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Macrophage NLRP3 inflammasome activated by CVB3 capsid proteins contributes to the development of viral myocarditis

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ABSTRACT

Viral myocarditis, mainly caused by enteroviruses specially coxsackievirus B3 (CVB3) infection, is a common clinical cardiovascular disease and characterized by cardiac massive inflammation. Our previous study showed that CVB3-induced myocardial NLRP3 contributed to the development of viral myocarditis. In this study, we found that beside of being up-regulated in myocardiocytes, NLPR3 was also obviously increased in the cardiac infiltrating macrophages. While whether this accumulated NLRP3 influences, macrophage inflammatory responses remains unknown. By adoptive transfer assays, we found that mice receiving NLRP3 up-regulated macrophages showed much more abundant cardiac IL-1β production and more severe myocardial inflammation, while those receiving NLRP3 down-regulated macrophages showed much less IL-1β production and milder myocarditis, indicating that NLRP3 up-regulated macrophages played a pathological role in CVB3-induced myocarditis. In addition, we further found that it was CVB3 capsid proteins VP1 (predominant) and VP2, but not viral RNAs, robustly triggered macrophage NLRP3 up-regulation and activation. Our study demonstrated macrophage NLRP3 inflammasome could be efficiently be activated by CVB3 capsid proteins, and contributed to the pathogenesis of viral myocarditis. It might provide some clues to the development of new therapeutic strategies based on macrophage NLRP3 modulation.

1. Introduction

Coxsackievirus B3 is a non-enveloped, positive-sense, single-stranded RNA virus belonging to the Picornaviridae virus family. It is considered as one of the most common causative agents leading to viral myocarditis, which is the main cause of sudden death in adolescents and adults less than 40 years old (Esfandiarei and McManus, 2008). However, to date, no effective and specific treatments are available due to the poorly elucidated pathological mechanisms.

Substantial evidence indicated that indirect immune-mediated tissue injury, beside of direct virus-mediated cell damage, is another important pathogenesis of viral myocarditis (Westermann et al., 2010). Macrophages have been proved to play an important role in the development of viral myocarditis. They are not only the first recruited inflammatory cells to heart tissues (Fairweather and Rose, 2007), but also represent the main infiltrating cell subset in the early stage of viral myocarditis (Li et al., 2009; Liu et al., 2014). In one way, they could influence the cardiac immune microenvironment by secreting pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and chemokine MCP-1 (Corsten et al., 2012). In another, they could modulate the strength, bias and persistence of the subsequent adaptive immune responses (Amoah et al., 2015). Therefore, elucidating the initiation and amplification mechanisms of macrophage inflammation is paramountly important for understanding the molecular basis of viral myocarditis.

Inflammasomes are intracellular multi-protein complexes activated upon infection or stress to promote pro-inflammatory cytokine maturation, and have been proven to play an important role in clearing infections and tailoring host immune responses (Hayward et al., 2018; Joshi and Morley, 2019). Among the growing number of inflammasomes, NLRP3 (NOD-like receptor family pyrin domain containing 3) inflammasome is the most well-characterized one, which consists of NLRP3 scaffold, ASC (apoptosis-associated speck-like protein containing a CARD) adaptor and pro-caspase-1. Strong association of NLRP3 inflammasome with the infectious (Mortimer et al., 2015; Tartey and Kanneganti, 2019) and inflammatory diseases (Liu et al., 2018) has been more and more evidenced. In previous studies, we and other researchers showed that NLRP3 expression was obviously up-regulated in CVB3-infected myocardiocytes (Miteva et al., 2018; Wang et al., 2014). And Tschöpe C and colleagues (Tschope et al., 2017) further showed that cardiac NLRP3 level was markedly decreased in the endomyocardial biopsies of healing CVB3-positive patients, further confirming the involvement of NLRP3 inflammasome in the pathogenesis

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of CVB3-induced viral myocarditis.

Actually, most reports characterizing NLRP3 inflammosomes have focused on myeloid cells such as macrophages, but not tissue constitutive cells (such as epithelia cells, myocardiocytes). Interestingly, these myeloid cells especially macrophages are an critical pathological inflammatory cell subset in CVB3-induced myocarditis. In addition, NLRP3 inflammasome could also influence macrophage functions such as adhesion, activation (Christo et al., 2016), and inflammation (Komada et al., 2018), therefore it prompted us to explore whether NLRP3 inflammasome could be activated in cardiac infiltrating macrophages, and how it impacted the development of viral myocarditis.

In this study, we found that NLRP3 inflammasome was robustly induced in cardiac infiltrating macrophages post CVB3 infection, and aggravated viral myocarditis via producing abundant IL-1β. Furthermore, we identified that it was CVB3 capsid proteins VP1 and VP2, but not other viral components, that triggered the NLPR3 expression in the cardiac infiltrating macrophages. Taken together, our study provided insights on the pathological role of macrophage NLRP3 in viral myocarditis.

2. Materials and methods

2.1. Mice

BALB/c male mice, 6–8 weeks old, were purchased from the Experimental Animal Centre of the Chinese Academy of Science (Shanghai, China). All mice were kept under conditions of specific-pathogen-free, and relating experiments suited for the Guide for the Care and Use of Medical Laboratory Animals (Ministry of Health, China, 1998) and permitted by the Laboratory Animal Ethical Committee of Soochow University.

2.2. Virus and cells

CVB3 was propagated and harvested from HeLa cells (ATCC number: CCL-2). Mice were infected with 10^3 PFU CVB3 through intraperitoneal injection.

RAW264.7 cells, HeLa cells were cultured in DMEM with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Bone marrow-derived macrophages (BMDMs) were obtained as previously reported (Martin et al., 2015).

2.3. Enzyme-linked immunosorbent assay

The expression levels of IL-1β in the heart homogenates and cell culture were measured by ELISA kits from eBioscience following the manufacturer's instructions.

2.4. Quantitative real-time PCR

Total RNA was extracted with TRIzol reagent (Takara) and then reversely transcribed to cDNA which was subjected to real-time PCR with SYBR green system (Takara) using the specific primers as follows: NLRP3 forward primer 5′-AGAAGAGACCACGGCAGAAG-3′, reverse primer 5′ − CCTTGGACCAGGTTCAGTGT-3′; IL-1β forward primer 5′ TCACAGCAGCACATCAACAA-3′, reverse primer5′-TGTCCTCATCCTGG AAGGT-3′; GAPDH forward primer 5′-GAGCCAAACGGGTCATCA TCT-3′, reverse primer 5′-GAGGGGCCATCCACAGTCTT-3′. Housekeeping gene GAPDH was used to normalize the expression of the target gene, and quantified for data as analyzed by the $2^{-\Delta\Delta Ct}$ method.

To determine the myocardial viral load, total RNA from CVB3-stimulated macrophages was reversely transcribed to cDNA using CVB3-specific positive-strand primer (5′-CACCGGATGGCCAATCCA-3′) or negative-strand primer (5′-GCGAAGAGTCTATTGAGCTA-3′), and then subjected to SYBR Green real-time PCR using CVB3 primers (5′-ATCA AGTTGCGTGCTGTG-3′ and 5′-TGCGAAATGAAAGGAGTGT-3′). The viral RNA load was normalized by mouse GAPDH expression.

2.5. Western blot

The same amounts of total proteins were separated by 10% SDS-PAGE and then transferred to a PVDF membrane (Bio-Rad). After blocked in 5% non-fat milk-PBST, membranes were incubated with polyclonal primary antibody rabbit anti-NLRP3 (R&D Systems), anti-Flag (Sigma) overnight at 4 °C. After washed with PBST 3 times, membranes were incubated with HRP-labeled donkey anti-rat (Jackson ImmunoResearch) or goat anti-rabbit secondary antibody (SouthernBiotech), respectively. Detection was used ECL chemiluminescence (Thermo Scientific) in accordance with the manufacturer's instructions.

2.6. Plasmid construction

Total RNA was extracted from CVB3-infected cells and then reversely transcribed. cDNA encoding CVB3 flag-tagged VP1, VP2, VP3 or VP4 protein was amplified by PCR respectively and then ligated to the eukaryotic expression vector pcDNA3.1.

To construct NLRP3-specific shRNA, the lentiviral-based shRNA expression plasmid pLL3.7 was used, and the double-stranded oligonucleotides containing the NLRP3 target sequence (5′- GGATCTTTGC TGCGATCAACA -3′) were applied as described previously (Ito et al., 2012; Rubinson et al., 2003).

2.7. PEI-mediated gene delivery in vivo

Polyplexes were prepared at Nitrogen-to-DNA phosphate (N/P) ratio = 7. Fifty μg pLL3.7-shRNA-NLRP3 were used for each mouse, where both the polymer and DNA were diluted in 5% dextrose to a volume of 100 μl (200 μl after mixing). Then DNA and polymer were mixed, whirled for 30 s and incubated for 30 min. The final PEI/DNA complexes were injected to the mice by high pressure tail vein injection.

2.8. Adoptive transfer of macrophages

NLRP3 up-regulated bone marrow-derived macrophages (BMDMs) were prepared by culturing in 5 μM NLRP3-specific activator nigericin (InvivoGen) for 3 h, NLRP3 down-regulated BMDMs were prepared by culturing in 1 μM NLRP3-specific inhibitor MCC950 (Sigma) for 30 min. Endogenous macrophages of recipient mice were depleted by Cl_2MBP-loaded liposomes treatment as described previously (Shen et al., 2004). For macrophage depletion, each mouse was intravenously continuously injected with 200 μl Cl_2MBP-loaded liposomes for 3 days prior infection (The depletion efficiency was > 70% as determined by FACS assays). And at day 3 post infection, the NLRP3 up-regulated or down-regulated BMDMs were transferred to mice at a dose of 5×10^6 cells each mouse.

2.9. Immunofluorescence assays

Macrophages were stimulated with CVB3 for 12 h, and then fixed, washed and permeabilized followed by incubating with the mouse anti-enterovirus VP1 antibody (Dako). After incubating with the secondary antibody Alexa Fluor 488-conjugated donkey anti-mouse IgG (H + L) for 90 min, sections were then stained with DAPI for nuclear identification.

2.10. Statistical analysis

Data were expressed as means ± SEM. GraphPad Prism version 5.0 was employed for statistical analysis. Student's *t*-test was used to compare differences between two groups. One-way ANOVA was followed by Bonferroni test to compare more than 2 groups. P values less than 0.05 were considered to be significant.

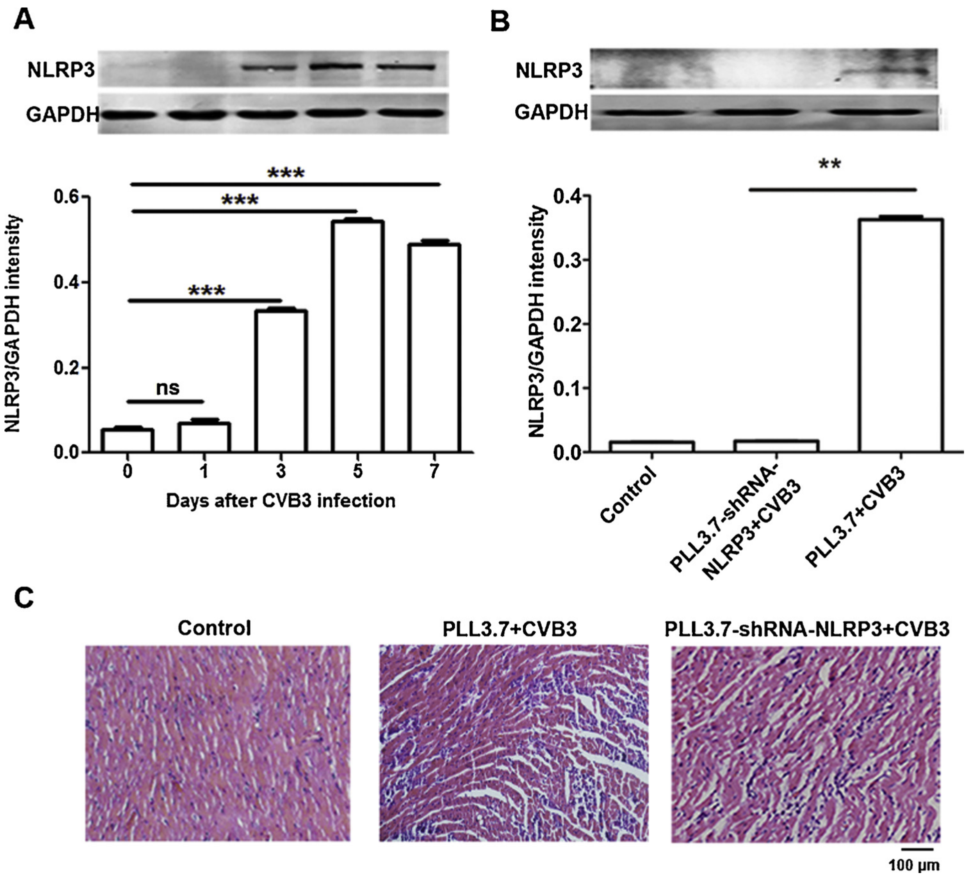

Fig. 1. NLRP3 participated in the development of CVB3-induced myocarditis. Mice were infected with CVB3 and hearts were collected at indicated time points. Then cardiac expression of NLRP3 was detected by western blot assays. NLRP3 and GAPDH were analyzed by grayscale scanning (A). Mice were intravenously injected with the control vector or shRNA-NLRP3 at the day before CVB3 infection and day 1 post infection. Cardiac expression of NLRP3 (B) and the paraffin sections of heart tissues were detected at day 7 post infection (C). Data were presented as means ± SEM of 5–6 mice per group. Individual experiments were conducted three times with similar results, and the representative data were shown. $^{**}P < 0.01$, $^{***}P < 0.001$.

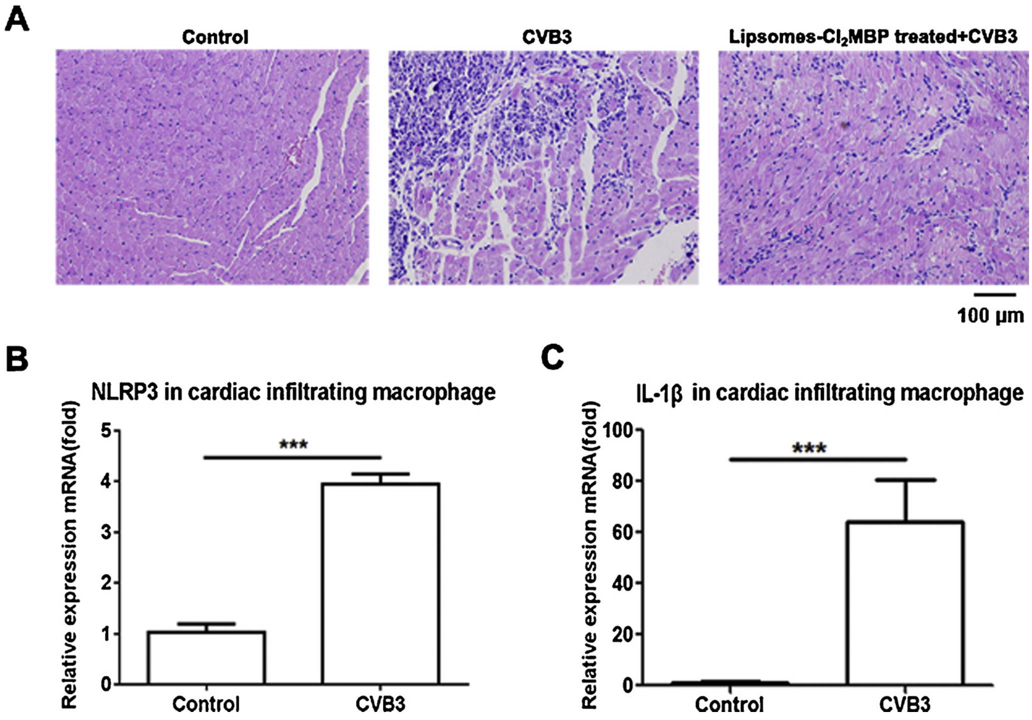

Fig. 2. NLRP3 was obviously elevated in the pathological macrophages in CVB3-induced viral myocarditis. (A) Macrophage depletion significantly alleviated CVB3-induced myocarditis. (B) The pathological cardiac infiltrating macrophages expressed a high level of NLRP3 in CVB3-induced viral myocarditis. Data were presented as means ± SEM of 5–6 mice per group. Individual experiments were conducted three times with similar results, and the representative data were shown. $^{***}P < 0.001$.

3. Results

3.1. NLRP3 contributed to the development of CVB3-induced myocarditis

To verify the role of NLRP3 in CVB3-induced myocarditis, we detected the dynamical cardiac NLRP3 expression at various time points post CVB3 infection. It showed that cardiac NLRP3 expression obviously increased at day 3 post infection and as time went by, its expression continued to increase and maintained at a high level until day 7, which was also the peak time point of viral myocarditis severity

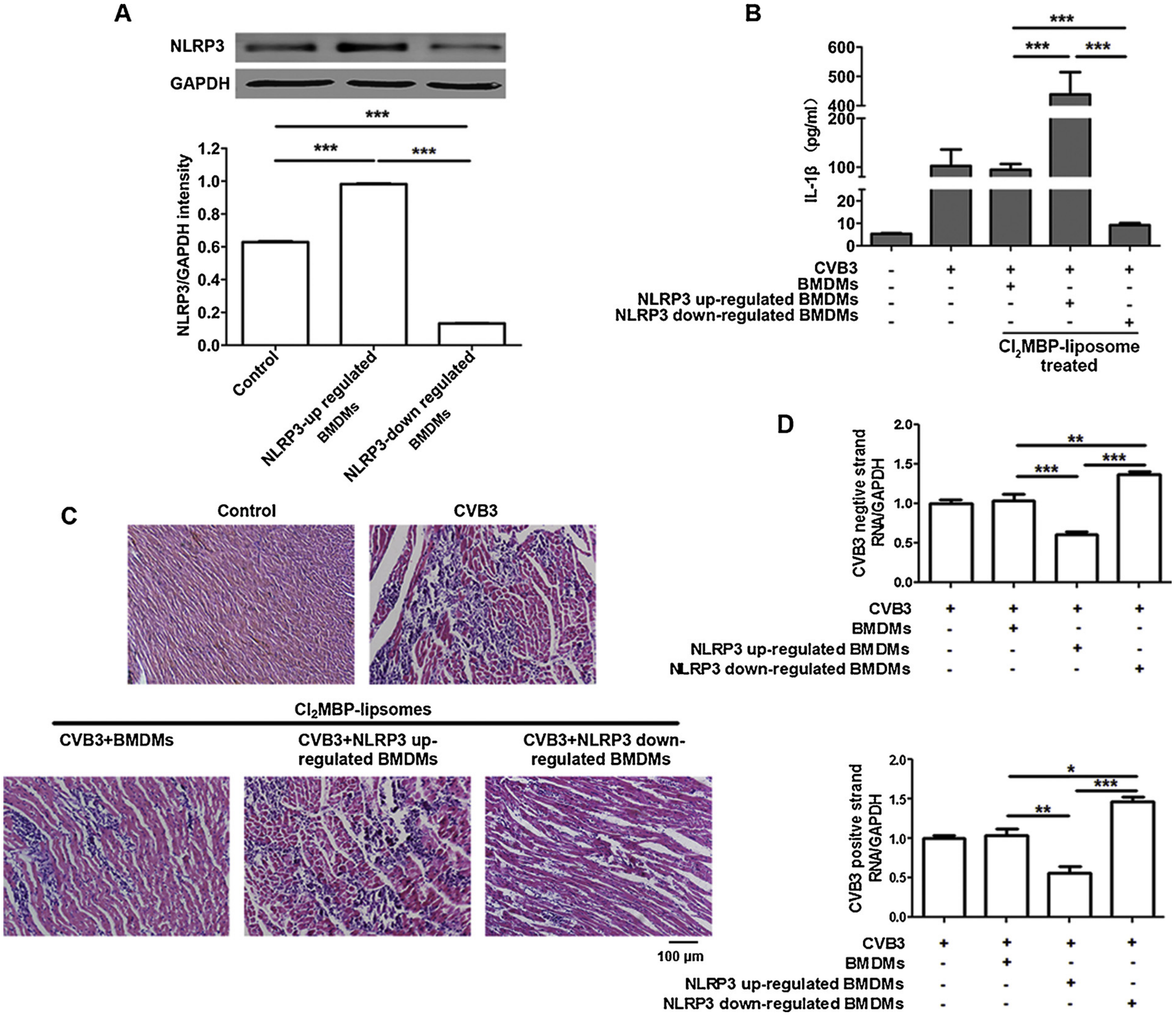

Fig. 3. Up-regulated NLRP3 in macrophages played an important pathological role in CVB3-induced myocarditis. A. NLRP3 expression in control, NLRP3 up-regulated or down-regulated BMDMs was detected by western blot assays and analyzed by grayscale scanning. After depleting endogenous macrophages with Cl_2MBP-liposomes treatment, recipient mice were given NLRP3 up-regulated, down-regulated or control BMDMs at day 3 after CVB3 infection. Heart tissues were collected and cardiac IL-1β levels (B) were detected by ELISA assays at day 7. Meanwhile, heart pathological observation was also detected by HE staining (C). Data were presented as means ± SEM of 5–6 mice per group. Individual experiments were conducted three times with similar results, and the representative data were shown. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

(Zha et al., 2015) (Fig. 1A). Consistently, down-regulating NLRP3 expression via specific shRNA administration could significantly alleviate cardiac inflammation and injury, illustrated by the fewer infiltrating inflammatory cells as well as limited necrosis foci compared with the control mice (Fig. 1C), indicating that NLRP3 played an important pathological role in CVB3-induced viral myocarditis.

3.2. NLRP3 was enriched in the pathological cardiac infiltrating macrophages in the CVB3-induced viral myocarditis

Cardiac macrophages have been proven to play a critical role for CVB3-induced viral myocarditis (Fairweather and Rose, 2007; Jaquenod De Giusti et al., 2015; Li et al., 2009; Liu et al., 2014), once macrophages were depleted, CVB3-induced viral myocarditis could be obviously relieved (Fig. 2A). Interestingly, meanwhile we also found that these pathological macrophages expressed high levels of NLRP3 (Fig. 2B) and IL-1β (Fig. 2C), indicating that NLRP3 up-regulated, IL-1β-producing macrophages might exert pathological effects on the progress of CVB3-induced viral myocarditis.

3.3. NLRP3 up-regulated macrophages significantly aggravated the severity of CVB3-induced viral myocarditis

Next, NLRP3 up-regulated or down-regulated bone marrow-derived macrophages (BMDMs) were prepared and adoptively transferred into CVB3-infected recipient mice in which endogenous macrophages have been depleted with Cl_2MBP-liposomes treatment. It was found that compared with the counterparts receiving the control macrophages, mice receiving NLRP3 up-regulated macrophages showed a significantly higher level of cardiac IL-1β (Fig. 3B), more severe myocarditis (Fig. 3C) as well as obviously reduced cardiac viral loads reflected by the decreased CVB3 positive and negative strand RNAs (Fig. 3D). In sharp contrast, mice receiving NLRP3 down-regulated BMDMs showed much lower cardiac IL-1β, less myocardial injury and greater cardiac viral loads. These data indicated that NLRP3 up-regulated macrophage significantly promoted the progression of myocarditis.

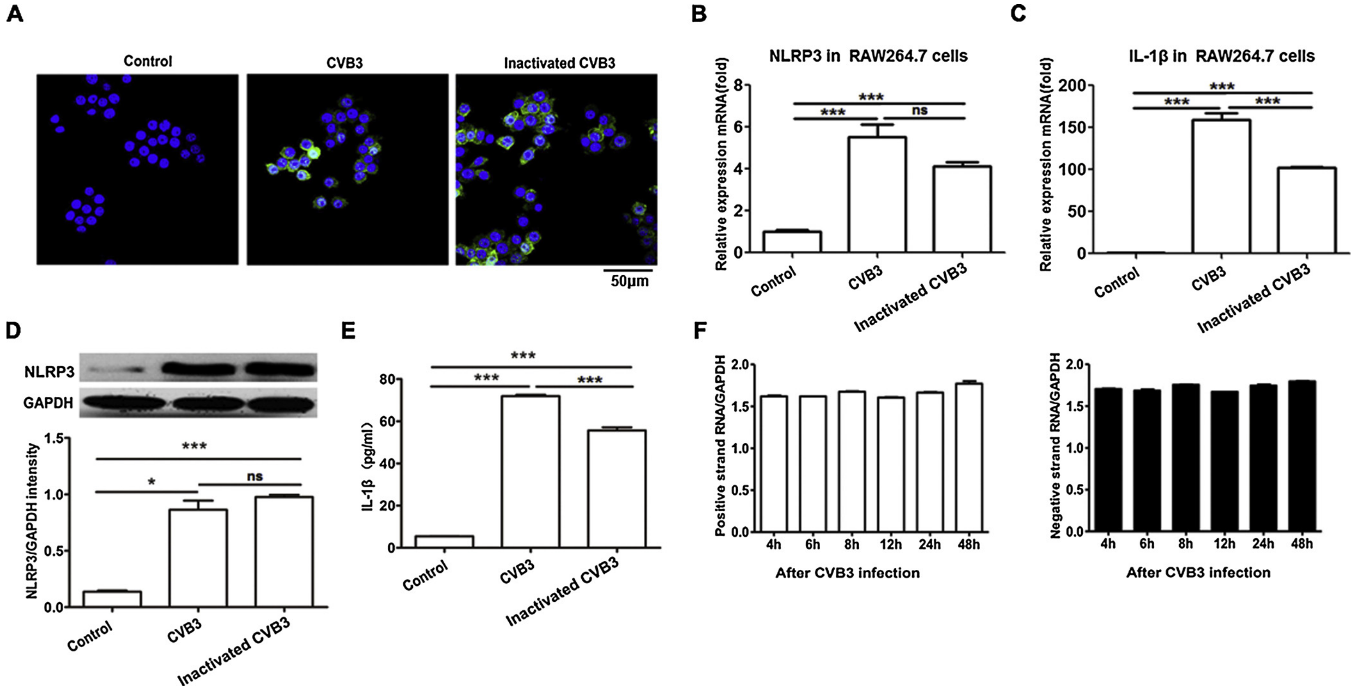

Fig. 4. NLRP3 up-regulation and activation were not dependent on CVB3 replication. Murine macrophage cell line RAW264.7 cells were treated with the same dose of CVB3 or heated-inactivated CVB3 for 12 h, and then cells were collected and subjected to detect the protein levels of NLRP3 (A) by western blot assays and the protein levels of IL-1β (B) by ELISA assays. To evaluate internalization of CVB3, RAW264.7 cells were stimulated with CVB3 or heated-inactivated CVB3 for 12 h respectively, and after removing the inoculum, cells were fixed and stained with anti-coxsackievirus VP1 antibody. CVB3 structural protein VP1 in RAW264.7 cells was detected by immunofluorescence assays (C). RAW264.7 cells were stimulated with CVB3 for indicated periods, and then cells were collected for the detection of CVB3 positive- (D) and negative- (E) strand RNAs detection by real-time PCR. Data shown were mean ± SEM of three independent experiments with similar results. $^{*}P < 0.05$, $^{***}P < 0.001$, ns: no significance.

3.4. Activation of macrophage NLRP3 inflammasome was not dependent on CVB3 infectivity and replication

To investigate whether activation of macrophage NLRP3 inflammasome relied on the CVB3 infectivity, macrophages were incubated with CVB3 or heated-inactivated CVB3, and then the NLRP3 and IL-1β expressions were detected. It was found that both live CVB3 and inactivated CVB3 could be equally uptake by macrophages (Fig. 4A) and efficiently increased the NLRP3 and IL-1β expressions (Fig. 4B–E), although the levels of these two proteins were slightly lower in macrophages incubated with the inactivated CVB3, indicating that the activation of CVB3-induced macrophage NLRP3 inflammasome was not dependent on the virus infectivity. Since the levels of positive and negative strand viral RNAs were barely changed in macrophages after incubating with live CVB3 for various periods of time (Fig.4F), these data were further supported by the previous studies (Girn et al., 2002; Zhang et al., 2017). Therefore activation of macrophage NLRP3 inflammasome was also independent on the virus replication.

3.5. CVB3 capsid proteins VP1 and VP2 robustly triggered the macrophage NLRP3 activation

Above data indicated that macrophage NLRP3 inflammasome activation was not attributed to the virus infectivity and replication, we next explored whether CVB3 structural components acted as the potential triggers for NLRP3 activation. Previous study reported that viral RNAs possessed the ability to stimulate macrophage NLRP3 expression and activation (Kanneganti et al., 2006), while in our study no significantly increased NLRP3 or IL-1β expression was evidenced in CVB3 genomic RNA-stimulated macrophages (Fig. 5A–D), suggesting that the triggers might be the viral structure proteins. Consequently, plasmids encoding flag-tagged viral capsid proteins (VP1, VP2, VP3, VP4) were constructed and transfected into macrophages respectively. It was found that NLRP3 and IL-1β expressions were only significantly increased in macrophages expressing VP1- or VP2 protein, but not other capsid proteins (Fig. 5E and F). Of note, VP1 compared with VP2 possessed the more potent ability to activate macrophage NLRP3 inflammamsome. All these data indicated that CVB3 capsid proteins VP1 and VP2 were the robust triggers for the macrophage NLRP3 inflammasome activation in the context of viral myocarditis.

4. Discussion

The NLRP3 inflammasome is a key intracellular multiprotein signaling platform that tightly controls the caspase-1 activation and the subsequent pro-inflammatory cytokines (IL-1β and IL-18) production. It comprised the sensor protein NLRP3, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase-1. Upon activation, NLRP3 interacts with ASC and the oligomerized complex bind pro-caspase-1 to form active inflammasome that produces mature IL-1β and IL-18. An ever increasing number of studies link the undue NLRP3 activation to many infectious and inflammatory diseases (Liu et al., 2018; Patel et al., 2017; Xiao et al., 2019) including viral myocarditis (Wang et al., 2019, 2014).

The prerequisite for the NLRP3 inflammasome activation is the obvious NLRP3 and pro-IL-1β induction, and this inflammasome prime process could be highly influenced by integrated pro-inflammatory signaling pathways such as TLR ligation triggered by microbial components (Lin et al., 2014) or endogenous cytokine like TNF-α (Schroder and Tschopp, 2010). In this study, we found that in the CVB3-induced murine viral myocarditis model, NLRP3 was obviously enriched in the cardiac infiltrating macrophage, and adoptive transferring NLRP3 up-regulated macrophages could robustly amplify the cardiac IL-1β production and obviously aggravated the myocarditis severity, and the phenomenon was totally reversed in the mice receiving the NLRP3 down-regulated macrophages. All these data clearly demonstrated the

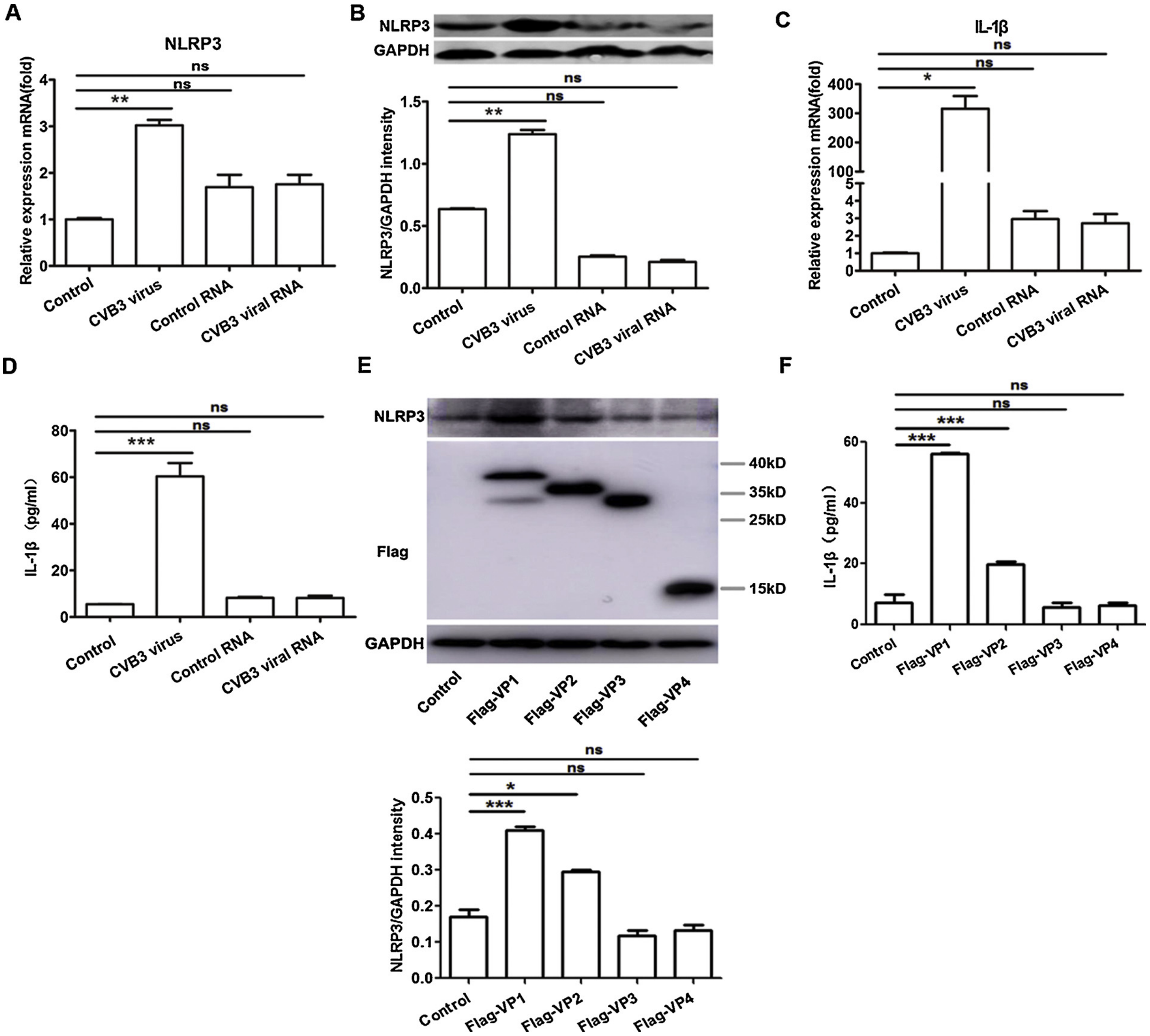

Fig. 5. NLRP3 activation relied on the capsid protein VP1 and VP2 of CVB3 virus. RAW264.7 cells were stimulated with CVB3 viral RNA or control RNA for 12 h. NLRP3 mRNA expression (A) was determined by real-time PCR assays. NLRP3 protein expression (B) was detected by western blot assays, and the expression of NLRP3 and GAPDH were analyzed by grayscale scanning. The mRNA expression level of IL-1β (C) was determined by real-time PCR assays. The protein level of IL-1β expression (D) was determined by ELISA assays. RAW264.7 cells were transfected with the plasmid encoding flag-tagged CVB3 VP1, VP2, VP3, VP4 or control vector, and 24 h later NLRP3 expression (E) was detected by the western blot assays and IL-1β expression (F) was determined by ELISA assays. Data shown were mean ± SEM of three independent experiments with similar results. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns: no significance.

pathological role of macrophage NLRP3 inflammasome in CVB3-induced myocarditis. In support of our data, other researchers also evidenced the robust macrophage NLPR3 up-regulation after distinct RNA virus infections including influenza A virus (Son et al., 2017), vesicular stomatitis virus (VSV) (Rajan et al., 2011) and flock house virus (Kanneganti et al., 2006). This indicates that NLRP3 inflammasome is a common immune sentinel surveillance mechanism for RNA virus infection.

Next, we tried to elucidate the activation mechanism of macrophage NLRP3 inflammasome in viral myocarditis. Virus infectivity might be important for macrophage NLRP3 activation, as the macrophage NLRP3-inducing abilities of encephalomyocarditis virus (EMCV) and VSV could be obviously deprived by virus inactivation (Rajan et al., 2011). To our surprise, herein we found that no matter being live or inactivated, CVB3 could consistently and significantly induce macrophage NLRP3 and IL-1β expression, indicating that unlike EMCV or VSV, CVB3 infectivity was not essential for macrophage NLRP3 up-regulation and activation. In addition, CVB3 replication was not required for NLRP3 up-regulation neither. Previous study showed that influenza virus RNAs can induce macrophage NLRP3 (Kanneganti et al., 2006), while in our study, CVB3 genomic RNAs failed to induce macrophage NLRP3 or IL-1β expression. In support of our data, Ito M and colleagues (Ito et al., 2012) also showed that EMCV RNAs, another member of Picornaviridae as CVB3, was insufficient to activate macrophage NLRP3 inflammasome neither. Therefore, the macrophage NLRP3-inducing abilities by viral RNAs might be different in distinct viruses.

These data suggested that it might very likely be the CVB3 proteins that trigger the macrophage NLRP3 up-regulation. We found that two CVB3 structural proteins capsid VP1 and VP2 could robustly induce macrophage NLRP3 expression and led to a high level of subsequent IL-1β production, indicating that VP1 and VP2 proteins were potential triggers for macrophage NLRP3 inflammasome activation. Other groups also showed that virus proteins could potently induce macrophage NLRP3, such as the membrane protein M2 and virulence protein PB1-F2 of influenza virus (Ichinohe et al., 2010; Pinar et al., 2017).

It has been reported that interaction of pathogen-associated molecular patterns (PAMPs) with pattern recognition receptors is required for the NLPR3 up-regulation. Although so far there was no direct evidence about the interaction of CVB3 VP1 or VP2 with host PRRs, increasing literature showed the possible association of TLR4 with CVB3 capsid proteins. For example, Satoh M et al (Satoh et al., 2004) demonstrated the extensive co-localization of CVB3 VP1 and TLR4 proteins in the cytoplasm of myocardiocytes were obtained from DCM patients. Marchant D et al (Marchant et al., 2010) found that CVB3-induced MAPK/ERK pathway activation, which was involved in the NLRP3 activation, corresponded with the onset of CVB3 VP1 production. Therefore, it is reasonable to deduce that VP1 and VP2 proteins might induce macrophage NLRP3 up-regulation by interacting with PRRs like TLRs, of course the detailed mechanism still needs to be further explored.

Actually, only NLRP3 up-regulation is insufficient for NLRP3 inflammasome activation, signal activating caspase-1 and promoting mature IL-1β formation is also required. Factors such as K^+ efflux, Ca^{2+} signaling, ATP, P2 × 7 and reactive oxygen species (ROS) have been reported to be involved in the NLRP3 inflammasome activation (Horng, 2014; Katsnelson et al., 2015; Mariathasan et al., 2006; Yamada et al., 1994; Yu and Finlay, 2008). We previously reported that CVB3 infection could induce ROS production and K^+ efflux in myocardiocytes (Wang et al., 2014), therefore these factors might also contribute to the macrophage NLRP3 inflammasome activation in this study. Recently, several studies also revealed that enterovirus non-structural proteins could activate NLRP3 inflammasome via disturbing intracellular ionic concentrations (Ito et al., 2012) or binding to NLRP3 to facilitate inflammasome assembly (Wang et al., 2017). In terms of CVB3, viral proteinases 3C and 2A has been reported to inactivate NLRP3 inflammasome in infected myocardiocytes, Hela as well as Jurket cells via cleaving NLRP3 and its upstream serine/threonine-protein kinase receptor-interacting protein 1/3 (RIP1/3) (Wang et al., 2019). While in our experiments, we did not observe such NLRP3 degradation phenomena in macrophages, this might be attributed to the very limited virus replication in macrophages. As shown in the previous studies (Wang et al., 2014, 2019), myocardioctyte NLRP3 inflammasome was activated as early as several hours post CVB3 infection, and led to the robust IL-1β production at the early stage of viral myocarditis to control virus infection. While amplified CVB3 virus adopted a strategy to counteract it by degrading myocardiocyte NLRP3 with their enriched proteases 3C and 2A, thereby attenuating the inflammation response. Fortunately, recruited cardiac macrophages became another important source of NLRP3 inflammasome. Since macrophages cannot support efficient virus replication, no abundant CVB3 3C and 2A proteases would accumulate in the cells, which led to the massive NLRP3 activation and IL-1β production. This will benefit the infection control, but over-reactive pro-inflammatory responses will further increase myocardial damage and aggravate viral myocarditis. Therefore, myocardiocyte and macrophage NLRP3 seemed to play roles in different periods of viral myocarditis. Miteva et al. (2018) reported that CVB3-activated myocardiocyte NLRP3 inflammasome could also be inhibited by mesenchymal stromal cells. These information indicated that NLPR3 inflammasome activation was under a complicated and precisely control. Whether other CVB3 non-structural proteins participated in the macrophage NLRP3 inflammasome activation calls for further study.

Taken together, our study revealed that CVB3 capsid proteins VP1 and VP2 could trigger macrophage NLRP3 up-regulation and inflammasome activation, which robustly promoted the macrophage pro-inflammatory cytokine IL-1β production and contributed to the viral myocarditis development. Our study may not only assist in understanding the pathogenesis of CVB3-induced myocarditis, but also suggest that manipulating macrophage NLRP3 inflammasome might represent a novel therapeutic strategy against viral myocarditis.

Declaration of Competing Interest

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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