REVIEW

Resident macrophages as potential therapeutic targets for cardiac ageing and injury

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Abstract
Cardiac-resident macrophages (CRMs) play critical roles in maintaining cardiac homeostasis and removing senescent and dying cells. Recent preclinical data have re-energised the area of cardioimmunology and provided improved understanding of the modulation of compositional and functional phenotypes of CRMs. These data can aid in achieving improved cardiac regeneration, repair and functional remodelling following cardiac injury. In this review, we discuss the composition and renewal of various subsets of CRMs. Specific attention has been given to delineate the roles of various CRM subsets with respect to (1) facilitation of cardiac development and maintenance of physiological function such as electrical conduction and rhythm; (2) promotion of cardiac regeneration, inflammation resolution and functional remodelling following a cardiac injury; and (3) therapeutic potential. We have also highlighted the relationship between CRM replenishment and cardiomyocyte senescence as well as cardiovascular diseases development. Finally, we have addressed future perspectives and directions in basic research and potentially clinical applications of CRMs.

Keywords: cardiac development, cardiac injury, cardiac regeneration, cardiac-resident macrophages, cardiomyocyte senescence

INTRODUCTION

Macrophages play important roles in maintaining tissue homeostasis, tissue-specific functions and protecting tissues from stress. Needless to say, they are also involved in the pathogenesis of multiple diseases such as cancers and autoimmune diseases as well as in tissue injury and remodelling.\textsuperscript{1} Previously, all macrophages were thought to be derived from bone marrow-derived monocytes. However, recent data demonstrate that most tissue-resident macrophages are established during the embryonic development and persist throughout life, independent of their replenishment by bone marrow-derived monocytes at the steady state.\textsuperscript{2-5} Nevertheless, several investigations have now revealed that the renewal of tissue-resident macrophages in some tissues is dependent on the bone marrow-derived monocytes. The prenatally derived-resident macrophages in the heart are gradually replaced by bone marrow-derived monocytes.\textsuperscript{5} The
possibility of such a turnover of cardiac-resident macrophages (CRMs) to affect cardiac function, accelerate cardiomyocyte senescence and influence cardiac remodelling following an injury remains to be understood. Understanding the characteristics, physiological roles, renewal mechanisms and homeostatic processes of CRM subsets is fundamental for designing intervention strategies to modulate cardiac function, delay cardiac ageing and benefit cardiac remodelling following an injury.

THE SUBSETS AND REPLLENISHMENT OF CRMS

Recently, remarkable progress has been made in identifying the diversity and understanding the process of replenishment of CRMs. The majority of the CRMs are defined as CD45\(^+\), CD11b\(^+\), F4/80\(^-\) or CD64\(^+\). The CRMs have been further assessed using multiple approaches, such as the use of a variety of markers to label the cell subsets. In neonatal mice, the CD45\(^+\), CD64\(^+\) and CD11b\(^+\) macrophages contain only the CCR2\(^{-}\)/MHCII\(^{lo}\) subset.\(^6\) However, in adult mice, a heterogeneous population has been identified containing four subsets: MerTK\(^+\)/Ly6C\(^{-}\)/MHCII\(^{hi}\)/CX3CR1\(^{hi}\)/CD206\(^{hi}\)/CCR2\(^{-}\) (containing a small subset of CD11c\(^{hi}\)), MerTK\(^+\)/Ly6C\(^{-}\)/MHCII\(^{lo}\)/CX3CR1\(^{int}\)/CD206\(^{hi}\)/CD11c\(^{lo}\)/CCR2\(^{-}\), MerTK\(^+\)/Ly6C\(^{-}\)/MHCII\(^{hi}\)/ CX3CR1\(^{hi}\)/CD206\(^{hi}\)/CD11c\(^{hi}\)/CCR2\(^{-}\) and the MerTK\(^+\)/Ly6C\(^{-}\)/MHCII\(^{lo}\)/CX3CR1\(^{hi}\)/CD11c\(^{lo}\)/CD206\(^{-}\)/CCR2\(^{-}\). The last subset constitutes the resident monocytes.\(^5\) Under steady-state conditions, CRMs are renewed primarily by local proliferation. Conversely, under cardiac stress, CCR2\(^+\)/Ly6C\(^{hi}\) monocytes can replenish all the four subsets (Figure 1). Recent data indicate that there are four subpopulations of macrophages in the heart of adult mice based on fate mapping, single-cell transcriptomics and parabiosis: TIMD4\(^+\)/LYVE1\(^-\)/MHCII\(^{lo}\)/CCR2\(^{-}\) cells (maintained independent of monocytes), TIMD4\(^+\)/LYVE1\(^-\)/MHCII\(^{hi}\)/CCR2\(^{-}\) cells (partially dependent on monocyte input), TIMD4\(^-\)/LYVE1\(^-\)/MHCII\(^{hi}\)/CCR2\(^{-}\) subset (can be completely replenished by monocytes) and CCR2\(^^{-}\)/MHCII\(^{lo}\) (monocyte subset).\(^6\)–\(^8\) In our laboratory, by single-cell analyses, we have demonstrated the presence of four subsets that are different from those reported previously. Moreover, various subsets demonstrate a specific function on the cardiac transient regenerative potential, cardiac development and maintenance of cardiac physiology in mice (unpublished data). Furthermore, in humans, CD14\(^-\), CD45\(^-\) and the CD64\(^+\) cells in the

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**Figure 1.** A diagrammatic representation showing that cardiac-resident macrophages (CRMs) are seeded within the cardiac tissue at embryogenesis. During their lifespan, CRMs undergo three developmental waves. The first wave of embryonic macrophage development is from yolk sac around E9.5-E10 and disappears before birth. The second wave involves the erythremaloid precursors (EMPs) seeded in the foetal liver between E12.5 and E16.5. The third wave is bone marrow-derived HSC (hematopoietic stem cells) from E16.5 throughout life. Under steady-state conditions, foetal liver-derived CRMs are maintained through in situ proliferation and decrease with age. A substantial pool of adult CRMs is replenished by monocyte-derived macrophages and ultimately replaces embryo- and foetal liver-derived cardiac macrophages. Following cardiac injury, the neonatal cardiac macrophage population expands through proliferation. However, in adults, CRMs are initially lost and replaced by recruited monocytes.
heart can be classified into three subsets based on the expression of CCR2 and HLA-DR (homologue of MHCII): MerTK+/CCR2+/HLA-DRhi, MerTK+/CCR2-/HLA-DRlo, and MerTK−/CCR2+/HLA-DRhi; the last subset includes monocytes.9 Although various investigators have identified different CRM subsets in the heart using different methods, the present data suggest that CCR2+ macrophages are primarily derived from the embryonic yolk sac or foetal liver. They undergo a monthly turnover without requiring the participation of the Ly6C+CCR2+ monocytes.10 The CCR2+ CRM cells are replenished by both local proliferation and recruitment of the Ly6C+CCR2+ monocytes; they ultimately replace the yolk sac- or foetal liver-derived CRMs with ageing.11 Although investigators have confirmed the existence of CRMs in the heart under steady-state conditions, the results are not consistent. The differences may be due to the fact that the germlines and age of the selected mice are not consistent among the research groups and the methods used are also variable. Interestingly, the results obtained by single-cell sequencing are also variable. Such observations may be the result of cardiac region-related sampling variations. However, the data, at least, demonstrate that (1) CRMs have highly heterogeneous and different phenotypes and functions; (2) different subsets have their own renewal process that may be associated with cardiac ageing and functional remodelling following cardiac injury; and (3) in neonatal mice, CRMs replenish by self-proliferation. Conversely, in adult mice, the renewal of CRMs is mainly dependent on the Ly6C+CCR2+ monocytes (Figure 1).

**CRMS during Early Cardiac Development and Later Cardiac Ageing**

Tissue-resident macrophages have been shown to play essential roles in a variety of developmental processes.12 CRMs involved in cardiac development originate from the yolk sac-derived CX3CR1hi CRMs at E12.5 and are located in the subepicardial space; they contribute to the expansion of perfused blood vessels by inducing endothelial cell proliferation and remodelling. Furthermore, CX3CR1hi CRMs are important for the maturation of coronary arteries. The yolk sac-derived CX3CR1hi CRMs facilitate the remodelling of the primitive coronary plexus via selective expansion of the perfused vasculature.13 Yolk sac-derived CD206+ CRMs rapidly decrease after birth (P30) and CD206+ MHCII+ CRMs dramatically increase during that period. The transition of CRMs is accompanied with cardiac development, the elongation of heart valves and extracellular matrix (ECM) remodelling that facilitates the maturation of trilaminar leaflets (Figure 2).14,15

Cardiac ageing, characterised by cardiomyocytes loss or hypertrophy, hyperosmosis, inflammation and collagen deposition, leads to the development of cardiac muscle sarcopenia and deterioration of the left ventricular diastolic function. Such changes may underlie increases in the cardiovascular morbidity and mortality.16–18 The disruption of macrophage numbers or their functional state can lead to severe defects in the cardiac function and lead to early ageing. CRMs are key contributors in cardiac ageing as evidenced by the enhanced levels of proinflammatory factors such as matrix metalloproteinases (MMPs) and CCL2, both of which positively correlate with the increase in ageing-related cardiomyocyte hypertrophy and ventricular size.18,19 Flow cytometric analysis suggests that there is an increase in CD206+ CRMs and a decrease in CD206− CRMs with ageing20 that is prevented by MMP-9 deletion. This indicates that MMP-9 modifies age-related CRM transitions or reprogramming.21 Currently, ageing is demonstrated to cause an increase in Ly6C+CCR2+ monocyte-derived CRMs in heart, referred to as “inflammaging”.22,23 Recent studies have further shown that TLR2 signalling is important for preventing the heart from ageing-associated adverse remodelling and contractile dysfunction as TLR2−/− reduces the CRMs and impairs Akt signalling in the heart.24 However, the precise effects of various CRM subsets and their renewal on cardiac ageing need to be further investigated.

Cardiac ageing increases the incidence and mortality of cardiovascular diseases.25 Therefore, promoting cellular youthful vitality and appropriately maintaining cellular proliferation are key factors in maintaining healthy ageing of the population.26 Stress and cellular senescence exacerbate cardiac ageing. Both extrinsic and intrinsic factors such as lifestyle changes, aerobic exercise, energy intake restriction, healthy diet, pharmacological administration, molecular intervention and cell-based therapies may be required to avoid the deleterious consequences of cardiac ageing and achieve optimal cardiovascular health.27
Cardiac-resident macrophages are spindle cells with long cytoplasmic elongations. They are present in the interstitial space between the muscle cells, fibroblasts and endothelial cells. CRMs are widely distributed in the atria and ventricles and are intrinsic components of the normal heart. They are closely connected to the blood vessels and are abundant in the conduction system. Under steady-state conditions, CRMs are sentinels that detect stress and infectious agents such as bacteria and viruses. They are involved in cell and matrix turnover, removal of apoptotic cells, maintenance of the energy-intense state and adaption to altered and increased tissue strain. CRMs likely participate in the physiological remodelling of the cardiac tissue during pregnancy or endurance training. Normal cardiac rhythms are critical for coordination of the cardiac pumping function that is mediated by the electrical conduction system of the heart. This system is composed of specialised cardiomyocytes responsible for initiating and transmitting electrical signals. The sinoatrial node located in the right atrium sends electrical signals to the atrioventricular node (AVN) that subsequently transmits the signal to the ventricles. Electrical activities are transmitted from one cell to another.
via the connexin system, especially connexin-43 (CX43). CRMs are especially abundant in the AVN where they contribute to the formation of the CX43-containing gap junctions which connect adjacent cardiomyocytes enabling efficient electrical conduction to both the atria and ventricles (Figure 2).32,33

The cardioprotective roles of CRMs are not limited to the modulation of the electrophysiological properties of the coupled cardiomyocytes.30 CRMs also regulate conduction abnormalities outside the AVN like ischaemic atrial fibrillation or ventricular arrhythmia.34 Under pathological conditions, except for the deregulation of cardiomyocytes and specialised conduction system, CRMs can also participate in the formation of the atrioventricular block. In addition, CRMs are related to arrhythmias. CRMs secrete IL-1 after cardiac injury. IL-1 destroys the electrical activity of the cardiomyocytes by paracrine signalling, which may promote the occurrence of ventricular arrhythmia.34 Some studies have linked the number of CRMs to atrial fibrillation in humans at the age of 28 and 29. Moreover, CRM depletion experiments further confirm that these cells are associated with atrial fibrillation. This suggests that in situ CRM depletion may be a reliable strategy to ensure normal heart rhythm in patients with signs of arrhythmia.30,36

CRMS FACILITATE CARDIAC REGENERATION

It is well documented that certain fish such as the zebrafish and the amphibians retain a robust regenerative capacity of the heart throughout their life.37–39 However, the mammalian heart has a transient capacity for cardiac regeneration during early life;40 this fact overturns the long-standing view of the mammalian heart being a postmitotic organ.41–43 Cardiomyocyte proliferation and hypertrophy contribute to heart formation and growth during development and this replicative capacity continues for a short time after birth (P7, 7 days postnatal). It has been demonstrated that the cardiomyocyte proliferation mediates cardiac repair in the resected neonatal mouse heart.40,44 Epigenetic and transcriptional profiling of neonatal and adult cardiomyocytes further confirms the loss of the replicative and regenerative ability after birth.45 However, the underlying mechanisms that control this difference remain unclear. Emerging evidence suggests that adult cardiomyocytes withdraw from the cell cycle and become binucleated compared with neonatal cardiomyocytes that possess strong proliferative ability.46

In the mammalian heart, the CX3CR1+ CRMs are gradually replaced by the CCR2+ monocyte-derived CRMs with age. Such changes are associated with decreased cardiomyocyte proliferation. In the neonatal mice heart, depletion of macrophages following uptake of clodronate liposome abrogates cardiac regeneration.12 Moreover, in response to cardiac stress, the frequency of CX3CR1+ CRMs is predominant in the neonatal cardiac tissue and directly facilitate cardiomyocyte proliferation and coronary angiogenesis,14,35,47 possibly through the CRM-derived factors. Furthermore, adult rat cardiomyocytes, following co-culture with neonatal rat ventricular CRMs, form new cardiomyocytes via dedifferentiation, and proliferation. Such a process may be induced by intercellular Ca2+ signals from neighbouring functional cardiomyocytes through gap junctions.48 However, the molecular signals that indicate that CRMs coordinate cardiac regeneration remain unclear.

Extracellular matrix serves as a mechanical scaffold for acellular and cellular communication and also plays a key role in cardiac regeneration. The transcripts encoding ECM components and structural constituents of the cytoskeleton are influenced by different subsets and specific functional phenotypes of the CRMs and vary greatly with age.49 Reducing ECM stiffness through the inhibition of lysyl oxidase restores the regenerative ability in the older mice.50 Additionally, ECM can directly interact with cells or act as a reservoir of growth factors and ligands for growth factor receptors to control cell function.51 For example, VEGF binds to specific fibronectin type III domains that are prevalent in many ECM and forms a complex of integrin and VEGFR to promote cardiomyocyte proliferation.52 The dystrophin–glycoprotein complex, a multicomponent transmembrane complex, creates linkage between the intracellular cytoskeleton and ECM and directly binds to the Hippo pathway effector YAP to inhibit cardiomyocyte proliferation.53 However, the potential mechanisms remain unclear (Figure 3). Understanding how to regulate these processes by different macrophages...
in detail is the key to control regenerative competence.

Cardiac ECM contains two segments: the interstitial matrix, including collagen I/III and the basement membrane including collagen IV, V, VII, X, as well as laminins. Generally, collagen I/III maintains the structural integrity of the heart through tensile support. Moreover, ECM appropriately distributes the physical stress. During mechanical cardiac stress, the down-regulation of elastin in the ECM leads to a stiffer scar tissue instead of providing elasticity to the cardiac tissue. Furthermore, ECM also serves as a transducer of signals in heart.

**Figure 3.** Diagram showing the role of cardiac-resident macrophages (CRMs) in cardiac injury. During the early stage of cardiac injury, damaged myocardium releases danger signals. Ly6C<sup>hi</sup>CCR<sub>2</sub> monocytes are recruited at the injured sites and differentiate into inflammatory macrophages, promoting inflammation through production of proinflammatory cytokines and recruitment of neutrophils aggravating cardiac injury. During the resolution of inflammation, the resolving macrophages ameliorate the inflammation by mediating efferocytosis, secreting IL-10 or lipoxins and by suppressing monocyte recruitment. During the last stage, inflammatory or infiltrated macrophages differentiate into tissue repair macrophages. In adults, they improve tissue repair, promote angiogenesis, activate fibroblasts and are directly involved in extracellular matrix (ECM) remodelling. In neonatal mice, CRMs promote cardiac regeneration driven by cardiomyocyte proliferation without scar formation.

**CRM FATE FOLLOWING CARDIAC INJURY**

Following cardiac injury or stress, CRM and blood monocytes infiltrate into the damaged sites, undergo differentiation and polarisation, induce a maladaptive response and subsequently lead to adverse outcomes such as infarct expansion, left ventricle (LV) dilation and LV systolic dysfunction. With the infiltration of the monocytes, the CRM fate remains a complicated and confusing topic. The current major views are the following: in neonatal mice, the yolk sac-derived CX<sub>3</sub>CR<sub>1</sub> CRMs facilitate cardiomyocyte...
proliferation and angiogenesis following cardiac injury through local proliferation and by producing high levels of growth factors instead of inflammatory chemokines or cytokines; thus, the CCR2\(^+\) monocyte recruitment is reduced.\(^{14,61}\) Conversely, in the adult heart, CRMs also locally proliferate in response to angiotensin II infusion, myocardial infarction and transverse aortic constriction models.\(^{5,6,62}\) However, extensive research indicates that, in the adult mice heart, the yolk sac-derived CRMs are almost completely replaced by the CCR2\(^+\) monocyte-derived CRMs. Therefore, these resident CRMs have limited local proliferation ability. Following cardiac injury, a large number of CCR2\(^+\) monocytes infiltrate the injury sites. However, the fate of the original CRMs settled in the heart is not yet clear. There are several possibilities: (1) the CRMs are reprogrammed in the local microenvironment and cause inflammatory damage to the heart; (2) the cells leave the heart and return to the neighbouring peripheral immune organs; and (3) the cells may undergo apoptosis or necrosis.\(^{63-66}\) More research is, however, needed to establish specific mechanisms.

**CX3CR1\(^+\) CRMs FACILITATE THE RESOLUTION OF INFLAMMATION FOLLOWING CARDIAC INJURY**

Inflammatory resolution is an active process that is characterised by the production of pro-resolving factors like IL-10 and lipids.\(^{67}\) Inflammatory resolution starts with the removal of damaged cells and infiltrated inflammatory cells, especially, proinflammatory macrophages. During resolution, the proinflammatory macrophages undergo selective death via self-produced NO\(^*\) or functional conversion to resident cardiac CRMs.\(^{59}\) The involvement of CRMs in these processes is illustrated in Figure 3.\(^{66,70}\)

**CX3CR1\(^+\)CRM can produce IL-10**

Following cardiac injury, IL-10, an important anti-inflammatory factor, inhibits cardiac collagen deposition by decreasing the level of hyaluronidase 3.\(^{71}\) IL-10 also improves the LV function and facilitates cardiac wound healing.\(^{71}\) Some data demonstrate that IL-10, derived from the CX3CR1\(^+\) intestinal resident macrophages, is dispensable for the maintenance of colonic regulatory cells (Treg) and gut homeostasis.\(^{72}\) However, in the heart, the role of IL-10 derived from CX3CR1\(^+\) CRMs in cardiac homeostasis is unclear. Moreover, we have demonstrated that IL-10 derived from the regulatory B cells (B10 cells) is important for ameliorating myocarditis progression.\(^{73}\) Furthermore, some additional data also indicate that although CX3CR1\(^+\) peritoneal macrophages can produce lipoxins,\(^{74}\) it is unclear whether the CX3CR1\(^+\) CRMs can perform similar functions.

**CRMs mediate efferocytosis**

Efferocytosis is the process of removal of dying cells. This is an important characteristic of inflammatory responses and imperative for inflammatory resolution. Deficient or impaired efferocytosis induces chronic inflammatory disorders.\(^{75}\) Following cardiac injury, the damaged cardiomyocytes release numerous soluble factors and find-me signals. Some important find-me signals identified include CX3CL1, triphosphate nucleotides, lysophosphatidylcholine and sphingosine-1-phosphate.\(^{76-78}\) CX3CR1\(^+\) macrophages are attracted to the site of the dying cell by these soluble factors and induce efferocytosis. These macrophages can also mediate efferocytosis using the ‘bridging’ proteins such as milk fat globule-EGF factor 8 protein, growth arrest-specific 6, cellular communication network factor 1 and protein S interaction with phosphatidylserine expressed on the dying cells.\(^{79}\)

**CX3CR1\(^+\) CRMs inhibit monocyte recruitment**

It is evident that CX3CR1\(^+\) and CCR2\(^+\) CRMs play completely different roles in cardiac injury. As is well known, monocytes and monocyte-derived macrophage infiltration are the hallmark responses to cardiac injury. Following cardiac ischaemia-reperfusion injury, CCR2\(^+\) CRMs are activated in a TLR9-dependent manner; inflammatory cytokines are produced by the NLRP3 inflammasome complex. The chemokines CXCL2 and CXCL5 orchestrate homing of the CCR2\(^+\) monocytes and CCR2\(^+\) monocyte-derived macrophages and neutrophils to the ischaemic area contributing to an inflammatory storm, which negatively affects cardiac healing.\(^{63,80-82}\) CCR2\(^+\) CRMs are thought to be a checkpoint for governing the infiltration of neutrophils, monocytes and monocyte-derived macrophages. Conversely, the selective depletion of CX3CR1\(^+\)
CRMs before ischaemic injury results in a deteriorating systolic dysfunction and accentuation of the dilative remodelling following infarction. Syngeneic cardiac transplantation models further demonstrate that cardiac-resident CX3CR1+ and CCR2+ CRMs play different roles in monocytes recruitment and that depletion of cardiac-resident CCR2+ CRMs significantly ameliorates inflammation and interstitial graft fibrosis. Taken together, these data suggest a relatively simple conclusion: CCR2+ CRMs exacerbate cardiac injury, promote recruitment of the proinflammatory cells and mediate cardiac dysfunction. In contrast, the CX3CR1+ CRMs are sentinel cells, limit the proinflammatory cells recruitment, promote or help inflammation resolution, and protect the injured heart from adverse remodelling.

CRMs and cardiac biology

Cardiac fibrosis is characterised by systolic and diastolic dysfunction, arrhythmogenesis and other adverse outcomes because of over-accumulation of extracellular connective tissue proteins in the cardiac interstitium; it is a key driver of heart failure. Several cell types, including cardiomyocytes, fibroblasts, endothelial cells, pericytes, lymphocytes and mast cells, are involved in this process. CRMs play essential roles in cardiac fibrosis by releasing cytokines and growth factors with fibrogenic properties by producing matricellular proteins and by secreting proteases that are involved in matrix remodelling.

With ageing, turnover of CRM composition contributes to augmented collagen deposition

In the neonatal mice, the yolk sac- or foetal liver-derived CX3CR1+ CRMs have regenerative potential and the heart can completely regenerate without traces of scar tissue following a cardiac injury. However, after P7, the CRMs lose this ability to regenerate because of altered CRM populations. Additionally, ageing-associated changes in the CRMs impair the cardiac function because of ventricular and atrial fibrosis. Compared with younger mice, with ageing, the collagen levels in the interstitium are elevated and aged mice show the presence of dense collagen fibres in the myocardium. CRMs also contribute to age-related cardiac fibrosis without cardiac injury. Interestingly, normal ageing does not cause systolic dysfunction, but by promoting the deposition of cardiac interstitial ECM, there is a gradual increase in the ventricular hardness. Some studies have suggested that age-related fibrosis may be due to decreased matrix degradation rather than increased collagen synthesis. Macrophages secrete stromal cell proteins and are directly involved in ECM remodelling by producing MMPs and their inhibitors.

Cardiac fibrosis is ameliorated by recruiting reparative macrophages

Cardiac-resident reparative macrophage (M2 phenotype) infiltration can ameliorate cardiac fibrosis following a cardiac injury by the removal of neutrophils and avoiding neutrophil-triggered extensive matrix degradation, collagen deposition and cardiac rupture. Regenerating islet-derived 3β protein (Reg3β), an essential regulator of macrophage trafficking to the cardiac tissues following an injury, can be released by the dedifferentiation of the cardiomyocytes in response to oncostatin M stimulus. This stimulus is produced by the inflammatory monocytes and neutrophils, and it recruits the reparative macrophages. CD206+ CRMs expressed galectin-3 specifically infiltrate the infarcted myocardium and produce osteopontin (OPN). The IL-10-Stat3-galectin-3 axis is essential for OPN-producing reparative macrophage reprogramming, and these macrophages contribute to tissue repair by promoting fibrosis and clearing the apoptotic cells. Furthermore, hypoxia-induced signalling in the macrophages inhibits excessive fibrosis mediated by oncostatin M secretion following a cardiac injury.

Interstitial MHCIIhi and pericardial cavity gata6+CX3CR1+ CRMs can prevent cardiac fibrosis following a cardiac injury

Gata6+CX3CR1+ CRMs are present in the pericardial fluid of mice and contribute to the reparative immune response. Following a cardiac injury, these cells infiltrate into the epicardium and lose gata6 expression but remain to play anti-fibrotic functions. These cells are also detected in the human pericardial fluid; this has led to the notion that they have reparative function and are associated with human cardiovascular diseases.
These findings are suggestive of a novel role regarding cardioprotection of the pericardial tissue compartment and argue for the re-evaluation of pericardium removal. Recent data also indicate that tissue interstitial macrophages contain two subsets: LYVE1hiMHCIIloCX3CR1hi and LYVE1hiMHCIIloCX3CR1lo with distinct localisations, phenotypes, gene expression profiles and functions. Depletion of LYVE1hiMHCIIloCX3CR1lo CRMs exacerbates experimental lung fibrosis. We speculate that interstitial macrophages maybe also exist in the heart. However, additional studies are required to demonstrate their phenotypes, and specific roles in cardiac fibrosis.

**CRMs can regulate fibrosis via ECM remodelling following a cardiac injury**

In the context of cardiac stress or injury, the ECM undergoes adverse remodelling leading to disarray of physical stress and cardiac dysfunction. However, inflammatory tissue repair and resolving macrophages play distinct roles in ECM remodelling. It is difficult to delineate the detailed roles of different subsets in ECM remodelling, inflammation, healing and repair. However, it is unquestionable that CCR2+ CRMs contribute to the clearance of fibrin-derived provisional matrix, which prompts a proinflammatory response and maintains chronic inflammation. Conversely, depletion of tissue repair and resolving macrophages can lead to a worsened outcome because these cells can secrete IL-10 that exerts protection roles against cardiac fibrosis. It has also been suggested that a subset of macrophages may directly contribute to fibrosis through conversion to activated fibroblasts.

**FUTURE PERSPECTIVES**

Collectively, a growing body of literature has indicated that, in mice, a heterogeneous population of distinct CRMs exerts different roles under pathological or physiological heart conditions, such as cardiac development, function maintenance and cardiac remodelling after injury. However, many questions remain unclear such as (1) the exact number of subsets, phenotypes and functions – because these subsets are identified by various research groups using different techniques; (2) the location of the various subsets, their associated functions and renewal process under physiological conditions; (3) the roles of distinct subsets following cardiac injury as well as their phenotypic conversion; and (4) current research progress on human CRMs is insufficient. To further delineate the above questions, novel approaches, for example fate-mapping technique or parabiosis to trace the origin of CRMs, their physiological functions and roles in diseases may be studied. One can also establish cardiac organoids to study the role of human CRMs on cardiac biology. Such detailed analyses may ultimately lead to novel therapies for heart diseases.

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**AUTHOR CONTRIBUTIONS**

Shiqing Zhang: Resources; Writing-original draft. Rong Chen: Formal analysis; Writing-original draft. Subrata Chakrabarti: Writing-review & editing. Zhaoliang Su: Conceptualization; Funding acquisition; Project administration; Writing-review & editing.

**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

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