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Original Article

Increased fibrosis and progression to heart failure in MRL mice following ischemia/reperfusion injury

Dia Smiley [a], Margaret A. Smith [a], Vinicius Carreira [b], Min Jiang [a], Sheryl E. Koch [a], Melissa Kelley [a], Jack Rubinstein [a], W. Keith Jones [a,c], Michael Tranter [a,*]

[a] Department of Internal Medicine, Division of Cardiovascular Health and Disease, University of Cincinnati, College of Medicine, Cincinnati, OH
[b] Department of Environmental Health, University of Cincinnati, College of Medicine, Cincinnati, OH
[c] Department of Pharmacology & Cell Biophysics, University of Cincinnati, College of Medicine, Cincinnati, OH

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ABSTRACT

The cardiac regenerative capacity of MRL/MpJ mouse remains a controversy. Although the MRL mouse has been reported to exhibit minimal scarring and subsequent cardiac regeneration after cryoinjury of the right ventricle, multiple studies have been unable to replicate this cardiac regenerative capacity after both cryogenic and coronary ligation cardiac injury. Therefore, we evaluated the cardiac regenerative wound-healing response and functional recovery of MRL mice compared to C57 mice, in response to a clinically relevant left ventricular (LV) coronary ligation. Male MRL/MpJ+/+ and C57BL/6 mice underwent left coronary artery ligation followed by reperfusion. Cardiac function was evaluated by echocardiography [LV ejection fraction (LVEF), LV end-diastolic volume (LVEDV), LV mass, wall thickness] at 24 hours post-ischemia and weekly for 13 weeks thereafter. Hearts were also analyzed histologically for individual cardiomyocyte hypertrophy and cardiac fibrosis. Our results show that contrary to prior reports of cardiac regenerations, MRL mice progress to heart failure more rapidly following I/R injury as marked by a significant decrease in LVEF, increase in LVEDV, LV mass, individual myocyte size, and fibrosis in the post-ischemic myocardium. Therefore, we conclude that MRL mice do not exhibit regeneration of the LV or enhanced functional improvement in response to coronary ligation. However, unlike prior studies, we matched initial infarct size in MRL and C57 mice, used high frequency echocardiography, and histological analysis to reach this conclusion. The prospect of cardiac regeneration after ischemia in MRL mice seems to have attenuated interest, given the multiple negative studies and the promise of stem cell cardiac regeneration. However, our novel observation that MRL may possess an impaired compensated hypertrophy response makes the MRL mouse strain an interesting model in the study of cardiac hypertrophy.

1. Introduction

It is generally accepted that mammalian cardiomyocytes are terminally differentiated and show very little capacity for cell division. Following myocardial injury, the damaged cells cannot be replaced by new cardiomyocytes and a scar is formed [1]. Therefore, infarcts that result in a significant area of scar tissue respond with compensatory cardiac remodeling and hypertrophy, followed by a decrease in cardiac function, heart failure, and eventually death. There continues to be an active quest for preventing scar formation, and stimulating myocardial repair and regeneration following infarction. Interestingly, amphibians such as newt and zebrafish display exceptional myocardial regenerative capabilities following injury, demonstrating cardiomyocyte division and complete regeneration without formation of scar tissue [2–4]. It was only recently noted that the mammalian heart might retain this regenerative ability to a limited extent [1,5]. The MRL/MpJ (MRL) mouse achieved significant attention with the first report by Leferovich et al. of its ability to respond without scar following cardiac injury. In this study, MRL mice displayed extensive regeneration of cardiac tissue and restoration of cardiac function following cryoinjury to the right ventricle in comparison to control C57BL/6 mice [6]. The underlying mechanisms of the MRL regenerative response remain unclear, but initiation of cardiomyocyte proliferation and the presence of a novel stem cell population in the fetal livers of MRL mice have been implicated in this regenerative response [6–8]. Further, several factors have been implicated in the regenerative phenotype observed in the MRL mice, including changes in inflammatory and immune responses [8,9]; differential regulation

Abbreviations: LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; I/R, ischemia reperfusion; LAD, left anterior descending coronary artery.

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* Corresponding author at: Department of Internal Medicine, Division of Cardiovascular Health and Disease, University of Cincinnati, College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267. Tel.: +1 513 558 3507.
E-mail address: trantemc@ucmail.uc.edu (M. Tranter).

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Table 1

	First author and Year	Cardiac injury	Modalities used	Findings/Conclusions
Cardiac regeneration in MRL	Leferovich 2001	Transmural cryoinjury to RV with 2 sequential 10-s exposures.	TTE, BrdUrd labeling of dividing cells (to address mechanism of regeneration/ replacement of injured myocardium), histologic analysis (localization of BrdUrd and α-Actinin to prove cardiomycytes), hyderoxyproline assay (to quantify scarring), RT-PCR (to determine collagen type I message expression).	MRL cardiomyocytes are capable of growing and replacing wounded tissue without fibrosis, MRL granulation tissue resolves quickly with restoration of normal myocardial architecture and a markedly reduced extent of scarring, and myocardial function recovers from the injury.
	Bedelbaeva 2004	Transmural cryo-injury to RV with 2 sequential 10-s exposures. Mice were irradiated, MRL fetal liver cells injected into C57 mice, and C57 fetal liver c injected into MRL mice.	Histologic analysis (collagen deposition, scar); immunohistochemistry (human MEF-2, human Nkx 2,and troponin—to detect cardiomycytes); in-situ hybridization (to detect donor Y chromosomes).	MRL fetal liver cells transferred the healing phenotype to the C57 nonhealer with the appearance of Y-chromosome positive, donor-derived cardiomyocytes in the injury site and MRL-like healing with little scar. Similarly, C57 fetal liver cells transferred the nonhealing phenotype to the MRL with little cardiomyocyte growth and an acellular B6-like scar.
	Naseem 2007	1. Non-transmural cryogenic injury. 2. Transmiral cryogenic injury for 15 s. 3. Permanent LAD ligation.	TTE to calculate %FS; micromanometer conductance catheter to measure volume pressure hemodynamics; histological analysis of infarct area and scar formation; immunohistochemistry; BrdU labeling and detection of BrdU-labeled cells by fluorescence-activated cell sorting; RT-PCR; Affymetrix array hybridization and analysis.	Restoration of myocardial function in the superficial MRL cryoinjured heart and significantly less collagen deposition compared with the injured hearts of C5 mice. Following severe transmural myocardial injury, the MRL mouse has increased survival and decreased ventricular remodeling compared with the C57 mouse, but without evidence of complete regeneration.
No regeneration in MRLS	Oh 2004	Permanent LAD artery ligation, 4mm from origin of artery.	MRI to confirm MI and assess scar, histologic analysis (to assess infarct).	Ischemic myocardial injury in MRL mice is not followed by scarless recovery. C57 mice were not used as controls.
	Abdullah 2005	1. LAD ligation with 45 minutes ischemia. 2. Non-transmural cryoinjury to LV free wall utilizing two exposures for10-s. 3. Transmural cryoinjury to LV wall with 5 10-s exposures.	Histologic analysis (to assess infarct size).	MRL strain forms intense scar and wall thinning characteristic of myocardial infarction in a fashion similar to that of C57 mice.
	Grisel 2008	1. Cryoinjury to RV apex with 2 10-s exposures. 2. Modified cryo-injury to RV apex with 1 30-s exposure. 3. Cryoinjury to mid-ventricular RV with 1 30-s exposure. 4. Cryo-injury to midventricular portion of the antero-lateral LV wall with 3 1-min exposures. 5. Permanent proximal LAD ligation.	Histologic analysis (to determine infarct size, and collagen deposition within infarcted areas); BrdU labeling of dividing cells and microscopic localization.	MRL mice cryoinjury to apical RV, modified cryoinjury to apical RV, cryoinjury to midventricular RV, cryoinjury to LV, and ischemia to LV; all healed by scarring, and lacked cardiac regeneration.
	Robey and Murry 2008	1. Permanent LAD ligation. 2. Cryoinjury to RV and LV.	Histologic analysis (to determine infarct size, and collagen deposition within infarcted areas), BrdU labeling of dividing cells and microscopic localization.	Both types of injury in MRL mice heal similarly to controls by typical scar formation rather than muscle regeneration, and no differences in cell proliferation, angiogenesis or scar contraction between the two mouse strains were observed.
	Cimini 2008	Permanent LAD ligation	TTE and calculated %FS, LVEF, and %FAC of LV; used a micromanometer conductance catheter to measure LVESV, LVESP, LVEDV, and LVEDP, and minimal and maximal dP/dt were recorded; histological analysis; immunohistochemistry for anti-Ki67 (to assess cellular proliferation) and anti-CD31 (as an estimation of the total blood vessel density after MI)	No significant differences in infarct area and no evidence of cardiac regeneration in this model. Instead, MRL hearts exhibited prominent transmural infarcts with no repopulation by cardiomyocytes and did not recover ventricular function.
	Moseley 2011	Permanent LAD ligation.	LVEF was assessed using assessed using a miniature conductance micromanometer catheter; Immunoblot analysis; flow cytometry (to evaluate 3 known stem cell markers, CD34—marker of hematopoietic precursors, c-kit—receptor for stem cell factor, and Sca-1—expressed on multipotent hematopoietic stem cells in MRL and C57 fetal livers.	No intrinsic differences were observed in cell cycle control molecules or stem cell populations between MRL and C57 mouse hearts. Ischemic injury is not repaired more efficiently in MRL myocardium

BrdU, 1,5-bromo-2′-deoxyuridine; dP/dt, rate of LV pressure rise in early systole is one of the oldest measures of LV global contractility; %FAC, fractional area change; %FS, fractional shortening; LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; RV, right ventricle; TTE, transthoracic echo.