Regulatory Role of Dendritic Cells in Post-Infarction Healing and Left Ventricular Remodeling

Running title: Anzai et al.; Dendritic cells in LV remodeling after MI

Atsushi Anzai, MD¹; Toshihisa Anzai, MD, PhD¹,⁴; Shigenori Nagai, PhD²,⁵; Yuichiro Maekawa, MD, PhD¹; Kotaro Naito, MD, PhD¹; Hidehiro Kaneko, MD¹; Yasuo Sugano, MD, PhD¹,⁶; Toshiyuki Takahashi, MD, PhD¹; Hitoshi Abe, BSc³; Satsuki Mochizuki, PhD³; Motoaki Sano, MD, PhD¹; Tsutomu Yoshikawa, MD, PhD¹; Yasunori Okada, MD, PhD³; Shigeo Koyasu, PhD²; Satoshi Ogawa, MD, PhD¹,⁶; Keiichi Fukuda, MD, PhD¹

¹Division of Cardiology, Dept of Med, ²Dept of Microbiology & Immunology, ³Dept of Pathology, Keio University School of Med, Tokyo, Japan; ⁴Dept of Cardiovascular Med, National Cerebral & Cardiovascular Center, Osaka, Japan; ⁵Core Research for Evolutional Science & Technology (CREST), Japan Science & Technology Agency (JST); ⁶Cardiovascular Center, International University of Health & Welfare Mita Hospital, Tokyo, Japan

Correspondence:
Toshihisa Anzai, MD, PhD
Department of Cardiovascular Medicine
National Cerebral and Cardiovascular Center
5-7-1 Fujishiro-dai, Suita
Osaka 565-8565, Japan
Phone: +81-6-6833-5012 (ext.2321)
Fax: +81-6-6833-9865
E-mail: anzai@cpnet.med.keio.ac.jp

Journal Subject Codes: [148] Heart failure - basic studies
Abstract:

**Background**- Inflammation and immune responses are integral components in the healing process after myocardial infarction (MI). We previously reported dendritic cell (DC) infiltration in the infarcted heart. However, the precise contribution of DC in post-infarction healing is unclear.

**Methods and Results**- Bone-marrow (BM) cells from CD11c-diphtheria toxin receptor/GFP transgenic mice were transplanted into lethally irradiated wild-type recipient mice. After reconstitution of BM-derived cells, the recipient mice were treated with either diphtheria toxin (DC-ablation) or vehicle (control), and MI was created by left coronary ligation. CD11c⁺ GFP⁺ DCs expressing CD11b and MHC class II were recruited into the heart, peaking on day 7 after MI in control group. Mice with DC-ablation for 7 days showed deteriorated left ventricular function and remodeling. DC-ablated group demonstrated enhanced and sustained expression of inflammatory cytokines such as interleukin (IL)-1β, IL-18 and tumor necrosis factor-α, prolonged extracellular matrix degradation associated with a high level of matrix metalloproteinase-9 activity, and diminished expression level of IL-10 and endothelial cell proliferation following MI compared with control group. In vivo analyses revealed that DC-ablated infarcts had enhanced monocyte/macrophage recruitment. Among these cells, marked infiltration of proinflammatory Ly6C(high) monocytes and F4/80⁺ CD206⁻ M1 macrophages, and conversely impaired recruitment of anti-inflammatory Ly6C(low) monocytes and F4/80⁺ CD206⁺ M2 macrophages in the infarcted myocardium were identified in DC-ablated group than in control group.

**Conclusions**- These our results suggest that dendritic cell is a potent immunoprotective regulator during the post-infarction healing process, via controlling monocyte/macrophage homeostasis.

**Key words:** myocardial infarction, remodelling, inflammation, immune system, heart failure
Introduction

Left ventricular (LV) remodeling after myocardial infarction (MI) is the process of complex architectural myocardial alteration and is associated with a poor clinical outcome. Congestive heart failure due to post-infarction LV remodeling remains an unresolved problem in spite of aggressive revascularization and pharmacological therapy in recent clinical practice. Therefore, better understanding of the molecular and cellular mechanisms involved in this process and the search for alternative therapeutic targets against LV remodeling are matters of great importance.

Inflammation and immune responses are integral components of the host reaction to myocardial injury, and play a crucial role in infarct healing and subsequent LV remodeling.1-3 We have previously reported that elevated serum C-reactive protein concentration and peripheral monocytosis predict a poor clinical outcome after MI.4, 5 Moreover, our experimental study demonstrated that the enhanced infiltration of monocytes and macrophages into the infarcted myocardium induced by granulocyte-macrophage colony-stimulating factor leads to aggravated infarct expansion and LV dysfunction.6 These findings indicate that excessive immune-mediated inflammatory reactions have a deleterious effect on post-infarction LV remodeling. On the other hand, immunosuppressive therapy using corticosteroids resulted in increased catastrophic incidents such as cardiac rupture in clinical practice,7, 8 and recent experimental data showed that macrophage depletion with clodronate-containing liposomes (Clo-Lip) impaired infarct healing in a murine model,9 suggesting that controlled inflammation and immune response are prerequisite for appropriate cardiac repair after MI. However, the regulatory mechanism controlling these reactions during the post-infarction healing process remains to be determined.

The dendritic cell (DC) is a potent central immunoregulator, which orchestrates various
kinds of inflammatory cells in innate and adoptive immunity. After microbial infection or tissue injury, bone-marrow (BM) and splenic precursors and circulating monocytes are reported to differentiate into DCs and exert various influences on the immune system at the inflammatory site, such as priming of antigen-specific immune responses, induction of tolerance, and chronic inflammation. Zhang et al. first demonstrated infiltration of DCs into the infarcted heart in experimental MI. We have previously reported that an increased number of mature DCs in the infarcted heart was associated with deterioration of LV remodeling in a rat MI model. However, the causative effect of infiltrating DCs on LV remodeling and their origin in the post-infarction healing process are unclear.

The primary aim of this study was to clarify the role of DCs in tissue repair and LV remodeling after MI and the origin of DCs involved in the process. We employed transgenic mice expressing diphtheria toxin receptor (DTR) on DCs, enabling us to specifically deplete DCs by injecting diphtheria toxin (DT), which have already been proven to be a powerful and useful tool to manipulate DCs in vivo.

Methods

An additional detailed Methods section can be found in the online-only Data Supplement.

Animals

CD11c-DTR/GFP transgenic mice on a C57BL/6 background (CD45.2+) were provided by D. Littman (New York University, NY). B6.SJL congenic mice on a C57BL/6 background (CD45.1+) were obtained from Taconic (Germantown, NY). Wild-type (WT) C57BL/6 mice (CD45.2+) were purchased from Clea Japan (Tokyo, Japan). These mice were bred and kept...
under specific-pathogen-free conditions. All experiments were performed in accordance with the
Keio University animal care guidelines, which conform to the Guide for the Care and Use of
Laboratory Animals published by the US National Institutes of Health (NIH Publication No.
85-23, revised 1996).

**Bone-marrow (BM) Cell Preparation and BM Transplantation (BMT)**

A BMT model was used to evaluate the role of DCs by depleting CD11c+ DTR+ cells by
DT administration.\(^\text{19}\) The detailed methods to harvest BM cells and to perform BMT are
described in the online-only **Data Supplement**.

**DT Administration**

BMT mice were intraperitoneally inoculated with DT (Sigma, St. Louis, MO) in
phosphate buffered saline (PBS) at a dose of 4 ng/g body weight. BMT mice were randomly
assigned to DT (DC-ablation) and PBS (control) treatment groups. DT or PBS was administered
on the day before MI or sham operation and then 2 and 5 days after the operation. In this study,
control and DC-ablated BMT mice were compared in permanent coronary occlusion model
according to the methods section mostly described in online-only data supplement.

**Additional Methods**

The expanded Methods section in the online-only **Data Supplement** contains
information on BM cell preparation and BMT; creation of MI; echocardiography and
hemodynamics; morphometric analysis; real-time quantitative polymerase chain reaction;
Western blotting; gelatin zymography; immunohistochemical staining; immunofluorescent
staining; preparation of splenic and peripheral blood cells; isolation of infiltrating leukocytes
from the infarcted heart; and flow cytometry.

**Statistical Analyses**
All data were expressed as mean value ± SEM. Comparisons between two groups were performed using Wilcoxon rank sum test. When more than two groups were analyzed, Kruskal-Wallis test followed by Bonferroni’s post-hoc test was employed. Survival distributions were estimated using the Kaplan-Meier method and compared by the log-rank test. Difference in cause of death between control and DC-ablated mice after MI was assessed by Fisher’s exact test. A P value of < 0.05 was considered to be significant. All statistical analyses were performed with SPSS 15.0 statistical software package for Windows (SPSS Inc., Chicago, IL).

Results

Strategy of DC Depletion In Vivo

To minimize the effect of DT on non-hematopoietic cells expressing an integrin, CD11c, we employed a BMT model as described in Methods. The repopulation efficiency of hematopoietic cells after BMT was determined using B6.SJL mice (CD45.1+) as recipients. After reconstitution with donor CD11c-DTR/GFP transgenic marrow cells (CD45.2+), flow cytometric analysis of splenocytes revealed that the reconstitution rate of our BMT method was approximately 96% (Supplemental Figure 1A). Moreover, almost all the CD11c+ DCs were CD45.2+ (CD45.2+ CD11c+ recipient-derived DCs were absent) and more than 97% of these DCs were also GFP+ (Supplemental Figure 1B). These data indicated that cells such as DCs of BMT mice were almost totally replaced with donor BM-derived cells in our BMT mice.

We assessed the effect of DT inoculation in BMT mice by flow cytometry. Flow cytometric analysis of splenocytes revealed that CD11c+ GFP+ and CD11c+ MHC class II (MHCII)+ DCs were completely depleted by DT administration (Supplemental Figure 2A), whereas other immune cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, and CD11b+
monocytes/macrophages, were hardly affected (Supplemental Figure 2B). These data were compatible with other previously published papers using this transgenic mouse system.\textsuperscript{18, 19}

**Recruitment of DCs into the Infarcted Heart**

Peripheral circulating CD11c\(^+\) GFP\(^+\) DCs expressing MHCII rapidly increased after MI in control mice, and were almost completely abolished by DT administration in DC-ablated mice. Immunofluorescent staining showed that resident DCs were rarely observed in the control heart without MI and the number of heart-infiltrating CD11c\(^+\) GFP\(^+\) DCs in the infarcted myocardium reached its peak 7 days after MI in control mice, but they were absent in DC-ablated mice for 7 days after MI (Figure 1A through 1C). DCs were predominantly identified in the infarcted and border area, whereas there were few DCs in the non-infarcted area after MI in control mice. These DCs infiltrating the heart also expressed CD11b and MHCII, which almost completely disappeared in DC-ablated mice 7 days after MI (Figure 1D). These data indicate that infiltrating cardiac mature DCs originated from the BM and were recruited into the infarcted heart, and that DT treatment is effective for the abrogation of DC infiltration after MI in our BMT model.

No effects of DT on WT mice were confirmed by the data of echocardiographic and organ weight measurements 28 days after sham or MI operation, showing that LV function and heart/lung weight adjusted for body weight were almost similar in non-treated and DT-treated WT mice (Supplemental Figure 3).

**DC-Ablation Aggravates LV Dysfunction and Remodeling after MI**

Cardiac function was analyzed 28 days after MI (Table 1). Echocardiographic measurements revealed acceleration of cardiac dilatation and deterioration of LV function in DC-ablated mice compared with control mice. Hemodynamic data also indicated that maximum rate of isovolumic pressure development after MI was significantly lower in DC-ablated mice
than in control mice. Moreover, heart and lung weights adjusted for body weight 28 days after MI were significantly higher in DC-ablated mice compared with control mice.

**Acceleration of MI-Induced Death, Wall Thinning and Myocardial Fibrosis in DC-Ablated Mice**

The survival rate tended to be lower in DC-ablated mice than in control mice 28 days after MI, whereas all sham-operated animals in both groups survived throughout the study (Figure 2A). We found that the incidence of death from heart failure tended to be higher in DC-ablated mice than in control mice after MI (21.7% vs. 9.4%, \( P = 0.086 \)), although the occurrence of cardiac rupture was similar in the two groups (13.0% vs. 9.4%, \( P = 0.53 \)).

Masson’s trichrome staining revealed that there was no difference in the gross morphology in sham-operated hearts between control and DC-ablated mice (Figure 2B). Twenty-eight days after MI, DC-ablated mice demonstrated enhanced infarct expansion and wall thinning compared with control mice, although the infarct size was similar in the two groups (Figure 2B through 2D). Collagen volume fraction detected by picrosirius red staining in the non-infarcted area was significantly higher in DC-ablated mice compared with control mice 28 days after MI (Figure 2E and 2F). Myocardial fibrosis in the infarcted area did not significantly differ between DC-ablated and control mice 28 days after MI (Figure 2E and 2F). However, picrosirius red polarized microscopy of collagen fibers in the infarcted area showed that a predominance of loosely assembled green or yellow fibers were seen in DC-ablated mice, whereas the well aligned thick orange fibers were observed in control mice 28 days after MI (Figure 2G).

**Absence of DCs Enhances Inflammatory Cytokine but Suppresses Anti-Inflammatory Cytokine Expression after MI**

*Figure 3* demonstrates the temporal changes of interleukin (IL)-1\( \beta \), IL-18, tumor
necrosis factor (TNF)-α, IL-10, CCL2 and CX3CL1 expression after MI. In control mice, the expression of IL-1β, IL-18, TNF-α, and CCL2, which are proinflammatory, increased 3 days after MI and gradually decreased thereafter (Figure 3A through 3D). In contrast, DC-ablated mice had sustained elevation of IL-1β, IL-18, TNF-α, and CCL2 for 7 days after MI compared with control mice (Figure 3A through 3D). On the other hand, the expression of IL-10 and CX3CL1, which are anti-inflammatory, 7 days after MI was lower in DC-ablated mice than in control mice (Figure 3E and 3F). Western blotting analysis confirmed that expression of IL-10 at the protein level was significantly lower in DC-ablated mice compared with control mice 7 days after MI (Figure 3G and 3H).

**DC-Ablation Accentuates Matrix Metalloproteinase (MMP)-9 Activity and Inducible Nitric Oxide Synthase (iNOS) Expression after MI**

As shown in Figure 4, MMP-9 activity was increased in the infarcted heart 3 days after MI and decreased thereafter in control mice, whereas its activity in DC-ablated mice was persistently upregulated for 28 days after MI (Figure 4A and 4B). The activity of MMP-2 tended to be higher in DC-ablated mice than in control mice, although there was no statistical significance between the two groups (Figure 4A and 4C). Furthermore, iNOS was also increased by MI, and DC-ablation further enhanced the induction of iNOS 7 days after MI (Figure 4D and 4E).

**Post-Infarction Myocardial Angiogenesis is Impaired by DC Depletion**

To assess the influence of DC depletion on cardiac angiogenesis, we performed the immunoblot and histological analyses. As shown in Figure 5A and 5B, the expression of vascular endothelial growth factor (VEGF) at protein level, which was upregulated in control infarcts, was significantly suppressed by DC-ablation 7 days after MI. Moreover, our
immunohistochemical staining revealed that the number of blood vessels in the infarcted myocardium was smaller and the fraction of CD31\(^+\) Ki67\(^+\) proliferating endothelial cells was significantly lower in DC-ablated mice compared with control mice 7 days following MI (Figure 5C and 5D), suggesting that DC depletion might affect the cardiac neoangiogenesis during the post-infarction healing process.

Enhanced Infiltration of Inflammatory M1 Macrophages and Diminished Recruitment of Anti-Inflammatory M2 Macrophages into the Infarcted Myocardium in Response to DC-Ablation

We evaluated the degree of various inflammatory cell infiltration after MI in control and DC-ablated mice. The number of total CD45\(^+\) leukocytes in the infarcted heart was significantly higher in DC-ablated mice than in control mice 7 days after MI (Supplemental Figure 4A). Immunofluorescent staining also indicated that infiltration of CD45\(^+\) leukocytes into the infarcted myocardium 7 days after MI was greater in DC-ablated mice compared with control mice (Supplemental Figure 4B). Immunohistochemical staining for Mac3 showed that the number of infiltrating differentiated macrophages into the infarcted myocardium peaked on day 7 after MI and was significantly higher in DC-ablated mice 7 and 14 days after MI compared with control mice (Figure 6A and 6B). Flow cytometric analysis also demonstrated that the percentage of Mac3\(^+\) macrophages in the heart 7 days after MI in DC-ablated mice was significantly higher compared with control mice (Supplemental Figure 4C and 4D).

Furthermore, among tissue-infiltrating F4/80\(^+\) macrophages, DC-ablated infarcts had enhanced infiltration of F4/80\(^+\) CD206\(^-\) inflammatory M1 macrophages and diminished recruitment of F4/80\(^+\) CD206\(^+\) anti-inflammatory M2 macrophages compared with control mice 7 days after MI based on the flow cytometry (Figure 6C and 6D). The number of MPO\(^+\) neutrophils in the
infarcted heart 7 days after MI tended to be higher in DC-ablated mice compared with control mice (Figure 6E). However, the number of CD3\(^+\) T cells infiltrating the infarcted myocardium was comparable in the two groups (Figure 6F).

**DC-Ablation Causes Increased Ly6C\(^{\text{high}}\) Monocyte but Decreased Ly6C\(^{\text{low}}\) Monocyte Infiltration into the Infarcted Heart**

Monocytes, which play important roles in tissue repair after MI, were evaluated in peripheral blood and heart tissue. Flow cytometric analysis showed that the total number of peripheral blood monocytes was higher in DC-ablated mice compared with control mice (Supplemental Figure 5A). In addition, peripheral blood Ly6C\(^{\text{high}}\) monocytes were persistently increased while Ly6C\(^{\text{low}}\) monocytes were decreased in DC-ablated mice compared with control mice, especially 7 days after MI (Figure 7A and 7B). Moreover, among the inflammatory leukocytes isolated from the infarcted heart, the number of total monocytes and inflammatory Ly6C\(^{\text{high}}\) monocytes were markedly higher whereas the number of reparative Ly6C\(^{\text{low}}\) monocytes was lower in DC-ablated mice 7 days after MI compared with those in control mice (Supplemental Figure 5B, Figure 7C and 7D).

**Discussion**

We demonstrated here that mature, activated CD11c\(^+\) CD11b\(^+\) DCs originating from the BM infiltrate the infarcted heart during the healing process after MI. Selective depletion of DCs exacerbated post-infarction LV remodeling in association with enhanced inflammatory cytokine expression, iNOS production, and MMP-9 activation likely via marked infiltration of proinflammatory Ly6C\(^{\text{high}}\) monocytes and F4/80\(^+\) CD206\(^-\) M1 macrophages into the infarcted myocardium. Meanwhile, the number of anti-inflammatory Ly6C\(^{\text{low}}\) monocytes and F4/80\(^+\)
CD206+ M2 macrophages, myocardial expression of IL-10 and cardiac angiogenesis after MI were suppressed by selective ablation of DCs. These results indicated the protective role of DCs in the post-infarction inflammation and subsequent LV remodeling by regulation of cellular employment in the heart.

Leukocyte influx is a highly sophisticated system contributing to cardiac repair following MI. Inflammatory signals recruit neutrophils to the ischemic heart immediately after MI, and monocytes/macrophages shortly thereafter. A large number of reports have indicated that excessive cardiac macrophage infiltration is associated with facilitated infarct expansion and adverse LV remodeling with augmented inflammation and MMP activity.6, 21, 22 Macrophages can be divided into two populations, which have a classically (M1) and alternatively (M2) activated phenotype.23 In addition to macrophages, growing evidence has emerged that monocytes, classically recognized as the precursor of tissue-infiltrating differentiated macrophages, per se have an important role in the post-infarction healing process. In the mouse, there are two distinctive subsets of monocytes; CD11b\textsuperscript{high} Ly6C\textsuperscript{high} proinflammatory monocytes and CD11b\textsuperscript{high} Ly6C\textsuperscript{low} reparative monocytes.24, 25 Nahrendorf et al. reported that the post-infarction repair process consists of biphasic reactions, in which the early phase is dominated by Ly6C\textsuperscript{high} monocytes that are recruited in a CCL2-CCR2-dependent manner and exhibit phagocytic, proteolytic, and inflammatory properties, whereas the late phase is dominated by Ly6C\textsuperscript{low} monocytes that are recruited in a CX3CL1-CX3CR1-dependent manner and promote angiogenesis and resolution of inflammation.26 Moreover, they also demonstrated that Ly6C\textsuperscript{high} monocytosis observed in apolipoprotein E knockout atherosclerotic mice was associated with exacerbated post-infarction LV remodeling through increased inflammatory reaction and MMP activity, whereas in vivo depletion of neutrophils did not improve LV dysfunction after MI.27 On
the other hand, infiltrating macrophage depletion by intravenous injection of Clo-Lip markedly impaired wound healing and accelerated LV remodeling and mortality after myocardial injury.\textsuperscript{9, 26} Furthermore, depletion of circulating Ly6C\textsuperscript{high} monocytes by administration of Clo-Lip during the early phase of MI caused larger areas of debris and necrotic tissue, whereas depletion of Ly6C\textsuperscript{low} monocytes by late phase administration of Clo-Lip resulted in decreased collagen deposition and reduced neoangiogenesis,\textsuperscript{9, 26} suggesting that successful cardiac repair depends on well-coordinated recruitment of monocytes/macrophages in the injured heart. However, the molecular or cellular mechanisms upstream of this critical framework remain to be elucidated.

As the conductor of immune-mediated inflammatory reactions, since their discovery in 1973, DCs have attracted a great deal of attention because of their distinctive and wide variety of functions.\textsuperscript{28} DCs are of hematopoietic origin and are widely distributed in organs and tissues. They are characterized by their capability of antigen capture, migration, antigen presentation and activation of other immune cells, and control immunity and tolerance in various conditions, such as infection, autoimmune disease, tumor, and allergy.\textsuperscript{10-12, 29} However, there are few reports regarding the role of DCs in cardiovascular disease, especially in LV remodeling after MI. Maekawa et al. demonstrated that activation of IL-1 receptor associated kinase (IRAK)-4, which is a downstream molecule of IL-1 and toll-like receptor family members, in DCs adversely affected post-infarction inflammation and LV remodeling, using IRAK-4-deficient mice.\textsuperscript{30} However, the IRAK-4 signaling pathway is also operative in cardiomyocytes and other immune cells in addition to DCs.\textsuperscript{31, 32} Since the initial inflammatory signals after MI are known to involve cardiomyocytes and are further strengthened by neutrophils and monocytes/macrophages expressing IRAK-4, their results might reflect the involvement of these cells in the development of post-infarction LV dysfunction. Therefore, the precise contribution of DCs in the infarcted
heart remains unclear.

In the present study, we employed CD11c-DTR/GFP transgenic mice to examine the effect of DC depletion on post-infarction myocardial remodeling. Conditional DC depletion for 7 days after MI resulted in dysregulation of organized monocyte/macrophage employment, critical for the post-infarction repairing process. In vivo analyses revealed that the number of peripheral monocytes and tissue monocytes/macrophages was higher in DC-ablated mice than in control mice. Among these monocytes/macrophages, marked infiltration of inflammatory Ly6C\textsuperscript{high} monocytes and F4/80\textsuperscript{+} CD206\textsuperscript{-} M1 macrophages and suppressed recruitment of anti-inflammatory Ly6C\textsuperscript{low} monocytes and F4/80\textsuperscript{+} CD206\textsuperscript{+} M2 macrophages were identified in DC-ablated mice, and this was associated with increased expression of CCL2 and decreased expression of CX3CL1. Prolonged expression of inflammatory cytokines and iNOS and enhanced MMP-9 activation were also observed in DC-ablated mice, which were associated with increased peripheral blood and tissue inflammatory monocytes/macrophages. Consistent with previously published paper which demonstrated that thick collagen fibers were degraded by augmented MMPs and replaced by premature collagen fibers in the infarcted heart,\textsuperscript{33} our study based on the picrosirius polarized microscopy showed that DC-ablated infarcts had disorganized collagen fibers, which may lead to subsequent infarct expansion and heart failure. Moreover, cardiac angiogenesis, which has a favorable effect on tissue repair following MI, was inhibited in DC-ablated mice compared with control mice. As discussed above, Ly6C\textsuperscript{low} monocytes selectively express higher levels of VEGF,\textsuperscript{26} and M2 macrophages also have proangiogenic properties.\textsuperscript{34} Taken together, these findings indicate that the absence of DCs leads to activation of inflammatory monocytes/macrophages and suppression of reparative monocytes/macrophages, inducing enhanced inflammation, extracellular matrix degradation and apoptosis, and impaired...
neoangiogenesis in post-infarction healing process.

DCs, macrophages, and monocytes are members of the mononuclear phagocyte system, defined as non-granulocytic, myeloid cells that play important roles in tissue remodeling and homeostasis, as well as regulatory and stimulatory aspects of innate and adaptive immunity. Their origins are presumed to be the same BM precursor cells, named macrophage DC progenitors. Moreover, several reports have shown that peripheral monocytes transmigrate into inflamed tissue and differentiate into macrophages or DCs. Based on such tight interaction in the mononuclear phagocyte system, it is possible that selective DC depletion may change the subpopulations of the system and that DCs contribute to the maintenance of disciplined monocyte/macrophage organization following MI. Although the precise mechanisms of the anti-inflammatory effect of DCs through controlling monocyte/macrophage subsets are not clear, it may be very difficult to address the mechanism since immune cells communicate through the release of many secreted factors. Further studies are needed to investigate how DCs interact with monocytes/macrophages in the healing myocardium.

In summary, the present study demonstrated that DC is likely to play an immunoprotective role especially in monocyte/macrophage homeostasis during the post-infarction healing process, and suggests that modulation of DCs could be a new therapeutic target in LV remodeling after MI.

Acknowledgements: We thank Hiromi Kato (Keio University), Mayu Matsuda (Keio University), and Yukiko Baba (Keio University) for excellent technical assistance. Thanks are also due to Kyoko Hidaka for animal care.

Funding Sources: This work was supported by a Grant-in-Aid for Young Scientists (B) (23790875 to A.A. and 21790476 to S.N.), a Grant-in-Aid (20590872 to T.A.), and a Medical
School Faculty and Alumni Grant from the Keio University Medical Science Fund (T.A.).

**Conflict of Interest Disclosures:** S.K. is a consultant for Medical and Biological Laboratories, Co. Ltd.

**References:**


34. Lambert JM, Lopez EF, Lindsey ML. Macrophage roles following myocardial infarction. *Int J Cardiol.* 2008;130:147-158.


38. Dominguez PM, Ardavin C. Differentiation and function of mouse monocyte-derived


**Table 1.** Body and Organ Weights, Echocardiographic, and Hemodynamic Data 28 Days after MI

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DC-ablation</td>
</tr>
<tr>
<td><strong>Organ weight</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>BW, g</td>
<td>27.2 ± 0.3</td>
<td>26.8 ± 0.4</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>3.66 ± 0.06</td>
<td>3.63 ± 0.05</td>
</tr>
<tr>
<td>LW/BW, mg/g</td>
<td>4.19 ± 0.09</td>
<td>4.01 ± 0.12</td>
</tr>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>535.9 ± 4.8</td>
<td>532.0 ± 4.8</td>
</tr>
<tr>
<td>AWT, mm</td>
<td>0.66 ± 0.02</td>
<td>0.66 ± 0.02</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>0.66 ± 0.02</td>
<td>0.65 ± 0.02</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>2.74 ± 0.06</td>
<td>2.79 ± 0.06</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>1.36 ± 0.03</td>
<td>1.39 ± 0.03</td>
</tr>
<tr>
<td>FS, %</td>
<td>50.3 ± 0.4</td>
<td>50.2 ± 0.3</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>49.0 ± 2.23</td>
<td>50.0 ± 3.13</td>
</tr>
<tr>
<td>LV mass/BW</td>
<td>1.80 ± 0.07</td>
<td>1.87 ± 0.11</td>
</tr>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>508.9 ± 4.2</td>
<td>509.6 ± 4.9</td>
</tr>
<tr>
<td>LVESP, mmHg</td>
<td>105.7 ± 1.5</td>
<td>106.2 ± 1.3</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>11695 ± 946</td>
<td>11881 ± 847</td>
</tr>
<tr>
<td>-dP/dt, mmHg/s</td>
<td>9976 ± 456</td>
<td>9740 ± 367</td>
</tr>
</tbody>
</table>

MI, myocardial infarction; DC, dendritic cell; BW, body weight; HW, heart weight; LW, lung weight; HR, heart rate; AWT, anterior wall thickness; PWT, posterior wall thickness; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; FS, fractional shortening; LVESP, left ventricular end-systolic pressure. Results are presented as mean ± SEM. \*P < 0.05 vs corresponding sham group; †P < 0.05 vs control MI group.
Figure Legends:

**Figure 1.** Recruitment of DCs into the infarcted heart. **A**, Representative photographs of immunofluorescent staining for CD11c (red) and GFP (green) in the infarcted myocardium 7 days after MI in control and DC-ablated mice. Scale bars indicate 30 μm. **B**, High magnification view of rectangle in A. White arrows indicate CD11c⁺ GFP⁺ heart-infiltrating DCs (yellow). Scale bar indicates 30 μm. **C**, Time course of CD11c⁺ GFP⁺ DCs infiltration after MI in control and DC-ablated mice analyzed by immunofluorescent staining. Data were obtained from 5 independent experiments at each time point. **D**, Representative flow cytometric dot plots of heart-infiltrating CD45⁺ leukocytes for CD11c-APC and CD11b-PE or MHCII-PE in control and DC-ablated mice 7 days after MI. *P < 0.05 and **P < 0.01 vs sham of same group; †P < 0.05 and ††P < 0.01 vs control MI.

**Figure 2.** DC-ablation accelerates MI-induced death, wall thinning and myocardial fibrosis without affecting infarct size. **A**, Kaplan-Meier survival analysis in control and DC-ablated mice after MI or sham operation. **B**, Representative Masson’s trichrome staining of cardiac tissue sections in control and DC-ablated mice 28 days after MI or sham operation. Scale bars indicate 1 mm. **C**, Wall thickness of scar at papillary muscle level 28 days after MI in control (n = 11) and DC-ablated (n = 12) mice. **D**, Quantitative analysis of infarct size 28 days after MI in control (n = 11) and DC-ablated (n = 12) mice. **E**, Representative picrosirius red-stained sections of infarcted and non-infarcted areas 28 days after MI in control and DC-ablated mice. Scale bars indicate 100 μm. **F**, Quantification of fibrotic area in infarcted and non-infarcted area 28 days after MI in control (n = 11) and DC-ablated (n = 12) mice. **G**, Polarized microscopic view of picrosirius red-stained section demonstrating in E. †P < 0.05 and ††P < 0.01 vs control MI.

**Figure 3.** Ablation of DC enhances proinflammatory cytokine but suppresses anti-inflammatory cytokine expression in the infarcted myocardium. **A** through **F**, Time course of mRNA expression of IL-1β (**A**), IL-18 (**B**), TNF-α (**C**), CCL2 (**D**), IL-10 (**E**), and CX3CL1 (**F**) in the infarcted heart after MI in control and DC-ablated mice. Data were obtained from 8 to 10 independent experiments at each time point. **G**, Representative immunoblotting image for IL-10 in the infarcted heart of control and DC-ablated mice 7 days after MI or sham operation. **H**,
Quantitative analysis of IL-10 protein expression in control and DC-ablated mice 7 days after MI or sham operation (n = 5 per group). *P < 0.05 and **P < 0.01 vs sham of same group; †P < 0.05 vs control MI.

**Figure 4.** Increased MMP-9 activity and iNOS expression in DC-ablated mice after MI. A, Representative photograph of zymographic gel demonstrating time course of MMP-9 and -2 activities in the infarcted heart of control and DC-ablated mice. B and C, Quantitative analyses of MMP-9 (B) and -2 (C) activities after MI based on gelatin zymography in control and DC-ablated mice. Data were obtained from 5 independent experiments at each time point. D, Representative immunoblotting analysis for iNOS in the infarcted heart of control and DC-ablated mice 7 days after MI. E, Quantitative assessment for iNOS expression in the infarcted heart of control and DC-ablated mice 7 days after MI (n = 5 per group). *P < 0.05 and **P < 0.01 vs sham of same group; †P < 0.05 and ††P < 0.01 vs control MI.

**Figure 5.** Post-infarction myocardial angiogenesis is impaired by DC depletion. A, Western blotting images for VEGF level in the infarcted myocardium 7 days after MI. B, Quantification data of VEGF based on immnoblotting experiment in control and DC-ablated mice (n = 4 per group). C, Representative immunohistochemical staining for CD31 (red), Ki67 (brown) and nuclei (blue) 7 days post-MI in control and DC-ablated mice. Scale bars indicate 30 μm. D, Quantitative assessment for blood vessels and CD31+ Ki67+ cells in control and DC-ablated infarcts 7 days after MI (n = 4 per group). *P < 0.05 and **P < 0.01 vs sham of same group; †P < 0.05 and ††P < 0.01 vs control MI.

**Figure 6.** Enhanced infiltration of inflammatory M1 macrophages and diminished recruitment of anti-inflammatory M2 macrophages by DC-ablation after MI. A, Representative photographs of immunohistochemical staining for Mac3+ macrophages in infarcted and border areas 7 days after MI. Scale bars indicate 100 μm. B, Time course of Mac3+ macrophage infiltration in control and DC-ablated mice after MI. Data were obtained from 5 independent experiments at each time point. C, Representative flow cytometric dot plots of CD45+ leukocytes in the infarcted hearts for CD206-Alexa 647 and F4/80-PE in control and DC-ablated mice 7 days after MI or sham operation. D, The percentage of heart-infiltrating F4/80+ CD206+ M1 (upper) and F4/80+ CD206+
M2 (lower) macrophages in total F4/80+ macrophages of control and DC-ablated hearts 7 days after MI or sham operation (n = 4 per group). E and F, Time course of MPO+ neutrophil (E) and CD3+ T cell (F) recruitment after MI in control and DC-ablated mice analyzed by immunohistochemical staining. Data were obtained from 5 independent experiments at each time point. *P < 0.05 and **P < 0.01 vs sham of same group; †P < 0.05 vs control MI.

**Figure 7.** Increased Ly6C<sup>high</sup> monocyte but decreased Ly6C<sup>low</sup> monocyte infiltration into the infarcted myocardium in DC-ablation mice. A and B, Temporal changes of counts of peripheral Ly6C<sup>high</sup> inflammatory (A) and Ly6C<sup>low</sup> reparative (B) monocytes based on flow cytometric analysis in control and DC-ablated mice. Data were obtained from 5 independent experiments at each time point. C, Representative flow cytometric dot plots for Ly6C-APC and CD11b-PE after sorting of CD45+ CD11b<sup>high</sup> cells from infarcted heart in control and DC-ablated mice 7 days after MI or sham operation. D, Flow cytometric evaluation of heart-infiltrating Ly6C<sup>high</sup> (upper) and Ly6C<sup>low</sup> (lower) monocytes in control and DC-ablated mice 7 days after MI or sham operation (n = 5 per group). *P < 0.05 and **P < 0.01 vs sham of same group; †P < 0.05 vs control MI.
A  

![Graph showing survival rates over days after MI for different groups: Control sham (n = 10), DC-ablation sham (n = 11), Control MI (n = 53), DC-ablation MI (n = 69). The survival rate is represented as a percentage, and the x-axis shows days after MI. The graph indicates a trend, but there is no significant difference (P = 0.09, Log-rank test).](image)

B  

![Images of heart sections for different groups: Sham, MI, Control MI, DC-ablation MI.](image)

C  

![Bar graph showing wall thickness (mm) for Control MI and DC-ablation MI.](image)

D  

![Bar graph showing infarct size (%) for Control MI and DC-ablation MI.](image)

E  

![Images of myocardial fibrosis for different parts of the heart: Infarct, Non-Infarct, Control MI, DC-ablation MI.](image)

F  

![Bar graph showing myocardial fibrosis (%) for Infarct and Non-Infarct regions in Control MI and DC-ablation MI.](image)

G  

![Images of fibrous tissue for Control MI and DC-ablation MI.](image)
A

<table>
<thead>
<tr>
<th>Sham</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>DC-ablation</td>
<td>Control</td>
<td>DC-ablation</td>
</tr>
</tbody>
</table>

MMP-9 (95 kDa)

Pro-MMP-2 (72 kDa)
MMP-2 (66 kDa)

B

C

D

E

iNOS / GAPDH

Sham Day 3 Day 7 Day 28

Control ControlDC-ablation Control DC-ablation Sham MI

MMP-9 activity / Sham

MMP-2 activity / Sham

iNOS (130 kDa)
GAPDH (37 kDa)

iNOS / GAPDH

Control DC-ablation Control DC-ablation Sham MI
A

![Image of Western blots showing VEGF and GAPDH levels with bands for Control, DC-ablation, Sham, and MI groups.]

VEGF (23 kDa)

GAPDH (37 kDa)

Control  DC-ablation  Control  DC-ablation

Sham  MI

B

![Graph showing VEGF/GAPDH ratios with bars for Control and DC-ablation groups in Sham and MI conditions.](http://circ.ahajournals.org/)

**  **  †

C

![Images of immunohistochemistry for Control MI and DC-ablation MI showing blood vessels and CD31^+ Ki67^+ cells.](http://circ.ahajournals.org/)

Control MI  DC-ablation MI

D

![Graphs showing Blood Vessels and CD31^+ Ki67^+ cells with bars for Control DC-ablation MI and DC-ablation MI conditions.](http://circ.ahajournals.org/)
Regulatory Role of Dendritic Cells in Post-Infarction Healing and Left Ventricular Remodeling
Atsushi Anzai, Toshihisa Anzai, Shigenori Nagai, Yuichiro Maekawa, Kotaro Naito, Hidehiro Kaneko, Yasuo Sugano, Toshiyuki Takahashi, Hitoshi Abe, Satsuki Mochizuki, Motoaki Sano, Tsutomu Yoshikawa, Yasunori Okada, Shigeo Koyasu, Satoshi Ogawa and Keiichi Fukuda

_Circulation._ published online February 3, 2012;
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2012/02/03/CIRCULATIONAHA.111.052126

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2012/02/03/CIRCULATIONAHA.111.052126.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL

Supplemental Methods

Bone-marrow (BM) Cell Preparation and BM Transplantation (BMT)

A BMT model was used to evaluate the role of dendritic cell (DC) by depleting CD11c\(^+\) diphtheria toxin receptor (DTR)\(^+\) cells by diphtheria toxin (DT) administration.\(^1\) To harvest BM cells, femurs and tibias were taken from 8-week-old male CD11c-DTR/GFP transgenic mice. BM was flushed out with a 24-gauge syringe, and a single cell suspension in phosphate buffered saline (PBS) was made through a 100-µm nylon mesh after red blood cell lysis using ACK lysis buffer solution (0.15 M NH\(_4\)Cl, 1.0 mM KHCO\(_3\), 0.1 mM Na\(_2\)EDTA [pH 7.2]). Five million BM cells were transferred intravenously into lethally irradiated (10 Gy) 6- to 8-week-old male wild-type (WT) C57BL/6 mice. The mice were allowed to rest for 8 weeks, and the success of BMT was checked by polymerase chain reaction (PCR) to confirm the presence of transgene in peripheral blood cells of the recipient mice before use. To evaluate the BM chimerism after BMT, lethally irradiated (10 Gy) CD45.1\(^+\) B6.SJL recipient mice were reconstituted with BM cells of CD11c-DTR/GFP donor mice (CD45.2\(^+\)) under the same protocols described above.

Creation of Myocardial Infarction (MI)

Experimental MI was induced as previously described.\(^2\)\(^,\)\(^3\) In brief, mice were anesthetized
with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg), then
intubated and connected to a rodent ventilator. The chest cavity was opened via left
thoracotomy to expose the heart, and the left coronary artery was permanently ligated with a
7-0 silk suture at the site of its emergence from the left atrium. Complete occlusion of the
vessel was confirmed by the presence of myocardial blanching in the perfusion bed. Mice
that died within 24 hours after surgery were excluded from the experiment. Sham-operated
animals in both groups underwent the same procedure without coronary artery ligation. The
decesed mice were performed an autopsy to determine the cause of death: cardiac rupture
was confirmed by the presence of blood coagulation around the pericardial sac and in the
chest cavity, and heart failure was diagnosed by lung congestion with chest fluid
accumulation.

Echocardiography and Hemodynamics

Echocardiographic and hemodynamic evaluations were performed on day 28 after the
operation. For echocardiography, mice were anesthetized with 1-2% of isoflurane. M-mode
tracings were recorded through the anterior and posterior left ventricular (LV) walls at the
papillary muscle level to measure anterior wall thickness (AWT), posterior wall thickness
(PWT), LV end-diastolic diameter (LVEDD), and end-systolic diameter (LVESD), using an
echocardiographic system (12-MHz linear transducer; EnVisor C, Philips Medical Systems,
Andover, MA). The following formulas were used to calculate LV fractional shortening (FS) and LV mass: 

\[
\text{FS} (\%) = \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}} \times 100 \text{ and } \text{LV mass} = 1.055 \times \left( \text{AWT} + \text{PWT} + \text{LVEDD}^3 \right) - \text{LVEDD}^3,
\]

respectively.

Cardiac catheterization studies were performed using a 1.4 French microtip catheter (SPR-671, Miller Instruments, Houston, TX) under sedation with intraperitoneal injection of pentobarbital sodium (40 mg/kg) with spontaneous respiration. LV end-systolic pressure, maximum rate of isovolumic pressure development and minimum rate of isovolumic pressure decay were measured using analysis software (PowerLab, AD Instruments, Bella Vista, NSW, Australia). Ten sequential beats were averaged for each measurement.

**Morphometric Analysis**

Heart tissue was fixed in formalin, embedded in paraffin, and cut into 5-µm-thick sections. Sections were stained with hematoxylin and eosin, Masson’s trichrome and picrosirius red to determine the infarct size and cardiac fibrosis, and identify their morphology. To evaluate the quality of collagen fibers, picrosirius red stained-sections were studied with polarized microscopy. The infarct size was assessed as total infarct circumference divided by total LV circumference times 100, as described previously. The wall thickness of the scar at the papillary muscle level was also measured. The fraction of collagen volume was assessed in 10 randomly chosen high-power fields (×200) in each section. These data were analyzed
using ImageJ software (version 1.38×, National Institutes of Health).

**Real-Time Quantitative PCR**

Total RNA was isolated by the acid-phenol extraction method in the presence of chaotropic salts (Trizol, Invitrogen, Carlsbad, CA) and subsequent isopropanol-ethanol precipitation as described previously.\(^5\) Reverse transcription was performed using a Super-Script First-Strand Synthesis System (Invitrogen) in accordance with the manufacturer’s protocol. The sequences of primer pairs were designed using Primer Express III software (Applied Biosystems, Foster City, CA) and are described in online-only Data Supplement Table 1. Real-time quantitative PCR was performed using an ABI Prism 7500 sequence detection system (Applied Biosystems). The obtained data were normalized using the expression levels of mouse 18S rRNA.

**Western Blotting**

Frozen tissue was homogenized in T-PER Tissue Protein Extraction Reagent (Thermo Scientific, Rockford, IL) with protease inhibitors (Thermo Scientific). After centrifugation, the supernatant was collected and protein concentration measured using a Bradford assay system. Equal amounts of protein samples (30 µg) were electrophoresed on 4-12% NuPAGE Bis-Tris gels (Invitrogen), and then proteins were electroblotted onto polyvinylidene
difluoride membranes. After blocking with 5% nonfat dried skim milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 at room temperature (RT) for 1 hour, the membranes were incubated with the primary antibodies anti-interleukin (IL)-10 (Abcam, Cambridge, MA), anti-inducible nitric oxide synthase (iNOS) (BD Bioscience, San Jose, NJ), anti-vascular endothelial growth factor (VEGF) (Santa Cruz Biotech., Santa Cruz, CA) and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotech.) at 4°C overnight or for 1 hour at RT, followed by exposure to the secondary horseradish peroxidase (HRP)-conjugated antibody for 1 hour at RT. The immunoblots were developed by enhanced chemiluminofluorescence method. The signals were scanned with MF-ChemiBIS (DNR Bio-Imaging Systems, Jerusalem, Israel) and analyzed using Quantity One software (Bio-Rad, Hercules, CA).

**Gelatin Zymography**

To evaluate the activity of gelatinase, matrix metalloproteinase (MMP)-9 and MMP-2, gelatin zymography was performed. Equal volumes of 30 µg protein were loaded onto each lane of 10% gelatin zymogram gels (Novex, Invitrogen). After running at 80V for 2 hours, the gels were incubated in 2.5% Triton-X 100 for 30 min at RT, washed and then further incubated in 50 mM Tris-HCl, pH 7.4, containing 200 mM NaCl and 5 mM CaCl₂ at 37°C overnight with gentle agitation. After washing with deionized water, the gels were stained with Coomassie
blue for 90 min followed by destaining with a solution containing 30% ethanol and 10% acetic acid. The presence of different MMPs was identified on the basis of their molecular weight. The gels were photographed using MF-ChemiBIS (DNR Bio-Imaging Systems) and analyzed using Quantity One software (Bio-Rad).

**Immunohistochemical Staining**

Immunohistochemical studies were performed by immunoperoxidase methods using paraffin-embedded tissue sections. After inhibiting endogenous peroxidase activity, the sections were incubated with primary anti-Mac3 (BD Bioscience), anti-MPO (Dako, Glostrup, Denmark) or anti-CD3 (Dako) antibodies at 4°C overnight. After incubation, a Vectastain ABC elite Kit (Vector Laboratories, Burlingame, CA) was used according to the manufacturer’s instructions. Following visualization with 3,3’-diaminobenzidine, the sections were finally counterstained with hematoxylin. The same methods were performed without the primary antibodies, as negative controls. The numbers of Mac3+ macrophages, MPO+ neutrophils, and CD3+ T lymphocytes were assessed by counting the total cell numbers in the infarcted and border areas in 20 randomly chosen fields in each section. For the assessment of endothelial proliferation in the infarcted heart, double immunohistochemical staining for CD31 (Santa Cruz Biotech.) and Ki67 (Dako) was performed. After antigen retrieval, tissue sections were incubated with primary antibodies followed by the reaction with secondary
antibodies and labeling with alkaline phosphatase (for CD31) and 3,3′-diaminobenzidine (for Ki67). Finally, sections were counterstained with hematoxylin. The number of blood vessels and CD31+ Ki67+ proliferating endothelial cells were counted per unit area.

**Immunofluorescent Staining**

For immunofluorescent staining, frozen tissue sections were used. The excised hearts were embedded in OCT compound, snap frozen in liquid nitrogen, and then cut into 6-µm-thick sections. After fixing in acetone at RT for 10 min, cryosections were blocked using Block Ace (DS Pharma Biomedical Co. Ltd., Osaka, Japan) at 37°C for 30 min, and then incubated with anti-CD11c-APC (BD Bioscience) and purified anti-GFP (Medical & Biological Laboratories Co. Ltd., Nagoya, Japan) antibodies at 4°C overnight to identify BM-derived DCs. Alexa-Fluor 488-conjugated secondary antibody (Molecular Probes, Carlsbad, CA) was added to detect GFP+ cells. To evaluate infiltrating leukocytes, primary purified anti-CD45 (BD Bioscience) and Alexa-Fluor 647-conjugated secondary (Molecular Probes) antibodies were used. Nuclei were identified with DAPI. As negative controls, the same procedures were performed without the primary antibodies. The sections were finally photographed under a confocal laser-scanning microscope (TCS-SP5, Leica).

**Preparation of Splenic and Peripheral Blood Cells**
Spleens were removed, mushed and then passed through a 100-µm nylon mesh in PBS. After addition of ACK lysis buffer solution to exclude erythrocytes, the single cell suspension in PBS was refiltered through a 100-µm nylon mesh to remove connective tissue. Peripheral blood was drawn via cardiac puncture and collected into a heparinized tube. ACK lysis buffer solution was also added to the collected peripheral blood, followed by washing in PBS.

**Isolation of Infiltrating Leukocytes from the Infarcted Heart**

On the indicated days after the operation, mice were deeply anesthetized and intracardially perfused with ice-cold PBS to remove blood cells before euthanasia. Infarcted tissue was dissected, minced with fine scissors, and enzymatically digested with a cocktail of type II collagenase (Worthington Biochemical Corporation, Likewood, NJ), elastase (Worthington Biochemical Corporation) and DNase I (Sigma, St. Louis, MO) for 1 hour at 37°C with gentle agitation. After digestion, the tissue was then triturated and passed through a 100-µm cell strainer. For the separation of leukocytes, cells were incubated with biotin-conjugated anti-CD45 antibody (BioLegend, San Diego, CA) at 4°C for 10 min, followed by incubation with streptavidin-conjugated microbeads (Miltenyi Biotec, Sunnyvale, CA) at 4°C for 15 min. Then, CD45+ leukocytes were positively collected by magnetic sorting with AutoMACS (Miltenyi Biotec). The purity of cells was generally > 97%.
Flow Cytometry

Isolated splenocytes, peripheral blood cells, and leukocytes isolated from the heart were analyzed by flow cytometry. To block nonspecific binding of antibodies to Fcγ receptors, isolated cells were first incubated with anti-CD16/32 antibody (BD Bioscience) at 4°C for 5 min. Subsequently, the cells were stained with a mixture of the following antibodies at 4°C for 20 min: anti-CD11c-PE, anti-CD11c-APC, anti-CD45.1-PerCP-Cy5.5, anti-MHCII-PE, anti-CD11b-PE, anti-Mac3-PE, anti-Ly6C-APC, anti-F4/80-PE, anti-CD4-PE, anti-CD8-PE, anti-CD19-PE (BD Bioscience), CD45.2-APC (BioLegend), and Alexa-Fluor 647-conjugated anti-CD206 antibody (eBioscience, San Diego, CA). Peripheral blood monocytes were defined as SSC\textsuperscript{low} CD11b\textsuperscript{high} cells as previously described. After staining with 7-amino-actinomycin D (Sigma) to discriminate dead cells, flow cytometry was performed on a FACS Calibur (BD Bioscience) and the data analyzed with FlowJo software (Tree Star, Ashland, OR).
References


Supplemental Table 1. Primers for real-time PCR in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>(F)TTGGGCCTCAAAGGAAAGAAT</td>
</tr>
<tr>
<td></td>
<td>(R)TGGGTATTGCTTGGGATCCA</td>
</tr>
<tr>
<td>TNF-α</td>
<td>(F)GCCAACGGCATGGATCTC</td>
</tr>
<tr>
<td></td>
<td>(R)GCAGCCTTGTCCTTTGAAGAG</td>
</tr>
<tr>
<td>IL-18</td>
<td>(F)CTGAAGAAAAATGGAGACCTGGAA</td>
</tr>
<tr>
<td></td>
<td>(R)TCCGTATTACTCGGTGTTGTACAGT</td>
</tr>
<tr>
<td>IL-10</td>
<td>(F)GCCAAGCCTTATCGGAAATG</td>
</tr>
<tr>
<td></td>
<td>(R)GGGAATTCAATGCTCCTTGAT</td>
</tr>
<tr>
<td>CCL2</td>
<td>(F)CCTGGATCGGAACCAAATGA</td>
</tr>
<tr>
<td></td>
<td>(R)ACCTTAGGGCAGATGCAGTTTTA</td>
</tr>
<tr>
<td>CX3CL1</td>
<td>(F)TCCCCATCTGCCCTGACA</td>
</tr>
<tr>
<td></td>
<td>(R)AGTTCCAAAGCAAGGTCTTCCA</td>
</tr>
<tr>
<td>18S</td>
<td>(F)TCGGAACTGAGGCCATGATT</td>
</tr>
<tr>
<td></td>
<td>(R)CCTCGACTTTCTTGTGTGATT</td>
</tr>
</tbody>
</table>

(F), Forward Primer; (R), Reverse Primer.
**Supplemental Figure Legends**

**Supplemental Figure 1.** Evaluation of BM chimerism of BMT mice. A, Representative flow cytometric dot plots of splenocytes for recipient-type CD45.1-PerCP-Cy5.5 and donor-type CD45.2-APC. B, Representative flow cytometric dot plots of splenocytes for CD11c-PE and CD45.2-APC, and histogram for GFP of CD45.2⁺ CD11c⁺ splenocytes.

**Supplemental Figure 2.** Depletion of DCs by DT injection. A, Representative flow cytometric dot plots of splenocytes for CD11c-APC and GFP or MHCII-PE in control and DC-ablated mice. B, Representative flow cytometric histograms of splenocytes for CD4, CD8, CD19 and CD11b in control (solid line) and DC-ablated (dotted line) mice.

**Supplemental Figure 3.** Inefficacy of DT on the WT mice heart. Echocardiographic measurements and heart/lung weight adjusted for body weight in non-treated and DT-treated WT mice are shown.

**Supplemental Figure 4.** Enhanced infiltration of CD45⁺ leukocytes and Mac3⁺ macrophages into the infarcted heart by DC-ablation. A, Time course of CD45⁺ leukocyte infiltration into the heart of control and DC-ablated mice after MI. Data were obtained from 5 independent experiments at each time point. B, Representative immunofluorescent staining patterns for
CD45 in infarcted and border areas 7 days after MI. Scale bars indicate 30 μm. C,

Representative flow cytometric dot plots of heart-infiltrating CD45⁺ leukocytes for Mac3-PE in control and DC-ablated mice 7 days after MI. D, Quantification of Mac3⁺ macrophages based on flow cytometry in control (n = 5) and DC-ablated (n = 5) mice 7 days after MI. *P < 0.05 and **P < 0.01 vs sham of same group; †P < 0.05 and ††P < 0.01 vs control MI.

**Supplemental Figure 5.** DC-ablation induces marked monocyte recruitment after MI. A,

Temporal changes in number of total peripheral blood monocytes by flow cytometry. B,

Number of tissue monocytes in control and DC-ablated mice 7 days after MI or sham operation. *P < 0.05 and **P < 0.01 vs sham of same group; †P < 0.05 vs control MI.
The online data supplement Figure 1.

A

B
The online data supplement Figure 2.

A

Control

DC-ablation

0.73%

0.01%

0.65%

0.02%

B

CD4

CD8

CD19

CD11b

Count
The online data supplement Figure 3.
The online data supplement Figure 4.

A

Control MI DC-ablation MI
DAPI / CD45
Infarct area Border area
Sham Day 1 Day 3 Day 7 Day 14

B

CD45+ leukocytes ($\times 10^4$)

0 20 40 60 80

Control DC-ablation

† ††

C

Mac3+ macrophages (%)

0 5 10 15 20

Control MI DC-ablation MI

9.25% 15.30%

D

Mac3+ macrophages (%)

0 5 10 15 20

Control MI DC-ablation MI

††
The online data supplement Figure 5.

A

B

Peripheral blood monocytes (%)

Tissue monocytes (%)

Sham   Day 3   Day 7  Day 14

Control  DC-ablation

control  DC-ablation

Sham MI

†  **  *

†  **

The online data supplement Figure 5.