The Chemokine Decoy Receptor D6 Prevents Excessive Inflammation and Adverse Ventricular Remodeling After Myocardial Infarction

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Objective—Leukocyte infiltration in ischemic areas is a hallmark of myocardial infarction, and overwhelming infiltration of innate immune cells has been shown to promote adverse remodeling and cardiac rupture. Recruitment of inflammatory cells in the ischemic heart depends highly on the family of CC-chemokines and their receptors. Here, we hypothesized that the chemokine decoy receptor D6, which specifically binds and scavenges inflammatory CC-chemokines, might limit inflammation and adverse cardiac remodeling after infarction.

Methods and Results—D6 was expressed in human and murine infarcted myocardium. In a murine model of myocardial infarction, D6 deficiency led to increased chemokine (C-C motif) ligand 2 and chemokine (C-C motif) ligand 3 levels in the ischemic heart. D6-deficient (D6−/−) infarcts displayed increased infiltration of pathogenic neutrophils and Ly6Chi monocytes, associated with strong matrix metalloproteinase-9 and matrix metalloproteinase-2 activities in the ischemic heart. D6−/− mice were cardiac rupture prone after myocardial infarction, and functional analysis revealed that D6−/− hearts had features of adverse remodeling with left ventricle dilation and reduced ejection fraction. Bone marrow chimera experiments showed that leukocyte-borne D6 had no role in this setting, and that leukocyte-specific chemokine (C-C motif) receptor 2 deficiency rescued the adverse phenotype observed in D6−/− mice.

Conclusion—We show for the first time that the chemokine decoy receptor D6 limits CC-chemokine–dependent pathogenic inflammation and is required for adequate cardiac remodeling after myocardial infarction. (Arterioscler Thromb Vasc Biol. 2012;32:XX-XX.)

Key Words: chemokines ■ immune system ■ inflammation ■ ischemic heart disease ■ leukocytes

The inflammatory response that develops in the ischemic heart after myocardial infarction (MI) is a major determinant of left ventricle (LV) remodeling. In mice, several leukocyte subsets infiltrate the ischemic heart: neutrophils massively infiltrate the myocardium within the first 24 hours and rapidly fade thereafter. Infiltration of the 2 monocyte subsets, Ly6Chi and Ly6Clo, is biphasic; Ly6Chi monocytes dominate the acute phase of injury during the first 4 days, whereas Ly6Clo monocytes are prevalent thereafter. Ly6Chi monocytes scavenge necrotic debris and control the early events of postinfarct remodeling through the combination of inflammatory mediator expression, phagocytosis, and proteolysis. Of note, serine proteases secreted by monocytes and neutrophils promote LV dilation and cardiac rupture. In this line, Ly6Chi monocytes in apolipoprotein E−/− (ApoE−/−) mice has been shown to be associated with high proteolytic activity in the myocardium and adverse LV remodeling after MI. In contrast, Ly6Clo monocytes are thought to have a pro-healing phenotype and promote vascular endothelial growth factor–dependent angiogenesis.

Hence, the inflammatory response appears as a double-edged sword because it is a protective attempt by the organism to initiate the healing process; however, inflammation itself can exacerbate scarring and loss of organ function. In this view, the successful resolution of inflammation appears as a key event in the repair of tissue damage, but little is known...
about the main mediators of this process in the infarcted cardiac tissue.

Leukocyte recruitment to the infarcted heart depends highly on chemokines of the CC-chemokine family. The chemokine (C-C motif) ligand 2 (CCL2)/chemokine (C-C motif) receptor 2 (CCR2) axis controls Ly6Chi monocyte infiltration in the infarcted myocardium, and several studies using genetic or pharmacological approaches have shown that disruption of CCL2/CCR2 signaling alleviates adverse LV remodeling after MI through the inhibition of inflammatory cell recruitment.5–7 In addition, expression of other CC-chemokines in the ischemic heart, such as CCL5 or CCL3,8 has been associated with excessive inflammation and poor LV remodeling.

Although extensive knowledge regarding the initiation of the inflammatory response after MI has been acquired over the past few years, little is known concerning endogenous mechanisms able to limit those inflammatory processes and the associated adverse tissue remodeling leading to loss of organ function. Here, we hypothesized that the chemokine decay receptor D6 could limit post-MI inflammation through the control of CC-chemokine levels. Indeed, D6 possesses the unique ability to specifically bind and internalize inflammatory CC-chemokines9 without triggering conventional cell activation or chemotaxis, and has been shown to limit in vivo inflammatory responses in settings of skin inflammation, cancer,10 Mycobacterium tuberculosis infection11 and intestinal inflammation.12 Recently, D6 has also been shown to prevent lymphangiogenesis, and D6 deficiency is associated with poor lymphatic drainage of inflamed tissues.13

Here, we show that D6 is expressed in human and murine infarcted myocardium and controls levels of CC-chemokines after MI. D6 deficiency was associated with increased pathogenic inflammation, cardiac rupture, and adverse LV remodeling after MI.

Materials and Methods

Immunohistochemistry on Human Samples

Apical ischemic myocardial tissue was removed through a normal surgical procedure for the implantation of LV-assist devices in 6 patients presenting cardiogenic shock attributable to acute MI (1–7 days). The clinical course of these patients was previously described and published.14 The tissues were submitted to surgical pathology diagnosis as previously reported.14 The remnant tissues in paraffin blocks were used for research purpose with informed consent of the patients. The samples consisted of chips of left myocardium measuring 0.5 to 2 cm of long axis and were samples of cardiac apical LVs removed for implantation of a LV-assist device. The selected cases were acute MI lasting for 24 to 48 hours and presenting with acute cardiac failure. In these samples, some areas showed figures of remote infarcted myocardium and controls levels of CC-chemokines after MI. D6 deficiency was associated with increased pathogenic inflammation, cardiac rupture, and adverse LV remodeling after MI.

RNA Extraction and Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from the infarcted area and its border zone with Trizol reagent according to the manufacturer’s instructions (Invitrogen, Paris, France). DNA synthesis was performed with QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). Polymerase chain reaction was performed on an ABI Prism 7700 with the use of Power SYBR Green PCR Master Mix (Applied Biosystems, Courtabeuf, France). Mouse GAPDH was used to normalize sample amplification. The following oligonucleotides (Applied Biosystems) served as primers: GAPDH forward: 5′-CGTCCTGGTAGAACAATGTGGA-3′; reverse: 5′-GGCCGTGATGGAATGACTGAAA-3′; D6 forward: 5′-GCAATCGGCCACTAAGTAAAG-3′; reverse: 5′-CAAGGCCAGCAGCAAGATCAG-3′.

Myocardial Infarction

Mice were anesthetized with ketamine and xylazine and placed under mechanical ventilation. Left thoracotomy was performed to expose the LV of the heart. The left anterior descending coronary artery was visualized and ligated just below the left auricular level with a 7/0 nylon suture. Mechanical ventilation was maintained until mice awakening, and mice were then allowed to recover for 1 hour on a heating pad.

Echography

Before ischemic injury, M-mode echocardiography was performed by a blinded investigator. On infarcted mice, B-mode echocardiography was performed by blinded investigators at the Small Animal Imaging Unit (Plateforme d’imagerie du petit animal, Hôpital Cochin, Université Paris 5).

Flow Cytometry

The infarcted area and its border zone were isolated from WT and D6−/− hearts, minced and digested in 450-U/mL collagenase I, 125-U/mL collagenase XI, 60-U/mL hyaluronidase, and 60-U/mL DNase for 1 hour, before gradient density centrifugation on histopaque 1083 (Sigma-Aldrich, St. Louis, MO). Infiltrating cells were stained with monoclonal rat anti-mouse CD45-allophycocyanin, CD11b-PerCPCy5.5 (clone M1/70), Ly6G-phycoerythrin (clone 1A8), natural killer 1.1-phycoerythrin (all from BD Biosciences, San Jose, CA), and 7/4-fluorescein isothiocyanate (Abd Serotec) and analyzed on a BD LSR II flow cytometer.

Chemokine Protein Levels and Zymography

Infarcts from WT and D6−/− mice were snap frozen in liquid nitrogen and homogenized in Regulation of Investigatory Powers Act buffer. The protein content of the samples was determined using a Lowry assay (BioRad). Total protein extracts (50 μg) were loaded onto CCL2, CCL3, CCL5, and chemokine (C-X-C motif) ligand 2 DuoSet ELISA kits (R&D Systems) to determine chemokine levels. For gelatin zymography, 10 μg of total MI protein extracts were loaded onto a 9% acrylamide gel containing 0.1% gelatin. After protein migration, gels were incubated overnight in a calcium-containing...
buffer and gelatinase activity was revealed by Coomassie Blue staining.

**Histology and Immunohistochemistry**

Infarcts from WT or D6−/− mice were snap frozen in liquid nitrogen. All stainings were performed on 7-µm cryostats slides. Masson Trichrome staining was performed according to standard procedures. Caveolin-1 immunostaining was performed using polyclonal rabbit anti-mouse caveolin-1 antibody (1/100; Santa Cruz) followed by Texas Red conjugated donkey anti-rabbit IgG secondary antibody (Jackson Immunology). Neutrophils and 7/4+ cells were stained with phycoerythrin-conjugated monoclonal rat anti-mouse Ly6G antibody (1/100; clone 1A8; BD Biosciences) and fluorescein isothiocyanate-conjugated 7/4 antibody (1/100; Abd Serotec).

**Statistical Analysis**

Results are expressed as mean±SEM. Values were compared with a Mann–Whitney U test, and $P<0.05$ was considered significant. Kaplan–Meier analysis was used to compare survival after MI.

**Results**

**D6 Is Expressed in Human Infarcted Myocardium**

We first analyzed D6 expression by immunohistochemistry in biopsies from human MI patients. Although D6 expression was not detected in remote, viable myocardium (Figure 1A), we observed strong D6 signal in necrotic and inflammatory areas on infiltrating leukocytes and vascular structures (Figure 1B and 1C). Further staining of serial MI sections showed D6 expression on CD31+ endothelial cells, CD68+ macrophages, and D2−40+ (podoplanin+) lymphatic endothelial cells (Figure 1D). These results show that D6 appears to be preferentially expressed in the ischemic human heart on cells of vascular endothelial, lymphatic, and macrophage lineages.

**D6 Is Expressed in Murine MI and Controls CCL2 and CCL3 Levels**

We then analyzed D6 mRNA expression in murine myocardium. D6 was expressed in normal mouse heart, although its mRNA levels were much lower than in positive control tissues such as lung and spleen11 (Figure 2A; heart versus spleen $P=0.08$; heart versus lung $P<0.001$). Interestingly, D6 mRNA levels were upregulated by 2.0-, 2.5-, and 2.5-fold at 5, 7, and 14 days after coronary artery ligation, respectively ($P<0.01$; Figure 2B).

To analyze the role of D6 in the control of CC-chemokine levels after MI, we measured CCL2 and CCL3 protein levels in WT and D6−/− hearts before as well as 1, 3, and 5 days after infarction. Although barely detectable in nonischemic WT or D6−/− hearts, CCL2 levels raised drastically and to a similar extent in WT and D6−/− hearts at 1 day after MI, indicating that the initial CCL2 burst is not altered in D6−/− hearts. At
day 3, we observed a slight but significant increase in CCL2 levels in D6−/− hearts ($P<0.05$ versus WT). Most importantly, while CCL2 decreased between day 3 and day 5 in WT hearts, CCL2 levels were maintained in D6−/− hearts ($P<0.01$ versus WT), indicating a defect in CCL2 clearance in D6−/− hearts (Figure 2C). We also observed a similar increase in CCL3 levels at 1 day after MI in WT and D6−/− hearts. CCL3 levels tended to remain higher at 3 days in D6−/− mice, although it did not reach statistical significance ($P=0.07$ versus WT). Of note, at 5 days, CCL3 levels were significantly increased in D6−/− hearts ($P<0.05$ versus WT; Figure 2D). Surprisingly, CCL5 levels were not affected by D6 deficiency (Figure I in the online-only Data Supplement).

Consistent with the specificity of D6 for inflammatory CC-chemokines, chemokine (C-X-C motif) ligand 2 levels were identical in WT and D6−/− hearts (Figure I in the online-only Data Supplement). Altogether, our human and murine data show that D6 expression is increased after MI, and that D6 deficiency in mice is associated with a defective clearance of specific CC-chemokines after MI, with a particularly marked effect on CCL2 levels.

Increased Inflammation in D6−/− Hearts After MI
At 5 days after MI, we collected infarcted hearts from D6−/− and WT mice and analyzed the inflammatory infiltrate by flow cytometry. In D6−/− hearts, infiltration of 7/4hi monocytes (equivalent to Ly6Chi monocytes) and Ly6G+ neutrophils was increased by 2.7- and 3.8-fold, respectively ($P<0.05$; Figure 3A and 3B), whereas 7/4lo monocyte (equivalent to Ly6Clo monocytes) levels were similar to those found in WT (Figure 3B). These results were further confirmed by immunofluorescence analysis of Ly6G+ and 7/4+ cell density in WT or D6−/− MI sections (Figure 3C and 3D). A similar infiltration pattern was observed at 7 days after MI: 7/4hi monocytes and neutrophil infiltration was still substantial in D6−/− MIs, whereas those leukocyte subsets had almost completely vanished from WT MIs. 7/4lo monocyte levels remained unchanged (Figure II in the online-only Data Supplement).

Neutrophils and inflammatory monocytes are a predominant source of proteases in the injured heart. We measured matrix metalloproteinase-2 and matrix metalloproteinase-9 activity in 5-day-old WT or D6+ mice by gelatin zymography and found strong increases of matrix metalloproteinase-2 and matrix metalloproteinase-9 activity in D6−/− hearts at 5 days after MI (2.3- and 2.1-fold versus WT levels, respectively; $P<0.05$; Figure 3C and 3D). Altogether, those data show that D6 deficiency leads to exacerbated infiltration of pathogenic Ly6Chi monocytes and neutrophils after MI, associated with high proteolytic activity in the ischemic heart.

Cardiac Rupture and Adverse LV Remodeling in D6−/− Mice After MI
We then analyzed the survival of D6−/− mice over a period of 14 days and assessed their cardiac function by echocardiography. Although mortality was low in infarcted WT mice, >40% of D6−/− mice had died by day 10 (Figure 4A; $P<0.05$). This increase in mortality was attributable to a high incidence of cardiac rupture in D6−/− mice (45% versus 16.6% in WT; $P<0.05$; Figure 4B). Although baseline cardiac function was identical in D6−/− and WT mice (Figure III in the online-only Data Supplement), echocardiography revealed features of adverse LV remodeling in surviving D6−/− mice at 14 days after MI: LV end-systolic and end-diastolic endocardial volumes were increased by 1.5- and 1.3-fold, respectively ($P<0.01$; Figures 4C and 3D), indicating substantial LV dilation, and ejection fraction was decreased by 36% in D6−/− hearts ($P<0.05$; Figure 4E; Figure III in the online-only Data Supplement). Further echocardiographic parameters such as average LV wall thickness and fractional area contraction also tended to be altered in D6−/− mice (Figure III in the online-only Data Supplement). Histological measures showed that D6−/− infarcts were 20% larger ($P<0.05$; Figure 4F). These data show that D6 deficiency leads to increased incidence of cardiac rupture and adverse LV remodeling in surviving mice.
Increased LV dilation and poor LV contractility occurred despite higher angiogenesis, as the capillary density in the border zone of D6−/− MI was slightly increased (Figure IV in the online-only Data Supplement).

**Adverse Remodeling in D6−/− Mice Depends on CCR2 Signaling**

We observed substantial D6 expression on leukocytes, including CD68+ macrophages, infiltrating human MI (Figure 1). To assess the putative role of D6 expressed by leukocytes, we generated BM chimeras by reconstituting lethally irradiated WT mice with D6 −/− BM. Mice with leukocyte-specific D6 deficiency did not show any significant mortality after MI (data not shown) and had LV end-diastolic, LV end-systolic, and LV ejection fraction identical to controls (irradiated WT mice reconstituted with WT BM; Figure 5A, 5B, and 5C; Figure III in the online-only Data Supplement). Nevertheless, those mice displayed a slight increase in neutrophil infiltration at day 7, although it did not reach statistical significance (Figure V in the online-only Data Supplement). These data suggest that although leukocyte born D6 might have an accessory role, it is clearly compensated by nonleukocyte D6 expression, at least in our model. To confirm the role of CC-chemokines, and most particularly that of CCL2, which local and systemic

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**Figure 3.** Increased inflammation in D6−/− hearts after myocardial infarction (MI). **A,** Representative fluorescence-activated cell sorting dot plots obtained from wild-type (WT) and D6−/− digested hearts at 5 days after MI. Cells were gated on CD11b-expressing leukocytes. **B,** Quantitative evaluation of neutrophils, 7/4hi monocytes, and 7/4lo monocytes infiltration in WT and D6−/− hearts at 5 days after MI. **P<0.01, *P<0.05 (n=6 per group). C,** 7/4 (green) and Ly6G (red) immunostainings and (D) quantitative analysis of 7/4+Ly6G− neutrophils and 7/4+Ly6G−monocytes per high power field (HPF) on day 5 WT and D6−/− MI cryosections, ×200 magnification, scale bar=10 μm. **P<0.01, *P<0.05. E,** Representative zymogram and (F) quantitative analysis of matrix metalloproteinase-9 (MMP-9) and MMP-2 activity in WT and D6−/− hearts at 5 days after MI. **P<0.05, *P<0.01 (n=10 per group). FITC indicates fluorescein isothiocyanate; NK, natural killer; PE, phycoerythrin.
levels were preferentially enhanced by D6 deficiency, lethally irradiated D6−/− mice were reconstituted with WT or CCR2−/− BM. At 14 days after MI, D6−/− mice reconstituted with WT BM displayed LV dilation and reduced ejection fraction (Figure 5D, 5E, and 5F; Figure III in the online-only Data Supplement). These data strongly suggest that leukocyte-borne D6 has no major role in the inflammatory response after MI, and that the adverse remodeling phenotype in D6−/− mice depends on CCL2/CCR2 signaling.

**Discussion**

Here, we have shown for the first time that CC-chemokine, and most particularly CCL2-scavenging by the decoy receptor D6, prevented excessive inflammation in the ischemic heart and limited the extent of myocardial damage caused by an overwhelming immune response. We propose a model where...
ischemia induces CC-chemokines in the ischemic heart, leading to the recruitment of pathogenic inflammatory Ly6Chi monocytes and neutrophils, and where D6 acts as a gatekeeper of uncontrolled inflammation through the regulation of CC-chemokine levels.

D6 is known to be expressed by lymphatic endothelial cells and some specific leukocyte population such as innate-like B cells and transforming growth factor-β-activated macrophages. Our data clearly suggest that D6 is preferentially expressed in the infarcted heart, as it was undetectable in viable human myocardium and its mRNA expression strongly raised after MI in mice. In our murine MI model, this increase in D6 levels could reflect expression by infiltrating leukocytes, increased expression in D6 expressing cells, or proliferation of D6 expressing cells after MI. The fact that high levels of D6 mRNA were maintained at 14 days after MI, when inflammation had almost completely faded (data not shown), suggests that this increase in D6 mRNA is at least partially leukocyte independent. In our immunohistochemical study on human MI biopsies, we surprisingly observed D6 expression on vascular endothelial cells (Figure 1), although these were previously described to be consistently D6 negative. Of note, these previous observations were mostly conducted on healthy tissues, and one could speculate that D6 expression could be induced in vascular endothelial cells in the hypoxic and highly pro-inflammatory environment of cardiac ischemic areas. In this line, transforming growth factor-β, which is predominantly expressed during MI healing, is able to induce D6 expression in a globin transcription factor 1–dependent manner in leukocytes.

Most interestingly, D6 controlled the level of the major inflammatory CC-chemokines CCL2 and CCL3. This might constitute a new mechanism by which the organism actively attempts to restrain post-MI inflammation and the associated tissue damage by diminishing CC-chemokine bioavailability. Indeed, various reports have shown that CCL2 or CCL3 were able to promote MI-associated inflammation. Most particularly, CCL2/CCR2 signaling is known to control Ly6Chi monocyte systemic levels after limb ischemia and controls the infiltration of this proinflammatory monocyte subset in the ischemic heart. In this line, we observed a strong increase of Ly6Chi monocyte infiltration in D6−/− MIs. Neutrophil levels were also increased, which was puzzling, as CC-chemokine are not classic granulocyte chemoattractants. Nevertheless, nuclear factor-κB–dependent expression of CCR2 in neutrophils can be induced during inflammatory diseases such as sepsis. CCL3, which levels were upregulated in D6−/− hearts, has also been shown to attract neutrophils in a CCR1-dependent manner. Furthermore, increased neutrophil infiltration has been noted in D6−/− mice in models of sterile skin inflammation and microbial lung inflammation. Moreover, we observed increased neutrophil counts in the blood of D6−/− mice at 5 and 7 days after MI (data not shown), further suggesting a clear effect of D6 deficiency on neutrophil systemic and local levels after MI. It is noteworthy that D6 deficiency led to a specific increase in the infiltration of potentially pathogenic neutrophils and Ly6Chi monocytes, although proangiogenic and more generally prohealing Ly6Clo monocytes levels were not affected. This might be explained by the fact that Ly6Clo monocyte recruitment to the infarcted heart depends on chemokine (C-X3-C motif) receptor 1 and its ligand, Fractalkine/chemokine (C-X3-C motif) ligand 1, but not on CCR2.

Previous reports have shown that neutrophils and Ly6Chi monocytes express proteases in the ischemic heart, which promote adverse remodeling and cardiac rupture. Nevertheless, leukocyte proteases are necessary for adequate myocardial angiogenesis after MI, and disruption of CCR2 signaling hampers angiogenesis in limb ischemia, emphasizing the need of an inflammatory balance for proper tissue remodeling. Here, D6 appears as an important regulator of this inflammatory balance, preventing Ly6Chi and neutrophil infiltration in the ischemic heart to reach pathogenic levels resulting in LV dilation and cardiac rupture.

Our observation of abundant D6 expression on leukocytes and CD68+ macrophages infiltrated in human MIs led us to hypothesize that chemokine scavenging in the ischemic heart might be mediated by inflammatory cell-borne D6. Nevertheless, our chimera studies in mice failed to evidence a role for leukocyte-borne D6. This is consistent with a previous study showing that despite strong D6 expression, leukocyte did not significantly participate to D6-mediated anti-inflammatory effects in a setting of gut inflammation. Nonetheless, it should be noted that those experiments have been conducted in young and otherwise healthy WT animals in which D6 expression by other cell types, including lymphatic endothelial cells, might compensate the chemokine scavenging defect of infiltrating leukocytes. Cardiovascular risk factors such as hypercholesterolemia, which is known to induce lymphatic vessels dysfunction, might reduce chemokine scavenging by D6 expressing lymphatics and reveal a role for leukocyte-borne D6.

Besides its well-described role in monocyte chemoattraction, CCL2 has been suggested to promote fibrosis via direct action on fibroblasts, and was also shown to induce cardiomyocyte apoptosis. Those 2 properties of CCL2 might participate to adverse LV remodeling after MI in D6−/− mice. Nevertheless, we did not observe any evidence of significant fibrosis in D6−/− hearts (data not shown), and deletion of CCR2 specifically on BM-derived leukocytes led to a total rescue of D6−/− mouse phenotype. Hence, it appears that D6 protective effects during post-MI LV remodeling preferentially depend on its anti-inflammatory properties.

In conclusion, we show in this work that D6-dependent CC-chemokine scavenging controls tissue homeostasis and cardiac remodeling after MI. Our study also provides a mechanism for the fine-tuning of the balance between protective and pathogenic immune responses, which could be a suitable target for innovative therapeutic approaches.

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Disclosures

None.

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Disclosures

None.

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None.

References

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Supplement Material
**Supplementary figure I.** CCL5 and CXCL2 protein levels in WT and D6-/- hearts 5 days after infarction.
Supplementary figure II. Inflammatory cells infiltration in ischemic cardiac tissue at 7 days after infarction. Infiltration of 7/4hi monocytes, 7/4lo monocytes and neutrophils in WT and D6-/- infarcts 7 days after myocardial infarction. *p<0.05, **p<0.01 vs WT (n=3/group)
Supplementary figure III. a) Baseline cardiac function in D6-/− vs WT mice as assessed by M-mode echocardiography; b) Representative B-mode echocardiography images of a WT and a D6-/− heart in diastole, with delineation of endocardium and epicardium, at 14 days after MI. Additional B-mode echocardiographic measurements 14 days after MI in c) WT vs D6-/− mice; d) WT BM>WT Host vs D6-/− BM>WT Host; e) WT BM>WT Host vs WT BM>D6-/− Host, CCR2-/− BM>D6-/− Host and CCR2-/− BM>WT Host.

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HR: Heart rate in bpm; IVS: Interventricular Septal Wall Thickness, d=diastolic, s=systolic; LVPW: Left Ventricle Posterior Wall thickness, d=diastolic, s=systolic; LVID: Left Ventricle Internal Diameter, d=diastolic, s=systolic; FS: Fractionnal Shortenning.
Supplementary figure IV. Capillary density in the infarct border zone 14 days after infarction. a) representative photomicrographs and b) quantitative analysis of Caveolin1+ capillaries per mm² in the border zone of WT and D6-/- infarcts, 14 days after MI. Scale bar 50 micrometers, *p<0.05 vs WT (n=6 to 8)
Supplementary figure V. Effect of leukocyte specific D6 deletion on MI inflammation. 7/4hi monocytes, 7/4lo monocytes and neutrophils infiltration in WT mice lethally irradiated and reconstituted with WT (WT BM) or D6-/- (D6-/- BM) bone marrow cells, 7 days after MI (n=5/group).