Macrophage migration-inhibitory factor (MIF) is one of the first cytokines to be identified more than 40 years ago.1 Today, MIF is known as a structurally unique, pleiotropic inflammatory cytokine with chemokine-like properties, which functions as a potent mediator of several inflammatory conditions.2-4 However, the role of MIF in cardiovascular diseases, especially in myocardial infarction (MI), has not yet been definitively clarified.

MIF is upregulated in endothelial cells and smooth muscle cells of atherosclerotic arteries, where it contributes to macrophage accumulation and plaque formation.5,6 It seems that MIF acts sequentially by first triggering monocyte arrest through the CXCR axis and then promoting monocyte transmigration through the intermediate production of CCL2.7,8 Being predominantly expressed in vulnerable plaques and inducing collagen-degrading matrix metalloproteinases,9,10 MIF has been implicated in plaque destabilization.

In the context of MI, the levels of MIF were found to be significantly elevated in patients with reversible myocardial ischemia-reperfusion injury.11-15 This phenomenon is likely mediated by the upregulation of CCL2 in miR-143/145 (miR)- silenced macrophages, leading to increased recruitment of monocytes and the stabilization of vulnerable plaques.16-18

Objective—Here, we aimed to clarify the role of CXC chemokine receptor (CXCR) 2 in macrophage migration-inhibitory factor (MIF)–mediated effects after myocardial ischemia and reperfusion. As a pleiotropic chemokine-like cytokine, MIF has been identified to activate multiple receptors, including CD74 and CXCR2. In models of myocardial infarction, MIF exerts both proinflammatory effects and protective effects in cardiomyocytes. Similarly, CXCR2 displays opposing effects in resident versus circulating cells.

Approach and Results—Using bone marrow transplantation, we generated chimeric mice with Cxcr2−/− bone marrow–derived inflammatory cells and wild-type (wt) resident cells (wt/Cxcr2−/−), Cxcr2−/− cardiomyocytes and wt bone marrow–derived cells (Cxcr2−/−/wt), and wt controls reconstituted with wt bone marrow (wt/wt). All groups were treated with anti-MIF or isotype control antibody before they underwent myocardial ischemia and reperfusion. Blocking MIF increased infarction size and impaired cardiac function in wt/wt and wt/CXCR2−/− mice but ameliorated functional parameters in Cxcr2−/−/wt mice, as analyzed by echocardiography and Langendorff perfusion. Neutrophil infiltration and angiogenesis were unaltered by MIF blockade or Cxcr2 deficiency. Monocyte infiltration was blunted in wt/Cxcr2−/− mice and reduced by MIF blockade in wt/wt and Cxcr2−/−/wt mice. Furthermore, MIF blockade attenuated collagen content in all groups in a CXCR2-independent manner.

Conclusions—The compartmentalized and opposing effects of MIF after myocardial ischemia and reperfusion are largely mediated by CXCR2. Although MIF confers protective effects by improving myocardial healing and function through CXCR2 in resident cells, thereby complementing paracrine effects through CD74/AMP-activated protein kinase, it exerts detrimental effects on CXCR2-bearing inflammatory cells by increasing monocyte infiltration and impairing heart function. These dichotomous findings should be considered when developing novel therapeutic strategies to treat myocardial infarction. (Arterioscler Thromb Vasc Biol. 2013;33:2180-2186.)

Key Words: CXCR2 receptors ■ ischemia-reperfusion injury ■ macrophage migration-inhibitory factors ■ myocardial infarction
ischemia. A model of acute MI revealed that both MIF mRNA and MIF protein are constitutively expressed at low levels in myocytes of normal and sham-operated rats but rapidly upregulated by surviving cardiomyocytes in the infarcted versus the noninfarcted regions, thereby increasing macrophage infiltration 1 day after acute MI. Furthermore, it seems that cardiomyocytes can secrete MIF through a protein kinase C–dependent pathway in response to reactive oxygen species and hypoxia in the myocardium. Of note, MIF released by cardiomyocytes in a model of myocardial injury induced by 15 minutes of ischemia followed by reperfusion (I/R) injury exerts cardioprotective effects via the CD74/AMPK/c-jun-N-terminal kinase axes, and the protective effect can be enhanced by S-nitrosylation of MIF. CD74 is the cell-surface form of the major histocompatibility complex class II–associated invariant chain (Ii) and was identified to bind MIF by high-affinity interaction and to mediate MIF-induced extracellular signal-regulated kinase 1/2 phosphorylation and cell proliferation. However, some of the cell types targeted by MIF (eg, neutrophils or the cell lines HEK293 and HeLa) do not express CD74 at the surface, raising the need to further clarify the molecular action and functional receptors of MIF in inflammation. Despite the beneficial role of MIF in cardiomyocytes, it was indeed shown that MIF deficiency protected the heart from prolonged, severe I/R injury by suppressing inflammatory responses. Thus, the exact role of MIF in healing after MI and the receptors involved therein are thus far from being completely understood.

Recent data have provided evidence that MIF is a noncognate ligand of the CXC chemokine receptors CXCR2 and CXCR4, thus extending the range of binding partners for MIF. MIF harbors ELR- and N-loop–like motives typically required for CXCR2 activation and has chemotactic activity toward monocytes and neutrophils through CXCR2, both crucial mechanisms in inflammatory pathologies such as atherosclerosis. Although MIF can bind to CXCR2 or CD74 individually, binding to a CXCR2/CD74 complex seems to further enhance G-protein–coupled receptor activation and atherogenic functions. In addition, MIF can also bind the CXC chemokine ligand 12 (CXCL12) receptor CXCR4, thereby promoting T-cell and endothelial progenitor cell recruitment, enhancing angiogenesis, and inducing phosphorylation of Akt and mitogen-activated protein kinase p42/44.

Notably, CXCR2 has been found to exert opposing effects on myocardial viability during I/R, with the recruitment of damaging inflammatory cells prevailing over direct myocardial protection promoted in resident cells, suggesting that CXCR2 may prominently contribute to mediating the effects of MIF in myocardial I/R injury. To shed light on the precise mechanisms enacted by MIF during the myocardial healing response after MI, we aimed to dissect and elucidate the compartment-specific role of CXCR2 in mediating the effects of endogenous MIF in a mouse model of I/R.

### Materials and Methods

Materials and Methods are available in the online-only Supplement.

### Results

#### Effect of Anti-MIF Antibody on Heart Parameters 1 Week After I/R

To examine the role of the MIF–CXCR2 pathway in healing after I/R, we obtained chimeric mice with wild-type (wt) resident cardiomyocytes and Cxcr2−/− bone marrow (BM)-derived inflammatory cells, mice with Cxcr2−/− cardiomyocytes and wt inflammatory cells, and mice with Cxcr2−/− mice after irradiation (50%) reduced the number of mice in these groups (4 per group).

One week after I/R, both wt/wt BM and wt/Cxcr2−/− BM mice treated with anti-MIF antibody showed a significant increase in the size of the infarcted area compared with isotype control, whereas Cxcr2−/−/wt BM mice displayed no effect with anti-MIF antibody (Figure 1). In contrast to a previous report, Cxcr2 deficiency in BM cells itself only slightly, but not significantly, reduced the size of the infarcted area after 1 week. In addition, heart function was evaluated by echocardiography before and after myocardial I/R. Before MI, no significant differences were observed among the groups. One week after I/R, anti-MIF antibody treatment significantly decreased the ejection fraction in wt/wt BM and wt/Cxcr2−/− BM mice compared with untreated mice (Table 2; Figure 2A). In contrast, treatment with anti-MIF antibody led to a significant increase in ejection fraction in Cxcr2−/−/wt BM chimera mice compared with isotype control (Table 2; Figure 2A). Analysis of cardiac output showed good compensation in all groups (Table 2). Heart rate and weight did not differ among BM chimeras (Table 2).

The analysis of cardiac function by Langendorff perfusion 1 week after myocardial I/R revealed corresponding results. Although treatment with anti-MIF antibody decreased left ventricular developed pressure in wt/wt BM and wt/Cxcr2−/− BM mice compared with isotype control, it significantly improved and restored the impaired left ventricular developed pressure in

### Table 1. Treatment Groups of Bone Marrow Chimeras

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Bone Marrow</th>
<th>Anti-MIF Antibody (100 μg/wk)</th>
<th>Appellation</th>
<th>Cells Resident/Recruited</th>
<th>CXCR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Wild type</td>
<td>−</td>
<td>wt/wt BM</td>
<td>Cardiomyocytes</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>Cxcr2−/−</td>
<td>−</td>
<td>wt/Cxcr2−/− BM</td>
<td>Cardiomyocytes</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cxcr2−/−</td>
<td>Wild type</td>
<td>−</td>
<td>Cxcr2−/−/wt BM</td>
<td>Cardiomyocytes</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
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</tbody>
</table>

BM indicates bone marrow; MIF, migration-inhibitory factor; and WT, wild type.
Table 2. Echocardiography and Functional Parameters

<table>
<thead>
<tr>
<th></th>
<th>Wt/wt BM</th>
<th>Anti-MIF</th>
<th>wt/Cxcr2−/− BM</th>
<th>Control</th>
<th>Anti-MIF</th>
<th>Cxcr2−/−/wt BM</th>
<th>Control</th>
<th>Anti-MIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejection fraction, %</td>
<td>56.5±2.5</td>
<td>41.8±2.7</td>
<td>61.2±4.5</td>
<td>46.0±3.5</td>
<td>41.6±2.2</td>
<td>41.6±2.2</td>
<td>54.6±4.8</td>
<td>54.6±4.8</td>
</tr>
<tr>
<td>Cardiac output, mL/min</td>
<td>10.9±1.5</td>
<td>10.8±0.9</td>
<td>10.5±1.7</td>
<td>9.6±0.7</td>
<td>9.5±0.5</td>
<td>9.7±0.9</td>
<td>434±19.0</td>
<td>407±5.0</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>388±11.8</td>
<td>413±14.5</td>
<td>377±15.0</td>
<td>425±25.7</td>
<td>434±19.0</td>
<td>407±5.0</td>
<td>88.3±20.2</td>
<td>93.7±6.7</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>89.4±8.8</td>
<td>84.5±9.7</td>
<td>88.4±6.3</td>
<td>83.8±2.6</td>
<td>88.3±20.2</td>
<td>83.8±2.6</td>
<td>93.7±6.7</td>
<td></td>
</tr>
</tbody>
</table>

BM indicates bone marrow; MIF, migration-inhibitory factor; and WT, wild type.
infarction size. Notably, the latter effect was not related to an infiltration with neutrophils, which did not require Cxcr2 or MIF, but was because of the control of monocyte infiltration in the infarcted area mediated by MIF and CXCR2. Because we did not observe significant differences between the infarction size in isotype-treated wt/wt BM and Cxcr2−/−/wt BM, it is conceivable that Cxcr2−/− mice harbor compensatory mechanisms, which may substitute for CXCR2 functions (eg, the CXCR4/CXCL12 axis, CCL2, tumor necrosis factor α, or hypoxia-inducible factor). The oxidoreductive function of MIF has been elegantly shown to provide an antioxidative and cardioprotective capacity in MI and could, therefore, be another compensatory MIF-based protective mechanism in myocardial ischemia/reperfusion injury.

It has previously been shown that MIF released by cardiomyocytes during I/R injury exerts cardioprotective effects by activating AMPK/c-jun-N-terminal kinase through CD74. We observed a reduction in the functional parameters of the heart (ejection fraction, LVED, contraction, and relaxation) and increased infarction size after anti-MIF antibody treatment in wt/wt BM and wt/Cxcr2−/− BM mice. MIF blockade improved ejection fraction, left ventricular developed pressure,
contraction, and relaxation without influencing infarction size in Cxcr2−/−/wt BM mice, which do not express CXCR2 on cardiomyocytes and, therefore, show impaired heart function after I/R injury compared with controls. It thus seems that protective effects of endogenous MIF secreted by cardiomyocytes can prevent severe functional loss in heart tissue in wt/Cxcr2−/− BM mice with Cxcr2-deficient circulating cells. Considering what is known about the CD74/AMPK-mediated MIF protection,15 these results are surprising. Hence, we can assume that CD74 and CXCR2 are both required for myocardial protection in ischemia injury. Although MIF is able to exert its biological effects by binding to CD74 and CXCR2 individually, it was shown that MIF binding to the CXCR2/CD74 complex enhances G-protein–coupled receptor activation and downstream cardiovascular effects.7 Accordingly, predictably the deletion of 1 or both of the receptors would attenuate the protective effect in cardiomyocytes exposed to ischemia. Therefore, we suppose that the deletion of both receptors similar to that of Cxcr2 alone would decrease the protective effect of MIF. However, because Cxcr2/Cd74-double deficient mice are not available, we currently cannot verify this notion and thus cannot exclude the compensation of this effect by other MIF-independent protective mechanisms in cardiomyocytes. Nevertheless, the CXCR2/CD74 complex should play a crucial role in mediating the protective effects of MIF in cardiomyocytes after MI.

Notably, deficiency of Cxcr2 in cardiomyocytes and BM-derived inflammatory cells caused only moderate alterations in the size of the infarcted area 1 week after I/R, which contrasts previous findings in BM chimeras after 1 day, as reported by Tarzami et al.27 Because 1 day after MI the infarction size is largely defined by cardiomyocyte death, this discrepancy may be explained by adaptive remodeling of the infarction area after the inflammatory reaction during the course of 1 week after I/R injury.

In general, MIF blockade reduced the number of inflammatory cells, namely monocytes, infiltrating the site of infarct. Mononuclear cells infiltrating the site of inflammation release cytokines and chemokines, which further enhances the recruitment and activation of these proinflammatory cells.8,32 It has been shown that MIF can induce monocyte arrest through CXCR2,7 and accordingly myocardial monocyte infiltration was also dependent on CXCR2 in our study. Studies in animal models characterized by impaired monocyte infiltration have consistently shown preserved heart function after experimental induction of MI.33–35 Because we performed our analysis 1 day after I/R, most of the recruited monocytes should be Gr-1high (also known as Gr-1+CCR2+CX3CR1lo) monocytes that dominate the early phase and exhibit phagocytic, proteolytic, and inflammatory features.28 Reducing inflammatory monocytes after MI seems to beneficially promote cardiac remodeling.30 Because Cxcr2 is absent in inflammatory cells in wt/Cxcr2−/−BM mice and because the number of infiltrating monocytes was not further reduced by MIF blockade in these mice, we can assume that MIF is able to control the recruitment of inflammatory monocytes. Indeed, MIF has been shown to recruit monocyte/macrophage in a CCL2-dependent manner, because Ccl2 deficiency or anti-CCL2 antibody treatment significantly inhibited MIF-induced monocyte adhesion and transmigration in mice.29 This reveals the importance of CXCR2 in the myocardial recruitment of inflammatory monocytes.

Surprisingly, infiltration of the infarcted myocardial tissue with neutrophils was unaffected by MIF blockade or Cxcr2 deficiency. Neutrophils have been found to express CXCR2 by guest on August 25, 2017 http://atvb.ahajournals.org/ Downloaded from
but not CD74. The moderate chemotactic activity toward MIF in neutrophils has been related to a lack of CD74 in these cells. Because the function of MIF depends on the CXCR2/CXCR4 complex, the absence of CD74 might explain why neutrophil infiltration was unaltered in our myocardial I/R model. Furthermore, this is consistent with findings showing that early neutrophil infiltration after I/R injury was greatly diminished by Ccr1 deficiency and is hence dependent on the presence of the CCL5 receptor CCR1.

Unexpectedly, our evaluation of neoangiogenesis in the infarcted tissue 1 week after myocardial I/R injury failed to reveal changes in coronary flow or CD31-positive capillaries in any of the experimental groups. This is surprising, given that MIF and CXCR2 have been implicated in angiogenic responses under hypoxic conditions by initiating tube formation and differentiation toward endothelial cell phenotypes or stimulating endothelial proliferation and capillary-like structure formation, respectively. We have shown that MIF can favor the differentiation of smooth muscle actin–positive cells in vivo. Furthermore, MIF in conjunction with CD74 can antagonize myocardial hypertrophy and fibrosis and experimental liver fibrosis. Thus, MIF may contribute to fibroblast differentiation toward smooth muscle actin–positive myofibroblasts, which is a crucial event in myocardial healing and scar formation. Because the number of myofibroblasts did not differ in our groups, MIF seems to exert a direct effect on myofibroblast function and collagen synthesis. However, the mechanism underlying MIF-dependent fibrosis and scar formation after MI remains to be clarified in detail.

In conclusion, this study provides novel insights into the mechanisms of MIF and its functional receptor CXCR2 in myocardial regeneration and scar formation. Without affecting neutrophil infiltration or angiogenesis, MIF protects cardiac tissue via CXCR2. This benefit of MIF is counteracted by the CXCR2-dependent recruitment of monocytes associated with an impaired heart function. Our findings should be taken into account when developing tailored therapeutic strategies for improved remodeling and preservation of heart function after MI.

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Disclosures

None.

References

particular importance when developing novel therapeutic strategies to preserve the heart function after myocardial infarction. Although migration-inhibitory factor exerts its effects after myocardial infarction. Although migration-inhibitory factor protects cardiac tissue via its receptor CXCR2 on resident monocytes, it also promotes leukocyte recruitment. This study provides novel insights into the mechanisms by which migration-inhibitory factor may play a role in cardioprotection and regeneration.

Significance

Myocardial infarction remains the most common cause of death in Western countries, despite the extensive research in the past decades. Developing new therapeutic strategies to prevent and treat myocardial damage after an ischemic insult represents a priority for the cardiovascular scientific community. To this end, more detailed and advanced knowledge about the mechanisms of cardiac cell protection and regeneration is urgently required. Macrophage migration-inhibitory factor was found to be significantly elevated in patients with myocardial ischemia and also seems to protect the heart, thus possibly serving as a suitable therapeutic concept. However, the underlying mechanisms and its implications for cardioprotection are not completely elucidated. This study provides novel insights into the mechanisms by which migration-inhibitory factor exerts its effects after myocardial infarction. Although migration-inhibitory factor protects cardiac tissue via its receptor CXCR2 on resident cells, it exerts detrimental effects on CXCR2-bearing inflammatory cells associated with an impaired heart function. These findings are of particular importance when developing novel therapeutic strategies to preserve the heart function after myocardial infarction.
Compartmentalized Protective and Detrimental Effects of Endogenous Macrophage Migration-Inhibitory Factor Mediated by CXCR2 in a Mouse Model of Myocardial Ischemia/Reperfusion

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Supplementary File

Compartmentalized protective and detrimental effects of endogenous MIF mediated by CXCR2 in a mouse model of myocardial ischemia/reperfusion

Materials and Methods

Bone marrow transplantation. BALB/c or Cxcr2\(^{-/-}\) eight-week-old male mice were irradiated and reconstituted with either BALB/c or Cxcr2\(^{-/-}\) bone marrow (BM), as shown in Table 1. Femurs and tibias were aseptically removed from donor BALB/c and Cxcr2\(^{-/-}\) mice, and BM cavities were flushed and single-cell suspensions were prepared. Donor cells (2×10\(^{6}\)) were administered to the recipient mice by tail-vein injection 24 h after an ablative dose of whole-body irradiation (2x6 Gray). The BM chimeras were split into three different groups, wild-type mice reconstituted with wild-type BM, wild-type mice reconstituted with Cxcr2\(^{-/-}\) BM and Cxcr2\(^{-/-}\) mice reconstituted with wild-type BM, yielding mice with Cxcr2\(^{-/-}\) inflammatory cells but wild-type resident cardiomyocytes, and mice with Cxcr2\(^{-/-}\) cardiomyocytes but wild-type inflammatory cells. Each of these groups was further divided into two subgroups, which were treated with either anti-MIF or isotype control antibody (n=4-6 per group, Table 1).

Myocardial ischemia and reperfusion (I/R). Six weeks after BM reconstitution, mice were intubated under general anesthesia (100 mg/kg Ketamine, 10 mg/kg Xylazine, i.p.) and positive-pressure ventilated with oxygen and 0.2% isoflurane using a rodent respirator. Hearts were exposed by left thoracotomy and MI was produced by suture occlusion of the left anterior descending artery over a silicon tube. After 60 min of ischemia, the tube and suture were removed to permit reperfusion. The muscle layer and skin incision were closed with a silk suture. Either one day or one week after I/R, the hearts were harvested for further analysis. All animal experiments and study protocols were approved by local authorities and complied with German animal protection laws.

Antibody treatment. The mice were treated with the monoclonal anti-MIF antibody NIH/III.D9, produced as described \(^1\). Anti-MIF or isotype control antibody (50 µg) were administrated intraperitoneally one day before I/R. The mice to be analyzed 1 week after I/R received an intraperitoneal injection of 100 µg anti-MIF or isotype control antibody one day after I/R. The efficiency of the anti-MIF antibody has been previously established in detail \(^1-3\).

Echocardiography. Two-dimensional and M-mode echocardiographic measurements (Vevo 770) were performed. Both procedures were performed both before and one week after I/R. Mice were anesthetized by mask with 1.5% isoflurane and placed in supine position on a warming pad. The ejection fraction, cardiac output, heart rate and heart weight were recorded and analyzed.

Langendorff perfusion. One week after I/R, the mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and heart function was analyzed with a Langendorff apparatus (Harvard Apparatus) in conjunction with Isowheat software at constant perfusion pressure (100mmHg) and with electrical stimulation to assure constant heart rates (600/min constant heart rate). Coronary flow, left ventricular developed pressure (LVDP), and increase (dP/dtmax) and decrease (dP/dtmin) in LV pressure were measured. Finally, hearts were fixed with 10% formalin, paraffin- embedded, and cut into 5 µm serial slices for analysis.

Histology and immunohistochemistry. To evaluate MI size, serial sections (10-12 sections per mouse, 400 µm apart, up to the mitral valve) were stained with Gomori’s 1-step trichrome stain. The infarcted area was determined for all sections using Diskus software (Hilgers) and
expressed as a percentage of total heart volume. Blue-stained collagen content was analyzed using Cell P Software (Olympus) by measuring the percent of blue pixels and expressed as a percentage of the infarcted area. Serial sections (3 sections per mouse, 400 µm apart) were stained to analyze the infarcted area for neutrophil content (specific esterase, Sigma), monocyte content (CD14, Sigma and CCR2, Abcam), CD31-positive capillaries (CD31, Santa Cruz) and myofibroblasts (α-smooth muscle actin, DAKO). Cells or vessels were counted in six different fields per section and expressed as cells or vessels per mm².

Statistical analysis. Data represent mean ± SEM. Statistical analysis was performed with Prism 4 software (Graph Pad) using unpaired Student-t test or 1-way ANOVA followed by a Newman-Keuls post-hoc-test, as appropriate. P-values of <0.05 were considered significant.

References:

