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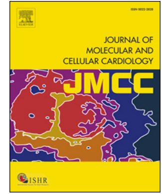

Bone marrow transplantation modulates tissue macrophage phenotype and enhances cardiac recovery after subsequent acute myocardial infarction

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ABSTRACT

Background: Bone marrow transplantation (BMT) is commonly used in experimental studies to investigate the contribution of BM-derived circulating cells to different disease processes. During studies investigating the cardiac response to acute myocardial infarction (MI) induced by permanent coronary ligation in mice that had previously undergone BMT, we found that BMT itself affects the remodelling response.
Methods and results: Compared to matched naive mice, animals that had previously undergone BMT developed significantly less post-MI adverse remodelling, infarct thinning and contractile dysfunction as assessed by serial magnetic resonance imaging. Cardiac rupture in male mice was prevented. Histological analysis showed that the infarcts of mice that had undergone BMT had a significantly higher number of inflammatory cells, surviving cardiomyocytes and neovessels than control mice, as well as evidence of significant haemosiderin deposition. Flow cytometric and histological analyses demonstrated a higher number of alternatively activated (M2) macrophages in myocardium of the BMT group compared to control animals even before MI, and this increased further in the infarcts of the BMT mice after MI.
Conclusions: The process of BMT itself substantially alters tissue macrophage phenotype and the subsequent response to acute MI. An increase in alternatively activated macrophages in this setting appears to enhance cardiac recovery after MI.
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1. Introduction

Bone marrow transplantation (BMT) is commonly used in experimental studies designed to investigate the specific contribution of BM-derived circulating cells to disease processes. The method involves irradiation of the recipient to ablate BM cells, followed by transplantation of donor BM, typically via intravenous infusion [1,2]. In mice after syngeneic BM transplantation, the BM, circulating and tissue pools of leukocytes are generally reconstituted within 2–3 weeks [1,2]. The use of BMT to generate chimeric mice with different gene expression in BM-derived cells versus host cells is a powerful approach in cardiovascular diseases [3–6].

Acute myocardial infarction (MI) evokes an initial inflammatory response involving infiltration by neutrophils, monocytes/macrophages and lymphocytes, during which the infarct undergoes repair and a fibrous scar is laid down. This is followed by a phase of infarct scar maturation, thinning and then gradual infarct expansion with adverse left ventricular (LV) remodelling. The latter results in ventricular dilatation and reduction in contractile function and involves significant changes in the non-infarcted myocardium, such as cardiomyocyte hypertrophy, interstitial fibrosis and other alterations in the extracellular matrix [7–9]. BMT in the context of acute myocardial infarction (MI) has been valuable in demonstrating the importance of macrophage-mediated inflammation [10,11] or the roles of BM-derived progenitor cells [12] in post-MI cardiac remodelling and dysfunction.

In the course of a study investigating responses to acute MI in mice that had undergone BMT, we found that the process of BMT itself substantially alters the response of the heart to acute MI. Here, we report

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that when acute MI is induced by permanent coronary ligation after BMT, there is a significantly enhanced cardiac contractile recovery and a reduction in adverse remodelling that is attributable to an altered tissue macrophage phenotype. This previously unrecognised effect may significant alter interpretation of studies involving BMT and MI and also provides new insights into the role of inflammatory cells in cardiac repair after MI.

2. Methods

Animal studies were conducted in accordance with the Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986 (UK Home Office) and institutional guidelines. Studies were performed on C57BL/6 mice. The experimental protocol is shown in Supplementary Fig. 1.

2.1. BMT

BMT was performed using standard methods [13]. Mice aged 8–11 weeks were irradiated with a lethal dose of 9 Gy (9000 mSv). At 24 h, BM isolated from donor C57BL/6 mice was injected into the recipient via the tail vein at a dose of 9×10^7 cells in 200 μl. Briefly, donor BM was harvested in DMEM, filtered through a 40 μm cell strainer, washed in fresh DMEM, and then re-suspended in PBS at a final concentration of 4.5×10^8/ml. Animals were allowed to recover for 4 weeks. Female mice were used for most experiments. Survival was assessed in both male and female C57BL/6 mice.

2.2. Induction of MI

Permanent left coronary artery ligation was performed as described previously [14]. Animals were anaesthetised with 2% isoflurane/98% oxygen and ventilated via endotracheal intubation. A lateral thoracotomy was made in the fourth intercostal space. The pericardium was removed and the left coronary artery was ligated 1–2 mm below the tip of the left atrium. The chest wall was repaired in layers. Mice were allowed to recover in a warmed chamber for at least 6 h and treated with intramuscular buprenorphine and subcutaneous flunixin for perioperative analgaesia.

2.3. Cardiac magnetic resonance imaging (CMRI)

CMRI was performed at 3, 10 and 21 days after surgery on a 7T horizontal scanner (Agilent, Varian Inc., Palo Alto, CA). The gradient coil had an inner diameter of 12 cm, gradient strength of 1000 mT/m (100 G/cm) and rise-time of 120 μs. A quadrature transmit/receive coil (RAPID Biomedical GmbH, Germany) with an internal diameter of 39 mm was used. Anaesthesia was maintained with 1.5% isoflurane and a mix of O_2/medical air. Body temperature was maintained at 37° using a warm air fan. The ECG was monitored via two metallic electrodes placed subcutaneously in the front paws. A pressure-transducer for respiratory gating was placed on the abdomen. Simultaneous ECG triggering and respiration gating (SA Instruments) was applied.

The CMRI protocol included (a) T1-weighted acquisition of functional/volumetric and anatomical parameters [15]; (b) T1-weighted acquisition for late gadolinium enhancement (LGE) after intraperitoneal (i.p.) injection of 0.75 mmol/kg of gadolinium DPTA (Magnevist, Schering Healthcare, UK) [15]; and (c) T2*-weighted acquisition for haemosiderin localisation and T2* evaluation. LGE scans were performed 20–30 min post-injection on day 3 post-MI to evaluate the infarcted area. Heart rates ranged from 400 to 500 bpm (cycle length 120–150 ms) with a fluctuation of ±10 ms per cardiac cycle.

Cine-FLASH was used as a T1-weighted method for temporally resolved dynamic short-axis cardiac images to quantify functional/volumetric parameters and LGE acquisitions. Imaging parameters were: repetition time (TR) = 1 RR-interval/number of frames (typically ~9–10 ms), effective repetition time (TReff) = RR-interval, echo time (TE) = 1 ms, field of view (FOV) = 25 × 25 mm, matrix size = 128 × 128, slice thickness = 1 mm; flip angle = 40°, 3 averages, 9–11 slices, 1 k-space line/frame, 9–12 frames equally distributed along the cardiac cycle. The acquisition time was 8 ± 0.5 min. Triggering was positioned at the peak of the QRS complex. Single ECG gating was used to maintain a steady-state during acquisition in T1 weighted scans.

Cine-FLASH was also used as a T2*-weighted method to locate areas of signal void and the related T2* value. Imaging parameters were: TR = 3 RR-intervals (≈400 ms); TE = 1, 2, 3, 5, 7 ms; single frame acquired, typically the diastolic frame; FOV = 25 × 25 mm^2; matrix size = 128 × 128; slice thickness = 1 mm; number of slices = 5; flip angle = 40°; 3 averages; scanning time = 12 ± 0.5 min. ECG and respiration triggering were used, although, during respiration, signal acquisition and RF pulses were turned off. The trigger was positioned at the peak of the QRS complex corresponding to the diastolic phase.

T2* values were achieved by applying a T2* fit to the multi-echo T2*-weighted cine-FLASH images (TE varying from 1 to 7 ms) using ImageJ (NIH, Bethesda, MD). The T2* fitted followed the following equation:

$$S = S0\, exp.(-TEn/T2^*).$$

where *S0* is the signal at full magnetisation, TE is the echo time and *n* identifies the echo time under study. Areas of rapid signal decay, affected by strong susceptibility effects, were compared to the control infarcted myocardium where a drop in signal was much less severe.

LV ejection fraction (EF), LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV) and LV mass were obtained from cine-FLASH T1-weighted images [16] using custom segmentation analysis software (www.clinicalvolumes.com). The initial infarct area (as a percentage of the LV) was analysed from cine-FLASH LGE images 3 days post-MI [15]. On subsequent scans, the extension of the infarct through the LV was quantified using a mid-line method [17]. The wall thickness along infarcted regions was evaluated by measuring the endocardium to epicardium distance using ImageJ software (NIH, Bethesda, MD) on the middle slice of CMRI images at 21 days post-MI.

2.4. Histology and immunostaining

Hearts were harvested and immediately immersed in 10% formalin for 48 h at 4 °C. Hearts were then embedded in paraffin and sectioned in 5 μm-thick transverse slices. After deparaffinisation and rehydration, sections were stained with haematoxylin-eosin (H&E), Prussian blue and Picrosirius red.

Cardiomyocytes were stained with an anti-troponin I antibody (Abcam, Cambridge, UK). The thickness of the infarct was evaluated in representative slices taken in the middle of the heart by measuring the endocardium to epicardium distance using ImageJ software (NIH, Bethesda, MD). Rhodamine-conjugated wheat germ agglutinin (WGA) was used to outline cell membranes. Large vessels and capillaries were labelled with anti-sm22α and isolectin B4 antibodies, respectively. Leukocytes were labelled using an anti-CD45 antibody (BD Biosciences, USA), detected with an HRP/DAB system followed by haematoxylin counterstaining. A similar procedure was used to detect CD163 (Bioss Inc., USA), a receptor involved in clearance and endocytosis of haemoglobin/haptoglobin complexes by macrophages [18], and VCAM-1.

Imaging was performed on an Olympus IX-81 microscope. Quantification was performed in a blinded fashion, using Volocity® software (PerkinElmer, USA).

2.5. Flow cytometry (FACS)

Quantitative analyses of LV macrophage number and phenotype were performed by FACS on tissue digests. Residual blood was first rinsed from the LV which was then dissected into infarcted and remote myocardium for separate analysis. Samples were digested in a mixture of collagenase IV, DNase and hyaluronidase at 37 °C for 30 min followed by trituration and filtration through a 70 μm nylon mesh. Cell suspensions were washed

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