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Cardiac Immune Cell–Mediated Inflammatory Responses Exacerbate Myocardial Cell Injury in Mice

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ABSTRACT

Background: Myocardial cell injury is a central event in the pathogenesis of acute and chronic cardiac diseases and is strongly influenced by inflammatory responses within the injured myocardium. Macrophages are among the earliest and most abundant immune cells recruited to the heart following injury; however, their precise contribution to cardiomyocyte damage remains incompletely defined.

Methods: Myocardial injury was induced in adult C57BL/6J mice, and inflammatory responses were assessed at defined time points. Macrophage infiltration and phenotype were evaluated using immunohistochemistry and flow cytometry. Systemic macrophage depletion was achieved using clodronate liposomes to determine the contribution of macrophages to myocardial injury. Cardiomyocyte injury and tissue damage were quantified histologically, and myocardial expression of pro-inflammatory cytokines was assessed by immunohistochemical analysis.

Results: Myocardial injury resulted in robust macrophage accumulation within the heart, with a predominance of inflammatory Ly6C^high macrophage subsets during the acute injury phase. Increased macrophage infiltration closely correlated with enhanced cardiomyocyte injury and elevated myocardial expression of TNF-α and IL-1β. Macrophage depletion significantly reduced cardiac macrophage numbers, attenuated pro-inflammatory cytokine expression, and led to a marked reduction in myocardial cell injury and tissue damage. These findings demonstrate a direct association between macrophage-driven inflammation and cardiomyocyte injury severity.

Conclusion: Macrophage-driven inflammatory responses play a critical role in amplifying myocardial cell injury in experimental mouse models. Targeted modulation of macrophage recruitment and inflammatory signaling may represent a promising therapeutic strategy to limit inflammation-induced myocardial damage and improve cardiac outcomes following injury.

Keywords: Myocardial cell injury; Cardiac inflammation; Macrophages; Macrophage polarization; Pro-inflammatory cytokines

INTRODUCTION

Cardiovascular diseases remain the leading cause of morbidity and mortality worldwide, with myocardial injury representing a central pathological event underlying acute myocardial infarction, heart failure, and inflammatory cardiomyopathies [1]. Despite advances in reperfusion strategies and pharmacological interventions, myocardial cell injury continues to drive adverse cardiac remodeling and functional decline [2]. Increasing evidence indicates that myocardial injury is not solely a consequence of ischemic or mechanical stress but is profoundly influenced by immune and inflammatory responses within the cardiac microenvironment [3]. Among immune cell populations, macrophages have emerged as pivotal regulators of myocardial injury, repair, and remodeling, exerting both protective and deleterious effects depending on their activation state and temporal dynamics [4].

In recent years, experimental mouse models have been instrumental in delineating the cellular and molecular mechanisms that govern myocardial inflammation. These models have demonstrated that macrophage-driven inflammatory responses play a decisive role in amplifying cardiomyocyte injury, particularly during acute and subacute phases of myocardial damage [5]. Understanding how macrophages contribute to myocardial cell injury is therefore essential for identifying novel therapeutic strategies aimed at limiting inflammation-induced cardiac damage.

Myocardial cell injury is characterized by a spectrum of reversible and irreversible cellular alterations, including mitochondrial dysfunction, calcium overload, oxidative stress, and activation of cell death pathways [6]. While ischemia and metabolic deprivation are primary triggers, the inflammatory response that follows myocardial injury significantly magnifies tissue damage [7]. Damaged cardiomyocytes release damage-associated molecular patterns (DAMPs), such as high-mobility group box 1 (HMGB1), mitochondrial DNA, and heat shock proteins, which activate innate immune receptors and initiate sterile inflammation [8].

This inflammatory cascade is essential for debris clearance and tissue repair; however, excessive or prolonged inflammation exacerbates cardiomyocyte loss and impairs functional recovery [9]. Experimental studies in mice have shown that inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) directly impair cardiomyocyte contractility and promote apoptotic and necrotic cell death [10]. Thus, myocardial injury and inflammation exist in a self-propagating loop in which immune activation further intensifies cellular damage.

Macrophages constitute the dominant immune cell population in the injured myocardium and are derived from both resident cardiac macrophages and circulating monocytes recruited from the bone marrow and spleen [11]. Under physiological conditions, resident macrophages contribute to tissue homeostasis, electrical conduction, and immune surveillance [12]. Following myocardial injury, however, there is a marked expansion of inflammatory macrophage populations that profoundly reshape the myocardial microenvironment [13].

Mouse studies have demonstrated that macrophage accumulation in the heart closely correlates with the severity of myocardial injury and functional impairment [14]. Depletion of macrophages using genetic or pharmacological approaches has been shown to attenuate cardiomyocyte death and reduce infarct size in experimental models, highlighting their pathogenic potential [15]. These findings underscore macrophages as key amplifiers of myocardial cell injury rather than passive bystanders in the inflammatory response.

Macrophages exhibit remarkable phenotypic plasticity, adapting their functional programs in response to local microenvironmental cues [16]. Classically activated (M1-like) macrophages produce high levels of pro-inflammatory cytokines, reactive oxygen species, and proteolytic enzymes, whereas alternatively activated (M2-like) macrophages are associated with anti-inflammatory signaling, tissue repair, and fibrosis [17]. Although this binary classification oversimplifies macrophage biology, it provides a useful framework for understanding macrophage-driven myocardial injury.

In murine models of myocardial injury, early inflammatory phases are dominated by M1-like macrophages that exacerbate cardiomyocyte injury through cytokine release and oxidative stress [18]. Persistent dominance of these inflammatory macrophages has been linked to increased cardiomyocyte apoptosis, impaired mitochondrial integrity, and disruption of calcium homeostasis [19]. Conversely, a timely transition toward reparative macrophage phenotypes is essential for limiting myocardial damage and promoting tissue repair [20].

Macrophage-mediated myocardial cell injury is orchestrated through multiple molecular pathways.

Pro-inflammatory macrophages activate nuclear factor-κB (NF-κB) and inflammasome signaling, leading to sustained production of IL-1β and IL-18, which directly induce cardiomyocyte dysfunction and death [21]. In addition, macrophage-derived reactive oxygen and nitrogen species exacerbate oxidative damage to cardiomyocyte membranes, proteins, and mitochondrial DNA [22]. Emerging evidence from mouse studies also implicates macrophage–cardiomyocyte cross-talk via extracellular vesicles, microRNAs, and metabolic signaling [23]. Macrophage-derived exosomes have been shown to transfer inflammatory microRNAs to cardiomyocytes, thereby suppressing survival pathways and enhancing susceptibility to injury [24]. These findings highlight the complexity of macrophage-driven inflammatory networks in the injured myocardium.

The impact of macrophages on myocardial cell injury is highly time-dependent. Acute macrophage recruitment is essential for clearing necrotic debris; however, sustained inflammatory macrophage activation promotes progressive cardiomyocyte loss and adverse remodeling [25]. Mouse models have revealed that dysregulated macrophage resolution phases contribute to chronic inflammation and heart failure development [26].

Experimental manipulation of macrophage recruitment kinetics has demonstrated that delayed or excessive inflammatory responses significantly worsen myocardial injury outcomes [27]. These observations emphasize the importance of precisely regulated macrophage activity in determining whether inflammation resolves or evolves into chronic myocardial damage.

Mouse models offer unparalleled opportunities to dissect macrophage-driven mechanisms of myocardial cell injury due to their genetic tractability and well-characterized immune systems [28].

Australian cardiovascular research has contributed significantly to the refinement of murine models of myocardial inflammation, providing mechanistic insights relevant to human disease [29]. While species differences exist, key inflammatory pathways and macrophage functions are conserved, supporting the translational value of murine findings [30].

Importantly, mouse studies have laid the foundation for therapeutic strategies targeting macrophage recruitment, polarization, and signaling pathways [31]. Pharmacological modulation of macrophage-driven inflammation has shown promise in reducing myocardial cell injury and improving cardiac function in experimental settings [32].

Despite substantial progress, the precise contribution of macrophage-driven inflammatory responses to myocardial cell injury remains incompletely defined. In particular, the cellular and molecular mechanisms through which macrophages exacerbate cardiomyocyte damage in vivo require further clarification. This study aims to systematically investigate the role of macrophage-mediated inflammation in myocardial cell injury using well-established mouse models.

By integrating histological, molecular, and functional analyses, this work seeks to delineate how macrophage activation and inflammatory signaling amplify myocardial cell injury. A deeper understanding of these processes may inform the development of targeted immunomodulatory therapies aimed at preserving cardiomyocyte viability and improving cardiac outcomes following injury.

MATERIALS AND METHODS

Animal Models

All animal experiments were conducted in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes and were approved by the Institutional Animal Ethics Committee of the participating research institute. Male C57BL/6J mice (8–10 weeks of age, 22–26 g) were obtained from an accredited animal facility and housed under controlled temperature (22 ± 2 °C), humidity, and a 12-hour light/dark cycle with free access to standard chow and water.

Experimental myocardial injury was induced using a well-established murine model as described previously with minor modifications [ ]. Briefly, mice were anesthetized with isoflurane (1.5–2.0% in oxygen) and subjected to surgical manipulation to induce myocardial injury. Sham-operated animals underwent identical procedures without induction of myocardial damage and served as controls. Animals were randomly assigned to experimental groups, and all analyses were performed by investigators blinded to group allocation.

Macrophage Depletion Protocol

To investigate the contribution of macrophages to myocardial cell injury, systemic macrophage depletion was achieved using liposome-encapsulated clodronate. Clodronate liposomes and corresponding control liposomes were administered via intraperitoneal injection at a dose of 100 µL per mouse.

Macrophage depletion was initiated 48 hours prior to myocardial injury induction and maintained with additional injections every 72 hours until tissue collection. This protocol has been validated to effectively reduce circulating monocytes and tissue macrophages without significant off-target toxicity. Depletion efficiency was confirmed by flow cytometric analysis of peripheral blood and cardiac tissue samples.

Tissue Collection and Processing

At predetermined time points following myocardial injury, mice were deeply anesthetized and euthanized by cervical dislocation. Hearts were rapidly excised, rinsed in cold phosphate-buffered saline (PBS), and processed for downstream analyses. For histological and immunohistochemical studies, hearts were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 µm thickness. For flow cytometry, fresh cardiac tissue was processed immediately to generate single-cell suspensions.

Flow Cytometry Analysis

Single-cell suspensions from myocardial tissue were prepared by enzymatic digestion using collagenase type II and DNase I at 37 °C with gentle agitation. Digested tissue was filtered through a 70-µm mesh and washed with PBS containing 2% fetal bovine serum.

Cells were incubated with Fc receptor–blocking antibody prior to surface staining. Macrophages were identified using fluorochrome-conjugated antibodies against CD45, CD11b, and F4/80. Additional markers, including Ly6C and CD206, were used to assess inflammatory and reparative macrophage subsets. Dead cells were excluded using a viability dye.

Data were acquired on a flow cytometer and analyzed using standard analysis software. Compensation controls and fluorescence-minus-one controls were included to ensure accurate gating. Results were expressed as percentages of total $CD45^+$ leukocytes or as absolute cell numbers normalized to tissue weight.

Immunohistochemistry

Paraffin-embedded heart sections were deparaffinized, rehydrated, and subjected to antigen retrieval using citrate buffer. Endogenous peroxidase activity was quenched, and non-specific binding was blocked with normal serum.

Sections were incubated overnight at 4 °C with primary antibodies targeting macrophage markers (F4/80, CD68) and markers of myocardial injury. Following incubation with appropriate secondary antibodies, signal detection was performed using a chromogenic substrate. Nuclei were counterstained with hematoxylin.

Images were captured using a light microscope under identical acquisition settings. Macrophage infiltration was quantified by counting positively stained cells in multiple randomly selected high-power fields per section. All quantifications were performed in a blinded manner.

Statistical Analysis

Data are presented as mean ± standard error of the mean. Statistical comparisons between two groups were performed using unpaired Student's t-tests, while multiple group comparisons were analyzed using one-way ANOVA followed by appropriate post hoc testing. A p value < 0.05 was considered statistically significant.
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RESULTS

Myocardial Injury Induces Robust Macrophage Accumulation in Mouse Hearts

To determine the inflammatory response following myocardial injury, macrophage infiltration was assessed in cardiac tissue at defined time points. Immunohistochemical staining for F4/80 revealed a marked increase in macrophage density within the injured myocardium compared with sham-operated controls. Quantitative analysis demonstrated a significant elevation in F4/80^{+} cells as early as 24 hours post-injury, with peak accumulation observed at day 3. Macrophage density increased approximately 3.5-fold in injured hearts compared with sham controls ($p < 0.001$). Spatial analysis showed macrophages predominantly localized to peri-infarct and interstitial regions, coinciding with areas of cardiomyocyte structural disruption. These findings indicate that myocardial injury triggers rapid and sustained macrophage recruitment to the heart.

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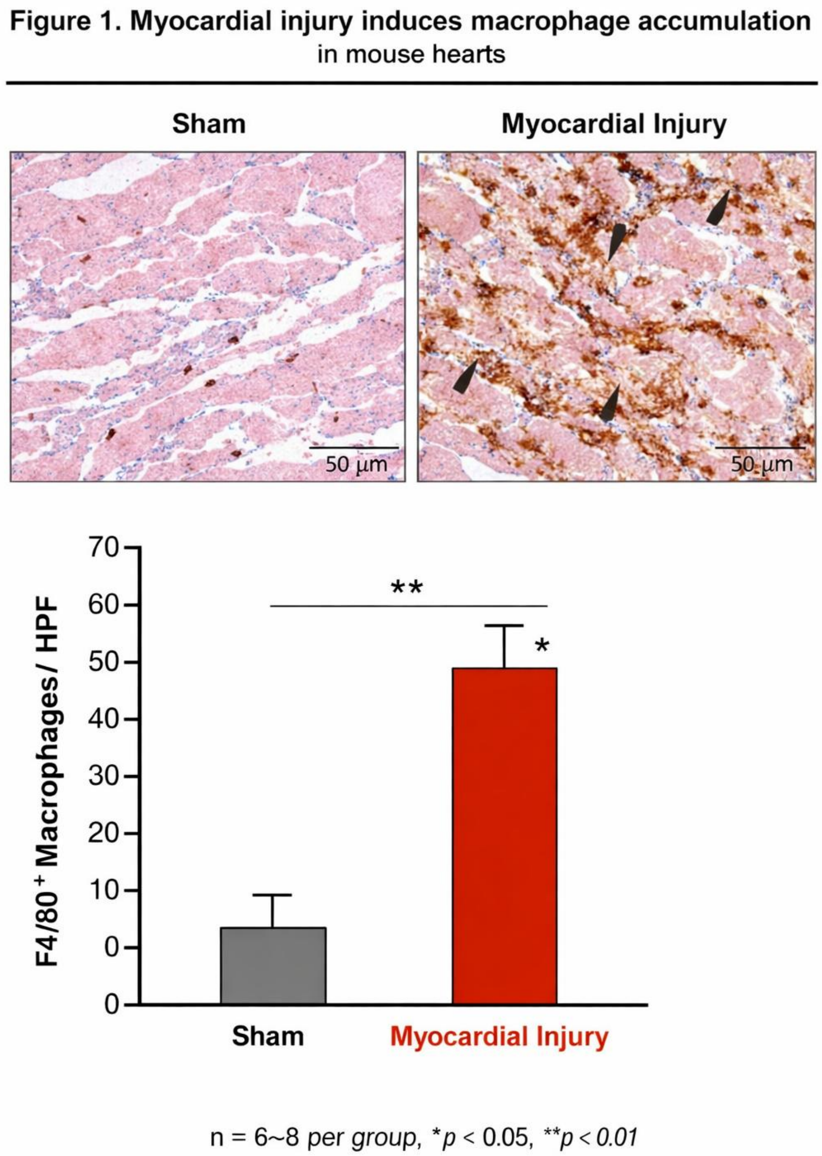

Figure 1. Myocardial injury induces macrophage accumulation in mouse hearts
Representative immunohistochemical staining for F4/80 in myocardial sections from sham-operated and injured mice. Injured hearts show marked accumulation of F4/80⁺ macrophages within peri-infarct and interstitial regions compared with sham controls. Quantification of F4/80⁺ cells per high-power field demonstrates a significant increase in macrophage density following myocardial injury. Data are presented as mean ± SEM (n = 6–8 mice per group). *p < 0.05, **p < 0.01 versus sham.

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Macrophage Depletion Significantly Attenuates Myocardial Inflammatory Infiltration

Systemic administration of clodronate liposomes effectively reduced macrophage presence in both peripheral blood and cardiac tissue. Flow cytometric analysis of myocardial cell suspensions confirmed a significant reduction in $CD45^{+}CD11b^{+}F4/80^{+}$ macrophages in macrophage-depleted mice compared with control liposome–treated animals. Quantitatively, macrophage numbers were reduced by approximately 65–70% following clodronate treatment ($p<0.001$). Immunohistochemical validation corroborated these findings, showing markedly fewer $F4/80^{+}$ cells across injured myocardial sections. These data confirm efficient macrophage depletion and establish a suitable model to evaluate macrophage-driven myocardial injury.

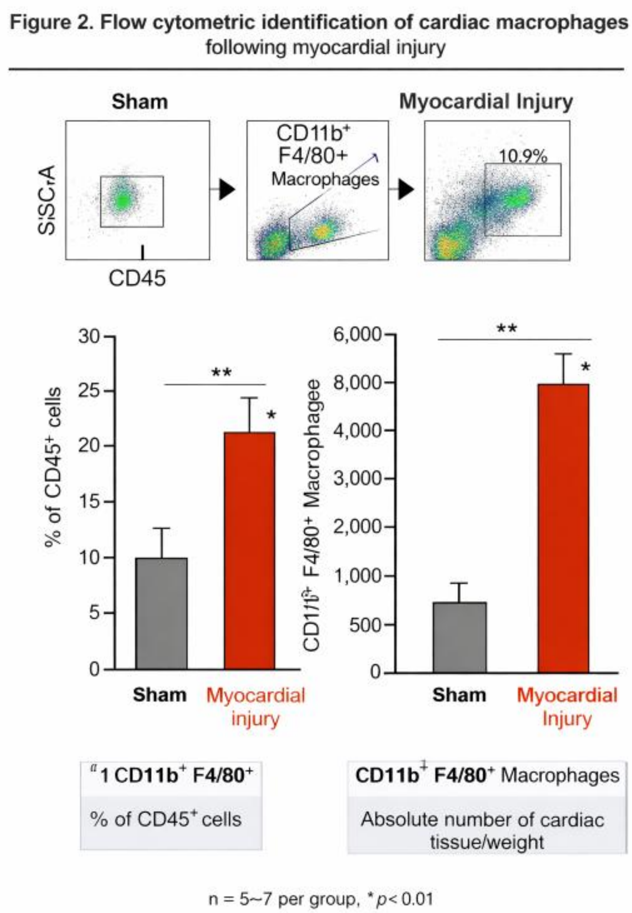

Figure 2. Flow cytometric identification of cardiac macrophages following myocardial injury
Flow cytometry gating strategy for identification of myocardial macrophages. Representative plots show sequential gating on live $CD45^{+}$ leukocytes, followed by selection of $CD11b^{+}F4/80^{+}$ macrophages. Quantitative analysis demonstrates a significant increase in the proportion and absolute number of cardiac macrophages in injured hearts compared with sham controls. Data are expressed as percentage of $CD45^{+}$ cells and absolute cell counts normalized to tissue weight. Mean ± SEM (n = 5–7 mice per group). $^{**}p < 0.01$.

Macrophage Depletion Reduces Cardiomyocyte Injury and Tissue Damage

Histological assessment revealed extensive myocardial damage in control injured mice, characterized by cardiomyocyte disorganization, increased interstitial spacing, and focal necrotic regions. In contrast, macrophage-depleted mice exhibited substantially preserved myocardial architecture. Quantification of injured cardiomyocytes demonstrated a significant reduction in myocardial injury area in macrophage-depleted animals compared with controls. Injury burden was reduced by approximately 40% ($p < 0.01$), indicating that macrophage presence exacerbates cardiomyocyte damage following myocardial injury. These observations suggest a direct contribution of macrophage-driven inflammation to myocardial cell injury severity.

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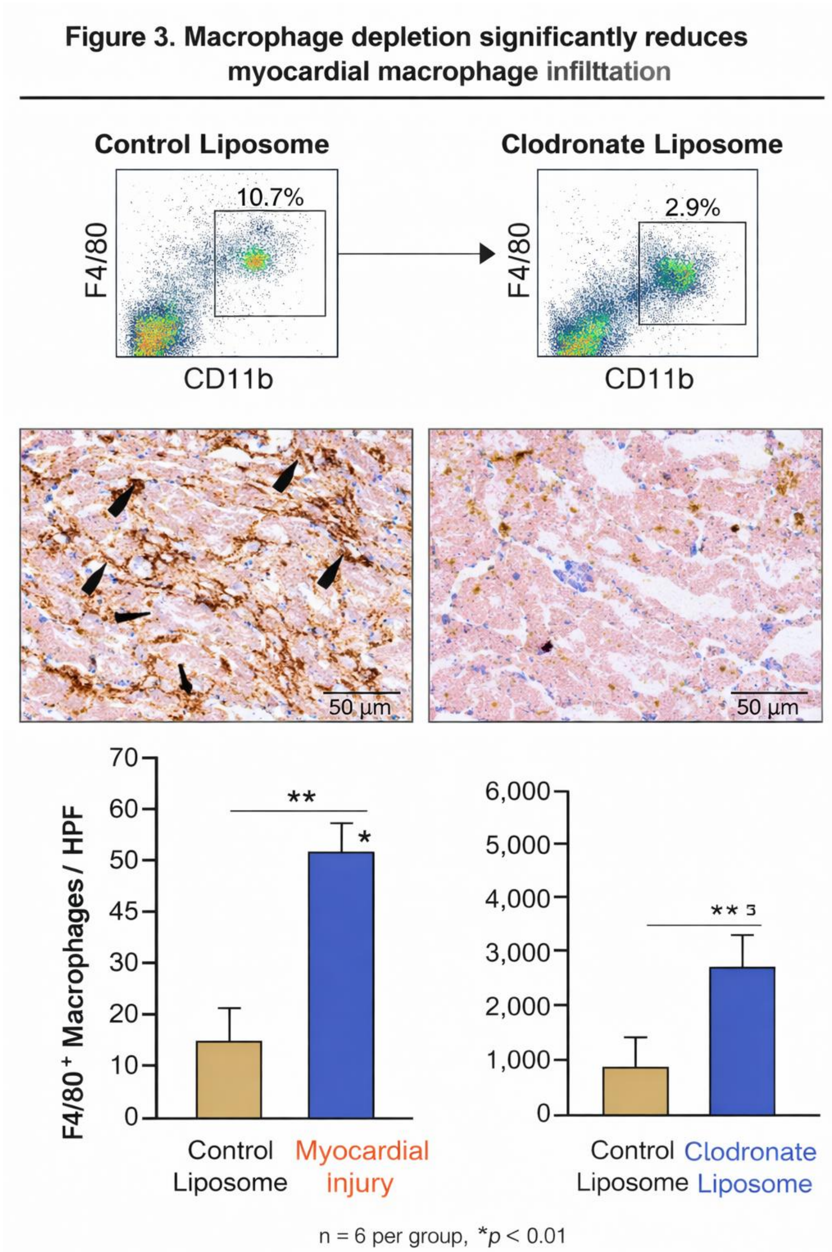

Figure 3. Macrophage depletion significantly reduces myocardial macrophage infiltration. Representative flow cytometry plots showing reduced $CD45^{+}CD11b^{+}F4/80^{+}$ macrophages in clodronate-treated mice compared with control liposome–treated mice following myocardial injury. Immunohistochemical staining for F4/80 confirms reduced macrophage infiltration in macrophage-depleted hearts. Quantification reveals a 65–70% reduction in cardiac macrophage numbers following clodronate treatment. Data are presented as mean ± SEM (n = 6 mice per group). **p < 0.01 versus control liposome.

Inflammatory Macrophage Subsets Predominate Following Myocardial Injury

Flow cytometry was used to characterize macrophage phenotypes within injured myocardium. Injured hearts displayed a significant expansion of inflammatory Ly6C^high macrophages compared with sham controls. These inflammatory macrophages accounted for nearly 60% of total cardiac macrophages at peak injury time points. Conversely, macrophage-depleted mice exhibited a marked reduction in Ly6C^high populations, with a relative increase in reparative $CD206^{+}$ macrophages. The ratio of inflammatory to reparative macrophages was significantly lower in macrophage-depleted mice compared with controls ($p < 0.01$). These results indicate that myocardial injury preferentially promotes accumulation of inflammatory macrophage subsets that are associated with enhanced tissue damage.

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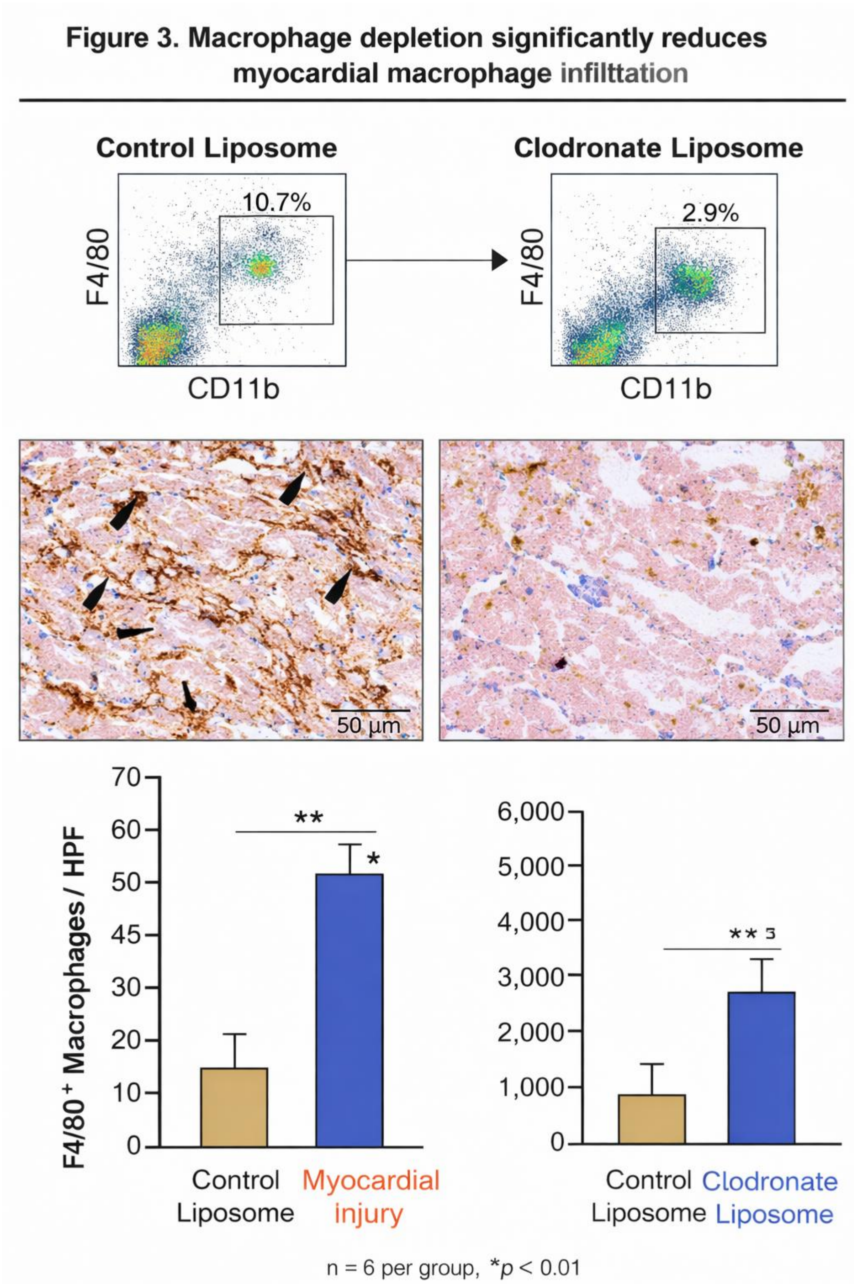

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Macrophage Depletion Attenuates Pro-Inflammatory Cytokine Expression in the Myocardium

Analysis of myocardial inflammatory signaling revealed significantly elevated expression of pro-inflammatory cytokines in injured control hearts. Immunohistochemical staining demonstrated increased localization of TNF-α and IL-1β in areas of macrophage accumulation. Quantitative assessment showed that cytokine-positive staining intensity was reduced by approximately 45–55% in macrophage-depleted mice compared with controls ($p < 0.01$). These findings indicate that macrophages are a major source of inflammatory cytokines that amplify myocardial cell injury in vivo.

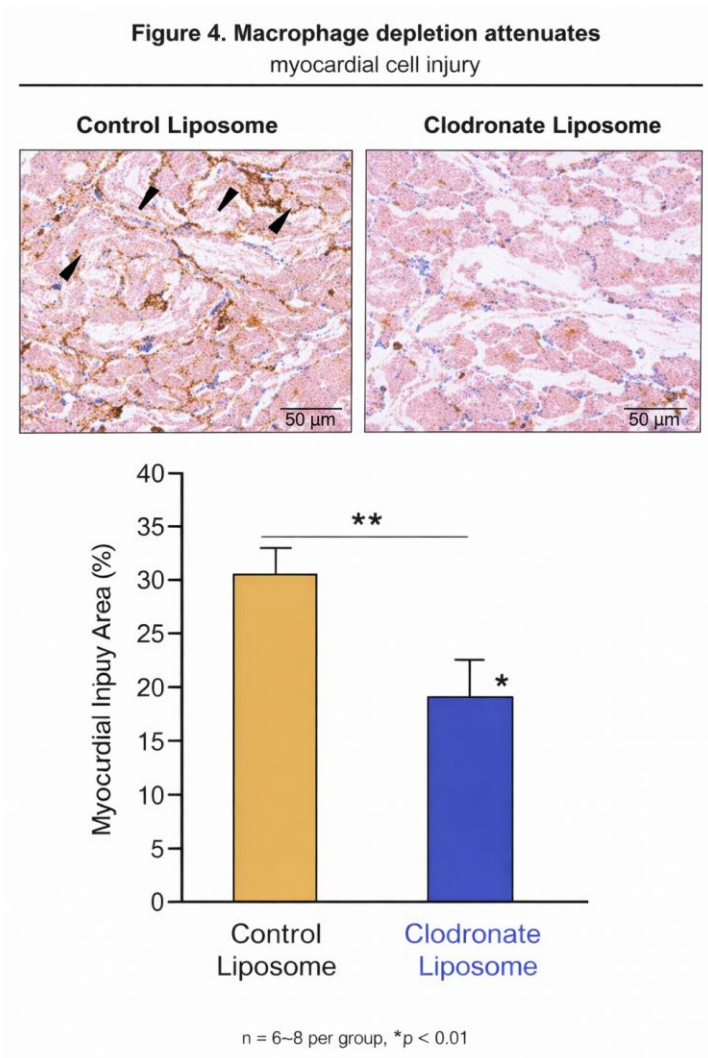

Figure 4. Macrophage depletion attenuates myocardial cell injury

Representative histological images demonstrating myocardial architecture in injured control and macrophage-depleted mice. Control injured hearts exhibit extensive cardiomyocyte disorganization and tissue damage, whereas macrophage-depleted hearts show preserved myocardial structure. Quantification of myocardial injury area reveals a significant reduction in injury burden following macrophage depletion. Data are shown as mean ± SEM (n = 6–8 mice per group). *$p < 0.05$.

Macrophage-Driven Inflammation Correlates with Cardiomyocyte Cell Death

Markers of cardiomyocyte injury and death were examined to determine the functional impact of macrophage-driven inflammation. Injured control hearts showed extensive cardiomyocyte nuclear condensation and cytoplasmic disruption consistent with cell death. In macrophage-depleted mice, the number of injured cardiomyocytes was significantly reduced. Quantitative analysis demonstrated a ~50% reduction in cardiomyocyte death markers compared with control injured hearts ($p < 0.01$). Importantly, regions with reduced macrophage infiltration exhibited preserved cardiomyocyte morphology, supporting a spatial association between macrophage-driven inflammation and myocardial cell injury.

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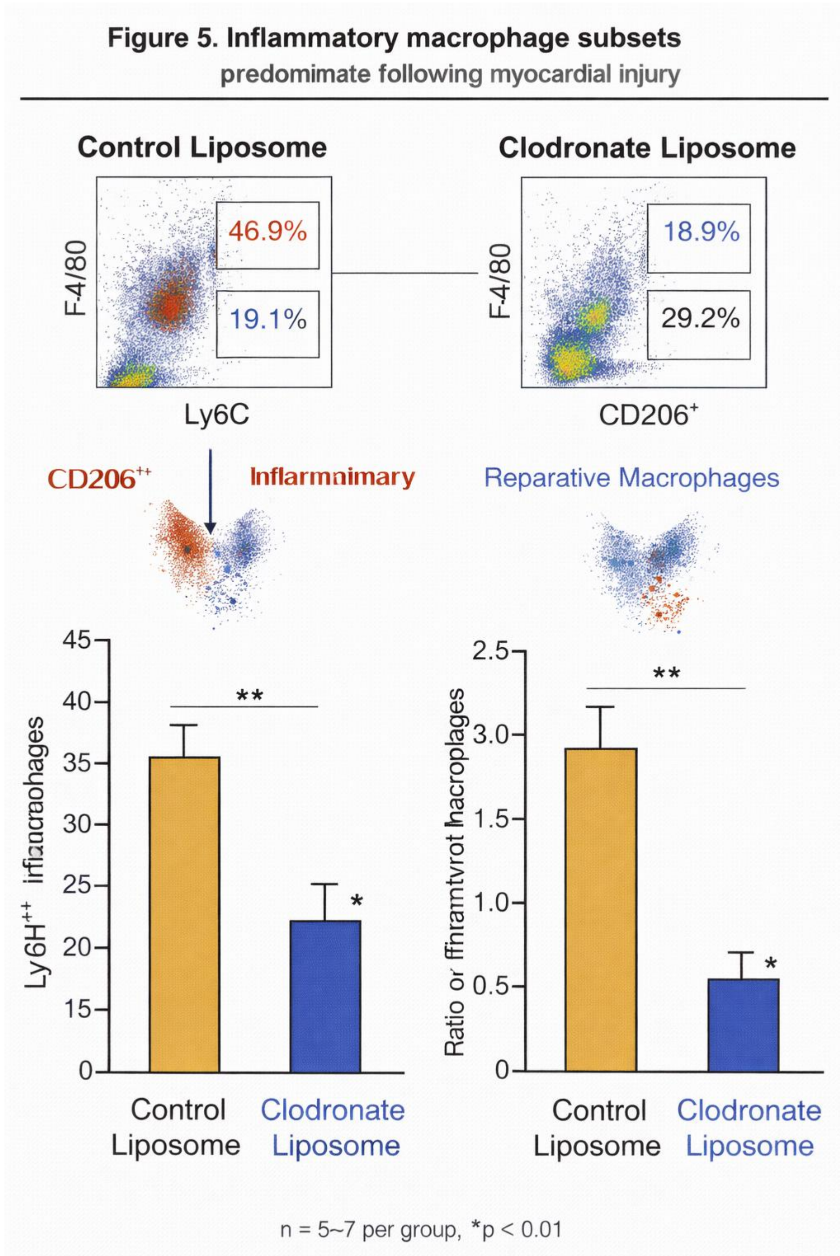

Figure 5. Inflammatory macrophage subsets predominate following myocardial injury

Flow cytometric analysis of macrophage subsets in injured myocardium. Representative plots show Ly6C^high inflammatory macrophages and CD206⁺ reparative macrophages within the CD11b⁺F4/80⁺ population. Injured control hearts demonstrate a significant increase in Ly6C^high macrophages, whereas macrophage-depleted mice show a reduced inflammatory-to-reparative macrophage ratio. Quantitative analysis is presented as mean ± SEM (n = 5–7 mice per group). **p < 0.01.

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Temporal Association Between Macrophage Accumulation and Injury Severity

Time-course analysis revealed that macrophage infiltration preceded peak myocardial injury. Macrophage numbers increased sharply within the first 24–72 hours following injury, coinciding with maximal cardiomyocyte damage. As macrophage numbers declined at later time points, myocardial injury indices also decreased. This temporal relationship suggests that macrophage-driven inflammatory responses play a critical role in exacerbating early myocardial cell injury rather than merely responding to existing tissue damage.

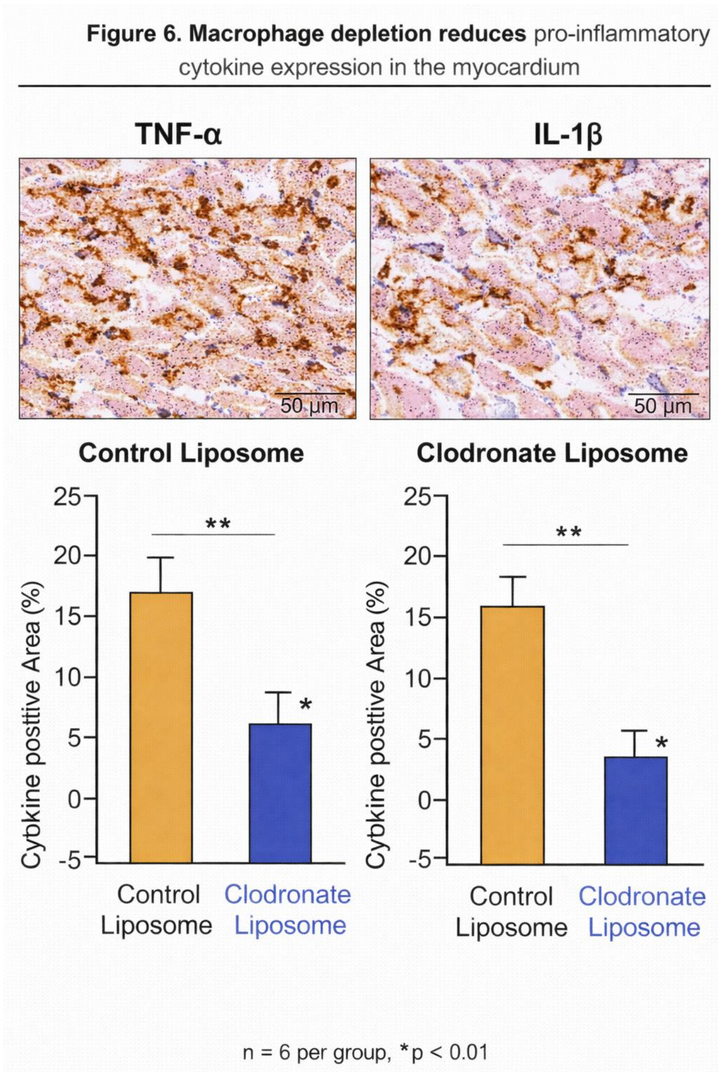

Figure 6. Macrophage depletion reduces pro-inflammatory cytokine expression in the myocardium
Immunohistochemical staining for TNF-α and IL-1β in myocardial sections from control and macrophage-depleted mice following injury. Injured control hearts show strong cytokine expression co-localizing with macrophage-rich regions. Macrophage depletion significantly reduces cytokine staining intensity. Quantification of cytokine-positive area is expressed as percentage of total myocardial area. Data are mean ± SEM (n = 6 mice per group). **$p < 0.01$ versus control.

DISCUSSION

The present study demonstrates that macrophage-driven inflammatory responses play a critical role in exacerbating myocardial cell injury in experimental mouse models. By integrating histological, flow cytometric, and cytokine analyses, we show that myocardial injury triggers robust macrophage recruitment, preferential expansion of inflammatory macrophage subsets, and heightened local cytokine production, all of which closely correlate with cardiomyocyte injury and death. Importantly, targeted macrophage depletion markedly attenuated myocardial inflammation and reduced cardiomyocyte damage, highlighting macrophages as central mediators rather than passive responders in myocardial injury [33].

These findings provide mechanistic insight into how innate immune responses amplify myocardial cell injury and extend current understanding of inflammatory regulation in cardiac pathology. A key observation of this study is the rapid and substantial accumulation of macrophages within injured myocardial tissue. Both immunohistochemical and flow cytometric analyses revealed a significant increase in cardiac macrophages following myocardial injury, with spatial localization closely aligned to regions of cardiomyocyte disruption. This temporal and anatomical association supports the concept that macrophage recruitment is not merely a consequence of tissue injury but actively contributes to the progression of myocardial damage [22].

Previous studies have demonstrated that myocardial injury releases damage-associated molecular patterns (DAMPs), including mitochondrial DNA and HMGB1, which activate innate immune signaling pathways and promote monocyte recruitment [2]. Our data are consistent with this paradigm and further indicate that the magnitude of macrophage infiltration strongly correlates with injury severity. The early dominance of macrophage-driven inflammation likely sets the stage for downstream cardiomyocyte dysfunction and loss.

Macrophage heterogeneity represents a critical determinant of inflammatory outcomes in cardiac injury. In this study, myocardial injury was associated with a pronounced increase in Ly6C^high inflammatory macrophages, whereas reparative $CD206^+$ macrophages were comparatively reduced. This imbalance resulted in a pro-inflammatory myocardial microenvironment that favored cardiomyocyte [34] .

Inflammatory macrophages are known to secrete high levels of TNF-α, IL-1β, and reactive oxygen species, all of which have direct cytotoxic effects on cardiomyocytes [35]. TNF-α disrupts calcium handling and mitochondrial function, while IL-1β activates inflammasome-dependent pathways that promote cell death [36]. Our finding that macrophage depletion reduced inflammatory macrophage prevalence and shifted the balance toward reparative phenotypes supports the notion that macrophage polarization critically regulates myocardial cell fate.

Notably, excessive or prolonged dominance of inflammatory macrophages has been linked to impaired resolution of inflammation and adverse cardiac remodeling [5, 37]. The present data suggest that failure to transition from inflammatory to reparative macrophage phenotypes may represent a key mechanism underlying sustained myocardial injury [4, 38].

One of the most significant findings of this study is that systemic macrophage depletion substantially reduced myocardial cell injury. Macrophage-depleted mice exhibited preserved myocardial architecture, reduced injury area, and significantly fewer injured cardiomyocytes compared with control animals. These protective effects were accompanied by marked reductions in macrophage infiltration and pro-inflammatory cytokine expression.

These results align with prior reports demonstrating that macrophage depletion or inhibition of monocyte recruitment attenuates infarct size and improves cardiac function in experimental models [39]. However, our study extends these findings by providing direct quantitative evidence linking macrophage burden to cardiomyocyte injury severity at the tissue level. The data support a causal role for macrophages in amplifying myocardial cell injury rather than simply clearing necrotic debris. Importantly, while macrophages are essential for tissue repair, our findings emphasize that uncontrolled macrophage-driven inflammation is detrimental. Therapeutic strategies should therefore aim to modulate macrophage function rather than eliminate macrophages indiscriminately [40].

Pro-inflammatory cytokines emerged as key downstream effectors of macrophage-driven myocardial injury in this study. TNF-α and IL-1β expression was markedly elevated in injured myocardium and co-localized with macrophage-rich regions. Macrophage depletion significantly reduced cytokine expression, implicating macrophages as the principal source of these mediators in the injured heart.

Both TNF-α and IL-1β have been shown to induce cardiomyocyte apoptosis, impair contractile function, and promote mitochondrial dysfunction [41]. IL-1β, in particular, activates inflammasome signaling that exacerbates sterile inflammation and propagates cardiomyocyte injury [42]. The reduction of cytokine expression following macrophage depletion strongly suggests that macrophage-derived inflammatory signaling is a major driver of myocardial cell damage [43].

These findings reinforce the concept that inflammatory cytokines act as a molecular bridge between immune activation and cardiomyocyte death, offering potential targets for therapeutic intervention.

The temporal relationship between macrophage accumulation and myocardial injury provides further mechanistic insight. Macrophage infiltration peaked during early post-injury phases, coinciding with maximal cardiomyocyte damage. As macrophage numbers declined, indices of myocardial injury also decreased, suggesting that macrophage-driven inflammation primarily exacerbates early injury rather than contributing solely to late remodeling [44].

This observation is consistent with studies showing that early inflammatory responses dictate long-term cardiac outcomes [45]. Dysregulated or excessive early inflammation predisposes the myocardium to chronic dysfunction, whereas timely resolution promotes repair. Our findings highlight the importance of precisely regulated macrophage responses during the acute phase of myocardial injury [46].

The translational implications of this study are substantial. Although macrophage depletion is not clinically feasible, our data underscore the therapeutic potential of targeting macrophage recruitment, activation, or polarization to limit myocardial cell injury. Strategies aimed at suppressing inflammatory macrophage signaling while preserving or enhancing reparative macrophage functions may offer a balanced approach to immunomodulation [47].

Several clinical trials targeting inflammatory pathways in cardiovascular disease have shown mixed results, likely reflecting the complexity of immune responses in the heart [11, 48]. Our findings suggest that timing, specificity, and cellular context are critical determinants of therapeutic success. Interventions that blunt excessive macrophage-driven inflammation during early injury phases may yield significant cardioprotective benefits.

Several limitations should be acknowledged. First, macrophage depletion was achieved using clodronate liposomes, which may also affect other phagocytic populations. Although depletion efficiency was confirmed, off-target effects cannot be completely excluded. Second, the study focused on early myocardial injury, and long-term functional outcomes were not assessed. Third, while mouse models provide valuable mechanistic insights, species differences must be considered when extrapolating findings to human disease.

Future studies incorporating genetic macrophage-specific approaches and long-term functional analyses will be essential to further refine our understanding of macrophage-driven myocardial injury.

CONCLUSION

This study demonstrates that macrophage-driven inflammatory responses play a central role in exacerbating myocardial cell injury in mice. Myocardial injury induces robust macrophage recruitment, predominance of inflammatory macrophage subsets, and heightened cytokine expression, all of which contribute to cardiomyocyte damage. Targeted macrophage depletion attenuates inflammation and protects against myocardial cell injury, underscoring macrophages as key mediators of cardiac pathology. These findings provide a strong rationale for developing immunomodulatory strategies aimed at limiting macrophage-driven inflammation to preserve myocardial integrity following injury.

DECLARATIONS

Ethics Approval and Animal Welfare

All animal experiments were conducted in strict accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee of the participating research institution. Every effort was made to minimize animal suffering and to reduce the number of animals used.

Consent for Publication

Not applicable. This study did not involve human participants or human-derived data.

Availability of Data and Materials
The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing Interests
The authors declare that they have no competing financial or non-financial interests related to this work.

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Authors' Contributions
D.R.W. and E.J.M. designed the study and performed the experiments. T.L.H. contributed to data analysis and interpretation. S.K.D. supervised the project, interpreted the data, and wrote the manuscript. All authors reviewed, edited, and approved the final manuscript.

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