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PRE-CLINICAL RESEARCH

Double-Edged Role of the CXCL12/CXCR4 Axis in Experimental Myocardial Infarction

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Objectives Here we assess the intrinsic functions of the chemokine receptor CXCR4 in remodeling after myocardial infarction (MI) using *Cxcr4* heterozygous ($Cxcr4^{+/-}$) mice.

Background Myocardial necrosis triggers complex remodeling and inflammatory changes. The chemokine CXCL12 has been implicated in protection and therapeutic regeneration after MI through recruiting angiogenic outgrowth cells, improving neovascularization and cardiac function, but the endogenous role of its receptor CXCR4 is unknown.

Methods MI was induced by ligation of the left descending artery. Langendoff perfusion, echocardiography, quantitative immunohistochemistry, flow cytometry, angiogenesis assays, and cardiomyocyte analysis were performed.

Results After 4 weeks, infarct size was reduced in $Cxcr4^{+/-}$ mice compared with wild-type mice and in respective bone marrow chimeras compared with controls. This was associated with altered inflammatory cell recruitment, decreased neutrophil content, delayed monocyte infiltration, and a predominance of $Gr1^{low}$ over classic $Gr1^{high}$ monocytes. Basal coronary flow and its recovery after MI were impaired in $Cxcr4^{+/-}$mice, paralleled by reduced angiogenesis, myocardial vessel density, and endothelial cell count. Notably, no differences in cardiac function were seen in $Cxcr4^{+/-}$mice compared with wild-type mice. Despite defective angiogenesis, $Cxcr4^{+/-}$ mouse hearts showed no difference in CXCL12, vascular endothelial growth factor or apoptosis-related gene expression. Electron microscopy revealed lipofuscin-like lipid accumulation in $Cxcr4^{+/-}$ mouse hearts and analysis of lipid extracts detected high levels of phosphatidylserine, which protect cardiomyocytes from hypoxic stress in vitro.

Conclusions CXCR4 plays a crucial role in endogenous remodeling processes after MI, contributing to inflammatory/progenitor cell recruitment and neovascularization, whereas its deficiency limits infarct size and causes adaptation to hypoxic stress. This should be carefully scrutinized when devising therapeutic strategies involving the CXCL12/CXCR4 axis. (J Am Coll Cardiol 2011;58:2415–23) © 2011 by the American College of Cardiology Foundation

In addition to governing hematopoetic cell trafficking, the CXCR4 ligand SDF-1α/CXCL12 has been shown to promote tissue regeneration by mediating recruitment of progenitor cells in ischemic areas (1–3). Therefore, the

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Abbreviations and Acronyms

EOC = early outgrowth cell
MI = myocardial infarction
PS = phosphatidylserine

interaction between CXCL12 and CXCR4 is increasingly exploited to enhance the efficacy of stem cell therapy after myocardial infarction (MI) (4). Exogenous CXCL12 applied by myocardial injection or overexpressed in transplanted cardiomyocytes, as well as overexpression of CXCR4 in mesenchymal stem cells, induces therapeutic angiogenic/progenitor cell homing (5–7), increasing capillary density and improving cardiac function after MI (8,9). On the other hand, CXCL12 directly activates the cell-survival factor protein kinase B (PKB/Akt) via CXCR4 and protects ischemic myocardium, decreasing scar formation and mediating neovascularization in mice and rats (10,11).

The intrinsic role of endogenous CXCL12/CXCR4 in MI, however, is far from being conclusively elucidated. For instance, administration of the selective CXCR4 antagonist AMD3100 reduced scar formation and improved cardiac contractility after MI (12). Moreover, CXCL12 can induce both survival and apoptotic signals via CXCR4, which may ultimately determine the fate of afflicted tissues (13). We therefore studied the function of CXCR4 in cardiac remodeling after MI in genetically modified mice to evaluate a potential relevance for unwanted effects of pharmacologic compounds (14). However, mice deficient in *Cxcr4* display profound defects in the hematopoietic and nervous systems and die perinatally. They have severely reduced B-lymphopoiesis, myelopoiesis in fetal liver, and a virtual absence of myelopoiesis in bone marrow (15). Therefore, we chose to assess the effects of reduced CXCR4 expression after MI in mice heterozygous for CXCR4 ($Cxcr4^{+/-}$), which appear normal and are viable and fertile (15), although CXCR4 surface expression on bone marrow–derived mononuclear cells from $Cxcr4^{+/-}$mice is significantly lower compared with that in wild-type BL6/J mice (16).

Methods

For the mouse model of MI and details of other methods (e.g., reverse transcriptase polymerase chain reaction analysis [Online Table 1]), please see the Online Appendix.

Results

Analysis of MI size and inflammatory cell content. Four weeks after MI, the infarct size was reduced in $Cxcr4^{+/-}$ mice by 42% compared with $Cxcr4^{+/+}$ littermates (Fig. 1A). As evident by tetrazolium/Evans blue staining, the area at risk 1 day after MI showed no difference in the 2 groups (Online Fig. 1A), indicating that the reduced infarct size likely reflects an enhanced wound contraction and alterations in reparative pathways rather than a difference in the initial extent of cardiomyocyte injury. Moreover, myofibroblast infiltration (2,600 ± 283/mm^2 vs. 1 011 ± 165/mm^2 in controls, $p < 0.001$) and collagen content in the infarcted area were significantly higher in $Cxcr4^{+/-}$ mice than in wild-type controls (Fig. 1B), indicating a more stable and robust scar formation.

We next analyzed the mobilization and recruitment of inflammatory cells after MI. The MI-induced and transient expansion of neutrophils in the circulation (Fig. 1C) and infiltration of the infarcted area with neutrophils (Fig. 1D) were severely reduced in $Cxcr4^{+/-}$ mice 1 day after MI. Thus, the initial inflammatory response differed markedly in $Cxcr4^{+/-}$ mice, indicating a prominent role of CXCR4 in post-infarction neutrophil recruitment. Further, peripheral blood monocyte levels did not differ between $Cxcr4^{+/-}$ mice and wild-type mice after MI (Online Fig. 1B), whereas the myocardial infiltration with monocytes/macrophages presented a slight delay in $Cxcr4^{+/-}$ mice compared with wild-type mice (Online Fig. 1C).

Analysis of monocyte subsets revealed fewer circulating Gr-1^{high} cells and relative expansion of Gr-1^{low} cells in peripheral blood of $Cxcr4^{+/-}$mice compared with wild-type mice after MI (Fig. 1E). These data correspond to diminished infiltration with proinflammatory tissue-degrading Gr-1^{high} monocytes 4 days after MI, whereas Gr-1^{low} monocytes, known to promote wound healing and collagen deposition (17), were increased in the hearts of $Cxcr4^{+/-}$ mice compared with wild-type mice (Fig. 1F). Thus, the inflammatory reaction after MI in $Cxcr4^{+/-}$ mice is shifted to an earlier termination of the acute response and onset of a repair process involving Gr-1^{low} monocytes. Notably, whereas CXCR4 expression on Gr-1^{low} monocytes from $Cxcr4^{+/-}$ mice (specific mean fluorescence intensity, 31.5 ± 3.0) was reduced by 43% compared with that on Gr-1^{low} monocytes from wild-type mice (specific mean fluorescence intensity, 61.9 ± 7.4), the low CXCR4 expression on Gr-1^{high} monocytes did not differ between wild-type mice and $Cxcr4^{+/-}$ mice (specific mean fluorescence intensity, 6.8 ± 3.5 vs. 5.2 ± 1.5). This suggests a strong adaptation of Gr-1^{low} cells to reduced CXCR4 expression and a possible role of other receptors in their recruitment.

Analysis of cardiac function after MI. Echocardiography (Online Table 2) and Langendorff (Table 1) measurements surprisingly failed to reveal changes in ventricular function and contractility after MI in $Cxcr4^{+/-}$ mice compared with wild-type mice. However, we observed a slightly decreased baseline ejection fraction as well as a moderate increase in the post-MI ejection fraction in $Cxcr4^{+/-}$ mice compared with wild-type mice. Moreover, the difference in ejection fraction before and after MI was significantly decreased in $Cxcr4^{+/-}$ mice compared with wild-type mice (7.6 ± 1.2% vs. 16.8 ± 2.4%, $p < 0.01$), implying a protective or adaptive mechanism in the hearts of $Cxcr4^{+/-}$ mice.

Moreover, coronary perfusion was markedly decreased in $Cxcr4^{+/-}$ mice compared with wild-type mice, as determined by coronary flow measurements in isolated perfused hearts. Coronary flow was already reduced under baseline conditions in $Cxcr4^{+/-}$ mice compared with wild-type mice (Table 1, Fig. 2A). Ligation of the left anterior descending artery decreased coronary flow by approximately 50% in

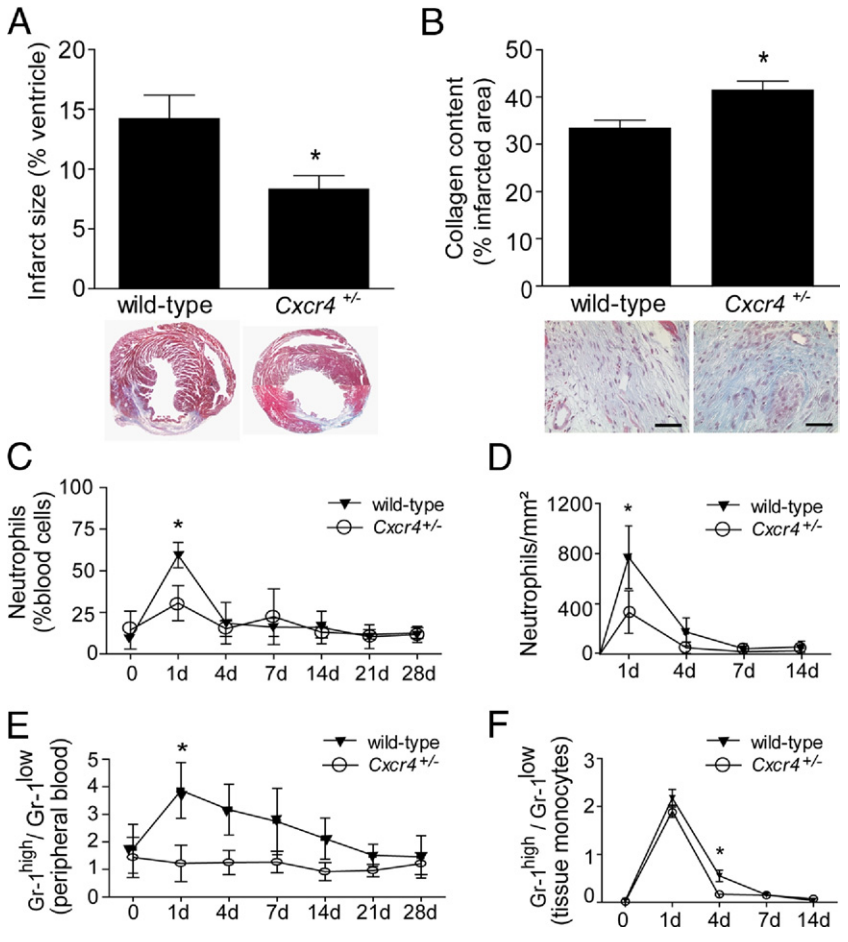

Figure 1 **Analysis of MI**

Compared with wild-type mice, *Cxcr4*$^{+/-}$ mice display a significantly smaller infarct size **(A)** and a significant increase in myocardial collagen content **(B)** (scale bar = 50 μm) 4 weeks after myocardial infarction (MI). Neutrophil counts in peripheral blood **(C)** and neutrophil infiltration in myocardium **(D)** are reduced in *Cxcr4*$^{+/-}$ mice compared with wild-type mice after MI. Analysis of the Gr-1^{high}/Gr-1^{low} ratio showed a relative shift toward Gr-1^{low} cells in blood of *Cxcr4*$^{+/-}$ mice compared with wild-type mice **(E)**. The Gr-1^{high}/Gr-1^{low} ratio in myocardium revealed a transient reversal after MI in both groups **(F)** and an earlier return to baseline levels in *Cxcr4*$^{+/-}$ mice compared with wild-type mice. *$p < 0.05$ versus wild type.

both groups (Figs. 2A and 2B). Four weeks after MI, the recovery of coronary perfusion was significantly impaired in *Cxcr4*$^{+/-}$ mice (Online Table 2, Fig. 2A).

To assess whether defective cardiac angiogenesis and neovascularization after MI contribute to the differences in coronary blood flow, myocardial endothelial cells and vessels were quantified. As determined by flow cytometry, the number of myocardial endothelial cells was intrinsically reduced in *Cxcr4*$^{+/-}$ mice compared with wild-type mice (Fig. 2C). Similarly, neovascularization after MI was impaired in *Cxcr4*$^{+/-}$ mice compared with wild-type mice, as evident by reduced formation of CD31^{+} blood vessels in infarcted myocardium (Fig. 2D). This might contribute to the defective recovery of coronary flow after MI in *Cxcr4*$^{+/-}$ mice. Moreover, the number of primary branches of coronary arteries (Fig. 2E) as shown by micro-angio-computed tomography, as well as the number of cremasteric artery branches as quantified by intravital microscopy (data not shown) was diminished in *Cxcr4*$^{+/-}$ mice compared with wild-type mice without evidence of disturbed endothelial permeability, as shown by perfusion of the cremasteric artery with albumin–fluorescein isothiocyanate. This indicates that the endogenous defect in angiogenesis was not restricted to the heart.

Moreover, to distinguish the influence of the CXCR4 heterozygous background and its role in circulating cells, bone marrow chimera experiments were performed after lethal irradiation. Four weeks after MI, the infarction area was significantly reduced in wild-type mice transplanted with *Cxcr4*$^{+/-}$ bone marrow and in *Cxcr4*$^{+/-}$ mice transplanted with wild-type bone marrow compared with the control group (Fig. 3A). In addition to the effects attributable to reduced leukocyte infiltration, these data suggest the existence of an additional intrinsic mechanism that can substantially influence scar formation in our model. Notably, neovascularization after MI was impaired in both groups compared with controls, as evident by CD31^{+} staining in infarcted myocardium (Fig. 3B), whereas heart function, as assessed by

Table 1 Functional Parameters of Wild-Type and *Cxcr4*$^{+/-}$ Hearts

Parameter	Langendorff Perfusion			
	Before MI		4 Weeks After MI	
	Wild Type (n = 3–4)	*Cxcr4*$^{+/-}$ (n = 3–5)	Wild Type (n = 4–5)	*Cxcr4*$^{+/-}$ (n = 5–6)
LVDP, mm Hg	86.7 ± 8.8	78.3 ± 17.0	40.0 ± 10.1	50.0 ± 11.5
Increase after dobutamine (Δ)	40.3 ± 4.0	46.2 ± 5.2	13.8 ± 4.3	10.3 ± 2.6
dPdt max, mm Hg/s	3,189 ± 99	3,058 ± 363	1,540 ± 97	1,590 ± 94
Increase after dobutamine (Δ)	2,410 ± 629	1,941 ± 187	770 ± 91	704 ± 74
dPdt min, mm Hg/s	−2,695 ± 244	−2,011 ± 411	−1,267 ± 111	−1,280 ± 79
Increase after dobutamine (Δ)	−1,756 ± 544	−1,407 ± 314	545 ± 28	484 ± 68
Coronary flow, ml	3.9 ± 0.2	2.0 ± 0.3*	3.2 ± 0.3	1.2 ± 0.3*
Increase after brief ischemia (Δ)	3.4 ± 0.2	2.8 ± 0.1	1.8 ± 0.4	0.2 ± 0.1*

Values are mean ± SD. *p < 0.05 versus wild type.
dPdt = derivative of pressure increase (maximum) and decay (minimum); LVDP = left ventricular developed pressure; MI = myocardial infarction.

echocardiography and Langendorff perfusion showed no significant differences between the groups (Fig. 3C). The reduction of neovascularization was more pronounced in *Cxcr4*$^{+/-}$ mice transplanted with wild-type bone marrow and correlated with decreased coronary flow (Fig. 3D). This may reflect that the vascularization of the scar is based mostly on vessel formation around pre-existing collaterals, which may explain the markedly reduced neovascularization in *Cxcr4*$^{+/-}$ mice despite reconstitution with wild-type bone marrow.

The myocardial infiltration with neutrophils, as well as blood leukocyte subsets (Fig. 3E) after MI in *Cxcr4*$^{+/-}$ mice transplanted with wild-type bone marrow emulate the pattern

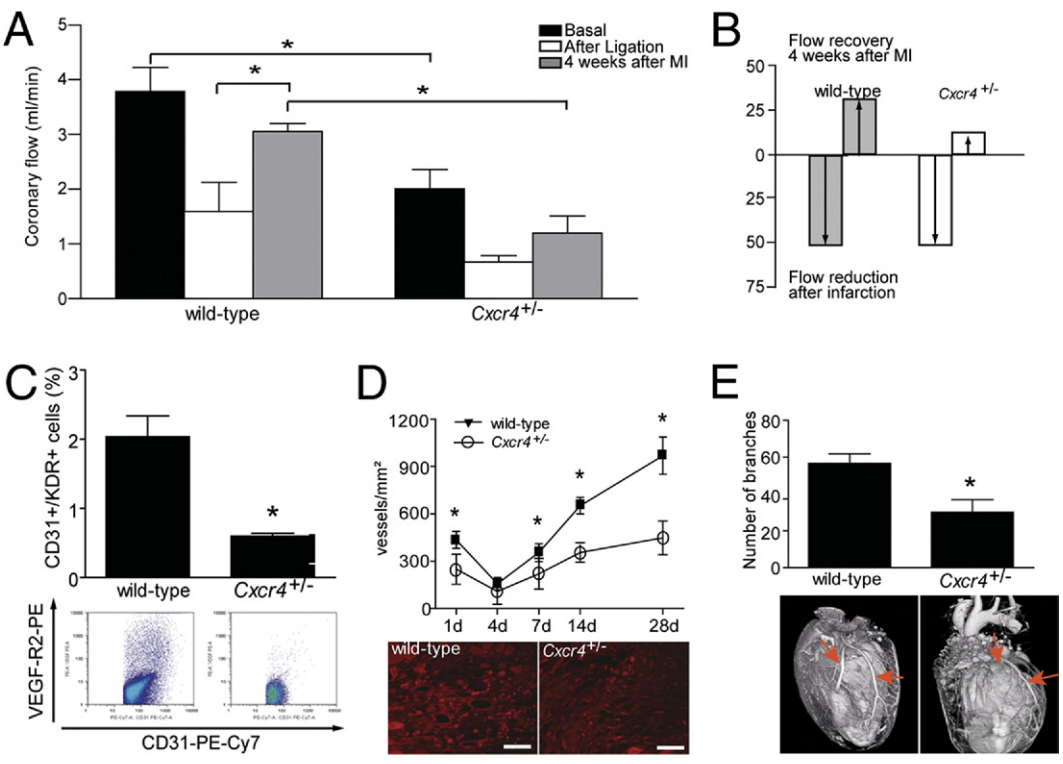

Figure 2 Analysis of Coronary Flow and Angiogenesis

(A) Langendorff perfusion revealed a reduction of coronary flow in *Cxcr4*$^{+/-}$ mice compared with wild-type mice at both basal conditions and 4 weeks after myocardial infarction (MI). **(B)** Despite equivalent flow reduction after MI, recovery of coronary flow after MI, represented as percentage of basal coronary flow, was impaired in *Cxcr4*$^{+/-}$ mice compared with wild-type mice. **(C)** Flow cytometry analysis indicated a decreased number of endothelial cells in myocardial tissue. **(D)** CD31 staining confirmed the impairment in neoangiogenesis in *Cxcr4*$^{+/-}$ mice compared with wild-type mice (scale bar = 50 μm). **(E)** The quantification of the branches of the coronary artery showed a marked impairment of vessel density in *Cxcr4*$^{+/-}$ mice compared with wild-type mice under basal conditions (**red arrows** indicate left anterior descending and circumflex arteries). *p < 0.05 versus wild-type.

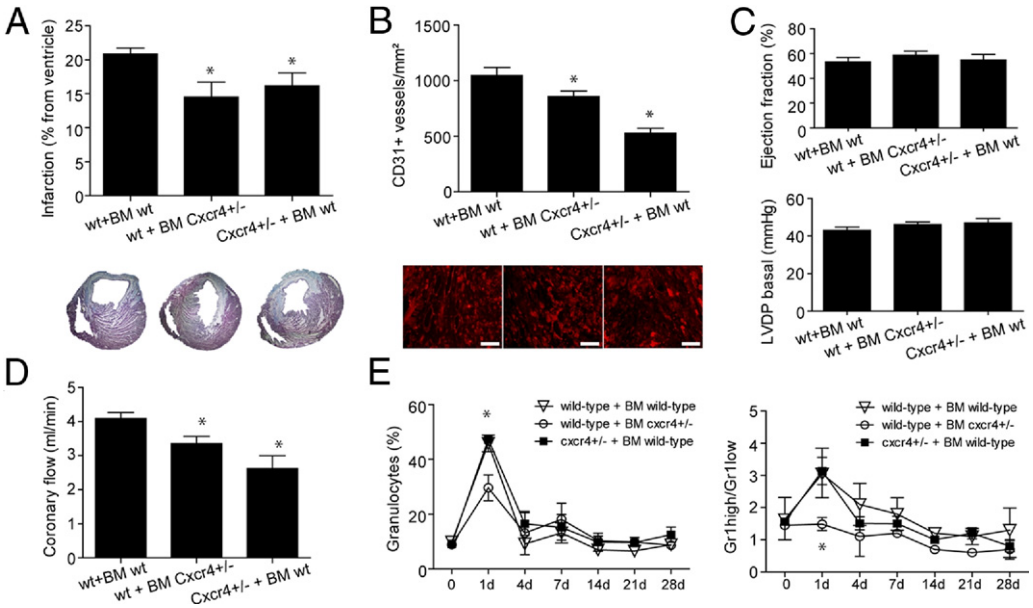

Figure 3 **Effect of CXCR4 Deficiency in Bone Marrow Cells After MI**

Four weeks after myocardial infarction (MI), the infarction area **(A)** and neovascularization **(B)** (scale bar = 50 μm) was reduced in wild-type mice transplanted with *Cxcr4*$^{+/-}$ bone marrow (BM) and in *Cxcr4*$^{+/-}$ mice transplanted with wild-type bone marrow, compared with controls. **(C)** Ejection fraction (echocardiography) and left ventricular developed pressure (LVDP) (Langendorff) were unaltered. **(D)** Coronary flow revealed reduced perfusion in wild-type mice transplanted with *Cxcr4*$^{+/-}$ bone marrow and in *Cxcr4*$^{+/-}$ mice transplanted with wild-type bone marrow compared with the control group. As analyzed by flow cytometry, blood leukocyte subset counts in peripheral blood showed a reduction in blood neutrophils and Gr-1^{high}/Gr-1^{low} ratio **(E)** in wild-type mice reconstituted with *Cxcr4*$^{+/-}$ bone marrow, assembly the *Cxcr4*$^{+/-}$ mice, but not in the other groups. *$p < 0.05$ versus control. wt = wild type.

in *Cxcr4*$^{+/-}$ mice, indicating a shift toward an earlier termination of the acute response and earlier onset of a repair process.

The role of CXCR4 for early outgrowth cells trafficking and function. Because early outgrowth cells (EOCs) contribute to post-infarction neoangiogenesis, the effect of Cxcr4 on EOC function was studied. Despite endothelium-like properties of both *Cxcr4*$^{+/-}$ EOCs and wild-type EOCs, the function of *Cxcr4*$^{+/-}$ EOCs was impaired, as shown in chemotaxis or Matrigel assays in vitro and in vivo. (Online Fig. 2). For more details, see the Online Appendix.

Myocardial apoptosis after MI. No difference in myocardial apoptosis after induction of MI was observed in *Cxcr4*$^{+/-}$ mice and wild-type mice after MI, as assessed by quantifying TUNEL (deoxyuride-5′-triphosphate biotin nick end labeling)-positive cells and by reverse transcriptase polymerase chain reaction for Bax and Bcl2 expression (Online Fig. 3). For more details, please see the Online Appendix.

Electron microscopy and characterization of lipid extracts. To detect structural alterations, we performed electron microscopy in the hearts of the *Cxcr4*$^{+/-}$ mice and wild-type mice before or 1 day after MI. Wild-type myocardium displayed extensive signs of necrosis with dramatic cellular disintegration (Fig. 4A). In infarcted *Cxcr4*$^{+/-}$ myocardium, signs of myofibril disorganization, and cardiomyocyte swelling were present, but cellular structures were still distinguishable (Fig. 4A). Notably, in uninjured *Cxcr4*$^{+/-}$ myocardium, we found atypical lipofuscin-like lipid accumulations with strong osmium tetroxide fixation (Fig. 4A), likely containing long-chain monounsaturated fatty acids and resembling those found after dietary fish oil feeding, known to reduce ischemic damage in rat hearts (18,19).

Subsequent lipid extraction and high-performance liquid chromatography analysis indicated a marked accumulation of phosphatidylserine (PS) (fractions 1 to 3) in *Cxcr4*$^{+/-}$ mouse hearts, which is not present in the hearts of wild-type mice (Fig. 4B). Using gas chromatography, we analyzed the unsaturated fatty acid index of cardiac lipid extracts (triglyceride, phosphatidylcholine/ethanolamine, PS). The triglyceride fraction obtained from *Cxcr4*$^{+/-}$ mouse hearts contained 10% more unsaturated fatty acids than wild-type mouse hearts, and 6 different unsaturated fatty acids appeared in the triglyceride fraction of *Cxcr4*$^{+/-}$ hearts. No differences were noted for saturated/unsaturated fatty acid content in phosphatidylcholine or ethanolamine. The fatty acid composition of the 3 PS fractions is detailed in Online Table 3.

To evaluate a potential contribution of the PS fractions to cardioprotection in *Cxcr4*$^{+/-}$ mice, isolated cardiomyocytes were pre-incubated with the PS fractions 1, 2, or 3 for 3 h, and the response to hypoxic stress was analyzed. As evident by dihydroethidium staining to monitor radical formation, all PS fractions protected cardiomyocytes against hypoxic injury (Fig. 4C). Notably, PS fraction 1 with the highest percentage of saturated fatty acids offered the best protection against hypoxic injury. These data indicate that *Cxcr4*$^{+/-}$ mouse hearts are intrinsically adapted to hypoxic injury.

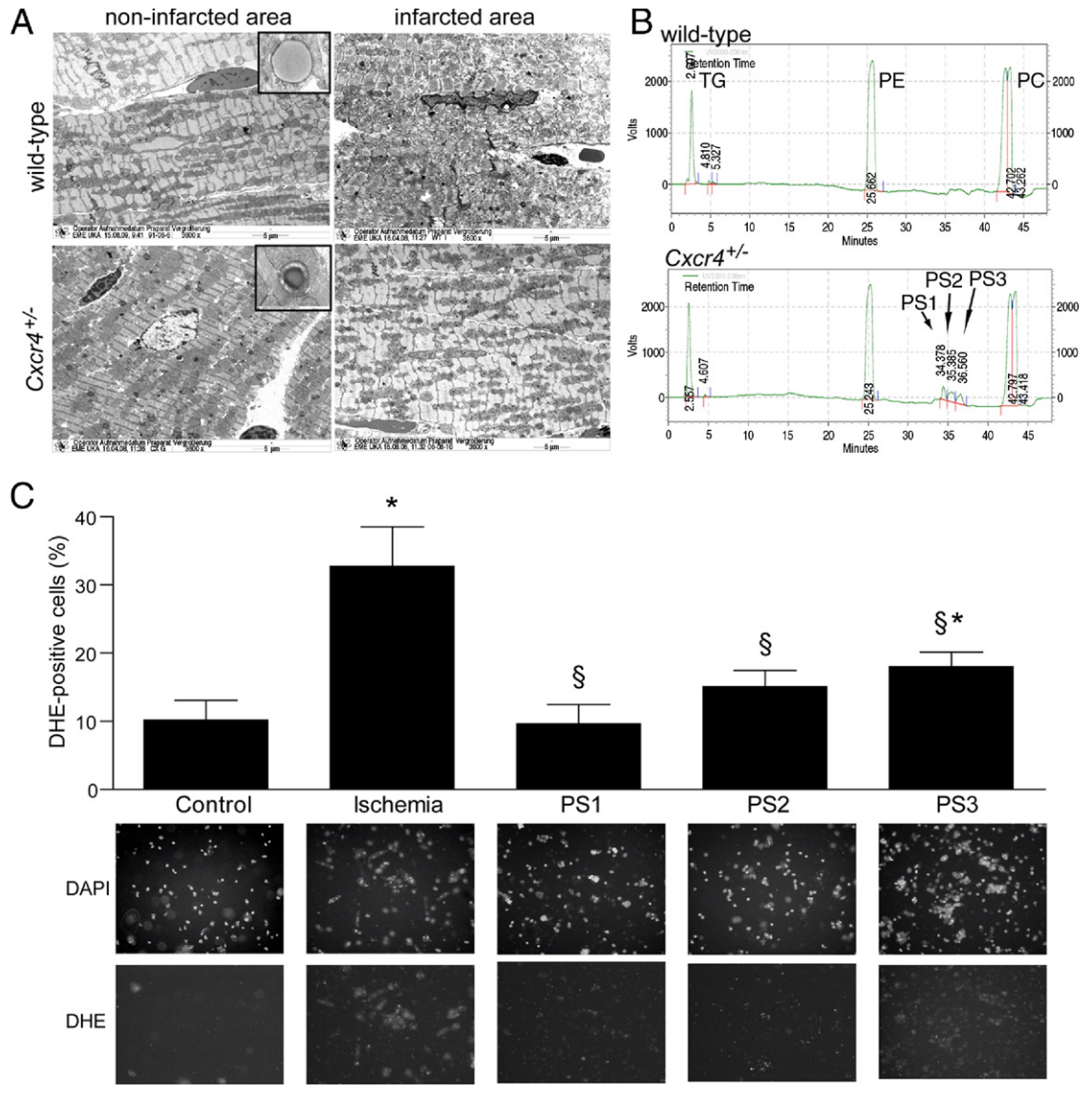

Figure 4 **Myocardial Electron Microscopy and Characterization of Lipid Extracts**

Electron microscopy showed atypical lipofuscin-like lipid accumulation with a strong osmium fixation (**A, left side**, 1 representative lipid vesicle shown in the inset). After myocardial infarction (MI), *Cxcr4*$^{+/-}$ myocardium exhibits signs of myofibril disorganization and cardiomyocyte swelling but is still distinguishable cellular structure, whereas wild-type myocardium shows signs of necrosis with dramatic structural disintegration **(A, right side)**. **(B)** Lipid extraction and high-performance liquid chromatography analysis indicate in addition to triglyceride (TG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC), an up-regulation of phosphatidylserine (PS) in *Cxcr4*$^{+/-}$ mouse hearts compared with wild-type hearts. **(C)** In in vitro hypoxia experiments, pre-incubation of isolated cardiomyocytes with PS fractions protects against hypoxic injury. *$p < 0.01$ versus control, §$p < 0.05$ versus ischemia, $n = 4$.

Discussion

Our data demonstrate the double-edged effects of CXCR4 on myocardial remodeling after MI and point to a variety of possible mechanisms with major clinical implications. Compared with wild-type mice, *Cxcr4*$^{+/-}$ mice revealed smaller and stable MI scars due to an attenuation of the acute inflammatory recruitment of neutrophils, a shift toward a more regenerative monocyte response, and better adaptation of cardiomyocytes to hypoxic stress. This was balanced by impaired EOC function, myocardial neovascularization, and coronary flow recovery, overall amounting to a lack of improvement in ventricular function. Given the major efforts to exploit the CXCL12/CXCR4 axis therapeutically to promote angiogenesis and cellular regeneration, our data provide important insights into endogenous function of CXCR4 after MI.

First, we found an altered inflammatory pattern in *Cxcr4*$^{+/-}$ mice after MI characterized by diminished neutrophils and tissue-degrading Gr1^{high} monocytes, myocardial infiltration, and earlier infiltration with Gr1^{low} monocytes, followed by collagen deposition (17). Reduced neutrophil infiltration itself may substantially affect myocardial injury and reduce MI size

(20,21) by reducing the release of reactive oxygen species, proteases, and inflammatory mediators. Recently, CXCR4 was identified as a central regulator of neutrophil homeostasis directing their release from bone marrow under stress conditions (20). Although complete disruption or deficiency of CXCR4 caused an expansion of less mature neutrophils in the circulation in the chronic context of atherogenesis (22), we found that an acute mobilization of neutrophils was blocked by the potent CXCR4 antagonist AMD3645 (23). Similarly, we observed that MI caused an acute expansion of circulating neutrophils and their myocardial recruitment, which was attenuated in *Cxcr4*$^{+/-}$ mouse hearts. This is in line with a recent study that failed to detect neutrophil mobilization after various forms of stimulation or infection when CXCR4 signaling was abrogated (20). Thus, our data confirm a role of CXCR4 in injury- or stress-induced neutrophil mobilization, allowing their subsequent recruitment.

Monocytes play an important and finely tuned role in cardiac repair (17). We found that after MI, overall monocyte/macrophage infiltration into the myocardium was delayed in *Cxcr4*$^{+/-}$ mice owing to a reduced infiltration with Gr-1^{high} inflammatory monocytes during the initial phase, in a process that may be governed by neutrophil secretory products (24,25). Preventing Gr-1^{high} monocytosis results in a delayed or inefficient removal of apoptotic cells and necrotic tissue but does not impede healing (17,26). Conversely, Gr-1^{low} monocytes, which promote healing via myofibroblast accumulation and collagen deposition, were more prevalent and recruited earlier after MI in *Cxcr4*$^{+/-}$ mice. This shift to a more robust repair may contribute to smaller and stable scar formation. Interestingly, we found that Gr-1^{high} monocytes from *Cxcr4*$^{+/-}$ mice did not display reduced CXCR4 expression. Whereas one may hypothesize that the reparative Gr-1 cells uses additional receptors to compensate for lower CXCR4 levels in recruitment, these data generally imply an important role of other receptors, namely, CCR2, in the recruitment of Gr-1^{high} monocytes.

Despite reduced MI size, ventricular function was not significantly improved in *Cxcr4*$^{+/-}$ mice. This could be due to the reduced basal coronary flow and to the impaired coronary flow recovery in *Cxcr4*$^{+/-}$ hearts 4 weeks after MI. As an underlying mechanism, we studied the function of EOCs as important contributors to neovascularization after MI. The SDF-1/Cxcr4 interaction is crucially involved in the mobilization and recruitment of stem and progenitor cells to the heart after MI (5,27). Despite appropriate acquisition of typical endothelial differentiation markers, splenic EOCs from *Cxcr4*$^{+/-}$ mice showed deficient chemotaxis toward CXCL12 (but not vascular endothelial growth factor) and reduced tube formation in vitro. Accordingly, myocardial vessel density, endothelial cell content, vessel invasion in Matrigel and arterial branching in vivo was impaired in *Cxcr4*$^{+/-}$ mice. This is in keeping with a previous study showing that EOCs from *Cxcr4*$^{+/-}$ mice were also significantly impaired to restore blood flow in ischemic nude mice compared with wild-type EOCs in the hindlimb ischemia model (16). Although we show in vitro that these effects are mostly due to a dysfunction of EOCs, we cannot exclude that a decrease in surrounding vascular density also plays a supportive role in our in vivo models through reducing the number of circulating cells available at the site of injury. Conversely, a lack of functional improvement in *Cxcr4*$^{+/-}$ mice cannot be explained by a modulation of cardiomyocyte contractility by CXCR4 because CXCL12 has been shown to exert negative inotropic effects (28) so that one would rather expect improved ventricular function on inhibition of *Cxcr4* deficiency.

Moreover, we performed bone marrow chimera experiments to distinguish the influence of the *Cxcr4* heterozygous background and its effect on circulating cells in normally developed wild-type mice. Despite a reconstitution with wild-type bone marrow, a reduction of infarction area and neovascularization persisted in *Cxcr4*$^{+/-}$ mice. Moreover, a significant, albeit less marked, reduction was observed in wild-type mice reconstituted with *Cxcr4*$^{+/-}$ bone marrow, indicating that reduced CXCR4 levels on circulating cells (namely, progenitor cells and leukocytes) may also contribute to the effects observed after MI in *Cxcr4* heterozygous mice, independently of their abnormal cardiovascular development.

Notably, the reduced basal and neovascularization of *Cxcr4*$^{+/-}$ hearts without any sign of physiologic dysfunction raises several questions. Diminished blood supply should lead to a series of histopathologic and structural changes of the myocardium with an increase in cardiomyocyte apoptosis, ventricular mass and volume, and progressive decline in left ventricular performance. None of these parameters, however, differ in *Cxcr4*$^{+/-}$ mice. Moreover, recent data indicate that CXCR4 expression on cardiomyocytes is not essential for cardiac development and has no major role in ventricular remodeling after MI (29). Because *Cxcr4*$^{+/-}$ myocardium is spared and hypoxic injury seems to be less extensive, compared with that in wild-type mice, we assume that the protective mechanism in *Cxcr4*$^{+/-}$ myocardium is mostly due to adaptive changes during embryogenesis. Using electron microscopy, we observed lipofuscin-like lipid accumulations, which resembled those found in rat hearts after dietary fish oil feeding (18). A diet enriched with n-3 fatty acids can reduce ischemic damage to the heart (30) and may represent a possible lead to protection, but this clearly requires further investigation into underlying mechanisms.

Another notable difference in the lipid extracts of *Cxcr4*$^{+/-}$ myocardium is the high levels of PS, generally known as a marker of cell death (31). However, PS supports other cell functions, including mitochondrial membrane integrity and activation of protein kinase C, which is important in hypoxia tolerance during late preconditioning (32), as well as in the inhibition of specific immune responses (33). In our study, the permanently decreased coronary flow in *Cxcr4*$^{+/-}$ mice may induce a chronic ischemia and thus may force cardiomyocytes to adapt even from early stages of embryonic development. An increase in

cardiac PS seems to be a possible cause mediating this adaptive mechanism because pre-incubation of cardiomyocytes with PS isolated from *Cxcr4*$^{+/-}$ mouse hearts protected cardiomyocytes against hypoxic injury. However, the exact mechanism remains to be established.

Extensive attempts have been made to directly affect the CXCL12/CXCR4 axis (e.g., by direct injection, nanofiber-mediated delivery of CXCL12, or overexpression of CXCL12/CXCR4 in cells transplanted into the myocardium (5–11), aiming to reduce MI size and to improve ventricular function after MI. The double-edged effects of CXCR4 are illustrated by an alteration of the inflammatory response and protection against hypoxic stress, as well as impaired EOC function, neovascularization, and coronary flow recovery. Pharmacologic antagonism of CXCR4 with AMD3100 has been reported to reduce infarct size and to improve ventricular function after MI in rats (12). Although the decrease in MI size is consistent with our findings, an improved contractility has been explained by a suppression of the hypertrophic response in the noninfarct area. This differs from *Cxcr4*$^{+/-}$ mice, which have intrinsically reduced coronary flow and can be considered as a model for congenitally impaired vascularization and adaptation to hypoxia.

Limitations and Conclusions

Although studies in bone marrow chimeras suggested a role of CXCR4 on cells infiltrating from the circulation in explaining the reduction in infarct size, one notable limitation of our study is clearly the lack of mice with specific and inducible deletion of CXCR4 in either circulating cells or resident myocardial cells to better dissect the underlying mechanisms. In addition, caution should be exerted when extrapolating these results to an inhibition of CXCR4 in the human system. Nevertheless, cell-specific, context-dependent, and long-term effects of CXCR4 interference or CXCL12 application need to be carefully taken into account when devising therapeutic strategies for MI and ischemic cardiomyopathy.

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Key Words: angiogenesis ■ chemokine receptor ■ inflammation ■ myocardial infarction ■ myocardial remodeling.

APPENDIX

For an expanded Methods section and supplemental tables and figures, please see the online version of this article.