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Letter to the editor

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Differential MHC-II expression and phagocytic functions of embryo-derived cardiac macrophages in the course of myocardial infarction in mice

The importance of the immune system in cardiac healing after myocardial infarction (MI) is increasingly recognized. However, due to the complexity of the remodeling process and the diverse functions of immune cells therein, therapeutic strategies precisely targeting the inflammatory process induced by MI have been lacking [1]. Macrophages are abundant in tissues and play an important role in maintaining organ homeostasis and function. Therefore, they could represent an attractive target to promote infarct healing. Cardiac macrophages derive from embryonic (yolk sac, fetal liver) as well as adult (bone marrow) precursor cells, and undergo phenotypic changes during life. They increase MHC class II (MHC-II) expression and decrease C-X3-C motif chemokine receptor 1 (CX3CR1) expression with age [2]. Importantly, MHC-II and CX3CR1 expression have been linked to functional changes in macrophages in non-cardiac tissues [3,4]. Therefore, we analyzed the expression of respective molecules on cardiac macrophages of different origins in the course of MI, and evaluated functional differences regarding their ontogeny and MHC-II expression.

We induced myocardial ischemia in mice by transient ligation of the left anterior descending (LAD) coronary artery for 60 minutes, and then analyzed infarcted hearts 2, 6, or 30 days post-MI (Fig. 1A). Following enzymatic digestion of the whole heart and single cell preparation of cardiac immune cells, we carried out flow cytometry using the gating strategy indicated in Fig. 1B ($CD45^+$, lineage [TER119, Ly6g, CD11c, TCRß, Nk1.1] negative, $CD64^+$, $F4/80^+$) to reveal the expression of MHC-II and CX3CR1 on cardiac macrophages. Macrophage numbers strongly increased early after MI and then gradually declined over time approximating baseline numbers after 30 days. MHC-II and CX3CR1 were highly expressed on cardiac macrophages at baseline and $MHC\text{-}II^{hi}/CX3CR1^{hi}$ macrophages represented the most frequent population. Myocardial infarction reduced MHC-II expression in both $CX3CR1^{hi}$ and $CX3CR1^{lo}$ cardiac macrophages, and consequently $MHC\text{-}II^{lo}/CX3CR1^{lo}$ macrophages were the most abundant macrophage population at day 2 post-MI. After 30 days, MHC-II and CX3CR1 expression was regained, reaching values similar to steady state (Fig. 1B). We also determined the association of macrophage phenotypes with the tissue milieu, and found that downregulation of macrophage MHC-II expression was most prominent in the infarct zone (Fig. 1C and D). To determine macrophage MHC-II phenotype in relation to their developmental origin, we carried out lineage tracing in *Rank*^{Cre} (a.k.a. *Tnfrsf11a*^{Cre}) mice crossed with a *Rosa26*^{eYFP} reporter mice, which efficiently label embryo-derived macrophages [5]. Post-MI, MHC-II expression decreased on both YFP^+ and $YFP^–$ macrophages (Fig. 1E). In analogy, we analyzed CCR2 surface expression to compare the phenotype of resident ($CCR2^–$) and recruited ($CCR2^+$) macrophages. Similar to the experiments in *Rank*^{Cre}*Rosa26*^{eYFP} mice, loss in MHC-II and CX3CR1 surface expression was comparable in both populations at baseline conditions as well as 2 or 6 days after MI (Suppl. Fig. 1). This indicates that I/R injury induces downregulation of MHC-II across cardiac macrophage populations and independently of their origin.

Phagocytosis of dead cardiomyocytes critically contributes to the resolution of inflammation after MI [6]. We therefore addressed phagocytic properties of $YFP^–$ and YFP^+ macrophages in *Rank*^{Cre} *Rosa26*^{eYFP} mice in dependence on their MHC-II expression as well as their anatomical relation to the infarct area (infarct and remote zone). In brief, we induced necrosis of cardiomyocytes by repeated freezing and thawing and incubated the debris with MACS®-sorted $CD45^+$ cells for 3 h, as previously described [7]. Embryo-derived (YFP^+) $MHCII^{lo}$ and $MHCII^{hi}$ cardiac macrophages displayed strong phagocytic activity of necrotic cardiomyocytes already at baseline (homeostasis), with no further increase after I/R-injury. On the other hand, YFP- $MHCII^{lo}$ macrophages provided low phagocytosis at baseline, which increased significantly in the infarct area (Fig. 1F; Supporting Information Fig. S2). Thus, BM macrophages are of key importance for the clearance of necrotic cardiomyocytes after myocardial infarction.

The importance of macrophages in tissue homeostasis and the detrimental effect of their absence have been

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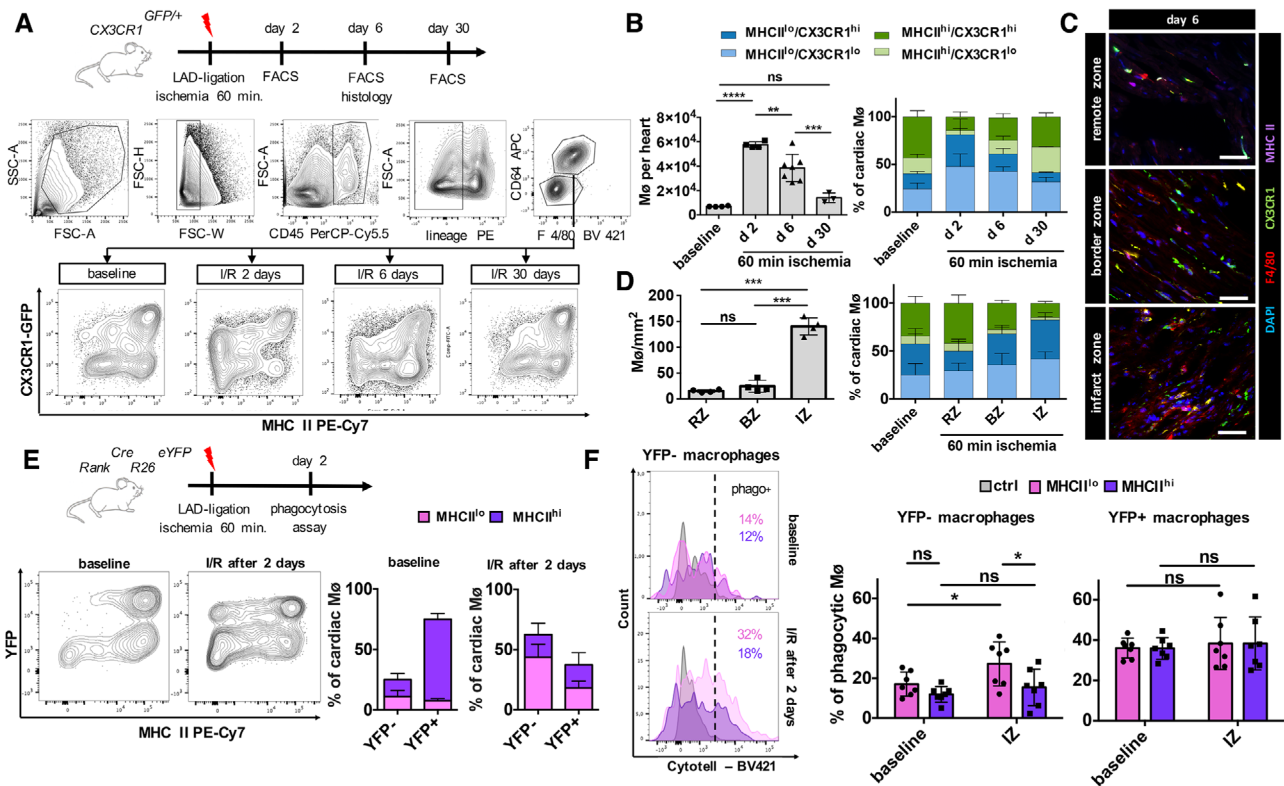

Figure 1. Phenotypic and functional changes of cardiac macrophages after MI. (A and B) Flow cytometry of cardiac macrophages of CX3CR1$^{GFP/+}$ mice before and after ischemia/reperfusion injury. A) Gating strategy and representative flow cytometry plots of MHC-II and CX3CR1 expression. B) Quantification at different time-points (baseline ($n = 4$) and 2 ($n = 4$), 6 ($n = 7$), and 30 days ($n = 3$) after I/R injury (two independent experiments for baseline, d2, d6, and 1 independent experiments for d30). All data are shown in a combined analysis. (C and D) Immunohistology of cardiac macrophages of CX3CR1$^{GFP/+}$ mice. (C) Representative images of CX3CR1^{+} MHC-II^{+} macrophages in remote, border, and infarct zones (RZ, BZ, IZ) 6 days after MI. Scale bars, 50 μm. (D) Quantification of cardiac macrophages in respective areas ($n = 4$; four independent experiments). Combined data are shown. (E) Flow cytometry of macrophage MHC-II expression in *Rank*^{Cre}*Rosa*^{eYFP} mice at baseline and 2 days after I/R-injury. Left, A representative flow cytometry experiments is shown. Right, Quantification of all data ($n = 7$ for each timepoint, three independent experiments for each time point). (F) Macrophage phagocytosis of necrotic cardiomyocytes; CD45^{+} cells were isolated from *Rank*^{Cre}*Rosa*^{eYFP} mice by MACS-separation at baseline and 2 days after I/R-injury, and macrophage phagocytic activity of necrotic cardiomyocytes (induced by freeze (liquid nitrogen) and thaw (37°C) treatment) labelled with Cytotell® BV421 was measured by flow cytometry. Left, A representative flow cytometry experiments is shown. Right, quantification of all data ($n = 7$ for each timepoint, three independent experiments for each time point). Statistical tests show unpaired *t*-test with Welch's correction or Fisher's LSD test and mean ± SD is depicted; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

demonstrated in various organs [8,9]. MHC-II^{lo} macrophages possess enhanced phagocytic capacities, however, the association with their developmental origin has been unclear. We show here that cardiac macrophages transiently change their phenotype and adapt functional changes after MI. While MHC-II^{hi} embryo-derived macrophages are most abundant at baseline conditions, bone marrow-derived MHC-II^{lo} macrophages represent the major population early after myocardial infarction. MHC-II and Cx3CR1 expression is modulated on macrophages by I/R injury and transiently downregulated after myocardial infarction independently of their ontogeny. In line with the importance in tissue homeostasis, embryo-derived macrophages display higher phagocytic activity at baseline compared to bone marrow-derived macrophages. Phagocytic activity of the latter is induced by ischemia and, together with their increasing abundance in the infarcted heart, they represent major effectors of necrotic cell removal in response to I/R injury. Targeting distinct macrophage populations may allow to refine anti-inflammatory responses and boost tissue repair after MI.

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