M2b Macrophages Regulate Cardiac Fibroblast Activation and Alleviate Cardiac Fibrosis After Reperfusion Injury

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Background: Macrophages play an important role in the development of cardiac fibrosis. However, the roles of different macrophage subtypes in cardiac fibroblast (CF) activation and cardiac fibrosis are unknown.

Methods and Results: Bone marrow-derived macrophages (BMDMs) were treated with different stimuli to induce differentiation into M1, M2a, M2b, and M2c macrophage subtypes. CFs were co-cultured with different subtypes of macrophages or cultured with macrophage supernatants. Results revealed that M2b macrophages significantly suppressed the proliferation and migration of CFs, the expression of fibrosis-related proteins (collagen I [COL-1] and α-smooth muscle actin [α-SMA]), and differentiation into cardiac myofibroblasts (MFs). The opposite effects were observed with M2a macrophages. A rat model of cardiac ischemia/reperfusion (I/R) injury was used to determine the effect of M2b macrophages transplantation. After cardiac I/R injury, transplantation of M2b macrophages improved cardiac function and reduced cardiac fibrosis. The effect of macrophage subtypes on p-ERK, ERK, p-p38, and p38 phosphorylation was examined by Western blotting. The results showed that M2b macrophages significantly inhibited the mitogen-activated protein kinase (MAPK) signaling pathway.

Conclusions: These study results demonstrate for the first time that different subtypes of macrophages have different roles in regulating CF activation. M2b macrophages inhibit CF activation, and thus can be considered anti-fibrotic macrophages. M2a macrophages promote CF activation, and thus are pro-fibrotic macrophages.

Key Words: Cardiac fibroblast; Cardiac fibrosis; Macrophage subtype; M2b macrophage; Myocardial ischemia/reperfusion injury

Cardiac fibrosis is associated with nearly all forms of heart disease, and there are limited treatment options.1 This condition is characterized by the excessive deposition of extracellular matrix (ECM), typically collagen. The proliferation of cardiac fibroblasts (CFs) and their conversion into synthetic myofibroblasts (MFs) are hallmarks of this process. There is accumulating evidence that the interaction of macrophages with fibroblasts is crucial to the development of fibrosis.3–6

Cardiac macrophages, including resident tissue macrophages and recruited monocytes from the bone marrow,7 are highly heterogeneous and exhibit functional and phenotypic versatility. They can be divided into classically activated macrophages (M1) and alternatively activated macrophages (M2).8 M1 macrophages, also called pro-inflammatory macrophages, are induced by Th1 cytokines and by lipopolysaccharide (LPS) recognition, and release large amounts of pro-inflammatory cytokines.9,10 M1 macrophages participate in the removal of pathogens, debris clearance, and neovascularization.6 M2 macrophages, also called anti-inflammatory macrophages, are polarized by Th2 cytokines and characterized by the production of high levels of anti-inflammatory cytokines and pro-fibrogenic factors.5,11 There are 3 subtypes of M2 macrophages: M2a, M2b, and M2c.9,12 All M2 macrophage subtypes are immunosuppressive, but are distinguished by marker expression, induction factors, the production of specific cytokines and chemokines, and their biological function.14,15 The main biological functions of M2a, M2b, and M2c macrophages are wound healing, immunological regulation, and effectorcytosis, respectively.13

However, Mosser and Edwards13 considered the M1/M2 classification to be an oversimplification and suggested a functional classification of macrophages that distinguishes between classical, regulatory and reparative subpopulations of macrophages. In this classification, M2b macrophages...
are considered regulatory cells. The different subtypes of macrophages have different functions in the process of cardiac fibrosis. Most studies have focused on the profibrotic actions of macrophages, especially M2a and M2c macrophages, which are often referred to collectively as the “M2 macrophage”. Given the different functions and cytokines secreted by the different subtypes of macrophages, we propose that M1 and M2b macrophages may be responsible for inhibiting the activation of CFs, and thus preventing or slowing the development of cardiac fibrosis. However, subpopulations of putative anti-fibrotic macrophages have not been characterized, and evidence supporting macrophage-dependent anti-fibrotic mechanisms is lacking.

Thus, in this work, we evaluated the effects of different subtypes of macrophages on regulating CFs in vitro, and found that M2b macrophages inhibit the activation of CFs. We then examined the effects of M2b macrophages on the myocardium after myocardial ischemia/reperfusion injury (I/R) in a rat model.

Methods
A detailed account of the Materials and Methods used in this study is provided in the Online Data (Supplementary File).

Results
Identification of Cardiac Fibroblasts and Macrophages
Immunofluorescence staining of vimentin was used to identify CFs. The results showed that the CF positive rate was 100% (Figure 1A; magnification ×600).

Bone-marrow-derived macrophages (BMDMs; M0) were stained and analyzed by flow cytometry to determine CD45 expression (Figure 1B). The results showed that 97.9% of non-polarized BMDMs (M0 macrophages) were CD45+ after 6 days of culture, indicating that the bone marrow cells had differentiated into macrophages.

M0 cells were further polarized into M1 and different M2 subtype macrophages. The M2b macrophages were stained to determine CD45 (APC-A750-conjugated Antibody) and LIGHT (TNF superfamily 14) (FITC-conjugated Antibody) expression (LIGHT is the marker of M2b macrophages) (Figure 1C). The results indicated that 94.4% of the cells were CD45+/LIGHT+ after 2 days of polarization.

The phenotypes of the polarized BMDMs were also identified by their gene expression using quantitative reverse transcription polymerase chain reaction (qRT-PCR) (Figure 1D). M1, M2a, and M2c macrophages expressed high levels of CXCL9, CCL17, and CXCL13, respectively. M2b macrophages expressed high levels of CCL1, Interleukin (IL)-10, and LIGHT.

Collectively, these data indicated that BMDMs were successfully activated and polarized into different macrophage subtypes.

Influence of Macrophages on CF Proliferation and Migration
CFs were co-cultured with macrophages (scratch-wound healing assay and Transwell migration assay), or cultured with macrophage supernatants (scratch-wound healing assay and Cell Counting Kit-8 [CCK-8] assay), or cultured with the same volume of culture medium (blank control).

The results of the CCK-8 assay are summarized in Figure 2A. When CFs were cultured with M2b macrophage supernatant, proliferation was significantly inhibited compared to culture with blank control. The proliferation of CFs was increased when the cells were cultured with M2a and M2c macrophage supernatant. The proliferation of CFs when cultured with M0 and M1 macrophages was not different than when cultured with the blank control.

The migration of CFs when cultured with different macrophage populations was examined with the Transwell assay (Figure 2B-C). The migratory capacity of CFs was impaired when co-cultured with M1 and M2b macrophages compared with the blank control, and the inhibitory effect of M1 and M2b macrophages was similar. The migratory capacity was increased when CFs were co-cultured with M2a macrophages. Co-culture with M0 and M2c macrophages had no significant influence on migration compared with the blank control.

The results of the scratch-wound healing assay are shown in Figure 2D and 2E. The migratory capacity of CFs cultured with M1 and M2b macrophage supernatant was impaired compared with that of the blank control, and was increased by culture with M2a macrophage supernatant. Culturing with M0 and M2e macrophage supernatant had no influence on migratory capacity. Co-culture with M0, M1, and M2b macrophages significantly impaired the migratory capacity of CFs, and the effect was especially prominent with M2b macrophages. M2a and M2c macrophages had no influence on the migratory capacity of CFs.

Taken together, these results indicate that M2b macrophages significantly inhibit the proliferation and migration of CFs. In contrast, M0, M2a, and M2c macrophages stimulate both the proliferation and migration of CFs, with M2a macrophages exhibiting the strongest effect.

Influence of Macrophages on the Expression of Fibrosis-Related Proteins in CFs
CFs were cultured with different macrophage supernatants, and the expression of collagen I (COL-1) and α-smooth muscle actin (α-SMA) in CFs was measured by immunocytochemistry (IHC). As shown in Figure 3A, the expression of COL-1 was decreased when CFs were cultured with M1 and M2b supernatant compared to the blank control, and was increased when cultured with M2a and M2c supernatant. As shown in Figure 3B, the expression of α-SMA was decreased when CFs were cultured with M1 and M2b supernatant, and was increased when CFs were cultured with M0, M2a, and M2c supernatant.

In addition, the expression of CCN2 (CTGF) and α-SMA was detected by Western blotting (Figure 3C,D). The expression of CCN2 was decreased when CFs were cultured with M2b supernatant compared with the blank control and was increased when cultured with M2a supernatant. The expression of α-SMA was decreased when CFs were cultured with M2b supernatant and was increased when cultured with M2a and M2c supernatant.

Culture with M0 macrophage supernatant showed no significant influence on COL-1 or CCN2 expression; culture with M1 macrophage supernatant showed no significant influence on CCN2 or α-SMA expression; culture with M2c macrophage supernatant showed no significant influence on CCN2 expression.

In summary, these results indicate that M2b macrophage supernatant had the strongest inhibitory effect on CF expression of COL-1, α-SMA, and CCN2; whereas, M2a macrophage supernatant increased the expression of all 3
Figure 1. Identification of cardiac fibroblasts (CFs) and macrophages. (A) Identification of CFs by immunofluorescence staining of vimentin. (B) Identification of bone-marrow-derived macrophages (BMDMs) by flow cytometry. BMDMs (M0 macrophages) were treated with macrophage colony stimulating factor (M-CSF) for 6 days. M0 macrophages were defined as CD45 positive. (C) Identification of M2b macrophages by flow cytometry. BMDMs were incubated with lipopolysaccharide (LPS)+immunoglobulin (Ig)G for 48h to promote differentiation into M2b macrophages. Cells that were positive for CD45 and LIGHT (TNF superfamily 14) were defined as M2b macrophages. (D) Gene expression profiles of different macrophage phenotypes detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR). M1, M2a, and M2c macrophages expressed high levels of CXCL9, CCL17, and CXCL13, respectively. M2b macrophages expressed high levels of CCL1, IL-10, and LIGHT (TNFSF14). Data are shown as fold-changes relative to the expression levels in untreated M0 cells and are the mean of 3 independent experiments. *P<0.05.
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In the sham operation, I/R injury, and M2b macrophage transplantation groups, 1, 5, and 2 rats experienced severe arrhythmia, cardiac arrest, or respiratory failure, respec-
Figure 3. M2b macrophages inhibit CF expression of fibrosis-related proteins. CFs were cultured with macrophage supernatant or the same volume of culture medium (blank control). (A) Expression of collagen I (COL-1) in CFs measured by immunocytochemistry (IHC) staining (400×). The expression of COL-1 was significantly decreased by M1 and M2b macrophage supernatant and was increased by M2a and M2c macrophage supernatant. (B) Expression of α-smooth muscle actin (α-SMA) in CFs measured by IHC staining (600×). The expression of α-SMA was significantly decreased by M1 and M2b macrophage supernatant, and was increased by M0, M2a, and M2c macrophage supernatant. (C) Expression of CCN2 (CTGF) and α-SMA detected by Western blotting. (D) Quantification of Western blots. The expression of CCN2 was significantly decreased by M2b macrophage supernatant compared with the blank control and increased by M2a macrophage supernatant. The expression of α-SMA was significantly decreased by M2b macrophage supernatant and increased by M2a and M2c macrophage supernatant. Data are representative of 3 independent experiments. *P<0.05 vs. M2b; **P<0.01 vs. M2b; *P<0.05 vs. the blank control; **P<0.01 vs. the blank control.
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Figure 4. M2b macrophages improved cardiac function and alleviated fibrosis after myocardial ischemia/reperfusion (I/R) injury. In rats that underwent the myocardial I/R procedure, either $1 \times 10^6$ M2b macrophages (M2b macrophage transplantation group) or normal saline alone (I/R injury group) were administered intramyocardially to the ischemic zone. Rats in the sham operation group underwent the operation without ligation of the coronary artery. (A) Representative echocardiography images at day 14 after injury. M-mode parasternal short axis (PSAX) echocardiography images showing changes in the anterior wall motion and ventricular diameter in the different treatment groups. (B) M2b macrophage transplantation improved cardiac function and reduced ventricular dimensions. LVEF (left ventricular ejection fraction) and LVIDd (left ventricular internal dimension at diastole) were measured using transthoracic echocardiography (n=9 rats in the sham operation group; n=15 rats in the I/R injury group; n=18 rats in the M2b macrophage transplantation group). (C) Sirius red staining of the heart section showing collagen deposition (20×). (D) Immunohistochemical (IHC) staining for COL-1 in heart sections (200×). (E) IHC staining for α-SMA in heart sections (400×). (F) Quantification of Sirius red staining and IHC staining. Collagen deposition and α-SMA expression were significantly increased in the I/R injury group compared to the sham operation group, and M2b macrophages transplantation significantly attenuated these increases (n=4 rats in the sham operation group; n=8 rats in the I/R injury group; n=8 rats in the M2b macrophage transplantation group). *P<0.05; **P<0.01.
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I/R injury group (Figure 4A,B). In addition, myocardial remodeling was decreased in the M2b macrophage transplantation group, which was demonstrated by LV enlargement, compared to the I/R injury group (Figure 4A,B).

Fibrosis in heart tissue was evaluated with Sirius red staining (Figure 4C,F) and IHC staining for COL-1...
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M2b Macrophages Influence the MAPK Pathway In Vitro and In Vivo

Indicators of mitogen-activated protein kinase (MAPK) signaling are p-ERK, ERK, p-p38, and p38. CFs were cultured with different macrophage supernatants, and the expression of p-ERK, ERK, p-p38, and p38 in CFs was measured by Western blotting. As shown in Figure 5A and 5B, phosphorylation of ERK and p38 was decreased when CFs were cultured with M2b macrophage supernatant, whereas the phosphorylation of p38 was increased when CFs were cultured with M0, M2a, and M2c supernatant.

The expression of p-ERK, ERK, p-p38, and p38 in total heart tissue was evaluated by Western blotting (Figure 5C). Compared with that in the sham operation group, the phosphorylation of ERK and p38 was increased after I/R injury, and was significantly decreased by M2b macrophage transplantation (Figure 5D). Furthermore, lower levels of ERK phosphorylation and p38 phosphorylation were observed in the M2b group than in the sham operation group.

Discussion

In this study, we examined the effects of different macrophage subtypes on CF activation. Importantly, the results showed that different macrophage phenotypes have different effects on CFs, especially the M2a and M2b phenotypes, which act as pro- and anti-fibrotic cells, respectively. M2b macrophages significantly suppressed the proliferation and migration of CFs, the expression of fibrosis-related proteins, and the differentiation of CFs into cardiac MFs. In contrast, M2a macrophages had the opposite effects; they promoted the proliferation and migration of CFs, the expression of fibrosis-related proteins, and the differentiation of CFs into cardiac MFs. The effects of M0 and M2c macrophages were similar to those of M2a macrophages, and the effects of M1 macrophages were similar to those of M2b macrophages; however, the degree of the effects did not reach statistical significance. In vivo experiments showed that transplantation of M2b macrophages into myocardium that had been subjected to I/R injury improved cardiac function and reduced the cardiac fibrosis and myocardial remodeling caused by I/R injury. Taken together, the results suggest that M2b macrophages are a subpopulation that possess “anti-fibrotic” characteristics. The knowledge that M2b macrophages are “anti-fibrotic” and can improve cardiac function after I/R injury opens the possibilities of treatments for cardiac injury such as promoting the differentiation of macrophages to the M2b phenotype or M2b macrophage transplantation.

Cardiac fibrosis is a common pathophysiological manifestation of many myocardial diseases, and is associated with deterioration of cardiac function, arrhythmogenesis, and adverse outcomes. Cardiac fibrosis is characterized by CF accumulation and activation, and excessive deposition of ECM proteins. CFs differentiate into cardiac MFs rapidly after injury to the heart, and MFs express the highly contractile protein, α-SMA. The expansion of MFs following cardiac injury is primarily driven through activation of interstitial cells. Many different types of inflammatory cells are involved in tissue repair and fibrosis after cardiac injury, and macrophages act as major effector cells. After cardiac injury, macrophages undergo marked phenotypic and functional changes, from a pro-inflammatory phenotype (M1 phenotype) to a pro-healing phenotype (M2 phenotype). Studies have suggested that subsets of activated macrophages may regulate fibrosis by producing fibrogenic mediators, secreting proteases that participate in matrix remodeling, producing matricellular proteins, and exerting contact-dependent actions on fibroblasts. However, comprehensive studies on the direct effects of macrophage phenotypes on CFs and cardiac fibrosis are lacking.

Macrophages are highly heterogeneous, exhibit functional and phenotypic versatility, and have historically been divided into undifferentiated (M0) macrophages, classically activated (M1) macrophages, and alternatively activated (M2) macrophages. Our results indicate that undifferentiated (M0) macrophages do not participate in CF activation, although the experiments suggest that they have a “pro-fibrotic tendency”.

The role of M2 macrophages with respect to cardiac injury and fibrosis has not been fully elucidated. Several studies using animal models and in vitro experiments have indicated that M2 macrophages accelerate the phenotypic transition of CFs, increase collagen and ECM secretion, and exacerbate the worsening of cardiac function. However, other in vitro and in vivo studies have demonstrated that M2 macrophages inhibit CF proliferation or fibrosis-related protein expression. Even if M1 macrophages could reduce cardiac fibrosis after injury, they are still believed to lack beneficial effects in the early period of injury due to their release of reactive oxygen species (ROS), inflammatory mediators, and proteases.

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We hypothesized that the conflicting results of the aforementioned studies may be because different M2 macrophage subtypes have different functional properties. The results of our study confirmed this hypothesis.

We found that M2a macrophages promote the activation of CFs, including proliferation, migration, collagen production, CCN2 (CTGF) expression, and differentiation into MFs. This finding indicates that the M2a macrophage phenotype is a fibrogenic phenotype, which is consistent with the results of prior studies. The activity of M2c macrophages was similar to that of the M2a macrophages, but their activity was weaker. M2c macrophages accelerated the proliferation of CFs and increased α-SMA expression, but they had no obvious effects on CF migration or CCN2 expression. In contrast, M2b macrophages exhibited prominent inhibition of the activation of CF, including proliferation, migration, collagen production, CCN2 expression, and differentiation into MFs. These results demonstrate that M2b macrophages are anti-fibrotic phenotypes and that M2b macrophages are crucial for inhibiting the activation of CFs.

M2b macrophages are a unique type of M2 macrophage and are distinctly different from M2a and M2c macro-
phages. M2b macrophages modulate inflammatory immune responses but do not appear to actively participate in wound healing, and as such, they do not cause fibrosis. In a prior study of M2b macrophages, we reported that they maintain a balance between anti- and pro-inflammatory functions, which indicates that they are regulatory macrophages, and this is consistent with the findings of Mosser et al. M2b macrophages have been shown to exhibit immunosuppressive activity in inflammatory diseases, and this activity primarily involves interleukin (IL)-10 production. Preclinical and early-stage clinical trials have shown that M2b macrophage therapy has significant therapeutic potential in patients who have received renal transplantation.

Few studies have examined the potential value of M2b macrophage therapy in heart disease. We previously demonstrated that M2b macrophages can ameliorate tissue injury and reduce cardiomyocyte necrosis and apoptosis in the early stage after myocardial I/R injury. Based on the results of the current study, we now propose that M2b macrophages can promote healing after myocardial I/R injury and reduce heart fibrosis. The current study showed that transplantation of M2b macrophages into myocardium that had undergone I/R injury improved cardiac function, alleviated myocardial remodeling, and reduced cardiac fibrosis. At the early phase (1–3 days) following acute myocardial infarction, M1 macrophages represented the predominant subtype, while M2 macrophages dominated at late phase (after 5 days). However, whether the M2b macrophages play a role in I/R-injured hearts is still unknown. In addition, whether and how M2b macrophages were kept activated after transplantation remain to be investigated.

A number of intracellular signaling pathways play important roles in the development of cardiac fibrosis. One important pathway that has been shown to promote cardiac fibrosis is the p38 MAPK signaling pathway. The current study showed that M2b macrophages inhibited the phosphorylation of p38 and ERK, which are indicators of MAPK signaling, in vivo and in vitro. In Figure 5, the p-ERK level was obviously elevated in the blank control. One possible explanation is that MFs can be activated to some extent after isolation from healthy myocardium. In contrast, M2a and M2c macrophages promoted the activation of MAPK signaling in vitro, which is consistent with a fibrogenic effect. Taken together, these results suggest that M2b macrophages inhibit CF activation and thus reduce cardiac fibrosis by regulating MAPK signaling. However, further studies are needed to fully elucidate the relations between M2b macrophages, MAPK signaling, and CFs due to the complexities of macrophage subtypes and differentiation and the interrelations between different signaling pathways.

Study Limitations

There are some limitations of this study that should be taken into consideration. First, no other macrophage subsets were used as controls to demonstrate the specificity of the beneficial effects of M2b macrophages. Part of the reason for this approach is that we extensively studied this topic in our previous research, from the acute phase after MI/R to convalescence. Second, we did not study the fate of M2b macrophages after transplantation. Finally, although we observed that transplantation of M2b macrophages improved cardiac function and reduced cardiac fibrosis, this method is of low clinical feasibility due to it being too time-consuming and the fact that the activated M2b macrophages could be difficult to maintain after transplantation. Therefore, inducing macrophages to differentiate into M2b subtypes in vivo by injection of M2b macrophage inducers or administration of downstream molecules of M2b macrophages may be an alternative method for the treatment of MI/R injury.

Conclusions

In conclusion, this study showed that M2b macrophages inhibited CF activation and thus reduced cardiac fibrosis. In contrast, M2a macrophages promoted the activation of CFs. Furthermore, M2b macrophages appear to have a protective therapeutic effect on the myocardium after I/R injury. Taken together, the results of this study suggest that therapy with M2b macrophages, or inhibition of M2a macrophages, may be potential treatment strategies for cardiac injury caused by different heart diseases.

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Disclosures

The manuscript is approved by all authors for publication. The authors have declared that no conflicts of interest exist.

References

1. Leask A. Getting to the heart of the matter: New insights into cardiac fibrosis. Circ Res 2015; 116: 1269–1276.
2. Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction: From inflammation to fibrosis. Circ Res 2016; 119: 91–112.
3. Kania G, Blyszczuk P, Eriksson U. Mechanisms of cardiac fibrosis in inflammatory heart disease. Trends Cardiovasc Med 2009; 19: 247–252.
4. Wang L, Li YL, Zhang CC, Cui W, Wang X, Xia Y, et al. Inhibition of Toll-like receptor 2 reduces cardiac fibrosis by attenuating macrophage-mediated inflammation. Cardiovasc Res 2014; 101: 383–392.
5. Falkenhämm A, de Antuerno R, Rosin N, Betsch D, Lee TD, Duncan R, et al. Nonclassical resident macrophages are important determinants in the development of myocardial fibrosis. Am J Pathol 2015; 185: 927 – 942.
6. Fujii K, Wang J, Nagai R. Cardioprotective function of cardiac macrophages. Cardiovasc Res 2014; 102: 232 – 239.
7. Honold L, Nahrendorf M. Resident and monocyte-derived macrophages in cardiovascular disease. Circ Res 2018; 122: 113 – 127.
8. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Front Biosci 2008; 13: 453 – 461.
9. Chinetti-Gbaguidi G, Colin S, Staelens B. Macrophage subsets in atherosclerosis. Nat Rev Cardiol 2015; 12: 10 – 17.
10. Mosser DM. The many faces of macrophage activation. J Leukoc Biol 2003; 73: 209 – 212.
11. Luch M, Anders HJ. Macrophages and fibrosis: How resident and infiltrating mononuclear phagocytes orchestrate all phases of tissue injury and repair. Biochim Biophys Acta 2013; 1832: 989 – 997.
12. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. J Leukoc Biol 2006; 80: 1298 – 1307.
13. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; 8: 958 – 969.
14. Gensel JC, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. Brain Res 2015; 1619:...
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1–11.

15. Liberale L, Dallegris F, Montecucco F, Carbone F. Pathophysiological relevance of macrophage subsets in atherogenesis. *Thromb Haemost* 2017; 117: 7–18.

16. Coli S, Chinetti-Gbaguidi G, Staels B. Macrophage phenotypes in atherosclerosis. *Immunol Rev* 2014; 262: 153 – 166.

17. Travers JG, Kamal FA, Robbins J, Yutzey KE, Blaxall BC. Cardiac fibrosis: The fibroblast awakens. *Circ Res* 2016; 118: 1021 – 1040.

18. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity* 2016; 44: 450 – 462.

19. Munoz-Canoves P, Serrano AL. Macrophages decide between regeneration and fibrosis in muscle. *Trends Endocrinol Metab* 2015; 26: 449 – 450.

20. Frangiogiannis NG. Cardiac fibrosis: Cell biological mechanisms, molecular pathways and therapeutic opportunities. *Mol Aspects Med* 2019; 65: 70 – 99.

21. Steffens S, Montecucco F, Mach F. The inflammatory response as a target to reduce myocardial ischaemia and reperfusion injury. *Thromb Haemost* 2009; 102: 240 – 247.

22. Ma F, Li Y, Jia L, Han Y, Cheng J, Li H, et al. Macrophage-stimulated cardiac fibroblast production of IL-6 is essential for TGF beta/Smad activation and cardiac fibrosis induced by angiotensin II. *PLoS One* 2012; 7: e35144.

23. Yan X, Anzai A, Katsumata Y, Matsuhashi T, Ito K, Endo J, et al. Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. *J Mol Cell Cardiol* 2013; 62: 24 – 35.

24. Mylonas KJ, Jenkins SJ, Castellan RFP, Ruckerl D, McGregor K, Phythian-Adams AT, et al. The adult murine heart has a sparse, phagocytically active macrophage population that expands through monocyte recruitment and adopts an ‘M2’ phenotype in response to Th2 immunologic challenge. *Immunobiology* 2015; 220: 924 – 933.

25. Moore JP, Vinh A, Tuck KL, Sakkal S, Krishnan SM, Chan CT, et al. M2 macrophage accumulation in the aortic wall during angiotensin II infusion in mice is associated with fibrosis, elastin loss, and elevated blood pressure. *Am J Physiol Heart Circ Physiol* 2015; 309: H1006 – H1017.

26. Urbina P, Singla DK. BMP-7 attenuates adverse cardiac remodeling mediated through M2 macrophages in prediabetic cardiomyopathy. *Am J Physiol Heart Circ Physiol* 2014; 307: H762 – H772.

27. Ghanta S, Cuzzzone DA, Torrisi JS, Albano NJ, Joseph WJ, Savetsky IL, et al. Regulation of inflammation and fibrosis by macrophages in lymphedema. *Am J Physiol Heart Circ Physiol* 2015; 308: H1065 – H1077.

28. Kondo H, Takahashi N, Gotoh K, Fukui A, Saito S, Aoki K, et al. Splenectomy exacerbates atrial inflammatory fibrosis and vulnerability to atrial fibrillation induced by pressure overload in rats: Possible role of spleen-derived interleukin-10. *Heart Rhythm* 2016; 13: 241 – 250.

29. Zhou X, Lao YC, Ji WJ, Zhang L, Dong Y, Ge L, et al. Modulation of mononuclear phagocyte inflammatory response by liposome-encapsulated voltage gated sodium channel inhibitor ameliorates myocardial ischemia/reperfusion injury in rats. *PLoS One* 2013; 8: e74390.

30. Duan J, Liu X, Wang H, Guo SW. The M2a macrophage subset may be critically involved in the fibrogenesis of endometriosis in mice. *Reprod Biomed Online* 2018; 37: 254 – 268.

31. Schnoor M, Cullen P, Lorkowski J, Stolle K, Robeneck H, Troyer D, et al. Production of type VI collagen by human macrophages: A new dimension in macrophage functional heterogeneity. *J Immunol* 2008; 180: 5707 – 5719.

32. Fleming BD, Mosser DM. Regulatory macrophages: Setting the threshold for therapy. *Eur J Immunol* 2011; 41: 2498 – 2502.

33. Yue Y, Yang X, Feng K, Wang L, Hou J, Mei B, et al. M2b macrophages reduce early reperfusion injury after myocardial ischemia in mice: A predominant role of inhibiting apoptosis via A20. *Int J Cardiol* 2017; 245: 228 – 235.

34. Gerber JS, Mosser DM. Reversing lipopolysaccharide toxicity by ligating the macrophage Fc gamma receptors. *J Immunol* 2001; 166: 6801 – 6808.

35. Broichhausen C, Riquelme P, Geissler EK, Hutchinson JA. Regulatory macrophages as therapeutic targets and therapeutic agents in solid organ transplantation. *Curr Opin Organ Transplant* 2012; 17: 332 – 342.

36. Hutchinson JA, Riquelme P, Sawitzki B, Tomiuk S, Miqueu P, Zuhayra M, et al. Cutting Edge: Immunological consequences and trafficking of human regulatory macrophages administered to renal transplant recipients. *J Immunol* 2011; 187: 2072 – 2078.

37. Nahrendorf M, Swirski FK. Monocyte and macrophage heterogeneity in the heart. *Circ Res* 2013; 112: 1624 – 1633.

38. Marber MS, Rose B, Wang Y. The p38 mitogen-activated protein kinase pathway: A potential target for intervention in infarction, hypertrophy, and heart failure. *J Mol Cell Cardiol* 2011; 51: 485 – 490.

39. Yokota T, Wang Y. p38 MAP kinases in the heart. *Gene* 2016; 575: 369 – 376.

40. Landry NM, Rattan SG, Dixon IMC. An improved method of assessing inflammatory and fibrotic response to Th2 immunologic challenge. *Immunobiology* 2015; 220: 924 – 933.