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IL-17A contributes to myocardial ischemic injury by activating NLRP3 inflammasome in macrophages through AMPKα/p38MAPK/ERK1/2 signal pathway in mice

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ABSTRACT

Background: Acute myocardial infarction (AMI) is followed by an acute inflammation involving inflammasome activation, thereby inducing cardiac dysfunction. Interleukin-17A (IL-17A) involves in many inflammatory diseases, but its roles in inflammation following AMI are still obscure. The aim of this study is to investigate the roles of IL-17A in the inflammatory response following AMI and its underlying mechanisms.

Methods and results: NLRP3 inflammasome and AMPKα/p38MAPK/ERK1/2 signaling pathway were significantly activated under the induction of IL-17A in mouse peritoneal macrophages, which could be inhibited by AMPK inhibitor compound C (CC). Both p38MAPK and ERK1/2 inhibitors could partially inhibit the activation of NLRP3 inflammasome in macrophages treated by IL-17A. In vivo, IL-17A knockout not only decreased the infiltration of macrophages and the activation of NLRP3 inflammasome and AMPKα/p38MAPK/ERK1/2 signaling pathway in ischemic myocardium, but also improved cardiac function and reduced infarction size after the ligation of descending segment from left coronary artery for 3 days in mice, while IL-17A administration further aggravated the myocardial ischemic injury, which were prevented by CC administration.

Conclusion: IL-17A aggravates inflammatory response during AMI by inducing macrophages infiltration and activating NLRP3 inflammasome through AMPKα/p38MAPK/ERK1/2 pathway.

1. Background

Acute myocardial infarction (AMI) is a common cardiac emergency, with the potential risk for substantial morbidity and mortality (Anderson and Morrow, 2017). The onset of acute myocardial ischemia induces cellular injury and death of myocardium and initiates an acute pro-inflammatory response. During the acute inflammatory period following AMI, the inflammatory monocytes were recruited to heart and differentiated into macrophages (Ong et al., 2018). Prolonged presence of inflammatory macrophages can extend the pro-inflammatory phase and cause the expansion of infarcted areas, thereby exacerbating the poor cardiac function following AMI (van Amerongen et al., 2007). In addition, during the acute ischemia period, lots of DNAs, ATP and cellular debris were released from damaged or dead cells, and lead to the activation of Nod-like receptor protein 3 (NLRP3) inflammasome, which can regulate the activation of caspase 1 and further amplify inflammatory responses by inducing the release of powerful pro-inflammatory cytokines, such as interleukin-1β (IL-1β) from cardiomyocytes, white blood cells or fibroblasts in the ischemic heart, as well as promote the adverse cardiac remodeling and failure (Ong et al., 2018; Elliott and Sutterwala, 2015; Kawaguchi et al., 2011; Mezzaroma et al., 2011; Takahashi, 2014; Toldo et al., 2015).

Interleukin-17A (IL-17A) is a pleiotropic cytokine that belongs to IL-17 family. IL-17A can be produced by multiple types of cells and execute its functions through binding with IL-17 receptor (IL-17R) (Kolls and Lindén, 2004). Most experimental evidences suggest the role of IL-17 during inflammatory process in inducing the release of pro-inflammatory cytokines. Mitogen-activated protein kinase (MAPK) signal pathway (Kolls and Lindén, 2004; Wuyts et al., 2005) and NLRP3 inflammasome activation (Yan et al., 2018; Zhang et al., 2016) may

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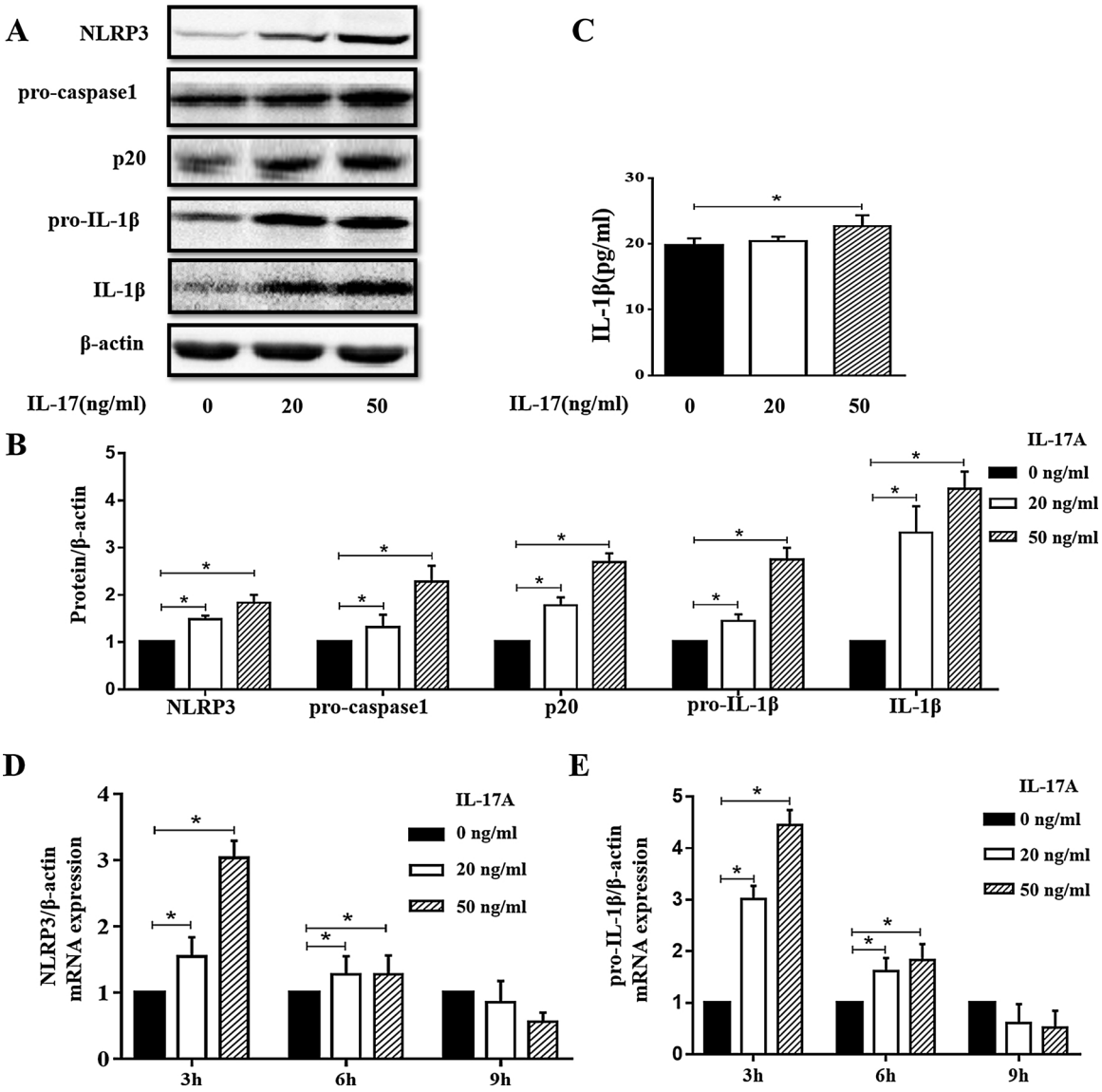

Fig. 1. IL-17A increased IL-1β release through activating NLRP3 inflammasome in macrophages. A–B, Representative western blot analysis of NLRP3, pro- and active caspase-1 (p20) and pro- and mature IL-1β in peritoneal macrophages treated with 0, 20 and 50 ng/ml IL-17A for 12 h. C, ELISA analysis for IL-1β in cell culture supernatant of peritoneal macrophages treated with 0, 20 and 50 ng/ml IL-17A for 12 h. D–E, The mRNA expression of NLRP3 and pro-IL-1β in peritoneal macrophages treated with 0, 20 and 50 ng/ml IL-17A for 3, 6 or 9 h. The β-actin was used as the loading control. All data are presented as mean ± standard deviation (M ± SD) from 3 independent experiments (n = 5 in each group for ELISA and Western blot, n = 9 in each group for PCR). *$P < 0.05$.

involve in the processes of inflammation induced by IL-17A. In addition, patients with AMI reveal a significant increase in peripheral Th17 number and blood IL-17A (Cheng et al., 2008; Liuzzo et al., 2013). And IL-17A contributes to myocardial ischemia/reperfusion injury and promotes ventricular remodeling after myocardial infarction by regulating cardiomyocyte apoptosis and neutrophil infiltration (Liao et al., 2012; Zhou et al., 2014). However, the relationship between IL-17A and inflammasome during acute inflammatory response following acute myocardial ischemia needs to be further addressed.

Adenosine monophosphate activated protein kinase (AMPK) is a kind of positive regulator for cellular energy and metabolic balance (Garcia and Shaw, 2017). The actions of AMPK are complicated and contradictive. In some cases, AMPK has emerged as an inflammation controller to protect vessels against inflammatory injury and reduce high-fat diet (HFD)-induced inflammation (He et al., 2015; Jung et al., 2015; O'Neill and Hardie, 2013; Thornton et al., 2016). But some other studies have demonstrated that up-regulated AMPK signaling is associated with the activation of inflammasomes and the suppressed AMPK activity can significantly attenuate ATP or lipopolysaccharide (LPS)-induced inflammasome activation in murine macrophages (Kim et al., 2017; Li et al., 2017; Zha et al., 2016). During myocardial ischemia-reperfusion, as an early adaptive mechanism, AMPK is activated in ischemic myocardium and this intrinsic AMPK activation has a beneficial role in preventing cardiac injury (Qi and Young, 2015). In contrast, recent reports have documented that AMPK activation during fatty acid oxidation also has the potential to exacerbate I/R injury and the suppression of AMPK may improve the recovery of cardiac function after ischemia through partial reduction of glycolysis in isolated mouse hearts due to the expression of a dominantly negative (DN) α2-subunit of AMPK (Folmes et al., 2009; Lopaschuk et al., 1993). The roles of AMPK during myocardial infarction are complicated due to its differences in different cell types, specific time point and specific microenvironment, which needs to be further elucidated. In addition, little is known about the association of IL-17A and AMPKα during the acute inflammatory response following AMI. Herein, we reported that IL-17A could amplify inflammation by increasing macrophage infiltration and

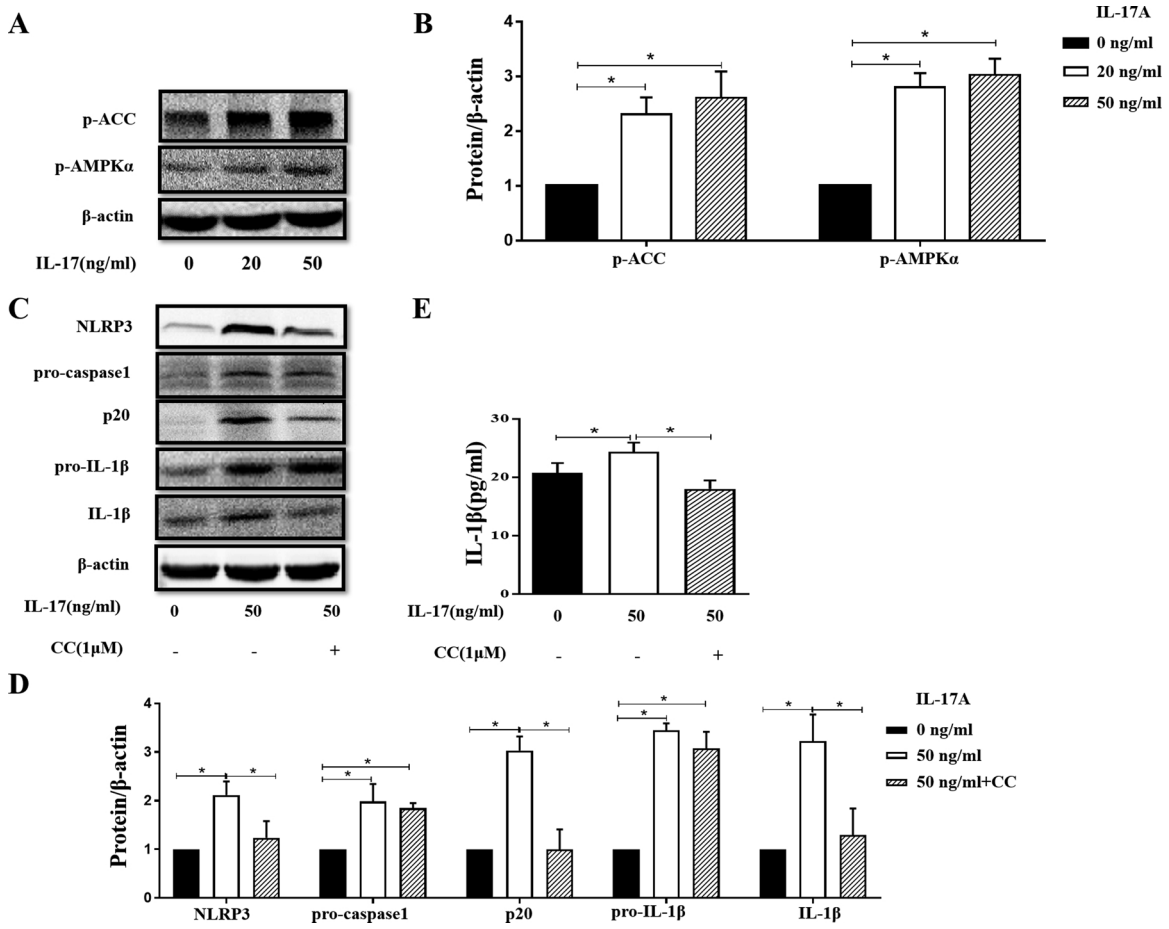

Fig. 2. IL-17A activated NLRP3 inflammasome via the phosphorylation of AMPKα in macrophages. A–B, Representative western blot analysis of p-ACC and p-AMPKα in peritoneal macrophages treated with 0, 20 and 50 ng/ml IL-17A for 5 min. C–D, Representative western blot analysis of NLRP3, pro- and active caspase-1 (p20) and pro- and mature IL-1β in peritoneal macrophages treated by 50 ng/ml IL-17A with or without CC (CC, compound C, a AMPK inhibitor) for 12 h. E, ELISA analysis of IL-1β in cell culture supernatant of macrophages treated by 50 ng/ml IL-17A with or without CC for 12 h. The β-actin was used as the loading control. All data are presented as M ± SD from 3 independent experiments (n = 5 in each group for ELISA and Western blot). *$P < 0.05$.

activating NLRP3 inflammasome in ischemic myocardium of mice with AMI via AMPKα/p38MAPK/extracellular signal regulated kinase 1 and 2 (ERK1/2) signal pathway.

2. Materials and methods

In the Supplementary materials.

3. Results

3.1. IL-17A increased IL-1β release through activating NLRP3 inflammasome in macrophages

In order to investigate the effect of IL-17A on inflammasome in macrophages, mouse peritoneal macrophages were treated with 0, 20 and 50 ng/mL IL-17A for 12 h or with 50 ng/mL IL-17A for 12, 24 or 36 h. As shown in Figs. 1A–B and S-2A–B, the expression of NLRP3 protein was increased. In addition, the amount of pro- and active caspase-1 (p20) and pro- and mature IL-1β was increased. We further investigated the mature IL-1β released into cultural supernatants from macrophages using ELISA assay. As shown in Fig. 1C, the amount of IL-1β in cell culture supernatant after 50 ng/mL IL-17A stimulation revealed an obvious increase when compared with the control group. Moreover, Fig. 1D and E exhibited that the expression of NLRP3 and IL-1β at the mRNA level was up-regulated after IL-17A treatment for 3 h and 6 h. These results suggested that IL-17A treatment could activate NLRP3 inflammasome and induce the release of IL-1β from macrophages.

3.2. IL-17A activated NLRP3 inflammasome via the phosphorylation of AMPKα in macrophages

According to a previous report (Mohamed et al., 2016), AMPK activation is involved in the protection of low-dose IL-17A administration on diabetic nephropathy in mice, which stimulates us to explore whether AMPK also plays a role in the activation of NLRP3 inflammasome in the presence of IL-17A. Then, we tested the level of phosphorylated AMPKα at Tr172 in peritoneal macrophages subjected to the treatment with 0, 20 and 50 ng/mL IL-17A for 5 min or with 50 ng/mL IL-17 for 5, 15 or 30 min. As shown in Figs. 2A–B and S-2C–D, both phosphorylated AMPKα and acetyl-CoA carboxylase (ACC) were increased obviously. On the other hand, upon the application of compound C (CC, an AMPK inhibitor), the increased expression of NLRP3, pro-caspase-1, p20 and pro- and mature IL-1β protein in macrophages and the release of IL-1β in cell culture supernatant induced by IL-17A were reversed (Fig. 2C–E). These results indicated that the activation of NLRP3 inflammasome induced by IL-17A in macrophages was mediated by the phosphorylation of AMPKα.

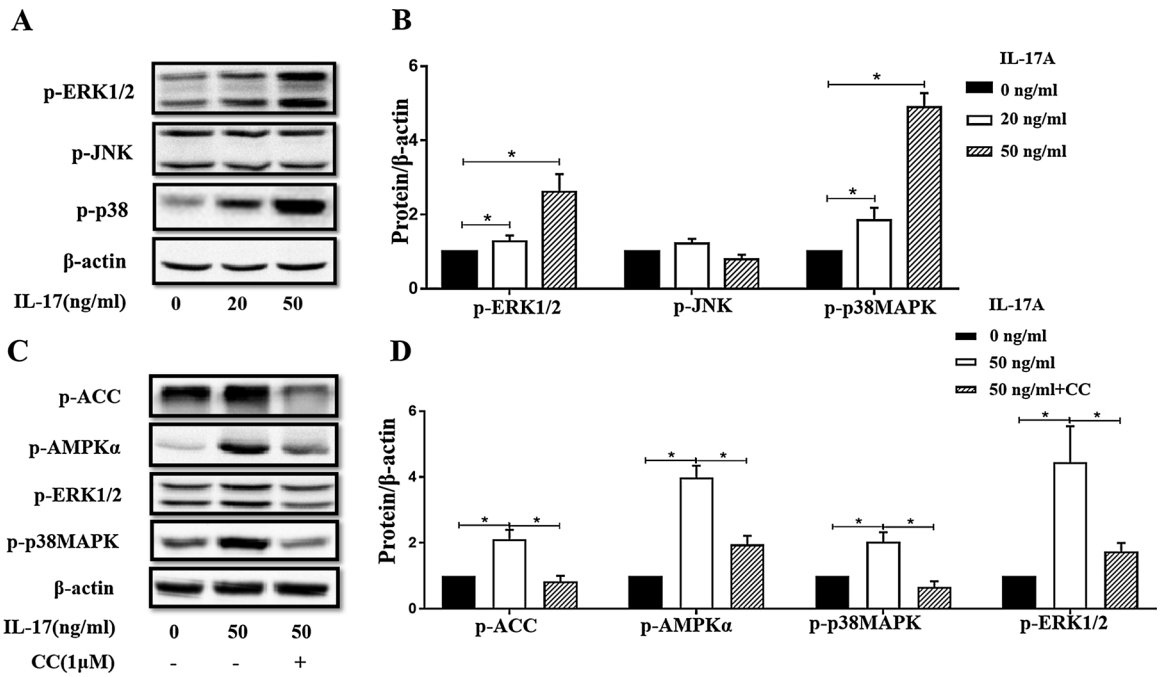

Fig. 3. p38MAPK and ERK1/2 were the downstream molecules of AMPKα activation. A–B, Representative western blot analysis of p-ERK1/2, p-JNK, and p-p38MAPK in peritoneal macrophages treated with 0, 20 and 50 ng/ml IL-17A for 5 min (A). C–D, Western blot analysis of p-ACC, p-AMPKα, p-ERK1/2 and p-p38MAPK in peritoneal macrophages treated by 50 ng/ml IL-17A with or without CC for 5 min. The β-actin was used as the loading control. All data are presented as mean ± standard deviation (M ± SD) from 3 independent experiments (n = 5 in each group for Western blot). *$P < 0.05$.

3.3. p38MAPK and ERK1/2 were the downstream molecules of AMPKα activation

MAPKs, especially p38MAPK and ERK1/2, are important mediators of IL-17A-induced inflammation (Iwakura et al., 2011; Wang et al., 2014a). We then tested the roles of MAPKs in macrophages treated with IL-17A. As shown in Fig. 3A-B and S-2C–D, the phosphorylation of ERK1/2 and p38MAPK, but not c-Jun N-terminal kinase (JNK), was increased rapidly in macrophages treated with 0, 20 and 50 ng/mL IL-17 for 5 min or with 50 ng/mL IL-17 for 5, 15 or 30 min. AMPK inhibitor CC almost reversed the upregulation of p-p38 and p-ERK1/2 induced by IL-17A (Fig. 3C and D). These data proved that IL-17A further activated p38MAPK and ERK1/2 through AMPKα in mouse peritoneal macrophages.

3.4. The activation of NLRP3 inflammasome and release of IL-1β were induced by IL-17A through AMPKα/p38MAPK/ERK1/2 signal pathway in macrophages

In order to further explore the roles of AMPKα and p38MAPK/ERK1/2 in NLRP3 inflammasome activation induced by IL-17A in macrophages, the macrophages were treated with 50 ng/mL IL-17A with or without SB203580 (SB, a p38MAPK inhibitor) or PD98059 (PD, an ERK1/2 inhibitor). As shown in Fig. 4A–D, the increased expression of NLRP3, pro-caspase-1, p20 and pro- and mature IL-1β protein induced by IL-17A in macrophages were reversed in the presence of CC, SB or PD. In addition, the increased expression of NLRP3 mRNA and the release of IL-1β in cell culture supernatant induced by IL-17A were similarly reversed when combined with CC, SB or PD (Fig. 4E and F). However, increased expression of pro-IL-1β mRNA induced by IL-17A cannot be reversed by CC, SB or PD (Fig. 4G), which suggested that IL-17A induced the release of IL-1β by activating NLRP3 inflammasome without affecting the transcription of pro-IL-1β. These results indicated that the activation of NLRP3 inflammasome induced by IL-17A was highly correlated with AMPKα/p38MAPK/ERK1/2 signal pathway in macrophages.

3.5. IL-17A deteriorated cardiac injury after AMI

To explore the involvement of IL-17A in acute inflammation following AMI, we investigated serum IL-17A levels at the third day post operation by using mouse AMI model (the treatment protocol for animals was shown in supplementary materials). As shown in S-Fig. 1, the serum IL-17A value increased significantly at the third day post AMI when compared with that in sham mice. We then detected the activation of NLRP3 inflammasome induced by IL-17A in macrophages during ischemia. As shown in Fig. 5A and B, when compared with the sham group, M-mode echocardiography images of the left ventricle on Day 3 post-operation showed that EF value and LV fractional shortening (FS) were decreased in AMI group and lower in IL-17A group. The administration of AMPK inhibitor CC increased EF and FS value obviously. Furthermore, when compared with the AMI group, IL-17A enlarged infarct size, which was detected by using Masson trichrome staining, but CC treatment reduced the infarct size sharply (Fig. 5C). We also evaluated the infiltration of macrophages in ischemic myocardial tissues using immunofluorescence staining. As shown in Fig. 5D–E, the amount of $CD68^+$ (CD68, a specific cell surface marker of macrophages) macrophages was increased in ischemic cardiac tissue from AMI group and was further increased in IL-17A group, while such an infiltration was notably reduced after CC treatment. These data indicate that IL-17A can exacerbate the infiltration of macrophages in ischemic myocardium tissue and the impairment of cardiac function after AMI through AMPKα signal pathway, which can be alleviated by CC administration.

3.6. IL-17A activated NLRP3 inflammasome in macrophages in ischemic cardiac tissues and increased IL-1β release via AMPKα signal pathway

The activation of inflammasomes from cardiomyocytes, white blood cells or fibroblasts has been demonstrated to play an important role in acute inflammation following AMI (Elliott and Sutterwala, 2015; Kawaguchi et al., 2011; Mezzaroma et al., 2011; Takahashi, 2014; Toldo et al., 2015). In order to further determine the roles of NLRP3

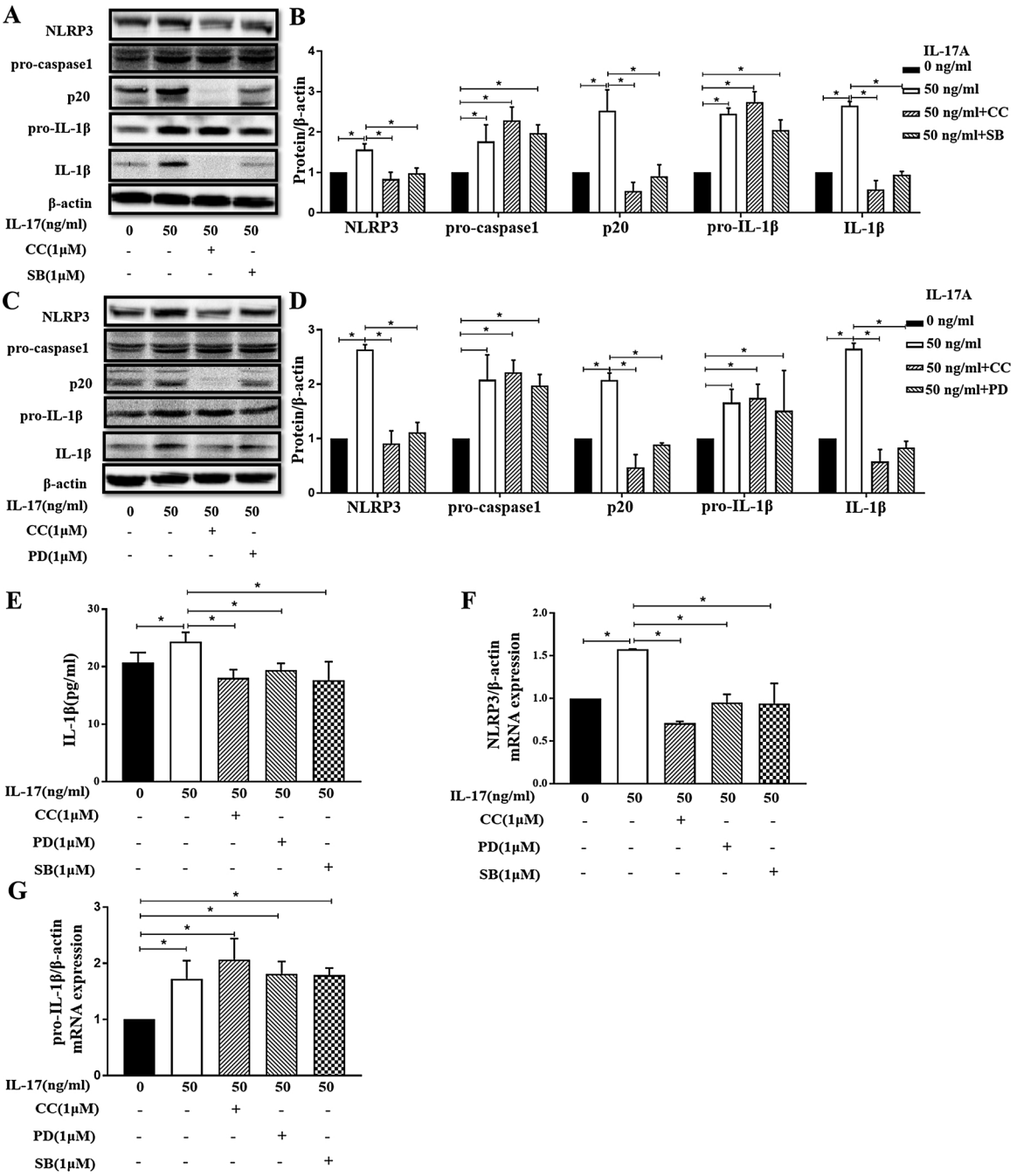

Fig. 4. The activation of NLRP3 inflammasome and release of IL-1β were induced by IL-17A through AMPKα/p38MAPK/ERK1/2 signal pathway in macrophages. A–B, Representative western blot analysis of NLRP3, pro- and active caspase-1 (p20) and pro- and mature IL-1β in peritoneal macrophages treated by 50 ng/ml IL-17A with or without CC or SB (SB, SB203580, a p38MAPK inhibitor) for 12 h. C–D, Representative western blot analysis of NLRP3, pro- and active caspase-1 (p20) and pro- and mature IL-1β in peritoneal macrophages treated by 50 ng/ml IL-17A with or without CC or PD (PD, PD98059, a ERK1/2 inhibitor) for 12 h. E, ELISA analysis of IL-1β in cell culture supernatant of macrophages treated by 50 ng/ml IL-17A with or without CC, SB or PD for 12 h. F–G, The mRNA expression of NLRP3 and pro-IL-1β in peritoneal macrophages treated by 50 ng/ml IL-17A with or without CC, SB or PD for 3 h. The β-actin was used as the loading control. All data are presented as M ± SD from 3 independent experiments (n = 5 in each group for ELISA and Western blot, n = 9 in each group for PCR). *$P < 0.05$.

inflammasomes in macrophages and the effects of AMPK inhibitor on myocardial injury after AMI, we labeled macrophages with CD68 and the components of NLRP3 inflammasomes (caspase-1, apoptosis speck-like protein containing a caspase-recruitment domain (ASC) or cryopyrin). Fig. 6A–B reveal that the number of $CD68^+caspase\text{-}1^+$, $CD68^+ASC^+$ and $CD68^+cryopyrin^+$ double positive macrophages was obviously increased in ischemic myocardial tissues on Day 3 post AMI when compared with the sham control group, which was further augmented by IL-17A administration. However, after CC treatment, the number of $CD68^+caspase\text{-}1^+$, $CD68^+ASC^+$ and $CD68^+cryopyrin^+$ double positive macrophages was significantly decreased. ELISA assay showed that IL-1β level in serum was increased at Day 3 post AMI and higher in IL-17A group, but was obviously decreased after CC treatment (Fig. 6G). Taken together, these results indicated that CC relieved the myocardial injury aggravated by IL-17A through reducing the activation of NLRP3 inflammasomes and IL-1β release of macrophages.

Moreover, we also evaluated the expression level of signaling molecules, such as p-AMPKα, p-ACC, p-p38MAPK and p-ERK1/2, and

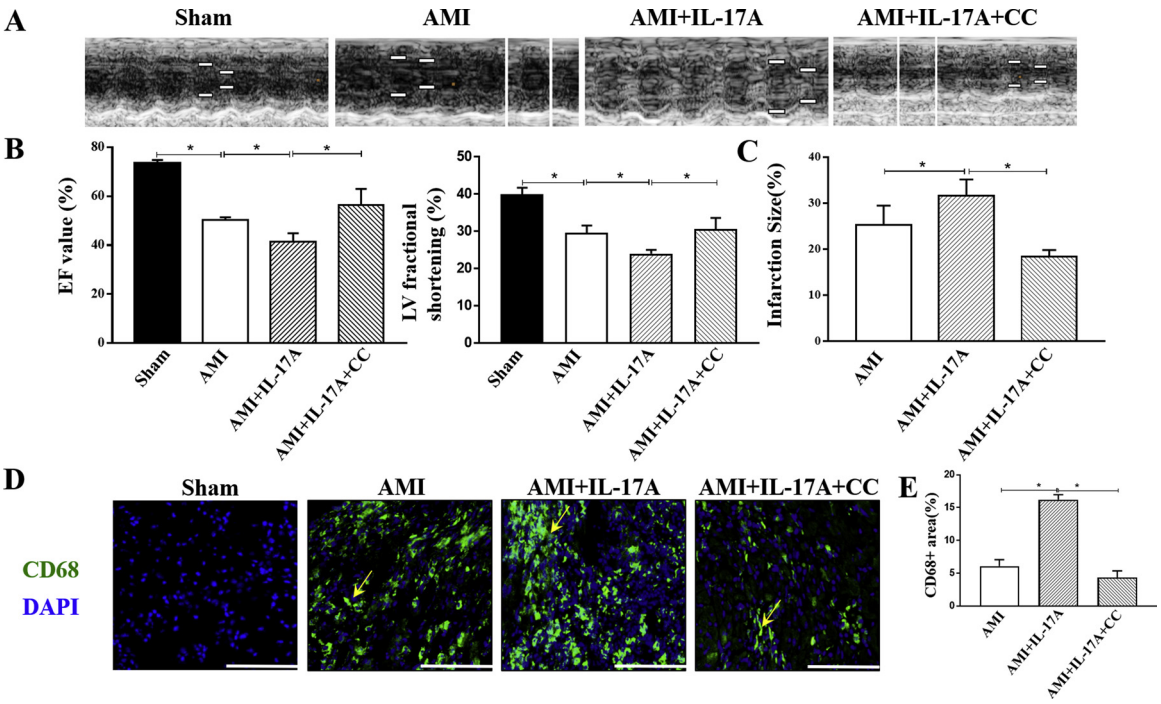

Fig. 5. IL-17A deteriorated cardiac injury after AMI. A, Representative M-mode images for LV of mice Day 3 post operation. B, Ejection fraction value (EF value) and LV fractional shortening (FS) of mice Day 3 post operation. C, Infarction size (Masson's trichrome) of mice Day 3 post operation. D–E, Immunofluorescent staining of cardiac tissue Day 3 post operation with antibodies against CD68 (green indicates $CD68^+$) and DAPI-stained (blue) cellular nuclei. $CD68^+$ positive macrophages were labeled with yellow arrows. (Scale bar, 100 μm.) All data are presented as M ± SD (n = 5–8 for each group). $^*P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inflammasome-related components, such as NLRP3, pro-caspase1, p20, pro-IL-β and IL-1β in injured myocardial tissues using western blot analysis. As shown in Fig. 6C–F, the above increased signaling molecules were further up-regulated and the NLRP3 inflammasomes were activated upon the administration of IL-17A, which revealed the opposite trend following CC treatment. These results also further confirmed the roles of AMPKα/p38MAPK/ERK1/2 signal pathway in myocardial injury after AMI induced by IL-17A.

3.7. IL-17A knockout ameliorated myocardial ischemic injury

To elucidate the impact of IL-17A on myocardial ischemic injury under IL-17A free situation, we constructed AMI model with $IL17A^{-/-}$ and compared the myocardial ischemic injury levels at Day 3 post operation with the wild-type C57BL/6 mice. As shown in Fig. 7A–C, $IL-17A^{-/-}$ mice had smaller infarction size and the improved cardiac function (EF and FS) after AMI than wild-type mice. In addition, the immunofluorescence staining showed that macrophage infiltration and NLRP3 inflammasome activation were also significantly relieved (Figs. 7D–E and 8 A–B). The western blot and ELISA analyses showed that the expression levels of signaling molecules (such as p-AMPKα, p-ACC, p-p38MAPK and p-ERK1/2), inflammasome-related components (such as NLRP3, pro-caspase1, p20, pro-IL-β and IL-1β) in injured myocardial tissues, and the serum IL-1β level were all decreased in the $IL17A^{-/-}$ AMI group than wild type AMI group (Fig. 8C–G). These results confirmed the roles of IL-17A in myocardial injury after AMI and its relationship with AMPKα/p38MAPK/ERK1/2 signal pathway.

4. Discussions

Although reperfusion therapy has been applied for many years in patients with AMI to salvage myocardium, there are still limited opportunities for these patients to receive percutaneous coronary intervention (PCI) operation in time due to various reasons. China PEACE Retrospective AMI Study has reported that there was no significant change in the rate of reperfusion treatment from 2001 to 2011 and only about 1/4 of STEMI patients received emergency PCI in 2011 (Wang et al., 2017). Therefore, it is still highly desired to explore multiple ways for the treatment of AMI. The acute inflammation following ischemia, even a short time, can result in the release of a variety of pro-inflammatory cytokines and chemokines and induce the recruitment of pro-inflammatory cells into the infarction zone, and then augment the pro-inflammatory response, which can aggravate myocardial injury (Ong et al., 2018). IL-17A involves in the pathological process of many inflammatory and autoimmune diseases and contributes to myocardial ischemia/reperfusion injury, but its roles in inflammation following AMI are still obscure (Kolls and Lindén, 2004; Liao et al., 2012; Liuzzo et al., 2013; Zhou et al., 2014; Basu et al., 2013). Our present study not only affirms the vital effects of IL-17A on acute inflammation following AMI, but also defines a novel regulatory role of AMPKα in inflammation following AMI. Accordingly, IL-17A may be a promising target to abate myocardial injury after AMI. In addition, the accurate roles of AMPKα in AMI need to be further explored and evaluated.

In the present study, we confirmed the proinflammatory role of IL-17A in macrophages by activating the NLRP3 inflammasome and releasing the strong proinflammatory cytokines in vitro. In vivo, we found that IL-17A treatment not only aggravated the infiltration of $CD68^+$ positive macrophages, but also accelerated the accumulation of $CD68^+caspase\text{-}1^+$, $CD68^+ASC^+$ and $CD68^+cryopyrin^+$ double positive cells in ischemic myocardial tissue, which further enlarged the infarction size and destroyed cardiac function induced by ischemia for 3 days (Figs. 5 and 6A–B). The activation of NLRP3 inflammasome and the amount of serum IL-1β was also up-regulated was increased after AMI and was higher with IL-17A treatment (Fig. 6E–G,). In addition, we compared myocardial ischemic injury levels between $IL17A^{-/-}$ and wild-type C57BL/6 mice at Day 3 following AMI, and observed that the $IL17A^{-/-}$ mice had smaller infarction size, the improved cardiac function, less macrophage infiltration and weaker NLRP3 inflammasome

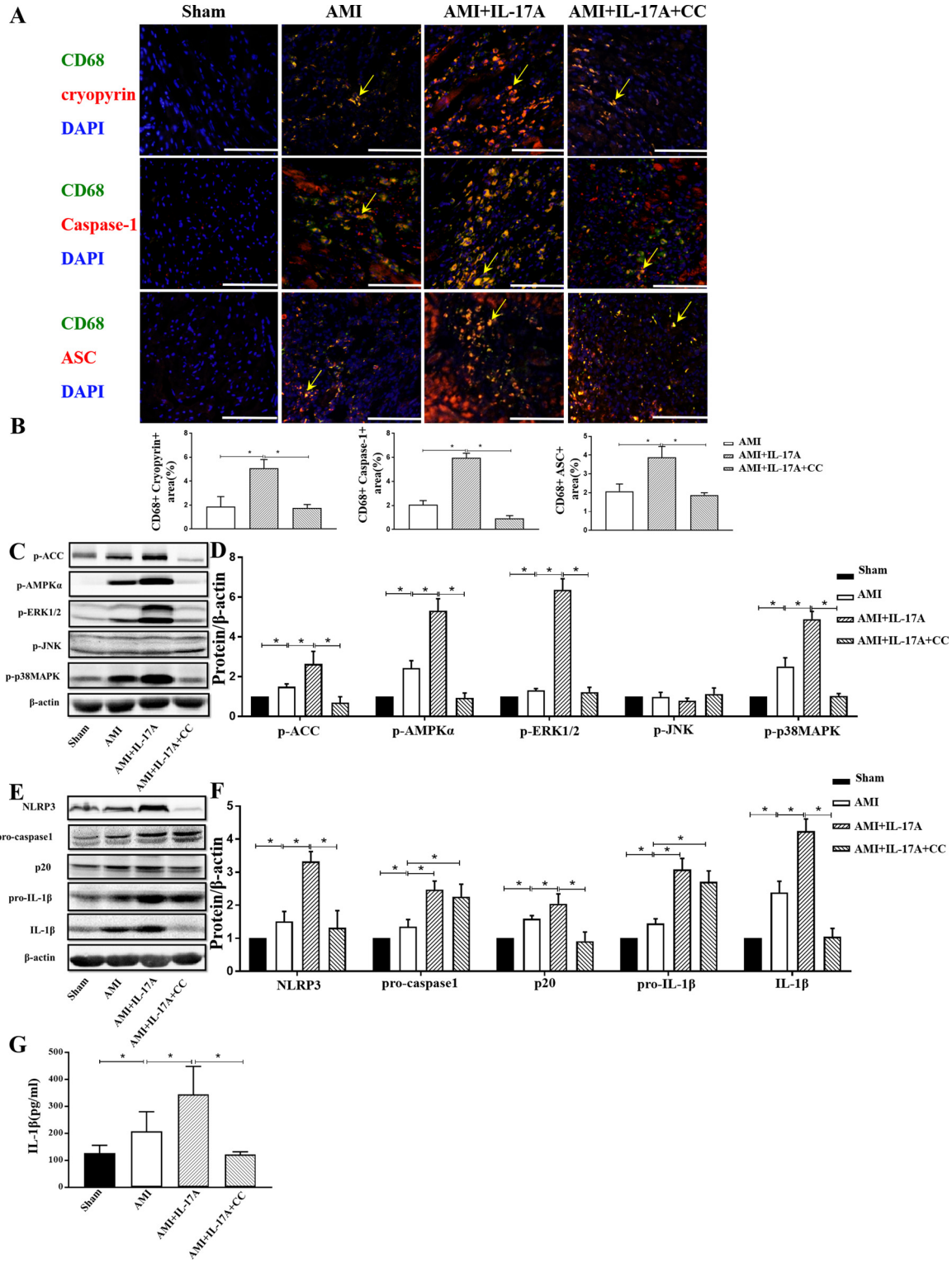

Fig. 6. IL-17A activated NLRP3 inflammasome in macrophages in ischemic cardiac tissues and increased IL-1β release via AMPKα signal pathway. A–B, Immunofluorescent double staining of cardiac tissue (control or ischemic) Day 3 post operation with antibodies against CD68 and caspase-1, ASC or cryopyrin. Green indicates CD68, red for caspase-1, ASC or cryopyrin and blue for DAPI-stained cellular nuclei. $CD68^+caspase\text{-}1^+$, $CD68^+ASC^+$ and $CD68^+cryopyrin^+$ double positive macrophages were labeled with yellow arrows. (Scale bar, 100 μm). C–D, Western blot analysis of p-ACC, p-AMPKα, p-ERK1/2, p-JNK MAPK, and p-p38MAPK in cardiac tissues (control or ischemic) of mice Day 3 post operation. E–F, Western blot analysis of NLRP3, pro- and active caspase-1 (p20) and pro- and mature IL-1β in cardiac tissues (control or ischemic) of mice Day 3 post operation. G, ELISA analysis of IL-1β in serum from mice Day 3 post operation. The β-actin was used as the loading control. All data are presented as M ± SD (n = 5–8 for each group). *$P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

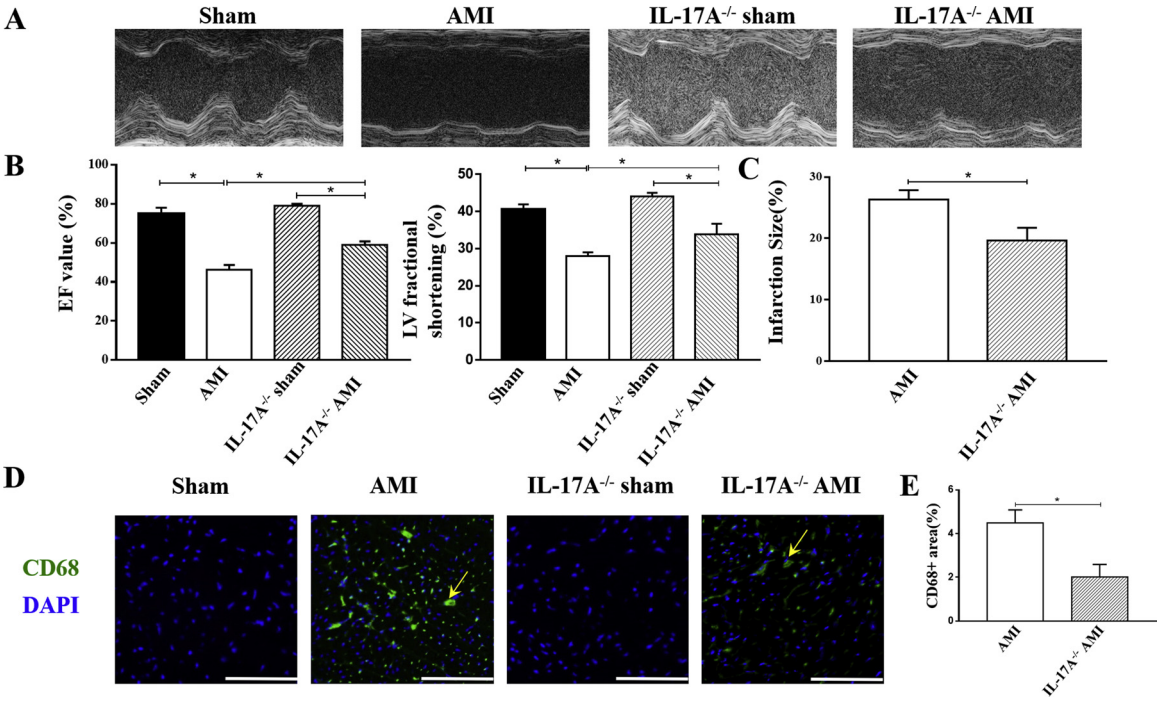

Fig. 7. IL-17A knockout ameliorated myocardial ischemic injury. A, Representative M-mode images for LV of mice on Day 3 post operation. B, Ejection fraction value (EF value) and LV fractional shortening (FS) of mice on Day 3 post operation. C, Infarction size (Masson's trichrome) of mice on Day 3 post operation. D–E, Immunofluorescent staining of cardiac tissue on Day 3 post operation with antibodies against CD68 (green indicates $CD68^+$) and DAPI-stained (blue) cellular nuclei. $CD68^+$ positive macrophages were labeled with yellow arrows, (Scale bar, 100 μm). All data are presented as M ± SD (n = 4–5 for each group). $^*P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

activation than wild-type mice (Figs. 7 and 8). These results suggested a pro-inflammatory role of IL-17A during AMI and its effects on the activation of NLRP3 inflammasome in macrophages.

As us well known, IL-17 plays an important role in cardiovascular diseases, but whether it is good or bad is still controversial. In the present study, we found that IL-17A revealed a significant increase after AMI in mice, promoted the release of inflammatory factors and aggravated cardiac dysfunction, which was consistent with several previous studies (Cheng et al., 2008; Jafarzadeh et al., 2009; Liao et al., 2012; Liuzzo et al., 2013; Zhou et al., 2014). However, contrary to the above-mentioned findings, Troitskaya et al. (2012) have used spleen cells as the model of circulating inflammatory cells to explore the role of IL-17 in MI and found that IL-17 may be beneficial through inhibiting monocyte chemotaxis. The inconsistency may result from the research methods and targets, because we measured the serum IL-17A level and checked the inflammatory response in the damaged myocardial tissues on the third day post AMI in mice, while they measured the expression of IL-17 in splenocytes. The decrease of circulating IL-17-producing splenocytes in MI mouse models does not contradict the increase of IL-17A within the damaged myocardial tissues. Moreover, they demonstrated that IL-17 reduced monocyte chemotaxis in vitro, while we found that IL-17A could promote the infiltration of macrophages in ischemic myocardium of mice. The complexity of in vivo environment may also play an important role. In another study, Simon et al. (2013) have found that the low serum level of IL-17 is associated with a higher risk of 2 years major cardiovascular events in Caucasian patients with AMI. To our knowledge, this study was a longitudinal follow-up study and the population in the present study was limited to coronary heart disease patients. While, the mouse AMI model used in our study was constructed by ligation of the anterior descending coronary artery and with the aim to explore the role of IL-17A in acute inflammatory response following acute myocardial ischemia. The different study subjects may be the major reason for causing the inconsistent conclusions. Currently, some scholars have proposed that the role of IL-17 in cardiovascular diseases is the local microenvironment-dependent and may vary according to cell types producing IL-17 and the cytokine profile in microenvironment where IL-17 works (Taleb et al., 2015). More evidence is needed to further clarify the role of IL-17A in myocardial ischemia.

The most important novelty of this study was that we investigated the signal transduction pathway under which IL-17A activated NLRP3 inflammasome in macrophages and found the unexpected proinflammatory role of AMPK. It is generally acknowledged that AMPK activation plays an essential positive role in modulating metabolism, mitochondrial function, endoplasmic reticulum (ER) stress, autophagy, and apoptosis (Zhou et al., 2014). Newly, the role of AMPK in regulating inflammation is gradually regarded highly by researchers. AMPK in macrophages can be activated rapidly upon the induction by anti-inflammatory cytokines, including interleukin-10 (IL-10) or transforming growth factor (TGF), whereas the stimulation with pro-inflammatory factors, such as lipopolysaccharide (LPS), can result in the inactivation of AMPK (Sag et al., 2008; Yang et al., 2010). In addition, it has been reported that pharmacological activation of AMPK prevents myocardial necrosis and contractile dysfunction during ischemia-reperfusion (Zhou et al., 2014). These data suggest the apparent anti-inflammatory and cardiovascular protective action of AMPK. However, in the present study, we found AMPKα mediated the activation of NLRP3 inflammasome and release of IL-1β in mouse peritoneal macrophage induced by IL-17A in vitro (Figs. 2 and 4), which reveals a novel regulatory effect of AMPKα on inflammation process. Besides, the in vivo experiments showed that AMPK inhibitor CC not only decreased the infiltration of $CD68^+$ positive macrophages, reversed the aggregation and activation of NLRP3 inflammasome induced by IL-17A administration, but also diminished infarction size and ameliorated cardiac function aggravated by IL-17A administration obviously (Figs. 5 and 6). The expression of p-ACC, p-AMPKα was significantly increased in ischemic myocardial tissue on Day 3 after AMI and was higher with IL-17A administration (Fig. 6C–D). What's more, AMPK was less active

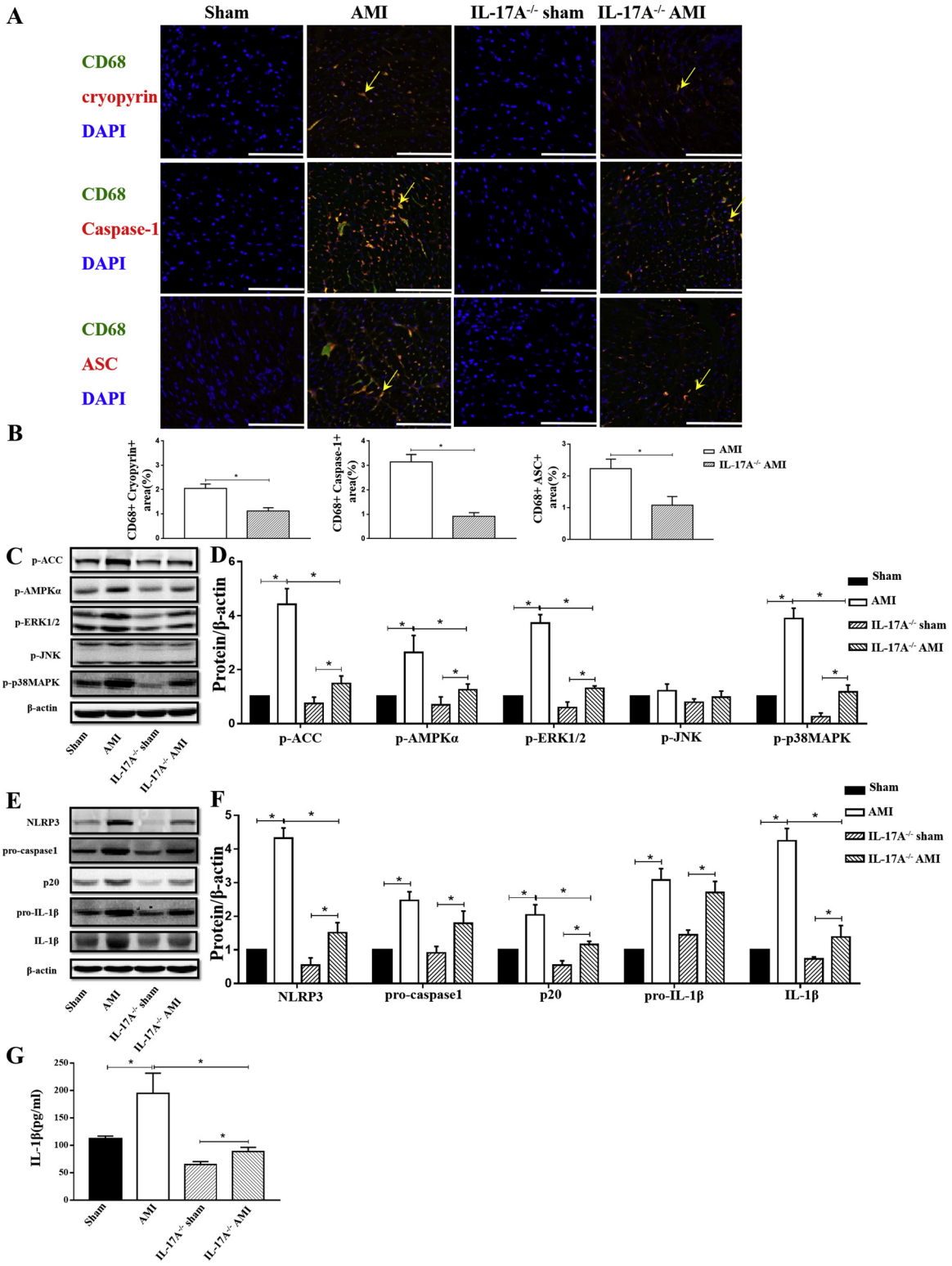

Fig. 8. IL-17A knockout reduced the NLRP3 inflammasome activation in macrophages from ischemic cardiac tissues and decreased IL-1β release and AMPKα/p38MAPK/ERK1/2 signal. A–B, Immunofluorescent double staining of cardiac tissue (control or ischemic) on Day 3 post operation with antibodies against CD68 and caspase-1, ASC or cryopyrin. Green indicates CD68, red for caspase-1, ASC or cryopyrin and blue for DAPI-stained cellular nuclei. $CD68^+caspase\text{-}1^+$, $CD68^+ASC^+$ and $CD68^+cryopyrin^+$ double positive macrophages were labeled with yellow arrows, (Scale bar, 100 μm). C–D, Western blot analysis of p-ACC, p-AMPKα, p-ERK1/2, p-JNK MAPK, and p-p38MAPK in cardiac tissues (control or ischemic) of mice on Day 3 post operation. E–F, Western blot analysis of NLRP3, pro- and active caspase-1 (p20) and pro- and mature IL-1β in cardiac tissues (control or ischemic) of mice on Day 3 post operation. G, ELISA analysis of IL-1β in serum from mice on Day 3 post operation. The β-actin was used as the loading control. All data are presented as M ± SD (n = 4–5 for each group). *$P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

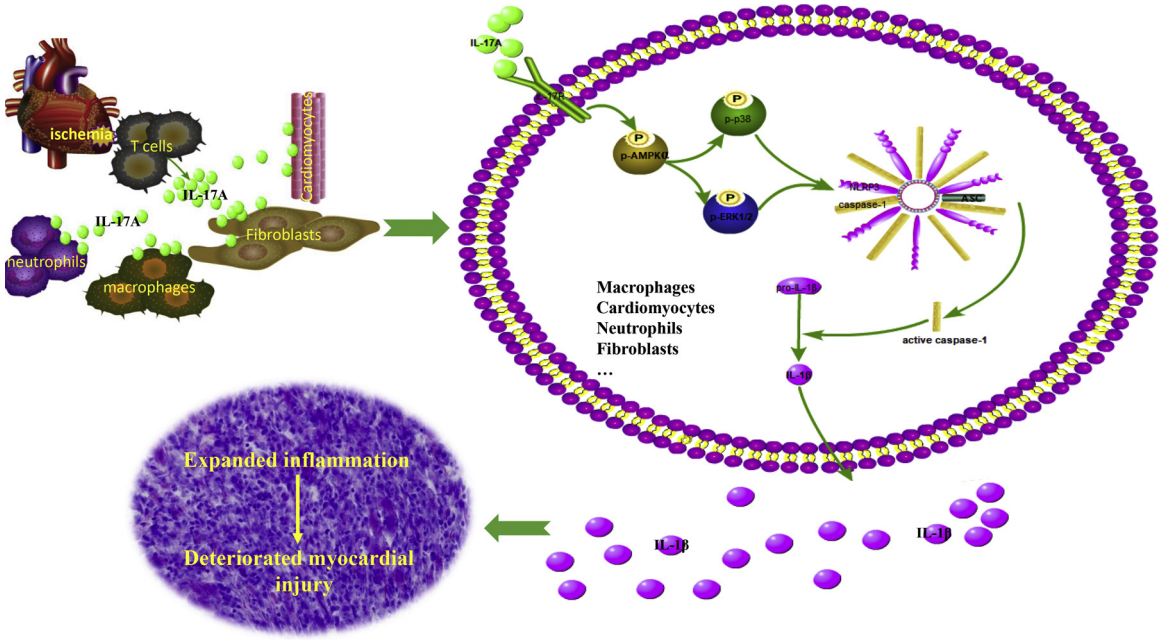

Fig. 9. Schematic diagram of molecular mechanisms for the aggravated inflammatory response and contribution to myocardial ischemia injury following AMI in the presence of IL-17A.

in IL-17A$^{-/-}$ mice than wild-type mice at Day 3 following AMI (Fig. 8C–D). These results indicated that IL-17A can activate NLRP3 inflammasome of macrophages via an AMPKα-dependent way in heart from ischemia mice Day 3 after AMI. Our study also demonstrated the destructive action of AMPKα activation in myocardial ischemia. In line with our research, there were studies demonstrating that up-regulated AMPK signaling is associated with the activation of inflammasomes and the suppressed AMPK activity can significantly attenuate ATP or lipopolysaccharide (LPS)-induced inflammasome activation in murine macrophages (Kim et al., 2017; Li et al., 2017; Zha et al., 2016). Folmes and Jaswal also demonstrate AMPK suppression may be helpful through increasing energy production or inhibiting p38 MAPK in isolated mouse hearts that are suffering from ischemic or ischemia-reperfusion injury (Folmes et al., 2009; Jaswal et al., 2007). The diverse outcomes of AMPKα activation may be due to different development periods and distinct source of cells in diseases. However, the specific mechanism for this is still unknown and deserves further investigation.

MAPKs, especially p38MAPK and ERK1/2, have been considered as the common downstream signaling molecules of IL-17A and play vital roles in inflammation (Kolls and Lindén, 2004; Griffin et al., 2012; Iwakura et al., 2011; Roussel et al., 2010; Wang et al., 2014a,b; Wuyts et al., 2005). AMPK can also activate MAPK in many disease (Lanna et al., 2014; Li et al., 2005). So we wondered if MAPKs also participate in the activation of NLRP3 inflammasome and the release of IL-1β induced by IL-17A in mouse macrophages. Fig. 3 showed that IL-17A promoted the phosphorylation of p38MAPK and ERK1/2, but not JNK, in mouse peritoneal microphages, which could be down-regulated by AMPK inhibitor CC. In addition, the mRNA or protein expression of NLRP3 and the production or the release of mature IL-1β induced by IL-17A were inhibited by treatment with AMPK inhibitor CC, p38MAPK inhibitor SB or ERK1/2 inhibitor PD, respectively (Fig. 4). The expression of p-ACC, p-AMPKα, p-p38MAPK and p-ERK1/2 signaling molecules was significantly increased in ischemic myocardial tissue on Day 3 after AMI and was higher with IL-17A administration, which was sharply reversed by AMPK inhibitor CC (Fig. 6C–D). And IL-17A knockout reduced the activation of AMPKα/p38MAPK/ERK1/2 signal pathway than wild-type mice on Day 3 following AMI (Fig. 8C–D). These results suggested that MAPKs play an important role in NLRP3 activation induced by IL-17A, during which, MAPK severed as the downstream molecules of AMPKα activation.

Taken together, our study reveals that IL-17A plays an important role in acute inflammatory response and has a harmful effect on cardiac function via AMPKα/p38MAPK/ERK1/2 signal pathway (Fig. 9), suggesting that IL-17A may be served as a new target for early intervention following myocardial infarction and more caution should be paid to the application of AMPK activators. There is still limitation in this study. As a specific AMPK inhibitor, CC generally can block AMPK signal pathway, but it may also induce some side effects, including the inhibition of bone morphogenetic protein (BMP) signaling and angiogenesis (Hao et al., 2008, 2010).

5. Conclusions

IL-17A can induce the exacerbated infiltration of macrophages, and the high activation of NLRP3 inflammasome, as well as the release of pro-inflammatory cytokine IL-1β from macrophages during the acute inflammatory response after AMI, which contributes to worse cardiac function and lager infarction size through AMPKα/p38 MAPK/ERK1/2 signal pathway.

Competing interests

The authors declare that they have no competing interests

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Author contributions

Z.Y. and J.Z. conceived and designed the experiments; Y.W. and L.Z. contributed to the design; L.Z. and P.L. performed experiments, X.B, W.W., Y.Z., M.L. and L.W. help to the experiments; L.Z., J.Z, and Y.Z. analyzed the data; L.Z. draft the paper; J.Z contributed to the drafting. All authors read, critically revised and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.molimm.2018.12.014.

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