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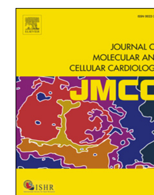

Original article

Differential roles of cardiac and leukocyte derived macrophage migration inhibitory factor in inflammatory responses and cardiac remodelling post myocardial infarction

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ABSTRACT

Myocardial infarction (MI) provokes regional inflammation which facilitates the healing, whereas excessive inflammation leads to adverse cardiac remodelling. Our aim was to determine the role of macrophage migration inhibitory factor (MIF) in inflammation and cardiac remodelling following MI. Wild type (WT) or global MIF deficient (MIFKO) mice were subjected to coronary artery occlusion. Compared to WT mice, MIFKO mice had a significantly lower incidence of post-MI cardiac rupture (27% vs. 53%) and amelioration of cardiac remodelling. These were associated with suppressed myocardial leukocyte infiltration, inflammatory mediators' expression, and reduced activity of MMP-2, MMP-9, p38 and JNK MAPK. Infarct myocardium-derived or exogenous MIF mediated macrophage chemotaxis in vitro that was suppressed by inhibition of p38 MAPK or NF-κB. To further dissect the role of MIF derived from different cellular sources in post-MI cardiac remodelling, we generated chimeric mice with MIF deficiency either in bone marrow derived-cells (WT^{KO}) or in somatic-cells (KO^{WT}). Compared to WT and KO^{WT} mice, WT^{KO} mice had reduced rupture risk and ameliorated cardiac remodelling, associated with attenuated regional leukocyte infiltration and expression of inflammatory mediators. In contrast, KO^{WT} mice had delayed healing and enhanced expression of M1 macrophage markers, but diminished expression of M2 markers during the early healing phase. In conclusion, global MIF deletion protects the heart from post-infarct cardiac rupture and remodelling through suppression of leukocyte infiltration and inflammation. Leukocyte-derived MIF promotes inflammatory responses after MI, whereas cardiac-derived MIF affects early but not ultimate healing process.

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1. Introduction

Myocardial infarction (MI) is the leading cause of cardiac death worldwide and its occurrence is expected to increase with population ageing [1]. A great challenge to modern cardiology is to limit cardiac damage and prevent adverse left ventricular (LV) remodelling and dysfunction after MI [2,3]. Many studies on the role of inflammatory responses to MI have highlighted the dual nature of the contribution of inflammation to disease progression. Following myocardial ischemia, necrotic cardiomyocytes release a wide range of inflammatory molecules such as reactive oxygen species and cytokines that stimulate regional inflammatory infiltration [4]. Recruitment of leukocytes to the injured myocardium is essential for healing via phagocytosis of cellular debris, activation of matrix metalloproteinases (MMPs) to remodel extracellular matrix (ECM), and secretion of growth factors to promote granular angiogenesis and deposition of ECM proteins [5,6]. However, either excessive or inadequate inflammation in the injured myocardium can lead to adverse outcomes such as cardiac rupture, adverse ventricular remodelling and heart failure [6,7].

Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine with a number of unique biological actions and is recognised as an important regulator of innate and acquired immunity [8,9]. Proinflammatory actions of MIF have been reported in various inflammatory diseases such as sepsis, rheumatoid arthritis and atherosclerosis [9–11]. MIF is released from intracellular stores in response to various

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cellular stressors, such as hypoxia or bacterial proteins [12]. MIF is rapidly released from the heart, if subjected to a brief period of ischemia, and enhances glucose uptake via activation of AMP-activated protein kinase (AMPK) [13,14], while it also inhibits c-Jun N-terminal kinase (JNK) [15] and attenuates oxidative stress [16], thereby reducing infarct size and preserving cardiac function. However, prolonged ischemia, which is frequently seen in clinical practice [1], provokes severe cardiac inflammatory responses. We recently reported that prolonged ischemia in mice leads to substantial myocardial damage and regional inflammation, effects which are attenuated in mice with a global MIF deletion (MIFKO) [17]. These distinct observations of the effects of MIF in different contexts highlight the complexity of pathophysiological processes following ischemic cardiac injury and warrant further investigations on the role of MIF, especially when anti-MIF therapies are to be considered for MI.

In the current study, we investigated the phenotype of MIFKO mice subjected to MI, by measuring post-MI inflammation, healing, and acute and chronic cardiac remodelling. MIF is expressed in multiple cell types including both leukocytes [18] and cardiomyocytes [19], but the relative contributions of these sources of MIF to myocardial injury are unknown. We hypothesized that MIF derived from different cellular sources would have different effects in the heart following ischemic injury. This hypothesis was investigated in chimeric mice generated by bone marrow transplant. The results confirm that MIF plays a vital role in mediating cardiac inflammatory injury after MI, and show that while leukocyte MIF enhances damage, non-leukocyte MIF enhances myocardial healing.

2. Methods

A detailed methodology section can be found in the online Supplementary Materials.

2.1. Animals

All animal investigations were approved by a local animal ethics committee complying with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition). Ten-week-old male global MIFKO mice and wild type (WT) littermates with C57Bl/6 genetic background were used [20].

2.2. Induction of MI

Mice underwent coronary artery occlusion to induce MI or sham operation, as previously described [21,22]. After surgery, mice were monitored daily for 4 weeks. Autopsy was performed for evidence of post-MI cardiac rupture or heart failure, as described previously [22]. Mice were killed at various time points following MI, infarct size was assessed and infarct and non-infarct myocardium were separated, snap frozen in liquid nitrogen and stored at −80 °C for molecular assays. Further, some LVs were fixed in 10% formalin or fresh frozen for histological analyses.

2.3. Echocardiography

Echocardiography was performed prior to surgery and 1, 2 or 4 weeks post-surgery, as previously described [17,23].

2.4. Immunofluorescence staining

Immunofluorescence was performed, as previously described [17]. Briefly, LV sections were stained with an anti CD45 antibody for leukocyte, and 4′,6-diamidino-2-phenylindole (DAPI) for nucleus, to identify leukocytes. Visualisation of capillaries in border zones was performed using Alexa Fluor® 568 isolectin GS-IB_4 conjugate. Images were acquired using an Olympus BX61 fluorescence microscope and densities of leukocytes and capillaries were analysed using Image Pro Plus software (Media Cybernetics, Inc., USA).

2.5. Quantitative real time-PCR

RNA was extracted from cardiac tissues. Gene expression of monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), interleukin-1β (IL-1β), IL-10, MMP-9, MMP-2 and transforming growth factor β1 (TGFβ1) was assessed by quantitative real-time PCR (qPCR) using Applied Biosystems 7500 fast real-time PCR system and normalised to GAPDH, as previously described [17,24].

2.6. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was performed, in duplicates, using a commercial mouse IL-1β (Life Research, Australia) and MIF (ElAab Science Co., Ltd. Wuhan, China) ELISA kits according to the manufactures' instructions.

2.7. Gelatin zymography

Proteins were extracted from infarct tissues and sham-operated hearts and concentrations were determined using Bradford protein quantification assay. Gelatin zymography was performed on a 7.5% acrylamide, 0.5% gelatine SDS-PAGE, as previously described [24].

2.8. Immunoblotting

Western blotting was performed with primary antibodies to phospho- and total-p38 mitogen-activation protein kinase (p-p38 and t-p38 MAPK), p-JNK and t-JNK, as reported previously [17]. Membranes were re-probed with GAPDH antibody to verify loading consistency.

2.9. Histology

Formalin-fixed paraffin-embedded LV sections were stained for hemotoxylin and eosin to assess remaining necrotic areas and scar thickness while picosirius red was used to determine collagen deposition in the infarct region. Images were captured using an Olympus light microscope and analysed using Image-Pro Plus 6.0 software, as described previously [21,25].

2.10. Cell culture experiments

To further understand potential influence of MIF on post-infarct healing, we studied effects of MIF on cardiac fibroblast biology in cell culture models. First, we examined whether fibroblasts and cardiomyocytes are able to release MIF under hypoxic stimulation. Second, fibroblasts were prepared from the infarct tissue of adult MIFKO and WT mice subjected to MI for 4 days, fibroblast proliferation, collagen deposition and fibrosis-related gene expression including TGFβ, α-smooth muscle actin (α-SMA), and collagen-1 and -3 were investigated.

2.11. Trans-well migration assay

Macrophages were isolated from the peritoneal cavities of WT and global MIFKO mice. Using a trans-well migration assay, cells were exposed to homogenised infarct or normal cardiac tissue, or recombinant human MIF (rMIF). Additionally, WT macrophages were pre-treated with either a p38 MAPK inhibitor, SB203580, or NF-κB inhibitor, Bay11-7082, prior to migration assay. The number of trans-well migrated macrophages was counted.

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