



# The Dynamic Role of Cardiac Macrophages in Aging and Disease

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## Abstract

**Purpose of Review** The cardiac immune landscape dynamically changes in response to aging, hemodynamic stress, and myocardial injury. Here, we highlight key cardiac immune cell types, their role in reshaping the cellular landscape and promoting tissue remodeling following cardiac insults, and how understanding of these processes uncovers novel disease mechanisms that contribute to cardiac pathology.

**Recent Findings** Distinct subsets of cardiac macrophages reside within the heart and exhibit divergent functions in response to myocardial injury. Parsing cardiac macrophages based on developmental origin has served as a valuable approach to define functionally divergent populations of reparative (embryonic-derived, tissue resident) and inflammatory (monocyte-derived, recruited) cardiac macrophages. Single-cell transcriptomics and elucidation of the effector mechanisms that orchestrate macrophage functions has provided new and therapeutically tractable insights into the pathogenesis of numerous cardiac diseases.

**Summary** The immune landscape of the heart is dynamic and represents an important mediator of disease pathogenesis across an array of cardiac pathology. Elucidation of mechanisms that drive inflammatory monocyte/macrophage recruitment, activation, and effector responses may lead to the identification of new therapeutic targets.

**Keywords** Cardiac immunology · Cardiac injury · Cardiac repair · Macrophages · Myocarditis

## Introduction

The role of the immune system during cardiac homeostasis, aging, and disease is an active area of investigation. In the steady state, the mouse and human heart is comprised of cardiomyocytes, fibroblasts, endothelial cells, smooth muscle cells, pericytes, endocardial cells, epicardial cells, and various immune cells including monocytes, macrophages, dendritic cells, T-cells, natural killer (NK)-cells, mast cells, and B-cells [1, 2, 3•]. Among these immune populations, macrophages represent the most prevalent cell type. The

cardiac immune landscape is dynamic and undergoes dramatic composition and phenotype shifts that differ based on the disease context (i.e., infection, sterile injury, hemodynamic perturbations, aging, myocarditis). In this review, we will discuss the cardiac inflammatory response following acute myocardial injury.

## Composition of the Cardiac Immune Landscape

The cardiac immune landscape dynamically changes as it transitions from homeostasis and surveillance to an inflammatory state. In the steady state, the heart contains immune cells that are interspersed within cardiac tissue. The bulk of these immune cells are tissue-resident macrophages [4]. As part of the innate immune system, macrophages are professional phagocytes with various capabilities including recognizing and destroying apoptotic cells and pathogens as well as communicating with surrounding stromal and immune cells [5].

Identification and classification of tissue-resident cardiac macrophage subtypes has been achieved through the use of cell surface markers (CCR2: chemokine receptor-2;

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LYVE1: lymphatic vessel endothelial hyaluronan receptor 1; and TIMD4: T-cell immunoglobulin and mucin domain containing 4), genetic fate mapping, transcriptomic profiling, and functional analysis [6–10, 11•, 12]. In the steady state, CCR2<sup>-</sup>LYVE1<sup>+</sup>TIMD4<sup>+</sup> tissue-resident macrophages comprise the bulk of all immune cells within the heart (Fig. 1). Cardiac tissue-resident macrophages (CRMs) are long-lived, originate from embryonic hematopoietic progenitors that arise within the yolk sac, and seed the heart during embryonic development [6, 7, 9, 10, 13, 14]. CRMs are maintained independent of monocyte input through local proliferation within the heart.

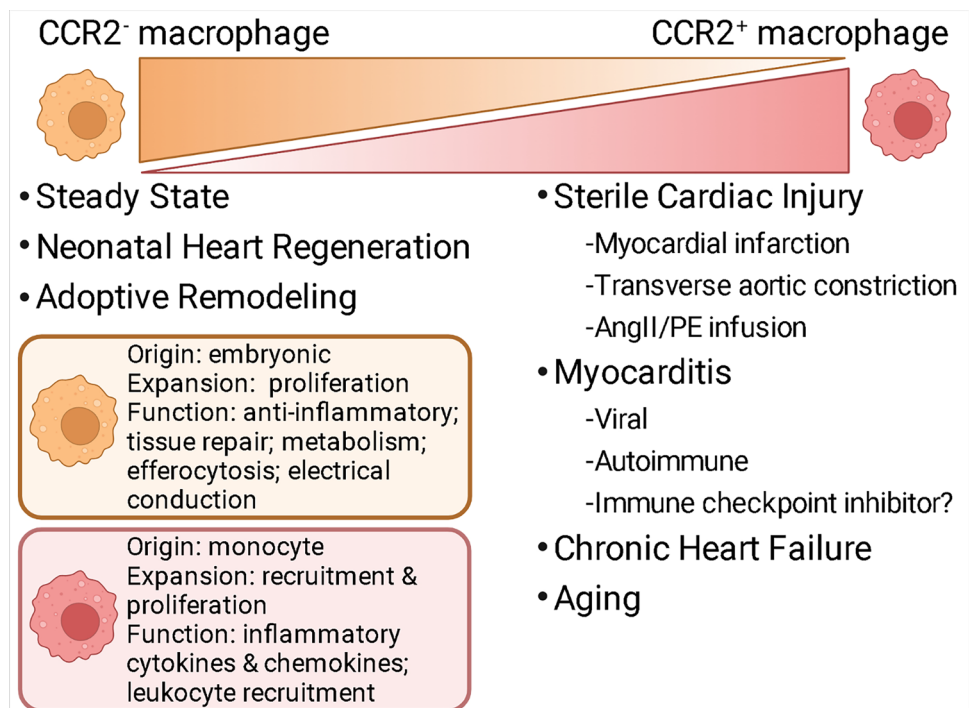
Multiple studies have demonstrated that CRMs have interesting and unique tissue specific functions, similar to what has been observed for other tissue-resident macrophage populations (i.e., microglia, Kuepfer cells, alveolar macrophages) [13, 15–18]. CRMs are active during development and homeostasis, where they function to clear apoptotic cells, promote coronary growth and patterning, clear damaged mitochondria, and facilitate electrical conduction [8, 11•, 19, 20]. CRMs suppress inflammation by preventing monocyte and neutrophil recruitment potentially through secretion of interleukin (IL)-10 and/or transforming growth factor (TGF)- $\beta$  [21]. CRMs orchestrate repair and regeneration of the neonatal heart by regulating myocardial proliferation and angiogenesis [9, 22, 23]. In the context of chronic heart failure, CRMs are activated by mechanical stretch through

transient receptor potential cation channel subfamily V member 4 (TRPV4) and trigger adaptations necessary to maintain cardiac output including reorganization of myocardial tissue and coronary angiogenesis [24••, 25, 26]. CRMs that express high levels of major histocompatibility complex (MHC)-II also have the capacity to present antigens; however, the functional relevance of this property is not known [8].

Under homeostatic conditions, CRMs are admixed with CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages, which are derived from monocytes (Fig. 1). CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages seed the heart postnatally (~2 weeks after birth) and are maintained through ongoing monocyte recruitment and subsequent proliferation. CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages have robust inflammatory potential and secrete chemokines that support neutrophil and monocyte recruitment [6–9, 14]. The heart also contains a small number of lymphocyte antigen 6 complex (Ly6C)<sup>hi</sup>CCR2<sup>+</sup> monocytes that can be distinguished from cardiac macrophages based on the expression of Mer proto-oncogene tyrosine kinase (MertK) [9, 27].

Lastly, the heart contains small numbers of dendritic cells, mast cells, memory T-cells, regulatory T-cells, and intravascular B-cells that contribute to immunosurveillance in the steady state. In this scenario, dendritic cells are thought to serve as gatekeepers of self-tolerance [28, 29]. The exact role of these populations remains to be fully elucidated.

**Fig. 1** Distribution and key features of macrophage subsets in the heart. The heart primarily contains chemokine receptor-2 (CCR2<sup>-</sup>) tissue-resident macrophages in the steady state, during neonatal heart regeneration, and in adoptive remodeling. There is a shift towards CCR2<sup>+</sup> macrophage expansion in sterile injury, myocarditis, chronic heart failure, and aging. Key features of each subset are highlighted in the colored corresponding boxes. AngII/PE, angiotensin II and phenylephrine (created with BioRender.com)



## Immune Response to Myocardial Injury

A diverse array of insults including ischemia, hypertension, autoimmunity, genetic mutations, chemical toxicity, and infection may lead to cardiac injury, cell death, and resultant inflammation. We define necroinflammation as the immune response to necrosis in a living organism. The coupling of cell death to inflammation and the immune systems response is referred to as necroinflammation [30]. Necrosis and programmed forms of necrotic cell death (ferroptosis, necroptosis, pyroptosis) promote release of intracellular materials that are recognized by immune cells and initiate inflammation. Collectively, these substances are referred to as alarmins, damage-associated molecular patterns (DAMPs), or pathogen-associated molecular patterns (PAMPs) depending on whether they are released by host cells or pathogens [31, 32] (Fig. 2). DAMPs and PAMPs consist of lipids, proteins, carbohydrates, or nucleic acids and are recognized by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs).

Within the heart, ferroptosis and necroptosis have been implicated as major forms of cardiomyocyte cell death [33]. There is increasing recognition that cardiomyocyte ferroptosis is a common form of cell death and is causally linked with myocardial inflammation and neutrophil recruitment [34]. The exact mediators (alarmins, DAMPs) released by ferroptotic cardiomyocytes remain an open area of investigation. Regardless, TLR4 and TLR9 appear to serve as PRRs, which are expressed on CRMs and CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages [34–36]. TLR signaling in CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages triggers downstream gene expression of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, and tumor necrosis factor (TNF) as well as chemokines such as CC-chemokine ligand 2 (CCL2), CCL3, CCL5, and CCL17 that are associated with negative inotropic effects, left ventricular (LV) dysfunction, and LV remodeling [37–42]. Cytokines and chemokines directly activate endothelial cells, recruit circulating monocytes and neutrophils from the bloodstream, and direct their extravasation into the myocardium. The role of TLR signaling in CRMs remains to be defined.

Acute myocardial injury triggers a dramatic shift in the cardiac immune landscape (Fig. 2). Loss of CRMs in conjunction with infiltration of neutrophils, monocytes, monocyte-derived macrophages, and dendritic cells has been observed in response to multiple forms of cardiac injury including permanent myocardial infarction, reperfused myocardial infarction, diphtheria toxin-mediated cardiomyocyte ablation, transverse aortic constriction, angiotensin II and phenylephrine infusion, and myocarditis [8, 9, 43–48]. Neutrophil infiltration and monocyte recruitment are initiated by CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages

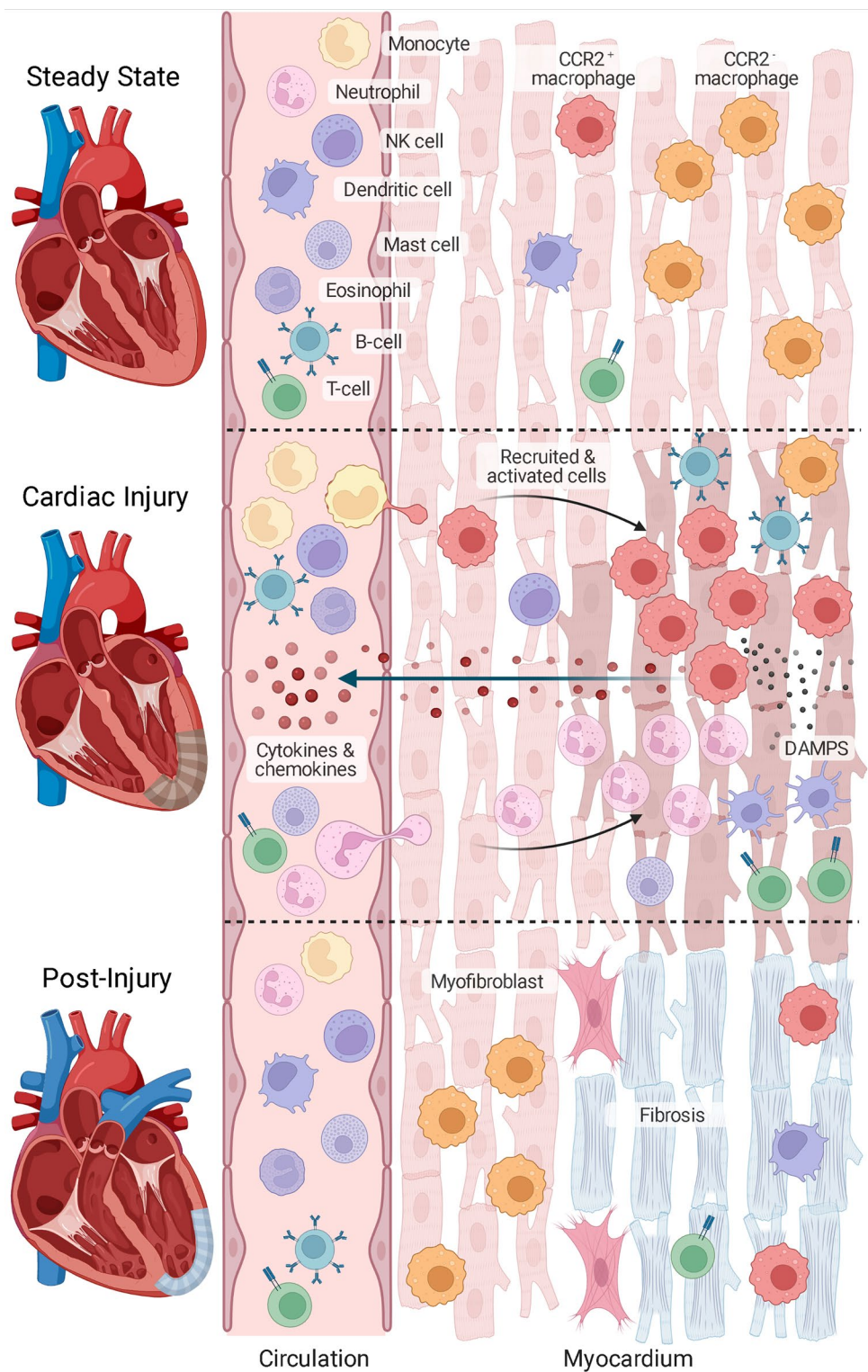
within the heart as depletion of this population results in marked reductions in neutrophil extravasation and monocyte recruitment [6, 36]. Following ischemia reperfusion injury, CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages generate and secrete multiple chemokines (CCL2, CCL7, CXCL2, CXCL5) and cytokines (TNF, IL-1 $\beta$ ) important for peripheral leukocyte recruitment. Each of these factors is dependent on myeloid differentiation primary response 88 (MYD88) signaling, an important mediator of TLR activation. CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages also generate and secrete CCL17, a pro-inflammatory factor that drives inflammation, myocardial fibrosis, and LV remodeling. CCL17 expression is triggered by granulocyte–macrophage colony-stimulating factor (GM-CSF) signaling and functions to prevent regulatory T-cell recruitment, highlighting the importance of crosstalk between macrophages and T-cells [42].

Intriguingly, CRMs appear to suppress neutrophil and monocyte recruitment through a mechanism that remains to be defined. In addition to their anti-inflammatory effects, CRMs also regulate lymphangiogenesis, a process that may be important for the resolution of inflammation [49]. Importantly, selective depletion of CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages or CRMs results in divergent effects on LV systolic function and cardiac remodeling following tissue injury highlighting the significance of cardiac macrophages in disease [6, 24••].

## Plasticity and Diversification of Monocytes and Monocyte-Derived Cells

Monocytes display extraordinary plasticity and may differentiate into a variety of cell types [8, 15–17]. Recent advances in single-cell RNA sequencing technologies have made it possible to resolve the myriad of cell states and phenotypes acquired by monocytes as they enter and expand within the injured myocardium. Following myocardial infarction, at least 7 morphologically and transcriptionally distinct subsets of monocytes, macrophages, and dendritic cells have been identified within the myocardium [6, 11•, 50]. The majority of these cell types are unique to the injured heart, highlighting how the cardiac immune landscape is reshaped by injury and disease. The exact function of these populations and mechanisms responsible for their differentiation and longevity is under active investigation. It is likely that monocyte fate decisions are heavily influenced by cues derived from their local environment. For example, monocytes that enter the infarct and peri-infarct zones are exposed to differing environments such as tissue hypoxia and oxidative intermediates, respectively [11•, 51]. Consistent with this premise, arginase 1 (Arg1)<sup>+</sup> macrophages are located within the infarct, while interferon-activated macrophages are found within the border zone [6].

**Fig. 2** Cardiac immune cells at steady state, following cardiac injury, and post-injury. In the steady state, the heart primarily contains chemokine receptor-2 ( $CCR2^{-}$ ) tissue-resident macrophages (CRMs) with few other immune cells including dendritic cells and T-cells. Activation of the cardiac immune response following cardiac injury begins with innate immune cells binding damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs). Pro-inflammatory cytokines and chemokines are released into the bloodstream, leading to recruitment and activation of  $CCR2^{+}$  major histocompatibility complex (MHC)-II<sup>hi</sup>, monocytes, and neutrophils, which then triggers activation of the adaptive immune system by recruiting B-cells and T-cells to the damaged myocardium. Additional immune cells such as mast cells, eosinophils, and natural killer (NK) cells may also be recruited to the site of injury. Finally, in a process that is dependent on CRMs, the post-injury heart undergoes remodeling through myofibroblast-generated extracellular matrix that is required for heart repair (created with BioRender.com)



Flow cytometry, immunostaining, and single-cell RNA sequencing studies have uncovered that the human heart harbors monocyte and macrophage populations that are functionally analogous to those found in the mouse heart. Under steady state conditions, the human heart contains

$CCR2^{-}LYVE1^{+}$  CRMs and  $CCR2^{+}MHC-II^{hi}$  macrophages with transcriptional profiles and inflammatory potential that resemble their mouse counterparts [7]. The abundance of  $CCR2^{+}MHC-II^{hi}$  macrophages is associated with poor outcomes including persistent LV dysfunction following

mechanical unloading. More recently, single-cell RNA sequencing of healthy human and heart failure tissue has revealed that the immune landscape of the human heart is also reshaped by disease. Chronic heart failure was associated with loss of CRMs and emergence of several monocyte and monocyte-derived macrophage populations [3•].

## Aging

Inflammaging is a phenomenon characterized by upregulation of the basal inflammatory response that is associated with aging [52]. As such, the cardiac immune landscape changes with aging, which is most notable with a decrease in CRM replication and a concomitant increase in pro-inflammatory CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages [53]. In addition to cardiac macrophage population changes, there is an increase in basal circulating pro-inflammatory cytokines (TNF- $\alpha$ ) and chemokines (CCL2), reflective of dysregulation of the innate immune system associated with aging [54, 55]. Research in clonal hematopoiesis reflects the recognition of the intersection between inflammaging and the expansion of mutated hematopoietic stem cell clones, reshaping the cardiac immune landscape with mutated pro-inflammatory myeloid cells [56]. In a mouse model of clonal hematopoiesis, ten-eleven translocation 2–mutant (Tet2)-deficient mice demonstrated cardiac expansion of CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages but not CRMs, which exhibited an inflammatory transcriptome profile in conjunction with age-related cardiac dysfunction [57].

In line with inflammaging, cardiac immunosenescence is the process by which the immune system deteriorates with aging. Macrophages have impaired phagocytic function as well as decreased polarization (pro- or anti-inflammatory) when exposed to pathogens [58, 59]. In addition, studies have shown impaired TLR expression and function in innate immune cells [60]. Collectively, inflammaging, clonal hematopoiesis, and immunosenescence within cardiac immune cells contribute to the notion that age is a risk factor in cardiovascular disease. The precise cell states associated with aging are incompletely understood and a topic of ongoing interest. Single-cell omic studies would be highly informative to clarify if cell states associated with aging are unique or reminiscent of those seen in acute or chronic inflammation.

## Non-invasive Imaging of the Cardiac Immune Landscape

A key limitation of studying human cardiac immune cells is the reliance on myocardial specimens and biases imposed by selectively studying individuals undergoing endomyocardial

biopsy, left ventricular assist device implantation, and/or heart transplantation. Molecular imaging offers a non-invasive solution to investigate cardiac immune cell composition, the natural history of inflammation, and establish associations between particular immune subsets and relevant patient outcomes. We have developed a positron emission tomography radiotracer that allosterically binds to CCR2, <sup>68</sup>Ga-DOTA [1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid]-ECL1i [extracellular loop 1 inverso]. <sup>68</sup>Ga-DOTA-ECL1i PET/CT readily visualizes CCR2<sup>+</sup> monocytes and macrophages in various cardiac injury mouse models [61]. Recently, we have translated this tracer for human investigation in pulmonary fibrosis [62]. We are currently assessing its utility in imaging patients that suffered an acute myocardial infarction. We envision that these studies will provide key information pertaining to the natural history of CCR2<sup>+</sup> monocyte and macrophage accumulation following human myocardial infarction and identify imaging features associated with clinical outcomes. Similar strategies are being developed to image activated fibroblast (FAPI-46), neutrophils, T-cells, and CRMs [63, 64].

## New Frontiers of Cardio-immunology

As the cardiac immune landscape continues to be defined within the confines of established cardiac injury models (myocardial infarction, pressure overload, viral myocarditis), it is imperative to also consider emerging disease entities with profound effects on survival and quality of life. For instance, immunotherapy continues to revolutionize the oncology field as it holds the potential to control and possibly cure previously untreatable cancers. However, immune checkpoint inhibitors, such as programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), are associated with myocarditis [65, 66]. Established immune checkpoint inhibitors function by blocking inhibitory receptor-ligand interactions to augment T-cell-mediated killing of cancer cells. Based on the success of these agents, there is an explosion of new immune checkpoint therapeutics, such as cluster of differentiation (CD40) and CD134 (also known as OX40) agonist antibodies and lymphocyte activating 3 (Lag3) neutralizing antibodies [67–71]. These therapies are expected to further enhance T-cell responses and augment activation of antigen presenting cells (macrophages, dendritic cells). The potential for immune checkpoint therapies to reshape the cardiac immune landscape is clear. Understanding the impact of checkpoint therapies on cardiac immune cell composition and function is needed to understand short-term and long-term toxicities as well as ramification on cardiac homeostasis and disease progression.

Recent work has established a mouse model that recapitulates checkpoint inhibitor-associated myocarditis, which demonstrated macrophage and T-cell myocardial infiltration that was associated with premature death [45]. It is unclear how immune checkpoint inhibitors promote macrophage and T-cell expansion and how these immune populations might differ from those found in the infarcted heart. In addition, the relevant contribution of T-cells and macrophages to myocarditis and the precise effector molecules involved remain to be defined. A detailed understanding of these mechanisms will be required to adequately treat this devastating disease without adversely affecting anti-tumor responses and long-term tumor control. Finally, little is understood regarding the chronic effects of immune checkpoint inhibitors on cardiac homeostasis, function, and aging. These examples highlight the clinical utility and importance of the growing field of cardio-immunology.

## Conclusion

Cardiac injury activates a complex inflammatory response comprised of the innate and adaptive immune systems. In the heart, CRMs are the most abundant immune cell in the steady state. However, following myocardial injury, the immune landscape shifts as a result of monocyte infiltration, diversification, and expansion of pro-inflammatory macrophage populations including CCR2<sup>+</sup>MHC-II<sup>hi</sup> macrophages. Single-cell technologies and downstream functional studies have allowed tremendous progress in defining the immune landscape of the heart and identifying therapeutic targets. Understanding the mechanistic basis for necroinflammation, monocyte infiltration, and fate decisions necessary for the specification of inflammatory populations is crucial to developing new therapies that may improve patient outcomes.

**Author Contribution** JJ and KJL conceptualized, wrote, and edited the manuscript and figures.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflicts of interest.

**Human and Animal Rights and Informed Consent** All reported studies/experiments with human or animal subjects performed by the authors

have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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