMI+VI mice than in those of sham-operated mice. Quantitative reverse-transcription polymerase chain reaction (RT-PCR) analysis showed a 7-fold increase in IL-6 mRNA expression in the AMI+VI group, as compared with the VI group, and a 500-fold increase, as compared with the sham-operated group (Figure 3A). Levels of MCP-1, VEGF, TGF-β, SDF-1α, and IL-1β mRNA were similar in both the AMI+VI and VI groups and higher than in the sham-operated group (supplemental Figure IA to IE, available online at http://atvb.ahajournals.org.). In addition, immunohistochemical analysis showed clear upregulation of IL-6 protein that paralleled the upregulation of mRNA expression in the neointimal region (Figure 3B).

Cardiac Expression of TNF-α After AMI

Because TNF-α reportedly stimulates IL-6 expression,22 we next used quantitative RT-PCR to examine expression of TNF-α mRNA in infarcted hearts in an effort to determine the reason why IL-6 mRNA was preferentially upregulated in injured arteries following AMI. As shown in supplemental Figure IIA, expression of TNF-α mRNA was significantly increased in infarcted hearts 1, 3, 7, and 28 days after AMI, as compared with sham-operated hearts. Moreover, immunohistochemical analysis showed clear upregulation of TNF-α protein that paralleled the upregulation of mRNA expression in the infarcted hearts (supplemental Figure IIB and IIC).
Femoral Arterial Expression of TNFR1 After Vascular Injury

Given the increased cardiac expression of TNF-α and circulating of TNF-α levels after AMI, we tested the possibility that TNF-α acts via locally expressed TNFR1 to upregulate expression of IL-6 within injured arteries. Consistent with that idea, quantitative RT-PCR analysis revealed that the level of TNFR1 mRNA expression was significantly higher in injured femoral arteries from both AMI VI and VI mice than in those from sham-operated or AMI mice (Figure 4).

Effect of Blockade of TNF-α Production on Vascular Remodeling

Finally, to confirm that the relationship between the increase in plasma TNF-α levels and the augmentation in neointima formation in remote injured arteries was causative, we tested the effects of PTX, an inhibitor of TNF-α synthesis. We found that plasma TNF-α levels were significantly higher in vehicle (Veh)-treated AMI+VI mice than in Veh-treated VI mice; but that TNF-α levels in AMI+VI mice were significantly diminished by PTX to a level similar to that seen in Veh-treated VI mice (Figure 5B). In addition, morphometric analysis revealed that neointimal areas and NI/M ratios in Veh-treated AMI+VI mice were significantly greater than in Veh-treated VI mice and that PTX significantly reduced neointimal areas and NI/M ratios (Figure 5A, 5C, and 5E). However, PTX treatment did not significantly affect neointimal areas or NI/M ratios in VI mice. Thus, prevention of the AMI-induced increase in plasma TNF-α levels by PTX attenuated neointimal hyperplasia in a remote artery after AMI.

Figure 2. BM-derived GFP-positive cells within injured arteries. Vascular injuries were induced in BMT GFP mice, after which the femoral arteries were fixed in 4% paraformaldehyde and embedded in plastic resin. Injured arteries from VI (A) and AMI+VI (B) mice were harvested after 4 weeks and observed under a confocal microscope. Arrowheads indicate the internal elastic lamina; arrows indicate GFP-positive cells; scale bars represent 25 μm. C, Immunofluorescent staining with Cy3-conjugated anti-α-SMA antibody (red) within injured arteries. The arrow indicates a GFP-positive SMC; scale bars represent 5 μm. D, Numbers of GFP-positive cells and total cell numbers within the injured arteries of VI (open bars) and AMI+VI (solid bars) mice. Bars are means ± SEM for 5 mice per group.

Figure 3. A, Effect of myocardial infarction on expression of the IL-6 within injured arteries: white bars, control; hatched bars, VI; black bar, AMI+VI. Tissue samples were prepared from injured and uninjured arteries 14 days after surgery (control). The result shown is representative of data obtained from 3 to 4 mice per group. IL-6 signal intensities were normalized to that of GAPDH; bars depict the fold increase relative to uninjured arteries (control). B, Immunohistochemical staining of IL6 within injured femoral arteries from VI and AMI+VI mice harvested 4 weeks after surgery. Scale bars represent 25 μm.
Discussion

The main findings of the present study are that: (1) the inflammatory response to AMI augments neointimal hyperplasia at sites of injury within distant arteries; (2) BM-derived cells contribute to neointimal hyperplasia after vascular injury, but not to the AMI-related augmentation of the response; (3) cardiac synthesis of TNF-α and circulating TNF-α levels are both increased after AMI, as is expression of TNFR1 mRNA in injured arteries; (4) IL-6 is preferentially upregulated in the neointima of injured arteries after AMI; and (5) treatment with PTX, an inhibitor of TNF-α synthesis, inhibited the AMI-induced increases in plasma TNF-α and attenuated neointima formation after vascular injury. It thus appears that the inflammatory response to AMI stimulates neointimal hyperplasia at sites of vascular injury at least in part by stimulating signaling via TNF-α, TNFR1, and IL-6.

Cardiac levels of several vasoactive cytokines are elevated after AMI. For instance, levels of VEGF are increased in both infarcted hearts and the plasma. We also have observed that plasma levels of placental growth factor (PIGF), another VEGF family cytokine, are elevated within infarcted hearts as a result of its synthesis in endothelial cells within the infarcted region.23 Earlier works by Hattori et al indicate that both VEGF and PIGF stimulate matrix metalloproteinase (MMP)-9 expression in BM stromal cells via the flt pathway, and that MMP-9 cleaves membrane bound Kit ligand into soluble Kit ligand, which in turn activates hematopoietic stem cells.24,25 In addition, numbers of CD34-positive cells also are increased after AMI.13 Based on these findings, we suggested that conditions directly caused by AMI stimulate neointimal hyperplasia at remote sites of vascular injury, and that BM-derived cells contribute significantly to the AMI-related augmentation of neointimal hyperplasia. In that regard, one recent report showed that BM-derived cells contribute to neointimal hyperplasia after mechanical vascular injury, especially after severe wire-induced injury.26

![Figure 4](image-url) Effect of vascular injury on arterial expression of TNFR1. Tissue samples were prepared from injured and uninjured (control) femoral arteries 14 days after surgery: A, A representative image of RT-PCRs for TNFR1 expression. B, Quantitative real-time PCR analysis of TNFR1 expression levels obtained from 3 to 4 mice in each group.

![Figure 5](image-url) Inhibitory effect of PTX in AMI+VI and VI mice. A, Hematoxylin and eosin-stained sections of femoral artery from Veh-treated AMI+VI, Veh-treated VI, PTX-treated AMI+VI, and PTX-treated VI mice (8 to 10 weeks old) harvested 4 weeks after vascular injury; scale bars represent 100 μm. B, Circulating TNF-α levels in AMI+VI and VI mice continuously infused for 4 weeks with Veh or PTX. C to E, Effect of PTX treatment on morphological changes. Morphometric analysis of injured femoral arteries in AMI+VI and VI mice, with or without PTX, 4 weeks after wire-induced injury. Values are mean±SEM; *P<0.05; **P<0.01; n=6 to 10; N.S indicates not statistically significant.
study, we confirmed the presence of BM-derived cells in the neointima and media of the affected artery after wire-induced vascular injury. But as shown in Figure 2, although the number of neointimal BM-derived cells tended to be higher in mice after AMI than after sham operation, the difference was not sufficient to explain the augmented neointimal hyperplasia seen after AMI. Apparently, AMI does not stimulate recruitment of BM-derived cells into the neointima at sites of vascular injury. Pathological significance of BM-derived cells in the setting of AMI is needed to be further elucidated.

Our analysis of cDNA arrays, performed to identify key molecules responsible for the AMI-related augmentation of neointimal hyperplasia after vascular injury, revealed upregulation of MCP-1, VEGF, TGF-β, SDF-1α, and IL-1β within injured arteries, with and without AMI. However, levels of IL-6 were ~7-fold higher in the injured arteries of AMI + VI mice than in those of VI mice. Studies have shown that IL-6 mRNA is expressed in the atherosclerotic lesions of apolipoprotein E knockout mice and humans and in the neointimas of injured arteries, and that STAT3, which is activated by IL-6, contributes to neointima formation by promoting neointimal SMC proliferation and survival. However, earlier reports mainly emphasized the importance of MCP-1, VEGF, TGF-β, SDF-1α, and IL-1β rather than IL-6 in neointima formation. The present study confirms the importance of IL-6 in neointimal hyperplasia after wire-induced injury and suggests that IL-6 plays a more important role in AMI-related neointimal hyperplasia than the other aforementioned mediators.

Bearing in mind, a key question is, what are the signals that stimulate the preferential elevation of IL-6 expression in injured arteries after AMI? A number of earlier reports have shown that TNF-α and IL-1β are strong stimulators of IL-6 expression. Consistent with that earlier work, we found that TNF-α expression is upregulated in infarcted hearts during the 2-week period after AMI and that plasma TNF-α levels were increased to 22.6 ± 4.2 pg/mL, which is sufficient to stimulate IL-6 expression. We also detected expression of TNFR1 mRNA in injured arteries, but not in healthy ones. TNF-α exerts its effects through both TNFR1 and TNFR2, but blockade of TNFR1 gene expression reportedly reduces neointimal hyperplasia after vascular injury by 2-fold, whereas blocking TNFR2 expression has no effect. Thus, in the setting of AMI, it is likely that TNF-α released from the infarcted heart binds to newly upregulated-TNFR1 on the surface of cells at remote sites of arterial injury, leading to IL-6 production and, ultimately, stimulation of neointimal hyperplasia.

To prove a cause–effect relationship between expression of TNF-α in heart and IL-6 in arteries, and one between AMI and augmented neointima formation, we demonstrated that prevention of TNF-α synthesis by PTX inhibited the AMI-induced increases in plasma TNF-α and attenuated neointimal formation after vascular injury. Collectively, these results are indicative of the important role played by TNF-α in experimental postangioplasty restenosis. An earlier report showed the short-term, exogenous administration of TNF-α did not increase neointima formation after balloon injury in rabbits, suggesting that prolonged and persistent elevation of circulating TNF-α is required to promote neointimal hyperplasia after vascular injury. In this study, we used PTX as an inhibitor of TNF-α synthesis instead of a neutralizing antibody or a soluble form of TNF-α receptor. Although both neutralizing antibody and soluble form of TNF-α receptor are more specific than PTX, they are immunogenic, and they are used in vivo experiments especially in the long-term experiment like the present experiment. PTX reduces the synthesis of TNF-α by blocking its transcription, and has been used successfully in earlier works. Therefore, we adopted PTX to inhibit TNF-α pathway. However, it might be possible that present findings were modulated by other molecules transcriptionally suppressed by PTX.

In the clinical point of view, primary purpose of the present study is to elucidate whether post-AMI conditions enhance the neointimal hyperplasia and restenosis after PCI, that is why we generated a mouse model of AMI plus vascular injury. However, further studies using model in larger animal such as porcine are necessary to get more directly evidence that AMI augments neointimal hyperplasia and restenosis after PCI of infarct-related artery.

In conclusion, the present findings provide experimental evidence supporting the idea that conditions directly resulting from AMI exacerbate neointimal hyperplasia after vascular injury through activation of TNF-α, TNFR1, and IL-6 network.

Acknowledgments
We thank Professor M. Okabe, Osaka University, Osaka, Japan, for providing us with GFP Tg mice.

Sources of Funding
This work was supported in part by research grants from the Japanese Ministry of Health, Labor and Welfare, the Japanese Society for the Promotion of Science, and the Japan Health Science Foundation, and a Japan Heart Foundation/Prizer Japan Grant on Cardiovascular Disease Research.

Disclosures
None.

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Arterioscler Thromb Vasc Biol. published online June 15, 2006;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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Material and Methods

Acute Myocardial Infarction

After anesthetizing mice with ether, they were intratracheally cannulated with a polyethylene tube and connected to a respirator (Shinano, Tokyo, Japan). Thereafter, the mice were anesthetized with 1.0% isoflurane, a left thoracotomy was performed, and the left coronary artery was occluded about 1 to 2 mm under the left auricle using an 8-0 nylon suture (Natsume Co., Tokyo, Japan). After ligation, the chests were closed, and the mice were weaned from the respirator. Finally, the intratracheal tube was removed and the mice were allowed to recover. The same procedure without coronary ligation was performed in sham operations.

Immunohistochemistry and immunofluorescent staining

Paraffin-embedded sections (5 µm thick) were deparaffinized and blocked first using 0.5% horse serum, after which endogenous biotin and biotin-binding proteins were blocked using a blocking kit (Vector Laboratories, Burlingame, CA, USA). The sections were then incubated with anti-IL-6 (sc1265, Santa Cruz, CA, USA), anti-TNF-α (sc1350, Santa Cruz, CA, USA) or alkaline phosphatase-conjugated anti-α-smooth muscle actin (SMA) (clone 1A4, Sigma, St. Louis, MO, USA), followed by incubation with avidin-biotin complex and Vector Red substrate (Vector Laboratories, Burlingame, CA, USA). Sections were counterstained with hematoxylin.

To preserve the GFP signal for histological analysis, the arteries were embedded in plastic resin (Technovit 8100, Heraeus Kulzer, Armonk, NY, USA) according to the manufacturer’s instructions. For immunofluorescent staining, plastic-embedded sections were incubated with Cy3-conjugated anti-αSMA (c6198, Sigma, St. Louis, MO,
USA). Nuclei were then counterstained with Hoechst 33258 (Sigma, St. Louis, MO, USA), and the sections were observed under a confocal microscope (FLUOVIEW FV300, Olympus, Tokyo, Japan).

**Figure Legends**

**Figure I.** Effect of myocardial infarction on expression of the proinflammatory cytokines MCP-1 (A), VEGF (B), TGF-β (C), SDF-1α (D) and IL-1β (E) within injured arteries: white bars, control; hatched bars, VI; black bar, AMI+VI. Tissue samples were prepared from injured and uninjured arteries 14 days after surgery (control). The result shown is representative of data obtained from 3 to 4 mice per group. MCP-1, VEGF, TGF-β, SDF-1α and IL-1β signal intensities were normalized to that of GAPDH; bars depict the fold increase relative to uninjured arteries (control).

**Figure II.** Time course of AMI-induced expression of TNF-α in the heart (A): open bars, sham-operated; solid bars, AMI. Results are means±SEM for 3 to 5 mice per group; *p<0.05, **p<0.01 vs. the sham-operated group. B, C, Immunohistochemical staining of TNF-α in infarcted (B) and sham-operated (C) hearts from mice harvested 3 days after surgery. Scale bars represent 100 µm.
A B

TNF-α/GAPDH

Day 1 Day 3 Day 7 Day 28

B C

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