Are activated B cells involved in the process of myocardial fibrosis after acute myocardial infarction? - An in vivo experiment

Fanrui Mo
Liuzhou Worker's Hospital
Ying Luo
Guangxi Medical University
Yuluan Yan
Liuzhou Worker's Hospital
Juan Li
Liuzhou Worker's Hospital
Shayi Lai
Guangxi Medical University First Affiliated Hospital
Weifeng Wu (drwuweifeng@126.com)
Guangxi Medical University First Affiliated Hospital https://orcid.org/0000-0003-0818-4070

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Abstract

Background: Inflammatory cells infiltrate into the ischemic and hypoxic myocardial tissue after myocardial infarction. B cells gather at the site of myocardial injury and secrete cytokines to regulate immune inflammation and fiber repair processes.

Methods: The animal experiment intended to use ligation of the left anterior descending branch of C57BL/6 mice to establish a mice AMI model to observe the changes of activated B cells and cytokines at different time points. 88 12-week-old C57BL/6 male mice were divided into the Sham group (24 mice) and acute myocardial infarction (AMI) group (64 mice) randomly. Besides, C57BL/6 Bmi-1 knock out (BKO) mice and C57BL/6 wild-type (WT) mice were used to establish AMI models to observe the expression levels of cardiomyocyte factors including TNF-α and IL-1β, IL-6, TGF-β1, COL1-A1, COL3-A1, TIMP-1, and MMP9. Moreover, pathological and collagen changes in the myocardium were analyzed. One-way ANOVA analysis of variance was used for comparison between multiple groups, and the LSD method was used for pairwise comparison between groups. $P < 0.05$ indicated statistical differences.

Results: AMI model of C57BL/6 mice was established successfully. The ratio of activated B cells and expression of TNF-α, IL-1β, IL-6, TGF-β1, and BAFF in 5 days subgroup was highest in the myocardium, spleen, and peripheral blood with the most obvious myocardial inflammatory cells infiltration. mRNA expression levels of TNF-α, IL-1β, IL-6, TGF-β1 in 5 days subgroup of the BKO group were decreased compared with the WT group ($P < 0.05$). Among 2 weeks subgroups of Sham, WT, and BKO groups, the LVEDd and LVESd in the BKO and WT groups were less than those in the Sham group ($P < 0.05$). The LVEDd and LVESd of the BKO group were less than the WT group,EF was higher than the WT group ($P < 0.05$).

Conclusion: Activated B cells participated in the sustained state of myocardial inflammation and immune system activation after AMI via promoting the secretion of cytokines, and may affect the metabolism of myocardial collagen. Moreover, B cells could damage the heart structure and left ventricular ejection function by promoting the expression of myocardial collagen Type I and type III.

Background

Acute myocardial infarction (AMI), which can lead to fibrosis after myocardial injury and morphological changes such as impaired left ventricular function, left ventricular remodeling, and heart enlargement [1]. Although the success rate of AMI treatment is getting higher and higher with the widespread implementation of the emergency percutaneous coronary intervention (PCI) and thrombolytic therapy, 40% of the patients have left ventricular remodeling and 14.2% have heart failure [2]. Studies have found that AMI vascular recanalization still cannot prevent the progression of myocardial fibrosis in some patients after recanalization [3]. Heart failure and arrhythmia caused by myocardial fibrosis seriously threaten human life and health. Once pathological remodeling occurs, it will be difficult to reverse. There is currently a lack of effective treatment methods, and new treatment methods are urgent to be found to solve this problem.

With the rise of precision medicine, immune-related treatments such as molecularly targeted drugs have become a research hotspot. More and more evidence suggests that the activation of myocardial inflammation and the immune system after AMI exacerbate the process of myocardial fibrosis [4]. After myocardial

infarction, a large number of inflammatory cells infiltrate into the ischemic and hypoxic myocardial tissue. Neutrophils and monocytes-macrophages are responsible for cleaning the necrotic tissue and secrete pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, TGF-β, etc., and then T cells and B cells gather at the site of myocardial injury and secrete cytokines to regulate immune inflammation and fiber repair processes. Although inflammation and fibrosis are the basic physiological responses for healing and repair after tissue injury, excessive inflammation, and fibrosis lead to left ventricular remodeling after myocardial infarction [5, 6]. The involvement of innate immunity in tissue fibrosis has been recognized by the public [7], and there have been many studies on the involvement of T cells in myocardial fibrosis in adaptive immunity. However, as another important member of the adaptive immune system, the role of B cells in myocardial injury and fibrosis has only been gradually concerned in recent years. B cell is an important type of adaptive immune cells, which is mainly involved in humoral immunity with regulatory effects on producing antibodies, presenting antigens, and secreting cytokines [8]. Recent studies have found that B cells are involved in the process of cardiovascular disease through an extensive immune regulatory network. Nish- Imura et al. [9] proved in the mouse model of dilated cardiomyopathy PD-1 deficiency that PD-1was an important factor related to B cells' specific differentiation, severe spontaneous dilated cardiomyopathy occurred in mice when it was deficient. Also, activated B cells can cause myocardial damage through regulating apoptosis signaling pathways and complement-mediated cytotoxicity [10]. Most of these studies focused on B cells about secreting antibodies to participate in myocardial infarction and myocardial injury, but the role of secreting cytokines, another important function of B cells, in this process is not well studied. The mechanism is not clear, especially in the study of immune response and fibrosis progression after acute myocardial infarction.

Previous studies have confirmed that B cells can promote the secretion of TGF-β1, TNF-α, IL-6, and other inflammatory factors [8, 11–16]. These factors are involved in myocardial injury and repair. Related studies have found that inflammatory cytokines play an important role in promoting or inhibiting tissue fibrosis in the process of fibrosis cascade reaction. TGF-β1 is the proinflammatory factor of major tissues and organs, which can phosphorylate Smad2/3 protein reactants, stimulate innate immune cells, and activate fibroblasts to produce more cytokines to promote the occurrence and development of tissue fibrosis [17]. Besides, IL-6 and TNF-α can also act indirectly through TGF-β1 inflammation and tissue fibrosis pathways [18]. In the AMI mouse model, it was found that NLRP3 inflammasomes cause myocardial damage and myocardial fibrosisactivate by promoting the formation of IL-1β [19]. In summary, B cells participate in the local inflammatory state of myocardial infarction after AMI, and secrete cytokines to participate in it, among which pro-inflammatory factors can promote fibrosis.

In recent years, it has been confirmed that B cells are involved in the process of tissue fibrosis in studies on transplantation immunity [20], autoimmune diseases [21], liver fibrosis [22], pulmonary fibrosis [23], and other diseases. In autoimmune diseases, the use of anti-BAFF and anti-CD20 antibodies can induce the depletion of the pre-B cell, which can reduce the degree of tissue fibrosis [24]. In non-cardiovascular diseases, anti-B cell therapy can reduce fibrosis, which gives us a new idea of whether anti-B-cell therapy can also reduce fibrosis in AMI. To answer this question, we first need to explore the effects of B cells on AMI myocardial fibrosis. Moreover, studies have found that there was a dynamic evolution of B cell infiltration in the injured local myocardial tissue in the AMI mice model. [25].

Therefore, we propose that activated B cells participate in the process of myocardial fibrosis after AMI. This subject intended to study it through animal experiments to find out the specific mechanism of myocardial fibrosis caused by the activation of myocardial fibroblasts, which might provide potential new targets in immunotherapy for the prevention and treatment of myocardial fibrosis caused by AMI.

Methods

2.1 Animal sample size and randomization

12-week-old C57BL/6 male mice and wild type (WT) C57BL/6 mice were provided by the Experimental Animal Center of Guangxi Medical University, and the animals were kept in the SFP animal room of the center. Bmi-1 knock out (BKO) mice were purchased from Jackson Laboratory, USA. Animal experiments follow the Guidelines for the Use of Experimental Animals formulated by the National Institutes of Health of the United States. The research plan was approved by the Experimental Animal Ethics Committee of Guangxi Medical University.

2.1.1. A total of 88 12-week old C57BL/6 male mice (20-25g) were randomly divided into the Sham group (24 Sham mice) and the acute myocardial infarction (AMI) group (64 AMI mice) via random number table method. The two groups were divided into 4 subgroups at time points of 3 days, 5 days, 1 week, and 2 weeks, respectively. There were 6 mice in each subgroup of the Sham group and 12 mice in each subgroup of the AMI group. In our study, observers did not know the specific grouping of each animal. At the same time, experimental operators and effect evaluators didn't not know the specific animal groups. Ensure that each AMI subgroup ended with at least eight mice alive.

2.1.2. The 30 WT C57BL/6 mice were divided into the Sham group (12 Sham mice) and AMI group (18 WT C57BL/6 AMI mice). 18 BKO mice were set into the BKO group. The three groups were divided into two subgroups respectively: 5 days and 2 weeks.

2.2 Models and treatment

Injected 1.25% avertin into the abdominal cavity of mice under general anesthesia at a dose of 0.2ml/10g. After the mice breathed smoothly, cut the skin on the left chest, bluntly separated the pectoralis major and pectoralis minor muscles, and exposed the thorax. Then opened the thorax between the side 3-4 intercostals to expose the heart with a mouse chest opener. 8-0 non-destructive sutures were used to sew from right to left on the anchor point 2mm lower edge of the left atrial appendage. The depth of the needle is about 1mm and the width is about 1-1.5mm. Next, the needle holder was knotted and the anterior descending branch (LAD) was permanently ligated. After the ligation, the myocardium became white and the movement was weakened at the bottom of the suture. 1ml syringe is pumped back into the thoracic cavity to form the negative pressure. Finally, the muscles and skin of the chest and neck are sutured layer by layer using 5-0 suture. When the mice regained their spontaneous breathing, the tracheal intubation was pulled out, and they were kept warm and returned to the cage for rearing. What's more, the mice of the Sham group were subjected to conventional open-chest surgery. The hearts were left open and threaded under the LAD without ligating the blood vessels. The rest of the operation was the same as before.

2.3 Preparation of peripheral blood monocyte suspension

1.5m peripheral blood of mice was collected to prefabricated heparin anticoagulant EP tubes by the method of taking blood from the eyeball. After 20 minutes' standing at room temperature, the peripheral blood was stratified. The upper yellow serum was collected into the EP tube and stored in the -80℃ refrigerator. The lower layer of blood cells was transferred into a 5mlBD flow tube, then 2ml diluted red blood cell lysate was added. Blown and mixed it until came to the mixed mixture, stood it for 5 minutes at room temperature subsequently. After centrifugation of the mixed mixture at 300×g for 5 minutes, added 2ml sterile PBS to it to stop the lysis. Then the supernatant was discarded after centrifuged it at 300×g for 5 minutes once again. And washed it with PBS and centrifuged it for the third time and discard the supernatant and 1ml PBS was added to it to resuspend cells. If the cells count up to standard, set it aside in reserve.

2.4. Preparation of spleen single cells suspension

Mice were sacrificed by neck dislocation. The spleen was removed aseptically and transplanted into a 1.5mL aseptic EP tube, followed by 300 L aseptic PBS into the EP tube. Grind the spleen gently with a sterile glass rod until it is white and transparent. The ground tissue solution is filtered through a 200 m filter screen, and the filtered spleen tissue cells solution is collected and transferred into a sterile test tube. After centrifugation of the spleen tissue cells solution at 300×g for 5 minutes, the supernatant was discarded. Next, 1ml diluted red blood cells lysate was added to it and then blow and mix it up until the cells mix well, after which stood it for 5 minutes at room temperature. Then centrifuged it at 300×g for 5 minutes, 1ml sterile PBS was added to it to stop the lysis. Then centrifuged it at 300×g for 5 minutes once again and discarded the supernatant, and washed it with PBS. Then centrifuged it for two more times and discard the supernatant and added 1ml PBS to it to resuspend cells in reserve.

2.5 Preparation of myocardial single cells suspension

After the heart was removed under aseptic conditions, it was rinsed with PBS buffer repeatedly. The blood inside the heart was gently squeezed until it got clean, then the heart was transferred into a 1.5mL sterile EP tube that had been added with 300 microliters of sterile PBS. The ventricular tissue was cut into pieces with ophthalmic scissors and subsequently been placed into a 15mL centrifuge tube. Next, added it with 3.0mL collagenase IV, and put the tube into a shaking table for digestion at 37°C for 30 minutes. Myocardial tissue was repeatedly blown with 3.0ml straw to make the tissue fully mixed with digestive enzymes. After filtration with 100-mesh aperture nylon mesh, the cells were collected and added into the BD flow tube. After centrifugation of the tissue solution at 1500rpm/min for 5 minutes, 1ml diluted red blood cell lysate was added to it to resuspended cells. Then stood it for 5 minutes at room temperature and added 1 ml PBS to neutralize it. Subsequently centrifuged it at 1500rpm for 5 minutes once again, discarded the supernatant, and then washed it with PBS and centrifugation twice. Finally, 1ml PBS was added to resuspend cells. If the cells count up to standard, set it aside in reserve.

2.6 Flow cytometry

100μl (containing about 1×10^6 cells) of single cells suspension of peripheral blood, spleen, and myocardial tissue were taken and placed in a centrifuge tube respectively. Then 1μl anti-MOUSE CD19-PERCp-CYANine5.5

antibody and 1μl anti-mouse CD69 PE antibody were added into the above 3 suspension tubes. The suspension was incubated at 4℃ for 30 minutes. Subsequently, we added 1 ml PBS to the suspension and centrifuged it at 300×g for 5 minutes. The cells were resuspended with 4% paraformaldehyde (300μl) after the supernatant was discarded. We adopted BD company FACS Canto instruments to conducted flow cytometry. The lymphocyte is P1, the CD19 is P2. The expression ratio of activated B cells was determined according to the proportion of CD69 in B cells. And the results of flow cytometry were analyzed with Flowjo7.6.1 software.

2.7 Real-time fluorescence quantitative polymerase chain reaction(RT-PCR)

Total RNA was extracted from myocardial tissue samples tissue of AMI mice and control mice by using TRIzol™ reagent according to the manufacturer's instructions (Fluorescent quantitative PCR kit were Provided by Bao Biological Engineering Co. LTD. Dalian, China). RNA was dissolved in sterile water and quantified by NanoDrop2000 ultraviolet spectrophotometry at 260nm/280nm (A260/A280 in 1.9-2.1 was considered as the qualified purity), after which it was reverse-transcribed using an All-in-One cDNA Synthesis SuperMix. RT-PCR was performed using the Reverse transcript kit (provided by Bao Bioengineering Co., LTD. Dalian, China). The PCR conditions were 30s at 95 °C followed by 40 cycles of 95 °C for 5 s and 58 °C for 30 s, and then was 30 s for 72 °C. The relative expression of the gene was calculated by ΔΔCt value. The primer sequences were presented is in Table 1.

2.8. Detection of TNF-α, Ll-1, Ll-6, BAFF, and TGF-β1 of peripheral blood.

TNF-α, Ll-1, Ll-6, BAFF, and TGF-1 concentrations were measured with enzyme-linked immunosorbent assay (ELISA, the kit was provided by Novus Biologicals) according to the instructions.

2.9. Echocardiography of heart

Mice in each group were intraperitoneally injected with 1.25% Afertin general anesthesia according to time points, the supine position was taken on the operating table of small animals, and the parasternal minor axis section examination was taken. LVEDd, LVESd, and EF values of mice were recorded under M-mode ultrasound.

2.10 Statistical analysis

All data were presented as mean±standard deviation. SPSS17.0 software was adopted to analyze results, one-way ANOVA was used for comparison between groups, and the LSD method was used for pairwise comparison between groups. $P < 0.05$ was considered statistically significant.

Results

3.1 Effect of activated B cells on myocardial fibrosis in mice with acute myocardial infarction

3.1.1. General situation of mice

After the AMI mice were fully awake, the mice showed with reduced activity and no obvious stimulus-response, while the mice in the Sham group generally responded normally. Ten mice in the AMI group died,

including 2 mice in the 3 days and 1-week subgroups, and 3 mice in the 5-day and 2-week subgroups. No mice died in the Sham group. Gross specimen and pathological findings of mouse LAD after ligation were shown in figure 1.

3.1.2. The ratio of activated B cells in mouse myocardium, spleen, and peripheral blood

In myocardial tissue, the proportion of activated B cells in the AMI group was higher than that in the Sham group at 3 days, 5 days, and 1 week (P 0.05), no significant difference from the Sham group at 2 weeks (P 0.05) and the highest level was represented at 5 days subgroup (P 0.05) In the spleen and peripheral blood, the proportion of activated B cells in the AMI group was higher than that in the Sham group on 3 and 5 days(P 0.05), and the highest level was represented at 5 days subgroup (P 0.05). There was no significant difference between 1 and 2-week and the Sham group (P>0.05). (Table 2, figure2-3).

3.1.3. Expression of cytokine mRNA in cardiomyocytes of AMI mice

In myocardial tissue, the mRNA levels of TNF-α, IL-1, IL6, and BAFF in the AMI group were higher than those in the Sham group at 3 days, 5 days, 1 week, and 2 weeks (P < 0.05), and were highest at 3 days and 5 days subgroups (P < 0.05), however, the mRNA levels of TNF-α, IL-1, IL-6 and BAFF in AMI 3 days and 5 days subgroups were not statistically significant (P > 0.05). The level of TGF-β1 was higher than that of the 4 subgroups in the Sham group, and the highest level appeared at the 5-day subgroup (P 0.05). (Figure 4)

3.1.4. Levels of cytokines in peripheral blood of AMI mice

The concentrations of TNF-α, IL-1, IL6, and BAFF of peripheral blood in the AMI group were higher than those in the Sham group including 3 days, 5 days, 1 week, and 2 weeks subgroups (P < 0.05), and were highest at 3 days and 5 days subgroups (P < 0.05). TGF- 1 concentrations were higher than the Sham group in 4 subgroups, with the highest concentration in the 5 days subgroup (P < 0.05). The results were shown in figure 5.

3.1.5. Pathological changes of mice myocardium

In the AMI group, myocardial cells necrosis and inflammatory cells infiltration occurred at 3 days. The inflammatory cells infiltration was the most obvious at 5 days, the inflammatory cells infiltration decreased and a little fibrosis appeared at the 1-week subgroup. A few inflammatory cells and obvious fibrosis appeared at 2 weeks. Masson staining indicated that myocardial collagen deposition increased with time and reached its peak at 2 weeks. No obvious abnormalities were found in the myocardial pathology of Sham group mice. The pathological changes were shown in figure 6.

3.2. The effect of B cells on myocardial collagen metabolism after acute myocardial infarction in mice and its possible mechanism

3.2.1. Cytokine mRNA levels in myocardial tissue differences among the Sham group, WT group, and BKO group.

In 5 days subgroup of AMI, mRNA levels of TNF-α(2.33± 0.35VS3.79 ±0.58), IL-1 (2.87±0.35vs 5.32±0.37), IL-6 (3.35±0.28 vS6.58 ±0.48), and TGF-β1 (2.87±0.62 vS4.35 ±0.35) in BKO group decreased compared with WT group ($P < 0.05$), and were higher than those in Sham group ($P < 0.05$). (Figure 7).

3.2.2. Peripheral blood cytokine concentration in myocardial tissue differences among Sham, WT, and BKO groups.

In 5 days subgroup of AMI, the concentrations of TNF-α, IL-1, IL-6, and TGF-β1 in peripheral blood of the BKO group decreased compared with the WT group (all $P < 0.05$), which were higher than the Sham group ($P < 0.05$), as shown in figure 8.

3.2.3. mRNA levels of collagen metabolism in the myocardium

The mRNA levels of COL1-A1 (3.60±0.65vs8.33±0.60), COL3-A1 (5.40±0.47vs2.46±0.30), MMP9 (3.84±0.71vs 8.19±0.99) of the AMI group, TIMP-1 (3.63±0.31vs6.23±0.56) of 2 weeks subgroup in BKO group were decreased compared with WT group ($P < 0.05$), but higher than those in Sham group ($P < 0.05$). (Figure 9).

3.2.4. Comparison of color doppler echocardiography in 2 weeks subgroup of Sham, WT and BKO group

The left ventricular end-diastolic diameter (LVEDd) left ventricular end-systolic diameter (LVESd) of the BKO group were smaller than those of the WT group (P□0.05), and the EF value was higher than that of the WT group (P□0.05). The LVEDd and LVESd were smaller than those of the Sham group, and the EF was lower than that of the Sham group. The difference was statistically significant ($P<0.05$). The results were represented in table 3 and figure 10.

3.2.5. Comparison of pathological findings in 2 weeks subgroup of Sham group, WT group, and BKO groups

Compared with the myocardial pathology of the mice in the WT group at 2 weeks, the myocardial fibrosis in the BKO group was reduced, and the collagen content decreased. The fibrosis in the BKO and WT groups was more obvious than that in the Sham group. The results are shown in Figure 11.

Discussion

In recent years, the incidence of AMI has been on the rise with high mortality and disability rate, which has become one of the main causes of heart failure [26]. The primary pathogenesis of Acute myocardial infarction (AMI) is the unstable and ruptured coronary atherosclerotic plaque, the thrombotic formation of platelet aggregation sustained coronary artery occlusion, and myocardial cells ischemia/anoxic necrosis. Myocardial necrosis after AMI triggers a local inflammatory response and activates the immune system. Myocardial infarction not only directly leads to myocardial necrosis, but a secondary inflammatory immune response also seriously damages cardiac function, which can cause heart failure and various arrhythmias [4, 27–29].

As acquired immune cells, B cells secrete cytokines such as transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), which promotes fibrosis progression. A large number of studies have shown that cytokines were involved in an inflammatory response and the fibrosis process of kidney, liver, and lung tissues [30, 31]. In recent years, there have been reports on the

involvement of B cells in the myocardial fibrosis process of cardiovascular diseases, but the mechanism of B cells and their cytokines in the myocardial infarction process after AMI is still unclear. Therefore, this study intends to establish a mouse AMI model by ligating the left anterior descending branch of C57BL/6 mice to observe the changes of activated B cells and cytokines at different time points and the relationship between them. The study of animal myocardial infarction models can help to explore its mechanism and treatment. Cardiovascular genes in mice are highly similar to those in humans [32], and gene knockout and other gene technologies are achieved in mice currently. However, due to small size, poor tolerance, not convenient for surgery and operation, the mouse myocardial infarction modeling has a high requirement. Only after a certain period of training, we can master the mouse AMI modeling technology. The descending branch before ligation is the mainstream method for the production of AMI models in mice [33], which requires a series of processes such as management anesthesia, endotracheal intubation, ventilator assisted breathing, thoracotomy, pre-exposure descending branch, pre-ligation descending branch, chest closing, resuscitation, removal of the ventilator and so on.

In a study of patients with heart failure after myocardial infarction and various reasons, it was found that various anti-myocardial antibodies exist in myocardial tissue. Endothelial cells are damaged after myocardial ischemia and hypoxia, secreting various cytokines, such as Chemical factors, inflammatory mediators factors, etc., the ischemic necrosis of myocardium exposes new antigens, which will trigger the immune response, antibody action, and immune cells infiltration [34]. Moreover, the congenital immune system plays an important role in the myocardial fibrosis process. Neutrophils are released in the damaged endothelial cells to accelerate the secretion of chemokines and growth factors such as chemical signals to the damaged heart muscle. Then natural killer cells, fine granulocyte, dendritic cells, and mononuclear-macrophage infiltrate into the damaged heart tissue to promote the secretion of cytokines and release oxygen free radicals, resulting in acute inflammation. By secreting cytokines, producing antibodies, and presenting antigens, B cells further aggravate myocardial injury under the combined action of inflammatory cytokines secreted by various activated immune cells [35–37]. Besides, B cells not only damage the myocardium but also are related to myocardial fibrosis after myocardial injury. In the B cell-deficient mouse cardiomyopathy model, it was found that with the decrease of TNF-α, serum collagen I and III levels decreased, and the number of collagen fibers deposited in the extracellular matrix decreased [23]. Thus, it can be inferred that B cells have the role of promoting myocardial fibrosis. Zouggari et al. found that myocardial B cells expressed Ccl7, a chemokine recruited to myocardial infiltration by CCR2 receptor-mediated monocytes, which could lead to myocardial damage. After the injection of the anti-CD20 antibody, monocyte infiltration was reduced and myocardial damage was alleviated [25]. However, Goodchild et al. reported that intramedullary injection of bone marrow-derived B lymphocytes into MYOCARDIAL infarction in SD rats was beneficial to cardiac function because it reduced in situ cells apoptosis and helped maintain ejection fraction [38]. The two studies drew different conclusions about B cells because Goodchild et al. used immature B cells, while Zouggari et al. used anti-CD20 antibodies to consume mature B cells.

In this study, Bmi-1 knock out (BKO) mice were used to investigate myocardial collagen deposition after AMI after B cell deletion. The BKO mice used in this study completely lack the entire B cells system, and the B cells are removed from the source, which is different from the elimination of B cells by drug depletion including

anti-CD20, anti-CD22, anti-BAFF, and other antigens that consume the blood circulation and express corresponding antigens, B cell factors could be completely excluded.

The results showed that B cell depletion reduced the expression of cytokines TNF-α, IL-1, IL-6, TGF-β1, decreased myocardial collagen synthesis after AMI, alleviated myocardial fibrosis, improved left ventricular remodeling, and maintained left ventricular ejection fraction. The BKO mice used in this study completely lack the entire B cells system, and the elimination of B cells from the source is different from the elimination of B cells by drug depletion. For example, anti-CD20, anti-CD22, and anti-BAFF, which deplete the expression of corresponding antigens in the blood circulation, B cell factors are completely excluded, which can be complementary to the study on drug depletion of B cells. Our study showed that the loss of B cells reduced the expression of cytokines TNF-α, IL-1β, IL-6, and TGF-β1, and reduced myocardial collagen synthesis after AMI. What's more, myocardial fibrosis in BKO AMI mice was degraded compared with WT AMI mice, which indicated that anti-B cell treatment could improve remodeling of the left ventricular and maintained left ventricular ejection fraction.

Conclusion

B cells could promote cytokine secretion and may participate in the pathological process of myocardial injury after AMI, which was related to myocardial fibrosis after AMI. Moreover, activated B cells could secrete cytokines, affected myocardial collagen metabolism after AMI, and promoted myocardial collagen I and III expressions, which seriously damaged cardiac structure and ventricular diastolic and systolic function, and caused heart failure eventually. However, further cell experiments as well as clinical experiments need to be conducted to verify our conclusion.

Declarations

Ethics approval and consent to participate

The study was approved by the Experimental Animal Ethics Committee of Guangxi Medical University and was carried out in accordance with BioMed Central editorial policies.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no conflict of interests.

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Authors' contributions

Wu WF and Mo FR designed the experiments and analyzed and interpreted the data. Yan YL, Li J, and Lai SY performed echocardiography date, Mo FR and Luo Y wrote the manuscript, and Wu WF helped to modify and edited the manuscript. All authors read and approved the final manuscript.

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Abbreviations

BKO: Bmi-1 knock out

COL1-A1: collagen alpha-1(I)

COL3-A1: collagen alpha-1(III)

ELISA: enzyme-linked immunosorbent assay

IL-1β: interleukin-1β

IL-6: interleukin-6

LSD: Least Significant Difference

LVEDd: left ventricular end-diastolic diameter

LVESd: left ventricular end-systolic diameter

MMP9: matrix metalloproteinase 9

RT-PCR: Real-time fluorescence quantitative polymerase chain reaction

M±SD: mean ± standard deviation

TIMP-1: tissue inhibitor of matrix metalloproteinase-1

TNF-α: tumor necrosis factor-α

TGF-β1: transforming growth factor-β

WT: wild-type

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Tables

Table 1 The primer sequences applied in our study.

Gene	Tm（°C）	Forward sequence	Reverse sequence
TGF-β1	62.5	ATGTCTCAGCCTCTTCTCATTC	TGGTGAATGACAGTGCGGTTATGG
TNF-α	58.2	ATGTCTCAGCCTCTTCTCATTC	GCTTGTCACTCGAATTTTGAGA
IL-6	60.5	CTCCCAACAGACCTGTCTATAC	CCATTGCACAACTCTTTTCTCA
IL-1β	60.5	TCGCAGCAGCACATCAACAAGAG	TGCTCATGTCCTCATCCTGGAAGG
BAFF	53.9	AACAAGATGTAGACCTCTCAGC	CTGCAGACAGTCTTGAATGATG
β-actin	60.5	CTACCTCATGAAGATCCTGACC	CACAGCTTCTCTTTGATGTCAC
COL1A1	54.3	AAAGATGGACTCAACGGTCTC	CATCGTGAGCCTTCTCTTGAG
COL3A1	52.4	GAAAGAATGGGGAGACTGGAC	TACCAGGTATGCCTTGTAATCC
TIMP-1	54.2	CTCCAGTTTGCAAGGGATAGAT	CAAAGACCTGAAAACCTCCAAC
MMP-9	53.9	CAAAGACCTGAAAACCTCCAAC	GACTGCTTCTCTCCCATCATC

Table 2. The percentages of $CD69^{+}CD19^{+}$ cells in myocardium, spleen, and blood in AMI and control mice.

Group	n	CD19+CD69+（%）		
		Myocardium	Spleen	Blood
AMI 3d	8	10.62±1.62*#	6.05±0.73*#	5.32±0.73*#
Sham 3d	6	3.72±0.51	3.62±0.36	2.29±0.43
AMI 5d	8	22.71±3.43*	12.40±2.07*	6.82±0.77*
Sham 5d	6	3.75±0.29	3.80±0.40	2.76±0.39
AMI 1W	8	7.62±1.74*#	4.02±0.58*#	3.60±0.73#
Sham 1W	6	3.53±0.65	3.71±0.56	2.84±0.55
AMI 2W	8	4.45±0.75#	4.13±0.82#	3.10±0.58#
Sham 2W	6	3.40±0.44	3.58± 0.59	2.58± 0.67

*P<0.05, the activated B cell ratio of AMI group was compared with the corresponding subgroups of the Sham group, #P<0.05，the activated B cell ratio of other subgroups of the AMI group was compared with AMI 5 days subgroups. Data are represented by mean ± standard deviation.

Table 3. Color Doppler echocardiography in 2 weeks subgroup of Sham, WT, and BKO group.

Indicators	Sham	BKO	AMI
LVEDd（mm）	3.70±0.12	4.97±0.19*#	5.51±0.19*
LVESd（mm）	1.09±0.04	2.57±0.06*#	3.67±0.07*
EF（%）	71.00±3.34	50.33±3.01*#	36.17±4.62*

*P<0.05，cardiac function indicators of BKO and AMI group was compared with Sham group；#P<0.05，cardiac function indicators of BKO group was compared with AMI group. Data are represented by mean ± standard deviation

Figures

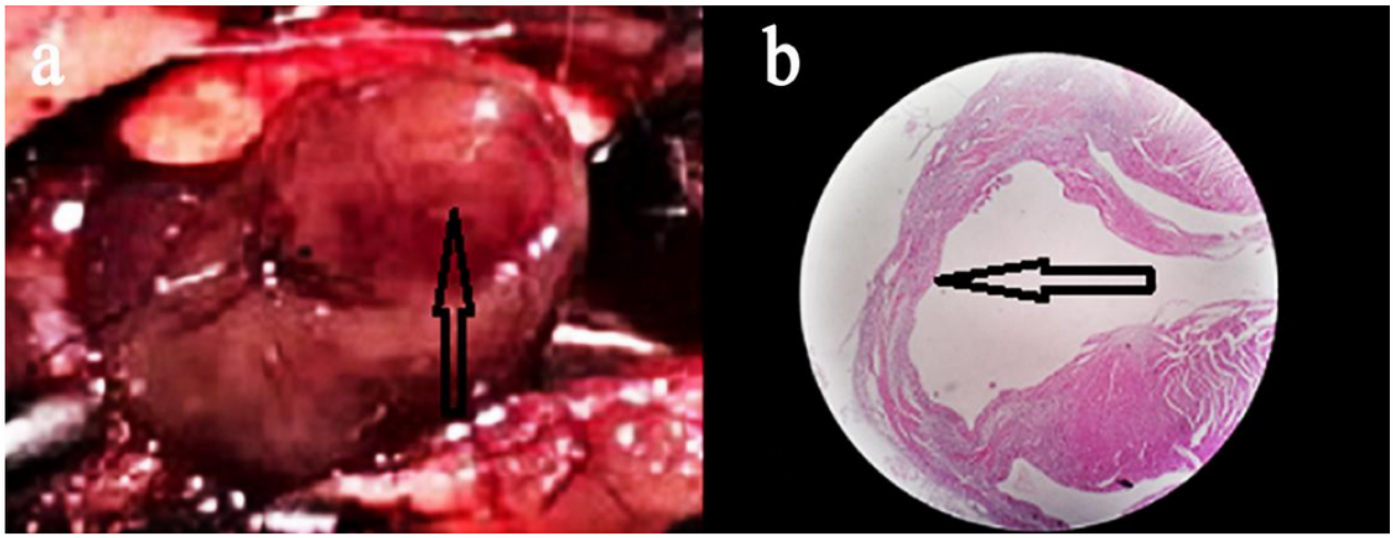

Figure 1

Gross specimen and pathological features after LAD ligation in mice. a: Myocardial whitening below ligation line after ligation of LAD in mice (arrow indication), b: HE staining after myocardial infarction suggested myocardium thinning (arrow indication).

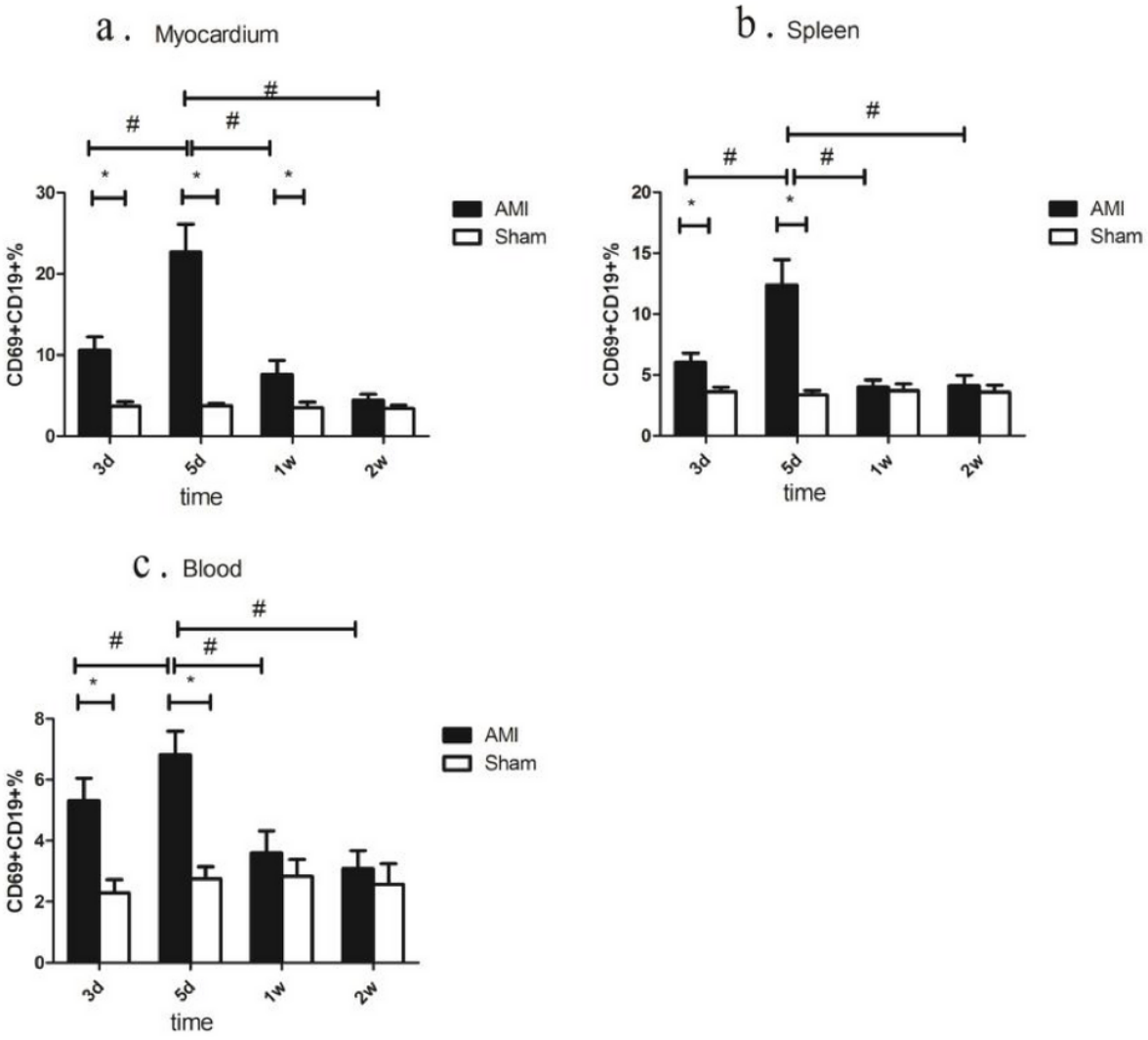

Figure 2

Results of activated B-cell flow in myocardium, spleen, and peripheral blood of the AMI group and Sham group in different subgroups. *P<0.05, the activated B cell ratio of AMI group was compared with the corresponding subgroups of the Sham group, #P<0.05the activated B cell ratio of other subgroups of the AMI group compared with AMI 5 days subgroups. Data are represented by mean ± standard deviation.

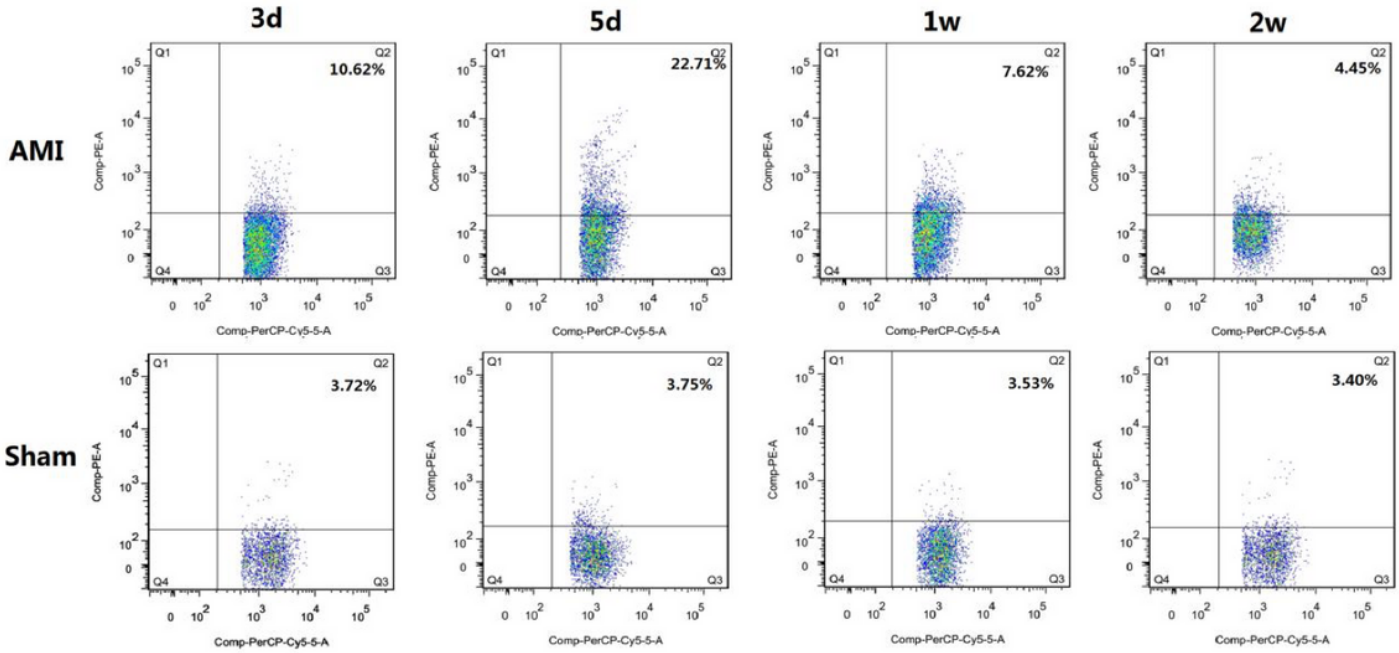

Figure 3

Flow cytometry of myocardial activation B cells in the AMI group and Sham group in different subgroups. The numbers in the upper right quadrant represent the mean proportion of activated B cells.

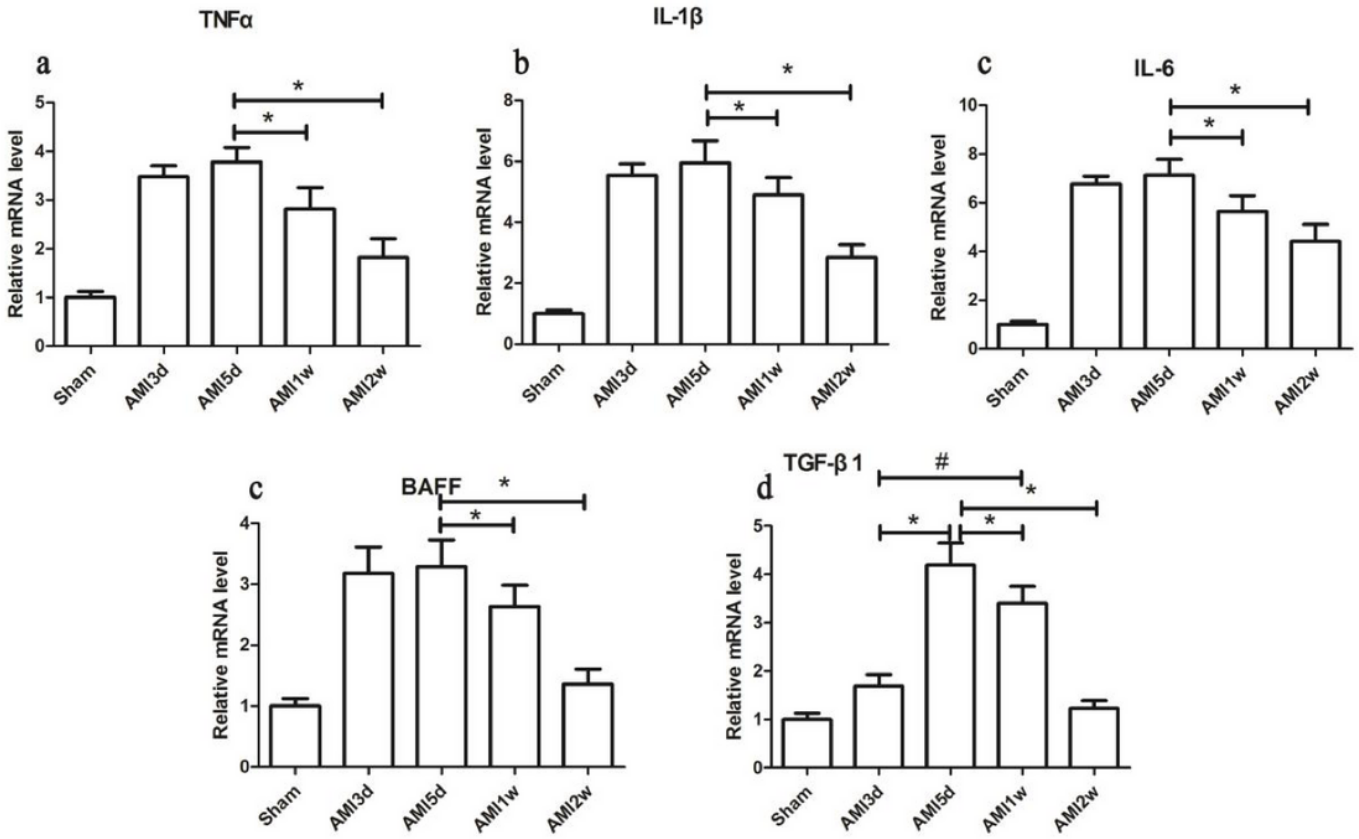

Figure 4

Relative expression levels of cytokine mRNA in AMI mice myocardium. *P<0.05，the relative expression levels of cytokine mRNA of other subgroup was compared with AMI group 5 days subgroup. #P<0.05，the relative expression levels of cytokine mRNA of AMI 1-week subgroup was compared with AMI 3 days subgroups.

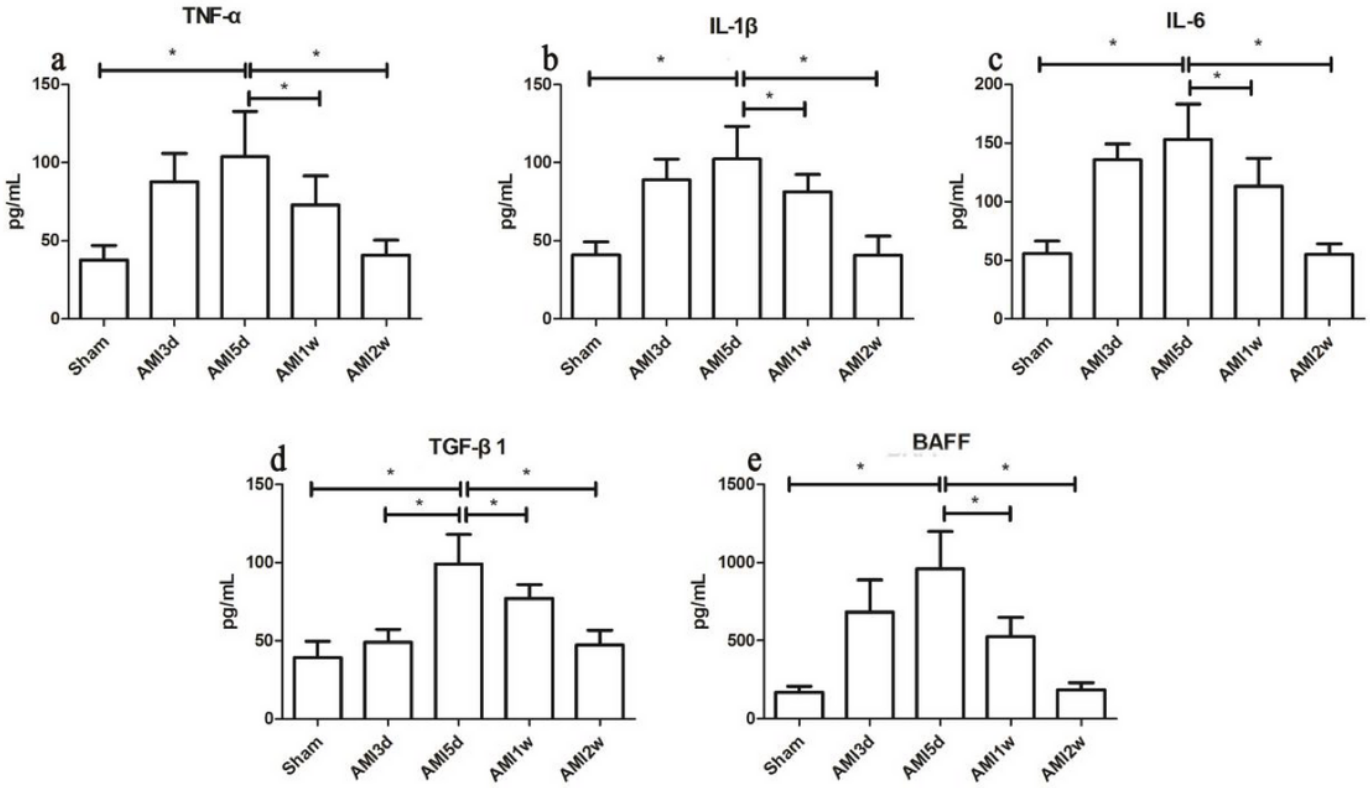

Figure 5

Peripheral blood cytokine concentration of AMI group. *P<0.05, the peripheral blood cytokine concentration of other subgroups were compared with AMI 5 days subgroups.

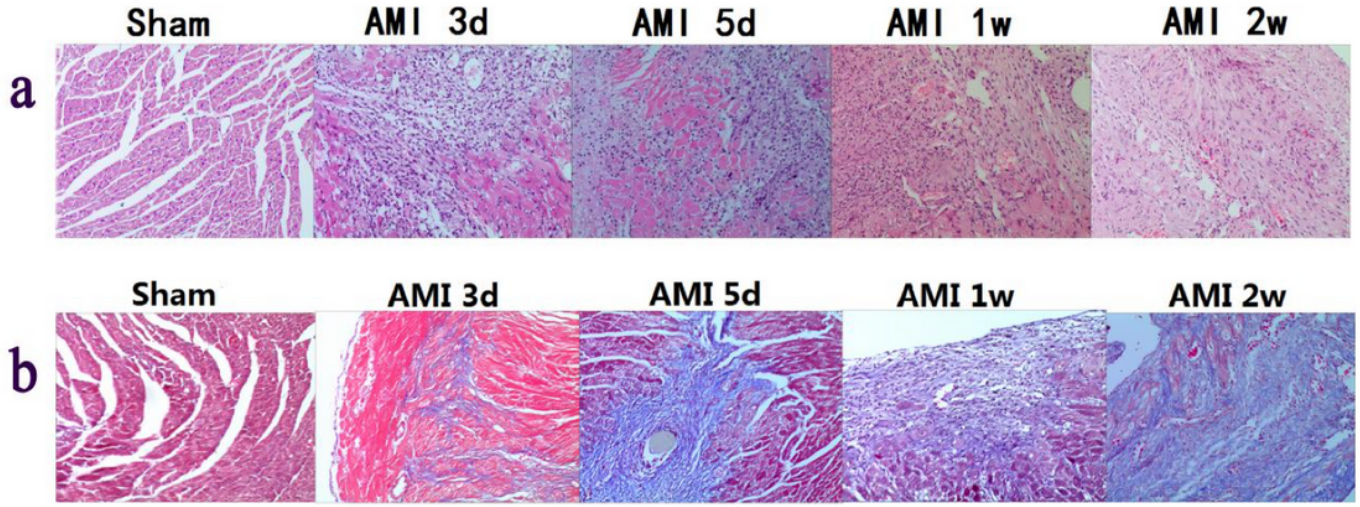

Figure 6

HE and Masson staining of myocardium AMI mice at different time points (200×). a: HE staining, b: Masson staining.

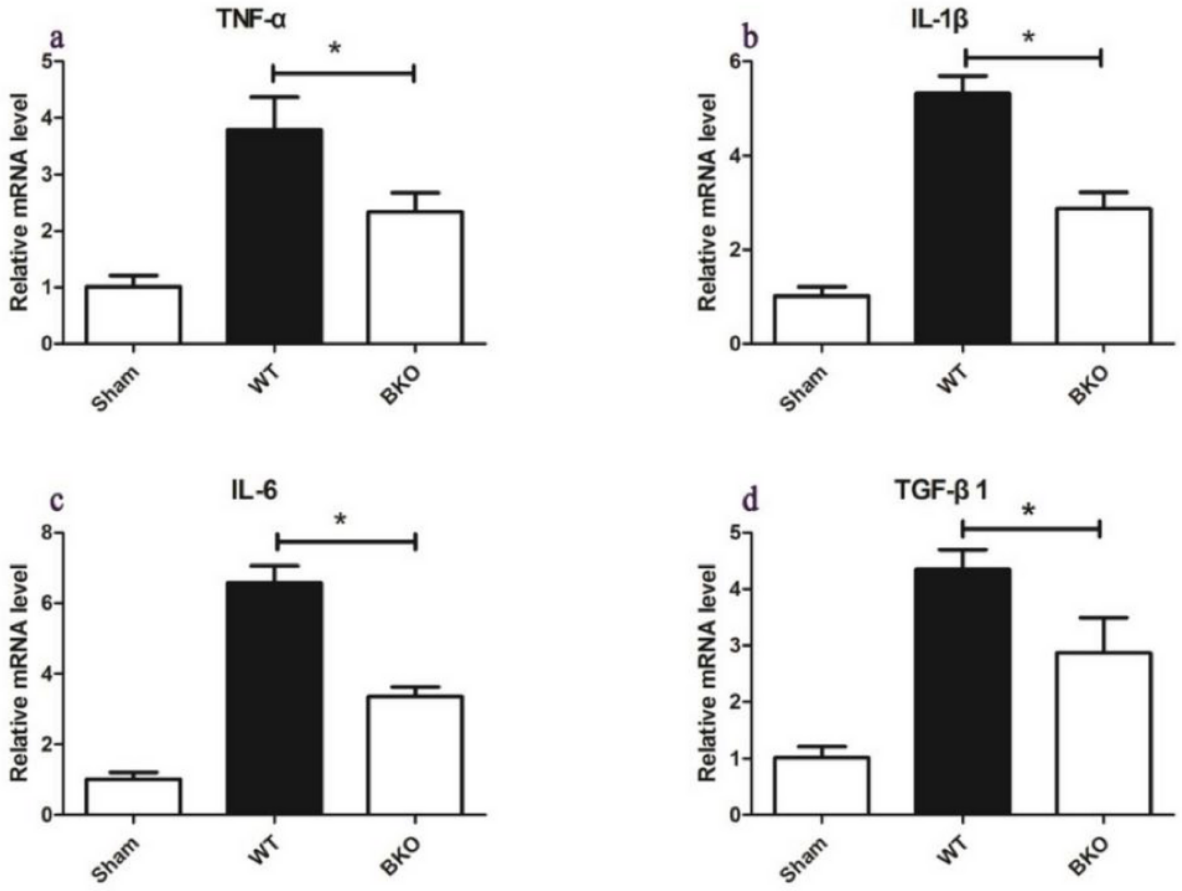

Figure 7

The relative expression level of cytokine mRNA in the myocardium of the Sham, WT, and BKO group. *P<0.05, the expression level of cytokine mRNA of the Sham group was compared with WT and BKO groups.

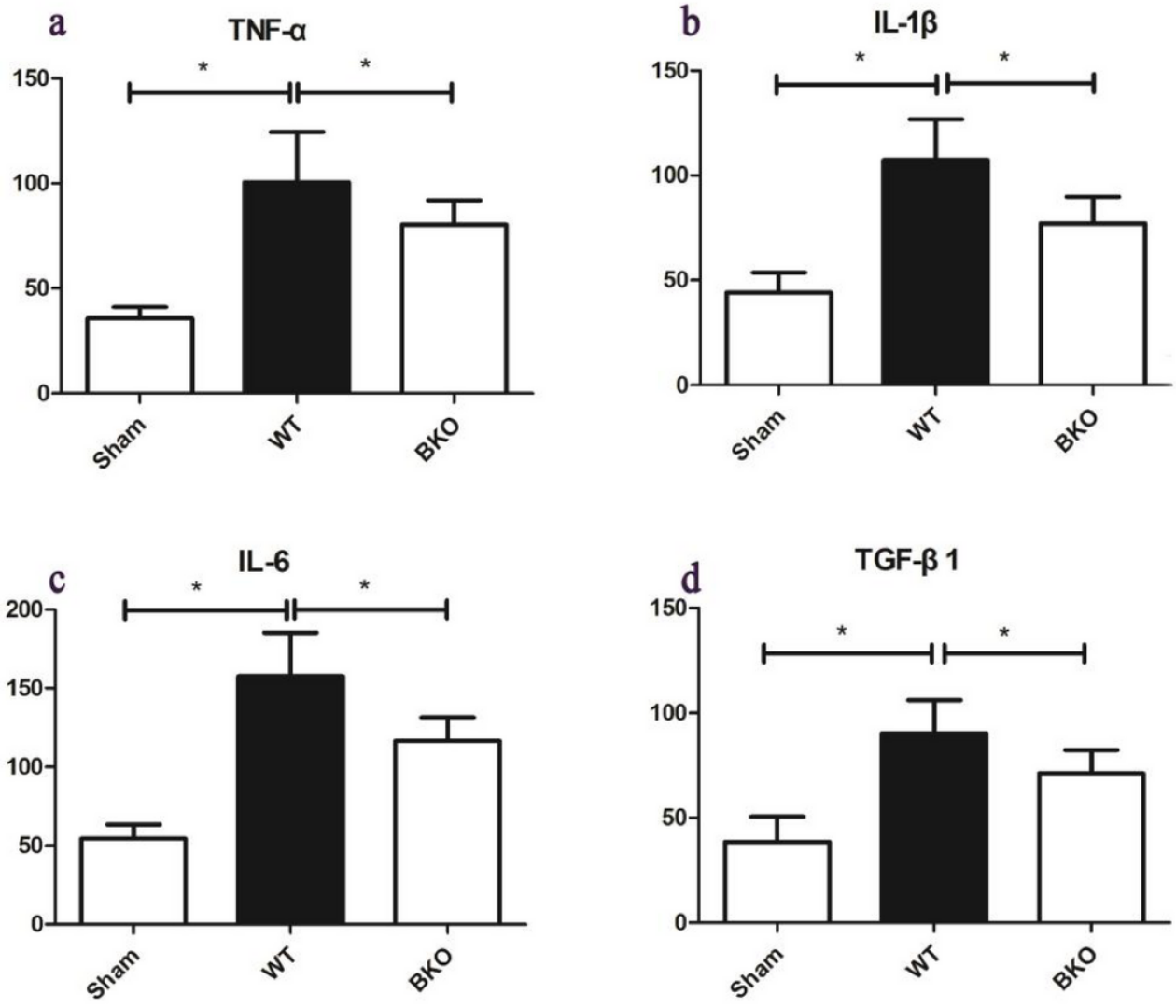

Figure 8

Peripheral blood cytokine concentrations in the myocardium of the Sham , WT, and BKO groups. *P<0.05, the concentration of peripheral blood cytokine of the Sham group was compared with WT and BKO groups.

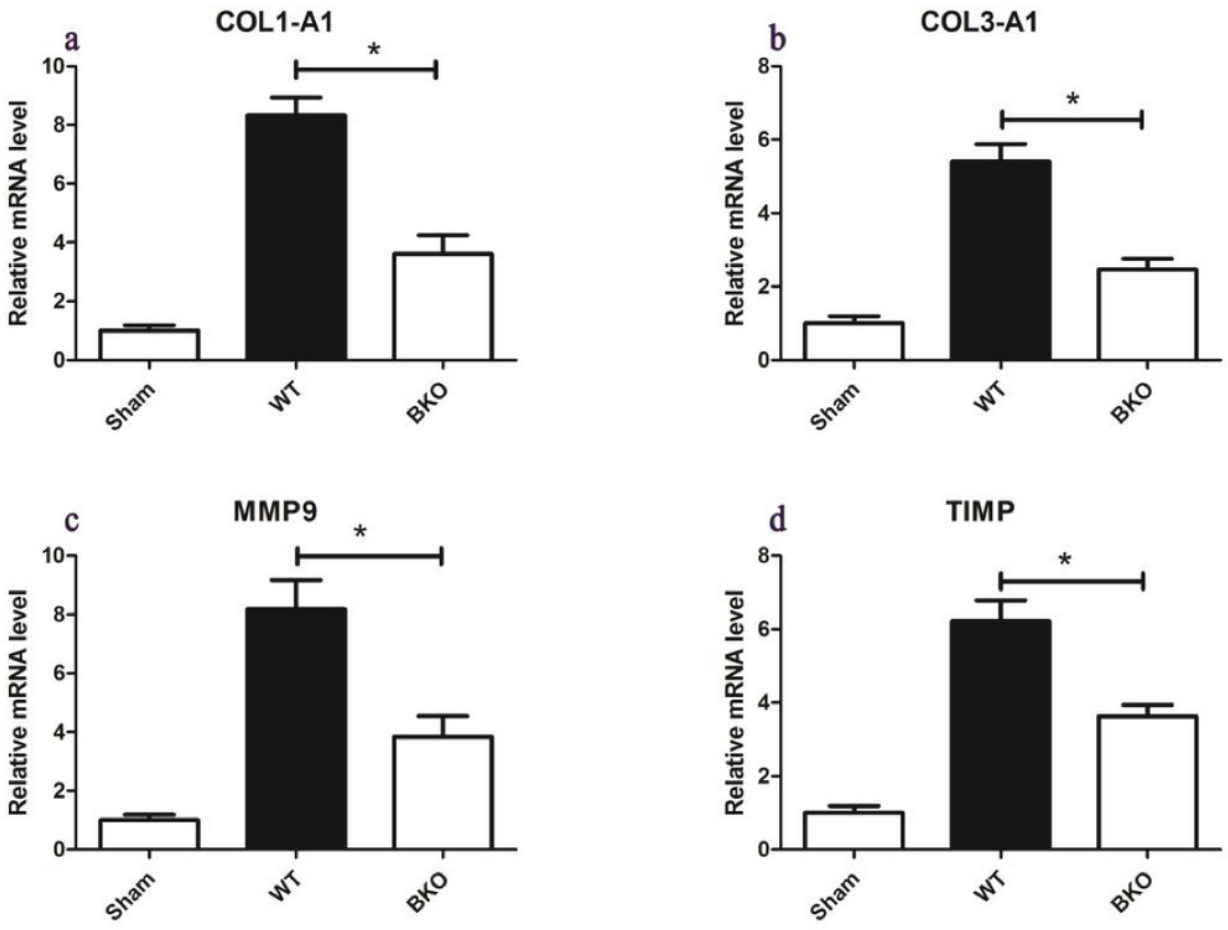

Figure 9

The relative expression level of collagen metabolism index mRNA in the myocardium of AMI mice*P<0.05, the relative expression level of collagen metabolism index mRNA of the Sham group was compared with WT and BKO groups.

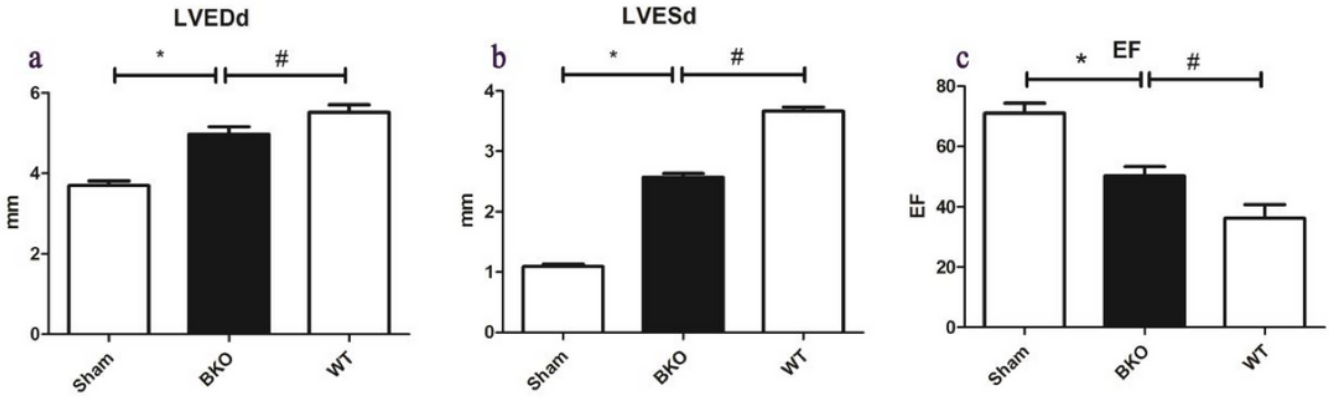

Figure 10

Color Doppler echocardiography in 2 weeks subgroup of Sham, WT, and BKO group. LVEDd: Left ventricular end-diastolic diameter, LVESd: left ventricular end-diastolic diameter, EF: Ejection fraction. *P<0.05cardiac function indicators of BKO and AMI group was compared with Sham group, #P<0.05cardiac function indicators of BKO group was compared with AMI group.

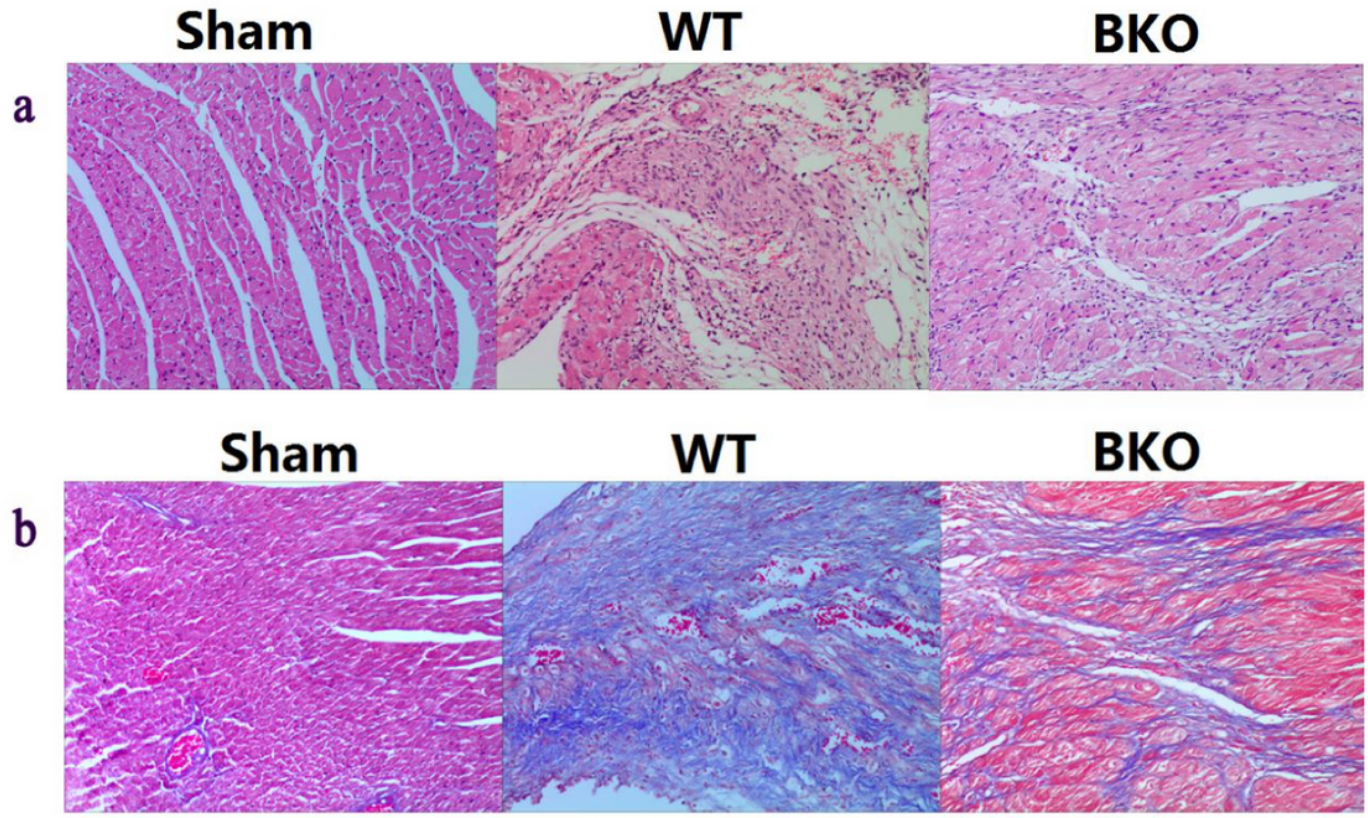

Figure 11

HE and Masson staining of the myocardium in 2 weeks subgroup of Sham, WT, and BKO group (200×). a: HE staining, b: Masson staining.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ARRIVEchecklist.pdf