Modulation of the inflammatory response following myocardial infarction

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Effects on cardiac function, remodeling, and inflammation following myocardial ischemia-reperfusion injury or unreperfused myocardial infarction in hypercholesterolemic APOE*3-Leiden mice

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ABSTRACT

Many novel therapies to treat myocardial infarction (MI), yielding promising results in animal models, nowadays failed in clinical trials for several reasons. The most used animal MI model is based on permanent ligation of the left anterior descending (LAD) coronary artery in healthy mice resulting in transmural MI, while in clinical practice reperfusion is usually accomplished by primary percutaneous coronary interventions (PCI) limiting myocardial damage and inducing myocardial ischemia-reperfusion (MI-R) injury. To evaluate a more similar murine MI model we compared MI-R injury to unreperfused MI in hypercholesterolemic APOE*3-Leiden mice regarding effects on cardiac function, left ventricular (LV) remodeling, and inflammation. Both MI-R and MI resulted in significant LV dilation and impaired cardiac function after 3 weeks. Although LV dilation, displayed by end-diastolic (EDV) and end-systolic volumes (ESV), and infarct size (IS) were restricted following MI-R compared to MI (respectively by 27.6% for EDV, 39.5% ESV, 36.0% IS), cardiac function was not preserved. LV-wall thinning was limited with non-transmural LV fibrosis in the MI-R group (66.7%). Two days after inducing myocardial ischemia, local leucocyte infiltration in the infarct area was decreased following MI-R compared to MI (36.6%), whereas systemic circulating monocytes were increased in both groups compared to sham (130.0% following MI-R and 120.0% after MI). Both MI-R and MI models against the background of a hypercholesterolemic phenotype appear validated experimental models, however reduced infarct size, restricted LV remodeling as well as a different distributed inflammatory response following MI-R resemble the contemporary clinical outcome regarding primary PCI more accurately, which potentially provides better predictive value of experimental therapies in successive clinical trials.

INTRODUCTION

Cardiovascular disease remains the main cause of death worldwide. Etiologically, atherosclerosis, a chronic inflammatory process, leads to the formation of lipid-rich lesions in the vascular wall, the atherosclerotic plaque. The vast majority of myocardial infarctions (MI) are a consequence of the rupture of such an inflamed atherosclerotic plaque resulting in sudden occlusion of a coronary artery[1]. Patients usually suffer from comorbidities like hypertension, hypercholesterolemia, and type 2 diabetes mellitus, amongst other risk factors of MI[2]. Current guidelines aim for timely reperfusion by primary percutaneous coronary interventions (PCI)[3] to limit myocardial damage and reduce infarct size resulting in a better clinical outcome[4]. Paradoxically, restoration of myocardial blood flow initiates myocardial reperfusion injury by a series of events, which apparently affects post-ischemic infarct healing, LV remodeling and effects of applied treatment opportunities[5,6].

To investigate the etiology of MI and treatment opportunities, animal models are indispensable. The selection of an appropriate animal model to ensure optimal translation of novel cardioprotective therapies from bench to bedside is of the utmost importance[7-9], which has additionally been underscored by a position paper of the European Society of Cardiology (ESC)[10] and united in several practical guidelines with the aim of increasing rigor and reproducibility in preclinical research[11,12]. Yet until recently, most data regarding MI have been generated in animals lacking human comorbidity, spared of atherosclerosis with its associated heightened inflammatory phenotype[13]. However, a close relation has been clarified, since leukocytosis predicts cardiovascular events and precipitates plaque rupture[14]. In addition, atherosclerosis is associated with chronic monocytosis associated with increased levels of lymphocyte antigen (Ly)-6C^{hi} monocytes in response to hypercholesterolemia[15]. Following MI, an inflammatory phenotype with Ly-6C^{hi} monocytes and M1-type macrophages initially dominates, which passes into a reparative phenotype with Ly-6C^{lo} monocytes and M2-type macrophages[16], making these cells main contributors in the murine post-ischemic acute inflammatory response affecting myocardial wound healing[17]. Even though these cells are necessary in post-ischemic wound repair, excessive Ly-6C^{hi} monocytosis resulted in impaired infarct healing and accelerated deterioration of ejection fraction (EF) in unreperfused MI, underscoring the need for a balanced and coordinated response[18,19]. In addition, most animal models used for studying novel therapeutic strategies are typically characterized by permanent LAD coronary artery occlusion, whereas in contemporary clinical practice patients with acute MI receive rapid reperfusion therapy defined in the guideline as within 90 minutes from first medical contact with the emergency medical system till reperfusion[3].

This scientific knowledge contributes to discussions about the relevancy of animal-derived data and whether these studies resemble the clinical setting with their human counterparts accurately, which has been explicitly addressed before[12,20]. As a result, promising experimental results derived from animal studies may be viewed with some caution. This is also supported by years of experience in which numerous therapeutic strategies, for several reasons, have failed their translation into successful clinical trials, deviating from promising results in preclinical

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animal studies[21,22]. When using preclinical animal experiments, it is of exceptional importance to guarantee reproducibility, pay attention to cardiovascular risk factors, comorbidities, and comedications, as well as addressing long-term effects of adjunct cardioprotection after completing the entire process of scar maturation[23]. Ideally, animal models resemble the clinical setting exhibiting a human-like atherosclerotic phenotype as a major comorbidity[24] and with respect to physiological cardiovascular parameters, in which they are exposed to temporarily myocardial ischemia followed by reperfusion experiencing human-like infarct distribution. Therefore, large animal studies remain most appropriate and are preferred over rodent models[25,26], however, these are expensive and practically more difficult to implement. This study aims to investigate the effects of MI-R compared to unreperfused MI on cardiac function, LV remodeling and the post-ischemic inflammatory response against the background of a hypercholesterolemic phenotype in APOE*3-Leiden mice, which are known to develop advanced aortic atherosclerotic lesions resembling their human counterparts when exposed to a hypercholesterolemic phenotype as a result of a cholesterol-enriched Western-type diet[27], and to test its suitability as an experimental murine MI-R model with respect to cost effectiveness and practical ease of use.

RESULTS

Plasma lipid profiles and animal characteristics

Plasma lipid profiles as expressed by total cholesterol (TC: 14.0±1.2 mmol/L vs. 15.8±1.1 mmol/L, p=0.94) and triglyceride (TG: 1.8±0.1 mmol/L vs. 1.9±0.2 mmol/L, p=1.00) levels did not differ between the MI-R and MI group 3 weeks after surgery (Table 1).

	T (wk)	sham	MI-R	MI
N		*13*	*15*	*16*
TC (mmol/L)	*0*	17.5 ± 1.7	16.8 ± 1.3	14.8 ± 1.0
	3	13.1 ± 1.1	14.0 ± 1.2	15.8 ± 1.1
TG (mmol/L)	*0*	2.5 ± 0.2	2.6 ± 0.2	2.8 ± 0.1
	3	2.4 ± 0.2	1.8 ± 0.1	1.9 ± 0.2
BW (g)	*0*	20.7 ± 0.5	21.1 ± 0.4	20.9 ± 0.5
	3	19.6 ± 0.3	20.2 ± 0.4	20.5 ± 0.4
BW change (%)		-4.7 ± 1.7	-3.8 ± 0.7	-1.6 ± 1.8
HW (mg)	*3*	144 ± 8	140 ± 7	167 ± 9
HW/BW ratio (mg/g)		7.3 ± 0.3	6.9 ± 0.3 [#]	8.2 ± 0.4

Table 1: Plasma lipid levels and animal characteristics. Plasma total cholesterol (TC), triglycerides (TG), body weight (BW), heart weight (HW), heart to body weight (HW/BW) ratio. Values are mean ± SEM. [#]p<0.05 vs. MI.

Cardiac hypertrophy was assessed by determination of the heart to body weight (HW/BW) ratio. The HW/BW-ratio was significantly less increased in the MI-R group compared to the MI group (6.9±0.3 mg/g vs. 8.2±0.4 mg/g, p=0.042), pointing to more subtle myocardial damage and limited LV remodeling and compensatory cardiac hypertrophy. Body weight (BW) was not affected between the MI and MI-R group (20.5±0.4 g vs. 20.2±0.4 g, p=1.00) (Table 1). Surgical survival rates were 78.9% in the MI-R vs. 76.2% in the MI group.

Infarct size, cardiac remodeling, and LV function

Sequential cardiac magnetic resonance imaging (MRI) after 3 weeks showed a significantly enlarged infarct size as assessed by contrast-enhanced MRI in the MI group when compared to MI-R (28.6±3.3% vs. 18.3±1.1%, p=0.008). This effect likely resulted from augmented LV remodeling in the 3 weeks following surgery, since initial infarct size after 2 days was similar in the MI and MI-R groups (35.2±2.9% vs. 30.6±2.1%, p=0.22; Figure 1). Moreover, reduced scar expansion after MI-R was accompanied by limited LV dilation after 3 weeks. LV volumes, both end-diastolic volume (EDV: 44.4±2.4 µl vs. 61.3±6.3 µl, p=0.002) and end-systolic volume (ESV: 26.6±2.2 µl vs. 44.0±7.0 µl, p=0.003) were significantly smaller in the MI-R group compared to
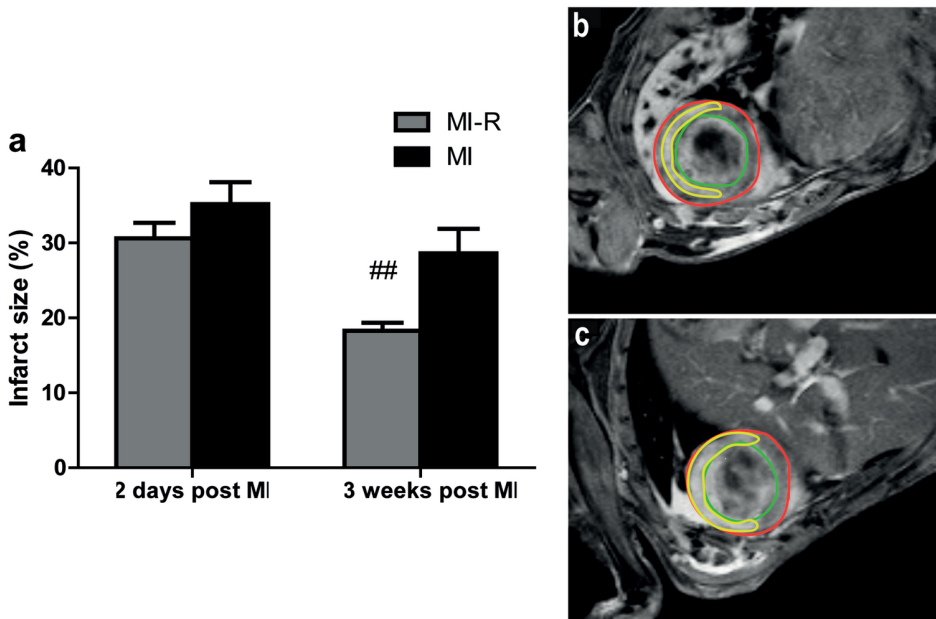

Figure 1: Contrast-enhanced MR imaging. After 2 days no significant difference in infarct size was observed between the MI and MI-R group. However, after 3 weeks the MI group displayed a significant increased infarct size as compared to the MI-R group (a; n=15-16 per group). Typical example of contrast-enhanced MR image 2 days following non-transmural MI-R (b) and transmural MI (c). Red line indicates epicardial border, green line indicates endocardial border and yellow line indicates infarct area. Individual data points are presented in Supplemental figure S1. Data are mean±SEM. [##]p<0.01 vs. MI.

the MI group, consistent with limited cardiac remodeling. Again, initial LV dimensions at day 2 showed no differences between both groups (EDV, p=0.81 and ESV, p=0.67), which implicates impaired or at least a difference in LV remodeling during the 3 weeks following MI (Figure 2a and 2b). The preserved LV volumes in the MI-R group were not associated with a significantly improved LV function, when expressed as ejection fraction (EF) following MI-R compared to unreperfused MI (40.8±2.9% vs. 34.4±5.1%, p=0.61), which was preceded by a similar EF (p=0.49) after 2 days at baseline (Figure 2c).
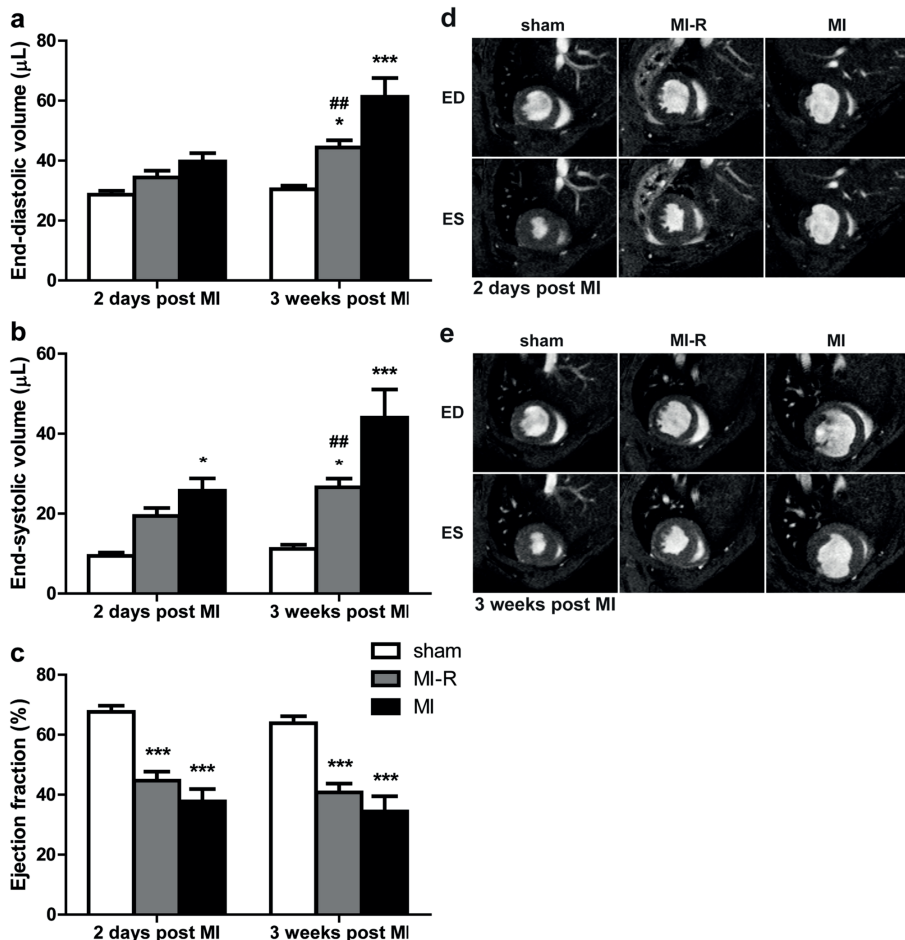

Figure 2: Cardiac MR imaging of LV volumes and function. Assessment of LV volumes and function after 2 days and 3 weeks (n=13-16 per group). MI-R caused a decreased EDV (a) and ESV (b) compared to MI. EF did not significantly differ between the MI-R and MI group but was reduced in both groups as compared to sham (c). Typical transversal short-axis MR images at end-diastole (ED) and end-systole (ES) 2 days (d) and 3 weeks (e) after infarction in the sham, MI-R and MI groups. Individual data points are presented in Supplemental figure S2. Data are mean±SEM. $^{##}$p<0.01 vs. MI; *p<0.05, ***p<0.001 both vs. sham.

LV fibrous content and wall thickness

Histological evaluation showed no significant difference in relative LV fibrous content after 3 weeks between the MI-R and MI group (19.8±1.8% vs. 25.5±3.4%, p=0.17, Figure 3a). However, analysis of the LV wall thickness (Figure 3b) demonstrated a substantial difference in distribution of fibrous content and LV wall thinning between the MI and MI-R group with a decreased LV wall thickness in the MI group compared to the MI-R group (0.45±0.08 mm vs. 0.75±0.04 mm, p=0.001). This observation explains the non-significant difference in relative LV fibrous content since transmural infarction in the MI group caused substantial LV wall thinning (Figure 3d), which underestimated the infarct size (IS) as a percentage of the total LV when compared to MI-R (Figure 3c). Furthermore, infarction caused a significantly thickened interventricular
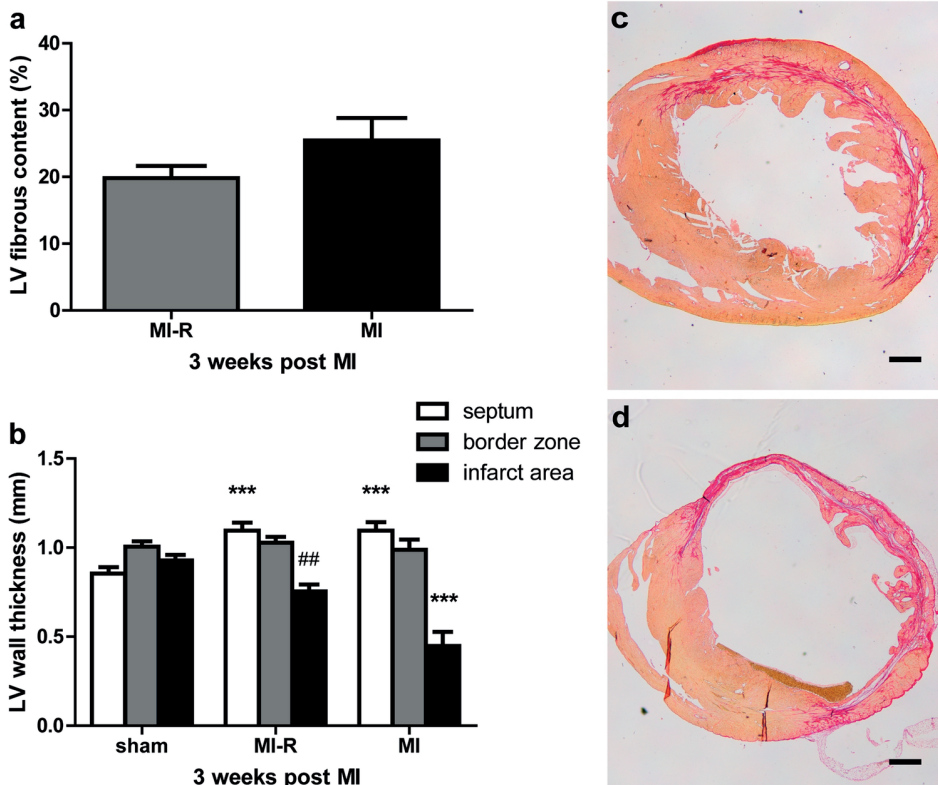
Figure 3: LV fibrous content and wall thickness. Histological analysis after 3 weeks (n=9-10 per group) showed no significant difference in LV fibrous content between the MI and MI-R group (a). This could possibly be explained since transmural infarction in the MI group caused substantial LV wall thinning, which underestimated the fibrous content as a percentage of the total LV when compared to MI-R. LV wall thickness was significantly decreased in the MI group as compared to the MI-R group (b). Sirius red staining of transversal short-axis sections showing typical non-transmural infarction in the MI-R (c) and transmural infarction with substantial LV wall thinning in the MI (d) group. Scale bar: 500 μm. Individual data points are presented in Supplemental figure S3. Data are mean±SEM. ##p<0.01 vs. MI; ***p<0.001 vs. sham.

septum in both the MI and MI-R group as compared to sham (1.10±0.05 mm and 1.10±0.04 mm vs. 0.85±0.04 mm, both p=0.001), indicating compensatory concentric hypertrophy (Figure 3b).

Systemic and local inflammatory response

To unravel the systemic inflammatory response following both MI-R injury and unreperfused MI we investigated circulating inflammatory cells. Fluorescence-activated cell sorting (FACS) analysis showed significantly increased circulating monocytes (% of total leukocytes) 2 days after MI-R (4.6±0.7%, p=0.02) and unreperfused MI (4.4±0.7%, p=0.03) compared to sham (2.0±0.5%; Figure 4a). After 2 days, as compared to sham, the percentage circulating pro-inflammatory Ly-6C^{hi} monocytes (Figure 4b) following MI-R showed a trend towards an increase (2.4±0.5% vs 0.8±0.2%, p=0.06). On the other hand, the percentage circulating reparative Ly-6C^{lo} monocytes (Figure 4c) showed a trend towards an increase in the MI group (2.1±0.5% vs. 0.9±0.2%, p=0.10). Although not significantly, Ly-6C^{hi} monocytes (of total leukocytes) seems to be overrepresented 2 days after MI-R compared to MI (2.4±0.5% vs. 1.7±0.7%) and Ly-6C^{lo} monocytes (of total
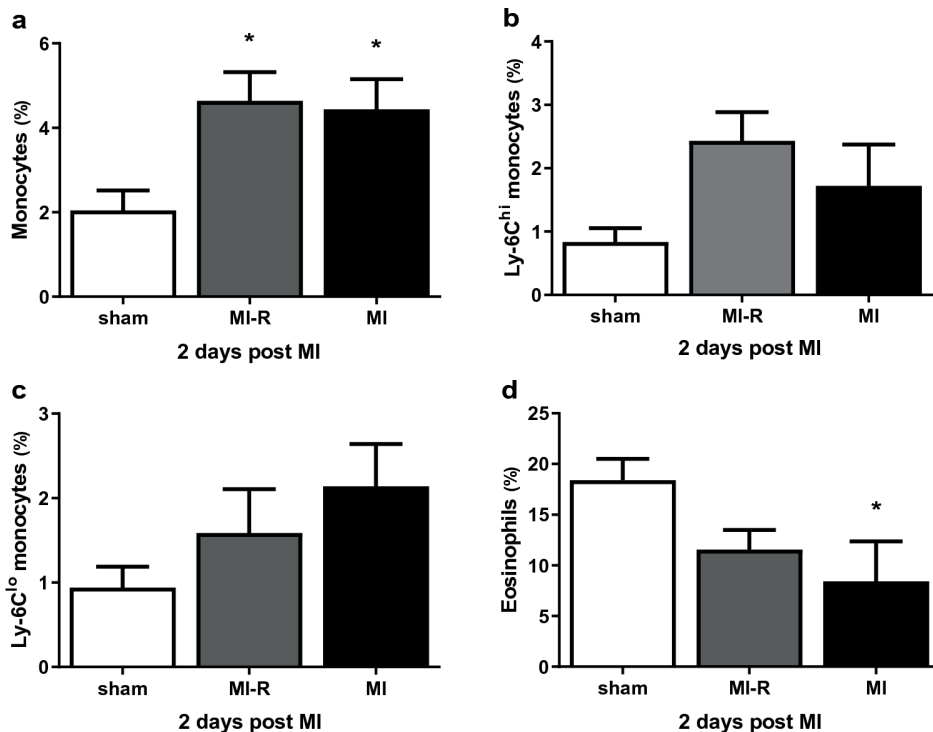

Figure 4: Systemic inflammatory response. FACS analysis after 2 days (n=5-8 per group) showed increased levels of circulating monocytes in both the MI-R and MI group as compared to sham (a) with a different distribution pattern of Ly-6C^{hi} and Ly-6C^{lo} monocytes in both groups (b and c). Circulating eosinophils were obviously decreased in both the MI-R and MI group compared to sham (d). Individual data points are presented in Supplemental figure S4. Data are mean±SEM. *p<0.05 vs. sham.

leukocytes) 2 days after MI compared to MI-R (2.1±0.5% vs. 1.6±0.5%). Circulating eosinophils were markedly decreased in the MI-R (11.6±1.9%, p=0.08) and MI (8.4±3.9%, p<0.05) group compared to sham (18.4±2.1%) animals (Figure 4d).

Histological analysis of the acute local inflammatory response after 2 days showed an increased local leukocyte infiltration (Figure 5a) in the infarct area of the MI group compared to the MI-R group (20.5±4.0 per 0.25mm^2 vs. 13.0±4.3 per 0.25mm^2, p=0.03). In addition, leukocyte infiltration compared to sham (4.9±0.4 per 0.25mm^2) was increased in both groups (p<0.001 and p=0.045, respectively). Furthermore, the distribution pattern in the chronic inflammatory phase after 3 weeks differed markedly regarding a resolution of infiltrated leukocytes in general as compared to 2 days following MI. Although, a significantly increased number of local leukocyte infiltration in the border zones and infarct area was demonstrated following MI-R (3.1±0.4 per 0.25mm^2, p=0.02, and 3.4±0.7 per 0.25mm^2, p=0.002) and MI (3.3±0.7 per 0.25mm^2, p=0.01, and 3.4±0.7 per 0.25mm^2, p=0.003) as compared to sham (1.0±0.2 per 0.25mm^2 and 0.8±0.1 per 0.25mm^2, respectively; Figure 5b).
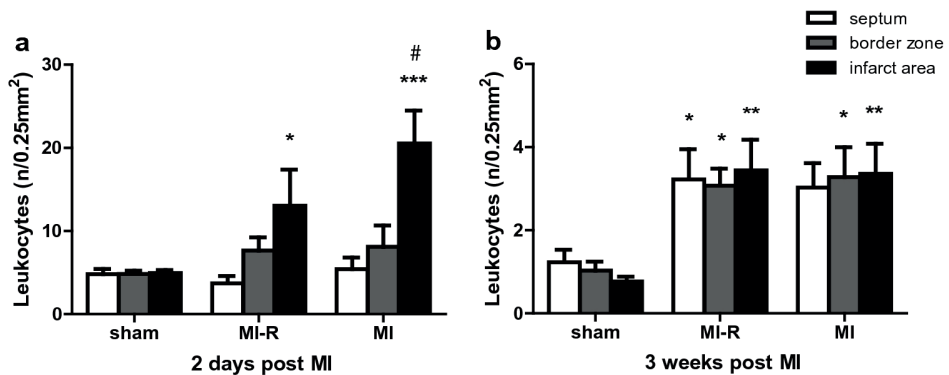

Figure 5: Local inflammatory response. Both MI-R and MI resulted in an increased number of infiltrated leukocytes 3 weeks after infarction as compared to sham (b; n=9-10 per group). After 2 days numbers of local infiltrated leukocytes in the infarct area were higher in the MI group compared to the MI-R group (a; n=5 per group). Individual data points are presented in Supplemental figure S5. Data are mean±SEM. #p<0.05 vs. MI-R; *p<0.05, **p<0.01, ***p<0.001 all vs. sham.

DISCUSSION

In this study, we provide a translational murine myocardial infarction model in which we consider both a chronic inflammatory hypercholesterolemic phenotype as well as reperfusion therapy followed by scar maturation during a 3 week follow-up period, aiming to resemble the clinical setting in patients and the contemporary clinical outcome using primary PCI more accurately. Key findings are that MI-R injury caused a reduced infarct size compared to unreperfused MI, which resulted in restricted LV dilation and less LV wall thinning of the infarcted area after 3 weeks. This did not preserve cardiac function as expressed by LV ejection fraction after 3 weeks

follow-up, which makes the MI-R model suitable to study hypothesized functional effects of novel therapeutic interventions. In addition, both infarction models caused an obvious local and systemic inflammatory response with subtle differences in inflammatory cell distribution illustrating different inflammatory responses following MI-R injury or unreperfused MI.

Although functional and structural long-term effects following rodent MI-R have been described before[28-30], unreperfused MI remained the most commonly used animal model. By comparing both MI-R injury and unreperfused MI, we showed the hypercholesterolemic MI-R model to be a suitable and reproducible murine infarction model to study long-term effects on cardiac function and LV remodeling following scar maturation, and paying attention to specific comorbidity what may be of interest to preclinical research as indicated in recent reviews[23] and guidelines[11,12]. This model potentially exerts better predictive value taking into account both reperfusion injury as hypercholesterolemia and their effects on post-ischemic cardiac function and remodeling, aiming for improved translation of novel cardioprotective therapies into the complex clinical reality after acute myocardial infarction including early reperfusion and a variety of comorbidities.

Cardiac MRI assessment of post-ischemic LV volumes showed significant LV dilation in both infarction models. In addition, MI-R demonstrated restricted LV dilation compared to unre-perfused MI accompanied by a reduced infarct size as assessed by cardiac MRI. Both infarction models caused an impaired cardiac function, however EF was not preserved in the MI-R model as compared to unreperfused MI despite of reduced LV dilation, which is in accordance with previous reported data[28]. Thus, LV remodeling was most severe in the unreperfused MI model, which was endorsed by an increased HW/BW-ratio indicating increased compensatory cardiac hypertrophy[31].

In the discussion of translating animal-derived data towards clinical trials, the degree of infarct size is a point of interest. Beneficial effects from animal derived studies usually regards large transmural MI sizes as a result of unreperfused MI. Treatment effects are therefore often overestimated and cannot be reproduced in clinical trials where patients, who suffered a MI, exhibited an infarct size between 13% to 16%, which limits the potential scope for cardiopro-tection[5]. This MI-R model provides an infarct size below 20% and histologically resembles the clinical setting in case of patients with timely reperfused non-transmural MI[32], making it more suitable to predict hypothesized beneficial clinical effects in preclinical studies as endorsed by previous reported reviews, guidelines and position papers[10-12,23]. Differences in distribution of LV fibrosis and scar formation between the MI-R, with non-transmural infarction and restricted LV-wall thinning, and unreperfused MI model, with transmural infarction and extensive LV-wall thinning, explain the non-significant difference in histologically determined LV fibrous content in this study. Transmural infarction in the unreperfused MI group underestimated the LV fibrous content as a percentage of the total LV because of LV-wall thinning when compared to the non-transmural MI-R group (Figure 3c and 3d). However, our results regarding infarct size and EF

are in agreement with previous reported data about the infarct size to be negatively correlated with EF[33].

Although both infarction models demonstrated a substantial post-ischemic inflammatory reaction, only subtle differences were observed regarding increased local leukocyte infiltration upon unreperfused MI in the acute inflammatory phase. This endorses the complexity and extensiveness of the post-ischemic inflammatory responses following MI-R injury and unreperfused MI, which are strictly regulated. Expression of inflammatory cells, and prior cytokine and chemokine levels all depend on mutual interaction and timing of peak levels[32], and previous studies already reported significant differences at other time points[32,34,35]. Moreover, the hypercholesterolemic phenotype, as a major comorbidity[24], influences the inflammatory response independently of myocardial ischemia, as we demonstrated before[36].

 As a result of either MI-R injury or unreperfused MI we also demonstrated locally infiltrated leukocytes and circulating monocytes to be increased and circulating eosinophil counts to be decreased as compared to non-infarcted sham controls. Our results suggest different timing and distribution patterns of the Ly-6C monocyte subsets regarding different trends of circulating pro-inflammatory Ly-6C^{hi} and reparative Ly-6C^{lo} monocytes consecutively following MI-R injury or unreperfused MI, which probably affects outcome since excessive Ly-6C^{hi} monocytosis impaired infarct healing and deteriorated cardiac function[19]. The fall in circulating eosinophils has been described before in patients with an acute MI. It has been suggested that eosinophils are attracted to the site of the lesion soon after the thrombotic event in human[37]. Differences in post-ischemic inflammatory responses will definitely affect therapeutic effects when using one of both translational animal models to study cardioprotective therapies[38] and finally influences LV remodeling in clinical trials as well[39].

The MI-R injury model in hypercholesterolemic APOE*3-Leiden mice provides a clinically relevant translational MI model with regard to timely reperfusion. We showed limitation of LV remodeling demonstrated by a reduced infarct size, restricted LV dilation and preservation of LV wall thickness, as well as a different distributed inflammatory response following MI-R injury as compared to unreperfused MI, resembling the contemporary clinical outcome using rapid reperfusion more accurately. As well as unreperfused MI, MI-R injury still caused a significant impaired cardiac function, LV dilation and both local and systemic inflammatory response as compared to non-infarcted sham controls, making it suitable to study promising novel cardioprotective therapies in a clinically more relevant setting of rapid coronary reperfusion as we have shown before[40].

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METHODS

See the Supplementary Information for an expanded Methods section.

Animals and diets

All animal experiments were approved by the Institutional Committee for Animal Welfare of the Leiden University Medical Center (LUMC) and conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 2011). *In vivo* studies were performed in hypercholesterolemic female APOE*3-Leiden mice[41] on a semisynthetic Western-type diet supplemented with 0.4% cholesterol (AB Diets, Woerden, The Netherlands) after completing a dietary run-in period of 4 weeks at the induction of myocardial ischemia. Female rather than male APOE*3-Leiden mice were used because of their higher and stable plasma cholesterol and triglyceride levels, confined to the VLDL/LDL-sized lipoprotein fraction[42,43]. Plasma levels of TC and TG were determined after a 4-hour fasting period using commercially available enzymatic kits according to the manufacturer's protocols (11489232; Roche Diagnostics, Mannheim, Germany, and 11488872; Roche Diagnostics, Mannheim, Germany, respectively).

Surgical myocardial infarction models

Myocardial infarction was induced with either MI-R injury or unreperfused MI model by ligation of the LAD coronary artery at day 0 in 12-14 weeks old female APOE*3-Leiden mice as described previously[28,44]. Briefly, after endotracheal intubation and ventilation mice were kept anesthetized with 1.5-2% isoflurane. Subsequently, a left thoracotomy was performed and the LAD coronary artery was ligated permanently in the MI group or during 45 minutes followed by permanent reperfusion in the MI-R group. Analgesia was obtained with buprenorfine s.c. pre- and post-operative. Sham operated animals were operated similarly but without ligation of the LAD (sham).

For short-term experiments, mice were euthanized 2 days after surgery to study the effects on the acute post-ischemic inflammatory response, resulting in the following groups: MI (n=6), MI-R (n=5), and sham (n=8). For long-term experiments, mice were followed for 3 weeks following surgery to assess the effects on cardiac function and post-ischemic inflammation, resulting in the following groups: MI (n=16), MI-R (n=15), and sham (n=13).

Cardiac magnetic resonance imaging

LV dimensions and function were serially assessed 2 days and 3 weeks after surgery with cardiac MRI by using a 7-Tesla MRI (Bruker Biospin, Ettlingen, Germany) equipped with a combined gradient and shim coil to obtain contrast-enhanced and cine MRI images. Initial infarct size was determined at day 2 to distinguish for any possible existing effect. Effects related to both infarction models regarding infarct size were determined by repetition of contrast-enhanced

MRI after 3 weeks. LV dimensions were measured after 2 days and 3 weeks and cardiac function was determined. Image reconstruction was performed using Bruker ParaVision 5.1 software.

LV endo- and epicardial borders were delineated manually with the MR Analytical Software System (MASS) for mice (Medis, Leiden, The Netherlands). End-diastolic and end-systolic phases and the contrast enhanced areas were identified automatically, and the percentage of infarcted myocardium, LV-EDV, LV-ESV, LV-EF, and LV stroke volume (SV) were computed.

Whole blood analysis

To study the systemic effects whole blood was analyzed for circulating inflammatory cells at day 2. Hematological values were obtained using a semi-automatic hematology analyzer F-820 (Sysmex; Sysmex Corporation, Etten-Leur, The Netherlands). For FACS analysis, whole blood was incubated on ice with directly conjugated antibodies directed against Ly-6C-FITC (AbD Serotec, Dusseldorf, Germany), Ly-6G-PE (BD Pharmingen, San Diego, CA, USA), CD11b-APC (BD Pharmingen, San Diego, CA, USA), CD115-PerCP (R&D Systems, Minneapolis, MN, USA), and CD45R-APC-Cy7 (eBioscience, San Diego, CA, USA).

LV fibrous content, wall thickness, and myocardial inflammatory response

After 3 weeks mice were euthanized and blood samples were collected for analysis. Subsequently, the heart and lungs were quickly excised. Hearts were weighted as an indication of congestive heart failure and immersion-fixated and embedded in paraffin.

Sirius Red staining was used to determine LV fibrous content, as a measure of infarct size, and LV wall thickness. All measurements were performed using the ImageJ2x 2.1.4.5 O software program (NIH, USA). For analysis of the cardiac inflammatory response sections were stained using antibodies against leukocytes (anti-CD45, 550539; BD Pharmingen, San Diego, CA, USA).

Statistical analysis

Values were expressed as mean±SEM. Comparisons of parameters between the MI, MI-R, and sham groups were made using 1-way analysis of variance (ANOVA) with Tukey's correction or 2-way ANOVA with repeated measures and Tukey's post-test in case of multiple time points. Comparisons between MI and MI-R were made using unpaired t-tests. A value of $p < 0.05$ was considered to represent a significant difference. Statistical procedures were performed using IBM SPSS 26.0 (SPSS Inc – IBM, Armonk, NY, USA) and GraphPad Prism 8.0 (www.graphpad.com, GraphPad Software Inc, La Jolla, CA, USA) also used for the representation of figures.

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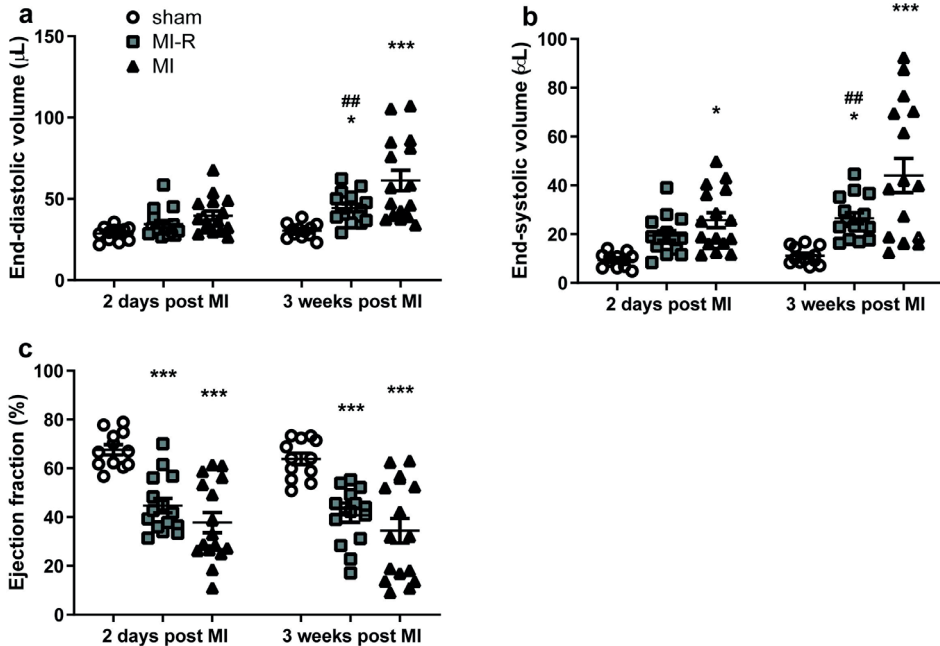
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SUPPLEMENTARY INFORMATION

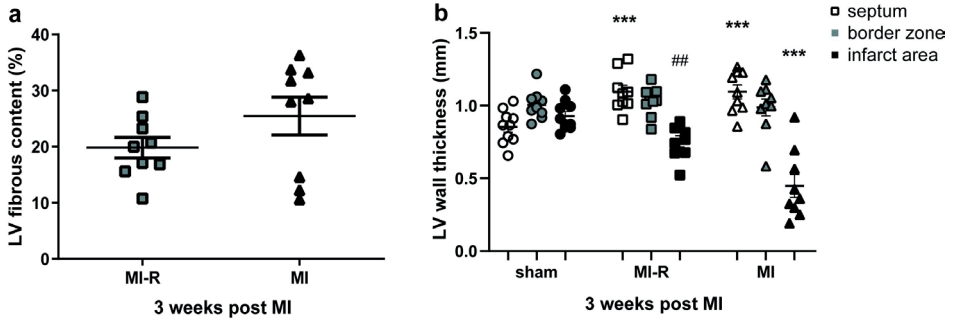### Supplementary figures with legends

This concerns all figures from the manuscript presented here as individual data points.
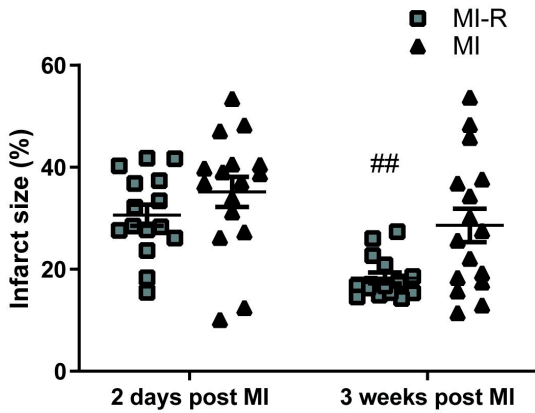
Supplementary figure S1: Contrast-enhanced MR imaging. Infarct size after two days and three weeks in the MI-R and MI group as assessed by MRI (n=15-16 per group). Data are mean±SEM. ##p<0.01 vs. MI.


Supplementary figure S2: Cardiac MR imaging of LV volumes and function. Assessment of LV volumes (a and b) and function (c) after two days and three weeks in the sham, MI-R and MI group (n=13-16 per group). Data are mean±SEM. ##p<0.01 vs. MI; *p<0.05, ***p<0.001 both vs. sham.

Supplementary figure S3: LV fibrous content and wall thickness. Histological analysis after three weeks of LV fibrous content (a) and LV wall thickness (b) in the sham, MI-R and MI group (n=9-10 per group). Data are mean±SEM. ##p<0.01 vs. MI; ***p<0.001 vs. sham.
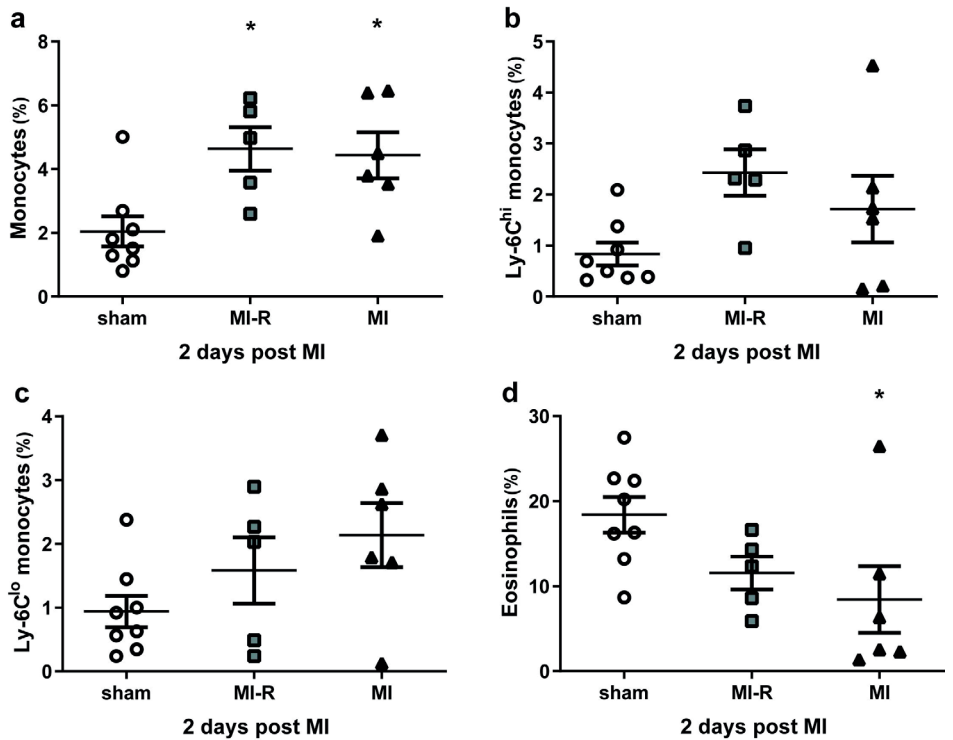
Supplementary figure S4: Systemic inflammatory response. FACS analysis after two days (n=5-8 per group) with levels of circulating monocytes (a), Ly-6C^{hi} (b) and Ly-6C^{lo} (c) monocytes, and eosinophils (d) in the sham, MI-R and MI group. Data are mean±SEM. *p<0.05 vs. sham.
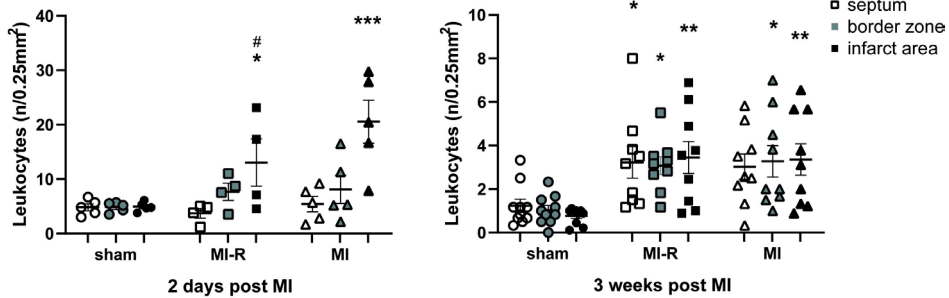
Supplementary figure S5: Local inflammatory response. Local infiltration of leukocytes in the septum, border zone and infarct area after two days (a; n=4-5 per group) and three weeks (b; n=9-10 per group) in the sham, MI-R and MI group. Data are mean±SEM. #p<0.05 vs. MI; *p<0.05, **p<0.01, ***p<0.001 all vs. sham.

Supplementary Methods – expanded version

Animals and diets

All animal experiments were approved by the Institutional Committee for Animal Welfare of the Leiden University Medical Center (LUMC) and conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 2011). Transgenic female APOE*3-Leiden mice[41], backcrossed for more than 40 generations on a C57Bl/6J background (bred in the animal facility of the LUMC), aged 8-10 weeks at the start of a dietary run-in period were used for this experiment. Mice were fed a semisynthetic Western-type diet supplemented with 0.4% cholesterol (AB Diets, Woerden, The Netherlands) 4 weeks prior to surgery, earlier proven to attain a stable hypercholesterolemic phenotype, and was continued throughout the complete experiment. Female rather than male APOE*3-Leiden mice were used because of their higher and stable plasma cholesterol and triglyceride levels, confined to the VLDL/LDL-sized lipoprotein fraction[42,43]. Mice were housed under standard conditions in conventional cages and received food and water ad libitum.

Plasma lipid analysis

Plasma levels of total cholesterol (TC) and triglycerides (TG) were determined for randomization one week before surgery and at the end of the experiment. After a 4-hour fasting period, plasma was obtained via tail vein bleeding (~50 μl) and assayed for total cholesterol (TC) and triglycerides (TG) levels using commercially available enzymatic kits according to the manufacturer's protocols (11489232; Roche Diagnostics, Mannheim, Germany, and 11488872; Roche Diagnostics, Mannheim, Germany, respectively).

Surgical myocardial infarction models

Myocardial infarction was induced with either a MI-R injury or an unreperfused MI model by ligation of the left anterior descending (LAD) coronary artery at day 0 in 12-14 weeks old female

APOE*3-Leiden mice as described previously[28,44]. Briefly, mice were pre-anesthetized with 5% isoflurane in a gas mixture of oxygen and room air and placed in a supine position on a heating pad (37°C). After endotracheal intubation and ventilation (rate 160 breaths/min, stroke volume 190 μl; Harvard Apparatus, Holliston, MA, USA), mice were kept anesthetized with 1.5-2% iso-flurane. Subsequently, a left thoracotomy was performed in the 4th intercostal space and the left anterior descending (LAD) coronary artery was ligated using a 7-0 prolene suture permanently in the MI group or during 45 minutes, knotted on a 2mm section of a plastic tube to protect the myocardium when removed, followed by permanent reperfusion in the MI-R group. Ischemia was confirmed by myocardial blanching. During this period muscle flaps were folded back and covered with a pre-warmed wet surgical mesh. Body temperature was kept constant between 35-37°C. In the MI-R group, mice received an intraperitoneal injection of lidocain (6 mg/kg) after 35 minutes of ischemia to prevent cardiac arrhythmias caused by reperfusion[39], and after 45 minutes of ischemia, permanent reperfusion was established. Subsequently, the thorax was closed in layers with 5-0 prolene suture and mice were allowed to recover. Analgesia was obtained with buprenorfine s.c. (0.1 mg/kg) pre-operative and 10-12h post-operative. Sham operated animals were operated similarly but without ligation of the LAD (sham).

Experimental groups

For short-term experiments, mice were exposed to MI or MI-R and euthanized two days after surgery to study the effects on the acute post-ischemic inflammatory response. The following groups were included in this study: MI (n=6), MI-R (n=5), and sham (n=8).

For long-term experiments, per group (n=20) mice were exposed to MI or MI-R and fol-lowed for three weeks to assess the effects on cardiac function and post-ischemic inflammation. As a result of mortality related to the invasive procedure and exclusion of non-infarcted mice (failed MI procedure) the following groups were included in this study: MI (n=16), MI-R (n=15), and sham (n=13).

Short-term experiments

Whole blood analysis

To study the systemic effects whole blood was analyzed for monocytosis at day 2. Hematological values obtained were white blood cell counts (WBC, x10^6/ml), red blood cell counts (RBC, x10^9/ml), and platelets (PLT, x10^6/ml) using a semi-automatic hematology analyzer F-820 (Sysmex; Sysmex Corporation, Etten-Leur, The Netherlands). For FACS analysis, 35 μl of whole blood was incubated for 30 min on ice with directly conjugated antibodies directed against Ly-6C-FITC (AbD Serotec, Dusseldorf, Germany), Ly-6G-PE (BD Pharmingen, San Diego, CA, USA), CD11b-APC (BD Pharmingen, San Diego, CA, USA), CD115-PerCP (R&D Systems, Minneapo-lis, MN, USA), and CD45R-APC-Cy7 (eBioscience, San Diego, CA, USA). To identify thresholds

for lineage-positivity 35 μl whole blood was incubated with an appropriate cocktail of isotype controls.

Myocardial inflammatory response

For analysis of the local inflammatory response, paraffin-embedded hearts (n=5 each group) were cut into serial transverse sections of 5 μm along the entire long-axis of the LV and mounted on slides. Paraffin sections of the mid-infarct region of the heart were stained using antibodies against leukocytes (anti-CD45, 550539; BD Pharmingen, San Diego, CA, USA). The number of leukocytes was expressed as a number per $0.25mm^2$ in the septum (2 areas), border zones (2 areas), and infarcted myocardium (3 areas).

Long-term experiments

Cardiac magnetic resonance imaging

Cardiac parameters were assessed two days and three weeks post MI using a 7-Tesla MRI (Bruker Biospin, Ettlingen, Germany) equipped with a combined gradient and shim coil, which is inserted into the magnet bore. Mice were pre-anesthetized as described above and kept anesthetized with 1.5-2% isoflurane. Respiratory rate was monitored by a respiration detection cushion, which was placed underneath the thorax and connected to a gating module to monitor respiratory rate (SA Instruments, Inc., Stony Brook, NY). Image reconstruction was performed using Bruker ParaVision 5.1 software.

Infarct size

To determine infarct size, contrast enhanced MR imaging was performed after injection of a 150 μl bolus (0.5 mmol/ml) of gadolinium-DPTA (Gd-DPTA, Dotarem, Guerbet, The Netherlands) via the tail vein. A gradient echo sequence (FLASH) was used to acquire a set of 14 contiguous 0.7 mm contract-enhanced slices in short-axis orientation covering the entire heart. Imaging parameters were: Imaging parameters were: echo time of 1.9 ms, repetition time of 84.16 ms, field of view ($33 mm^2$), and a matrix size of 192x256.

Left ventricular function

Assessment of cardiac function was performed with a high-resolution 2D FLASH cine sequence to acquire a set of 9 contiguous 1 mm slices in short-axis orientation covering the entire heart. Imaging parameters were: echo time of 1.49 ms, repetition time of 5.16 ms, field of view ($26 mm^2$), and a matrix size of 144x192.

Image analysis

All MR image data was analysed with the MASS for mice software package (Medis, Leiden, The Netherlands). The endocardial and epicardial borders were manually delineated and a reference

point was positioned by an investigator blinded to treatment. Subsequently, the infarcted area of the LV, end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), stroke volume (SV), cardiac output (CO), ED wall thickness, ES wall thickness, wall thickening and wall motion were computed automatically.

After three weeks mice were euthanized and blood samples were collected for analysis. Subsequently, the heart and lungs were quickly excised. Hearts were weighted as an indication of congestive heart failure and immersion-fixated in 4% paraformaldehyde for 24 hours and embedded in paraffin.

LV fibrous content and wall thickness

After selecting 10 mice of the mid-population of each experimental group, by using the functional MRI data after three weeks, paraffin-embedded hearts (n=10 each group) were cut into serial transverse sections of 5 μm along the entire long-axis of the LV and mounted on slides. To analyze collagen deposition as an indicator of the fibrotic area, every 50th section of each heart was stained with Sirius Red. LV fibrous content as a measure of infarct size was determined by planimetric measurement of all sections and calculated as fibrotic area divided by the total LV wall surface area including the interventricular septum.

LV wall thickness was measured in five different sections centralized in the infarct area. Per section, wall thickness was analyzed at three places equally distributed in the infarcted area, both border zones, and two places of the interventricular septum. Measurements were performed perpendicular to the ventricular wall. Corresponding areas were used for measurements in the non-infarcted sham group. All measurements were performed by an observer blinded to the groups, using the ImageJ2x 2.1.4.5 O software program (NIH, USA).

Myocardial inflammatory response

For analysis of the cardiac inflammatory response paraffin sections of the mid-infarct region of the heart were stained using antibodies against leukocytes (anti-CD45, 550539; BD Pharmingen, San Diego, CA, USA). The number of leukocytes was expressed as a number per $0.25mm^2$ in the septum (2 areas), border zones (2 areas), and infarcted myocardium (3 areas).

Statistical analysis

Values were expressed as mean±SEM. Comparisons of parameters between the MI, MI-R, and sham groups were made using 1-way analysis of variance (ANOVA) with Tukey's correction or 2-way ANOVA with repeated measures and Tukey's post-test in case of multiple time points. Comparisons between MI and MI-R were made using unpaired *t*-tests. A value of p<0.05 was considered to represent a significant difference. Statistical procedures were performed using IBM SPSS 26.0 (SPSS Inc – IBM, Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software Inc, La Jolla, CA, USA), also used for the representation of figures.