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BRIEF REPORT

Mycobacterium intracellulare mediates macrophage pyroptosis by activating AIM2 and NLRP3 inflammasomes

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Abstract
Clinically, the incidence of nontuberculous mycobacteria (NTM) lung disease is on the rise, and *Mycobacterium intracellulare* (*M. intracellulare*) has attracted much attention as a common opportunistic pathogen in clinical practice. So it is very important to study its immunopathogenic mechanism. In this study, the mechanism of *M. intracellulare* induced pyroptosis of macrophage was investigated. As shown in Fig. 1, the secretion of IL-1β and IL-18 in J774A.1 cells increased with time after *M. intracellulare* infection and was affected by caspase-1 activation and K + efflux, while caspase-1 was significantly expressed in infected cells. Further from Fig. 2, NLRP3,AIM2,ASC proteins were significantly expressed in J774A.1 cells after infection, indicating that the NOD-like receptor thermal protein domain associated protein 3 (NLRP3) and absent in melanoma 2 (AIM2) inflammasome were involved in the infection process. In addition, when caspase-1 activity and K + efflux were inhibited, the expression of related proteins was significantly reduced. It indicates that the activation of NLRP3 and AIM2 is regulated by caspase-1 and K+. Figure 3, the percentage of dead cells with cell membrane damage increases after infection and cleavage of GSDMD proteins occurs. In summary, infection of J774A.1 cells with *M. intracellulare* induces pyroptosis, and this process is mediated by caspase-1. Our study provides information for further understanding of the molecular mechanism of *M. intracellulare* infection.

Keywords Pyroptosis · Macrophage · NLRP3 · AIM2 · *Mycobacterium intracellulare*

Introduction

The nontuberculous mycobacteria (NTM), ubiquitous in soil and water, exhibits varying levels of pathogenicity. In recent years, NTM infections have been on the rise and are increasingly recognized as the cause of chronic lung disease, lymphadenitis, skin diseases, and disseminated infections, drawing increasing attention (Koh et al. 2002; Field and Cowie 2006). The nontuberculous mycobacteria species are divided into 4 groups: photochromogens, scotochromogens, nonchromogens, and rapidly growing mycobacteria. *M. intracellulare* is the most common slow-growing nontuberculous mycobacteria in clinical settings. It can be isolated from soil, water, and animal excreta. Infected birds, mammals, and soil rich in bird droppings may serve as natural hosts for this bacterium, which can invade the lungs and cause nontuberculous mycobacterial lung disease.

After the infection by an intracellular pathogen, the host is exposed to several ligands leading to activation of multiple signalling pathways (Pattanaik et al. 2022). Pyroptosis is a form of programmed inflammatory cell death that involves the formation of inflammasome macromolecular complexes mediated by inflammatory caspases.The signaling pathways of pyroptosis is shown in Fig. 1 of the Supplementary Material– quote from Wang et al. 2024. It is most commonly observed after intracellular infection and can be considered as part of the host's antimicrobial response. It is characterized by cytoplasmic swelling, plasma membrane rupture, and the release of pro-inflammatory cell contents (Lamkanfi and Dixit 2014; Geng et al. 2015). The inflammasome is a multiprotein complex assembled in the cytoplasm. Under the influence of internal or external danger signals, the pattern recognition receptor is activated to recruit the adaptor

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protein Accounting Standards Committee (ASC), assemble the cysteine protease caspase-1, and then initiate the formation of the inflammasome (Fang et al. 2019). Previous studies have shown that bacterial infection-induced secretion of IL-1β and IL-18 is associated with the activation of NLRP3 and AIM2 inflammasomes (Bittner and Preheim 2016). *Staphylococcus aureus*, *Pneumonia Klebsiella and Candida albicans* are among the many pathogens that activate NLRP3 inflammasome (Allen et al. 2009; Willingham et al. 2009; MuñozPlanillo et al. 2016). It has been shown that *Mycobacterium tuberculosis* EsxL induces cytokine secretion through activation of TLR2-dependent MAPK and NF-κB pathways (Pattanaik et al. 2021), and animals with NLRP3 or AIM2 deletion show higher susceptibility to *Mycobacterium tuberculosis* (Beckwith et al. 2020). By interfering with the NLRP3 inflammatory vesicle pathway, the inflammatory response and mortality of microglia during Mycobacterium tuberculosis H37Ra strain infection can be significantly reduced (Xie et al. 2021). AIM2-deficient mice are more susceptible to Mycobacterium tuberculosis infection, and secretion of IL-1β and IL-18 is significantly reduced during infection (Saiga et al. 2012).In the process of cell pyroptosis, caspase-1/4/5/11 are activated, the N-terminal domain of GSDMD is cleaved, and it oligomerizes on the cell membrane to form pores, resulting in cell membrane rupture (Cerretti and Kozlosky 1992; Thornberry et al. 1992; Zychlinsky et al. 1992; Chen et al. 1996).

This study goes through the following three questions: (1) *M. intracellulare* infection of J774A.1 cells induced secretion of IL-1β and IL-18 and activation of caspase-1; (2) *M. intracellulare* infection with J774A.1 assembly of cell-induced inflammatory vesicles; (3) Whether *M. intracellulare* can induce J774A.1 cells to undergo pyroptosis. To investigate the relationship between *M. intracellulare* infection and inflammasome activation and whether a pyroptosis occurs. The results show that *M. intracellulare* infection can stimulate the maturation and secretion of IL-1β and IL-18, induce the activation of caspase-1, and then induce the assembly of NLRP3 and AIM2, which is regulated by K + channel. These results indicate that the NLRP3 and AIM2 inflammasomes serve as immune defense mechanisms against the invasion of *M. intracellulare*, and pyroptosis is involved in the infection process. This may lead to a new research direction for understanding the infection mechanism of *M. intracellulare* and provide a scientific foundation for further investigation into its pathogenesis.

Materials and methods

Bacterial culture

M. intracellulare was preserved by the Laboratory of Basic Veterinary Medicine, Jilin Agricultural University.

M. intracellulare was cultured in 7H9 liquid medium at 37 °C for over 7 days. The composition of 7H9 liquid medium includes 7H9 solid powder (Solarbio, China), glycerol (Biosharp, China), Tween-80 (Biosharp, China) and distilled water. An appropriate volume of bacterial solution was taken, diluted with PBS, evenly spread on 7H9 solid medium, and then incubated in a constant temperature chamber at 37 °C for 3 days. The number of colonies was recorded, and the colony-forming units (CFU) were calculated.

Cell culture and infection

Mouse macrophages (J774A.1 cells) were derived from the Preventive Veterinary Laboratory of Jilin Agricultural University. J774A.1 cells were cultured in Dulbecco's modified eagle medium (DMEM, Solarbio, China) medium with 10% Fetal Bovine Serum (FBS, Gibco, USA) and 1% Penicillin/Streptomycin (Solarbio, China) in a humidified atmosphere containing 5%CO2/95% air at 37ºC. After the cells reached the logarithmic proliferation stage, they were infected with $3 \times 10^{\wedge 6}$ CFU/ml *M. intracellulare* for 2 h, and the negative control was added with the same amount of sterile PBS.

J774A.1 cells were divided into control group (PBS group), model group (*M. intracellulare* group), Ac-yVAD-CHO group and Glibenclamide group with concentrations of 0.1 μM, 1 M and 10 M. Ac-yVAD-CHO (Santa Cruz, USA) is a potent, specific and reversible caspase-1 inhibitor that completely blocks caspase-1 activity at certain concentrations. Glibenclamide (Santa Cruz, USA) is an orally active ATP-sensitive K + channel (KATP) inhibitor. Since the above two inhibitors were selected in the group's previous study of applying clinical isolates of *Mycobacterium neoaurum* and BCG to induce the assembly of inflammatory vesicles and cellular focalization in mouse macrophages, the same inhibitor types and concentrations were selected in the present study, which can be used for comparison. First, J774A.1 cells were inoculated on a 6-well plate with a density of 3 × 10^5 cells per well, and the bacteria were inoculated with MOI = 10. The inhibitor group treated cells with the inhibitor for 1 h prior to infection.

Cell transfection

J774A.1 cells were cultured in six-well plates until they reached a density of approximately 60–80%. Add 125 μL of

DEPC water to a 2.5 nmol tube and gently mix to create a 20 μM/L nucleic acid solution (ZETA, POL). The sequence of small interfering RNA is available in the supplementary material. The nucleic acid solution was directly mixed with the transfection reagent in a 1:1 ratio. The mixture was then agitated by pipetting up and down 10–15 times and left at room temperature for 10–15 min. Add 6 μL of the nucleic acid complex to each well of the cell plate and mix gently for transfection.

ELISA

Levels of IL-1β and IL-18 in J774A.1 cell lysates were measured by an ELISA kit (Solarbio, China) according to the manufacturer's in structions. The contents of IL-18 and IL-1β in the samples were calculated according to the optical density (OD).

Quantitative real-time PCR (qRT-PCR)

Using an RNA extraction kit (BioFlux, USA), the total RNA was extracted according to the manufacturer's protocol, and then reverse-transcribed into cDNA using a reverse transcription kit (TransGen Biotech China). Quantitative real-time PCR was performed using cDNA as template, GAPDH as internal reference, and $2^{-\Delta\Delta CT}$ method was used to calculate the relative mRNA expression multiple changes. The primers used are listed in the supplementary material.

Western blot analysis

Total proteins from J774A.1 cell lysate were collected and extracted with RIPA lysate buffer (Solarbio, China). The concentration was detected according to the BCA protein assay kit (Solarbio, China). GAPDH was used as a control. Samples in different groups were separated with SDS-PAGE and transferred to polyvinylidene difluoride (PVDF, Milipore, USA) membrane. After block ing with 5% nonfat milk for 2 h. After blocking, the membranes were washed five times with Tris-buffered saline with Tween −20 detergent (TBST, Yazyme, China) for 5 min each time and incubated overnight at 4 ◦C with primary antibodies. After washing five times with Tris-buffered saline with TBST at a 1:5000 dilution ratio in 5% non-fat milk, the membranes were incubated with horseradish peroxidase (HRP)-linked goat anti-rabbit IgG secondary antibody for 1 h at room temperature. Washed with Tris buffered saline and TBST for 5 times, the prepared ECL color developing solution (Yazyme, China) was added to the PVDF membrane after antibody hybridization, and the protein bands were detected by chemiluminescence imager after static for 2 min.

Lactate dehydrogenase release assay (LDH release assay)

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme that is released by cell membrane lysis during cell death in vitro. After treating the cells with 0μM, 0.1 μM, 1 μM, and 10 μM Ac-yVAD-CHO, the cells were infected with a 10:1 infection complex number (MOI). 2 h after infection, LDH levels were measured using an LDH release kit (Promega, USA) according to the manufacturer's instructions.

Statistical analysis

The Prism 8 software (GraphPad) was used to analyze the differences between the experimental and control groups. Data are expressed as mean ± SEM($n = 3$). The value p was considered significant at $p < 0.05$, and not significant (ns) at $p > 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$.

Results

M. intracellulare induces the activation of caspase-1 and maturation of IL-1β and IL-18

The production of IL-1β and IL-18 at different stages of infection was measured by ELISA, and the optimal infection time was 2 h (Fig. 1a, b). At the optimal time of infection, the expressions of IL-1β, IL-18, and Caspase-1 in macrophages were measured by qRT-PCR and WB. Compared with the PBS group, the expressions of IL-1β, IL-18 and Caspase-1 in the *M. intracellulare* group were increased (Fig. 1c, d). Next, we treated the cells with 0.1 μM, 1 μM, and 10 μM of Ac-yVAD-CHO and Glibenclamide, the secretion levels of IL-1β and IL-18 were detected by ELISA. The results showed a significant decrease in the secretion levels of IL-1β and IL-18. As the inhibitor concentration increased, the secretion of IL-1β and IL-18 decreased even more significantly (Fig. 1e–h). The above results show that, *M. intracellulare* induces the activation of Caspase-1 and the secretion of IL-1β and IL-18, and the secretion of IL-1β and IL-18 is regulated by caspase-1 and K+.

M. intracellulare induces the assembly of NLRP3 and AIM2 inflammasomes

So far, a variety of inflammasomes have been discovered, among which the NLRP3 and AIM2 inflammasomes play an important role in the process of bacterial infection(Zhang et al. 2021). To investigate the mechanism by which NLRP3 and AIM2 inflammasomes induce the secretion of IL-1β

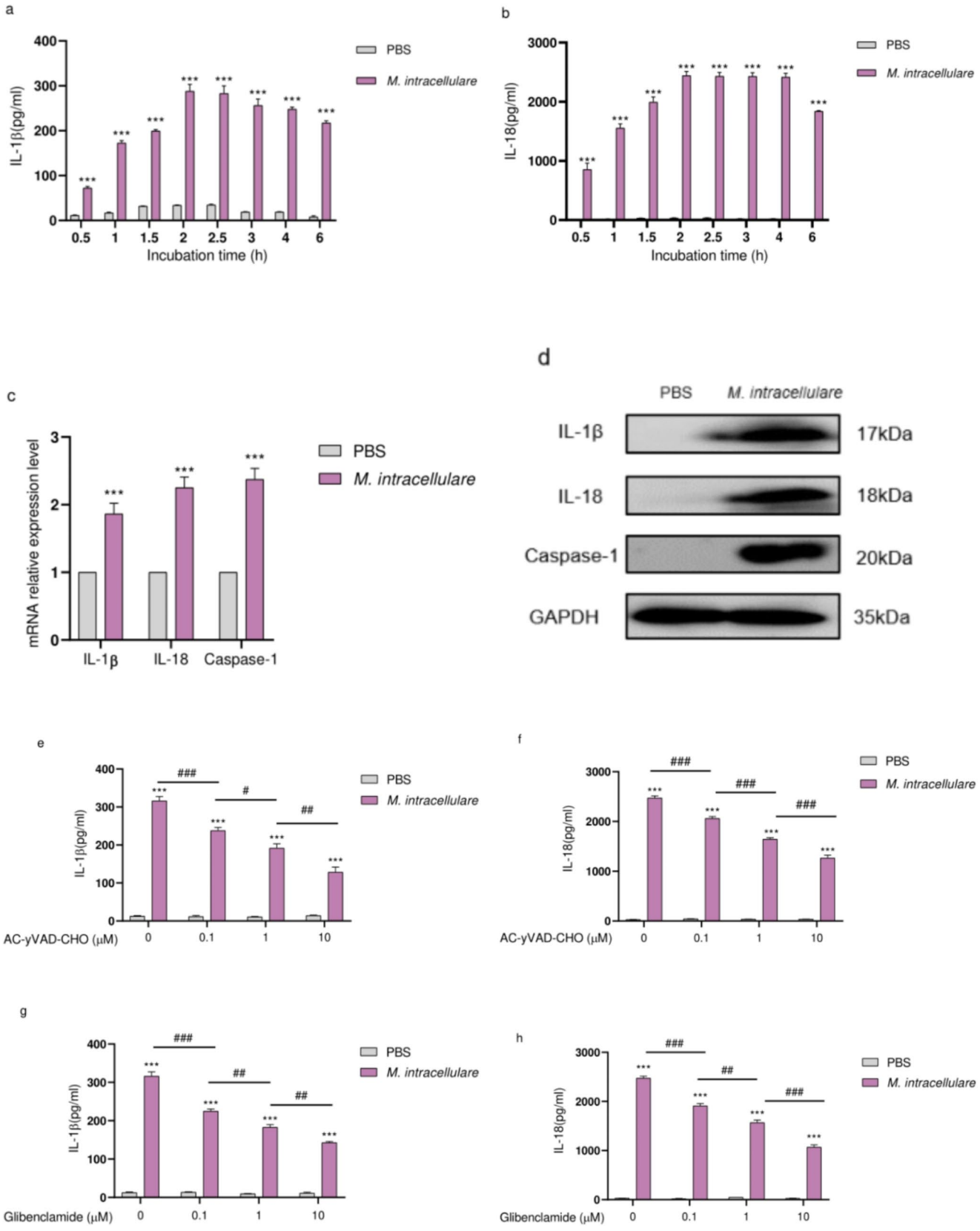
a
IL-1β(pg/ml)
Incubation time (h)
PBS
M. intracellulare
b
IL-18(pg/ml)
Incubation time (h)
PBS
M. intracellulare
c
mRNA relative expression level
IL-1β
IL-18
Caspase-1
PBS
M. intracellulare
d
PBS
M. intracellulare
IL-1β
17kDa
IL-18
18kDa
Caspase-1
20kDa
GAPDH
35kDa
e
IL-1β(pg/ml)
AC-yVAD-CHO (μM)
PBS
M. intracellulare
f
IL-18(pg/ml)
AC-yVAD-CHO (μM)
PBS
M. intracellulare
g
IL-1β(pg/ml)
Glibenclamide (μM)
PBS
M. intracellulare
h
IL-18(pg/ml)
Glibenclamide (μM)
PBS
M. intracellulare

Fig. 1 The secretion of IL-1β and IL-18 induced by *M. intracellulare* infection of mouse macrophages depends on the activation of caspase-1. **a**, **b** The secretion of IL-1β and IL-18 was detected by ELISA at different infection times. **c** The mRNA expression of IL-1β, IL-18 and caspase-1 was detected by qRT-PCR. **d** Western blot analysis of IL-1β, IL-18 and caspase-1 protein expression. **e**, **f** Cells were treated with Ac-yVAD-CHO (caspase-1 specific inhibitor) at different concentrations, and IL-1β and IL-18 secretion were detected by ELISA. **g**, **h** Cells were treated with different concentrations of glibenclamide (an ATP-dependent selective K + channel inhibitor), and IL-1β and IL-18 secretion were detected by ELISA. Data are expressed as the mean ± SEM ($n = 3$), $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$

and IL-18 by *M. intracellulare*, we initially transfected mouse macrophages with small interfering RNA to silence the expression of NLRP3 and AIM2 mRNA genes. Subsequently, we measured the levels of IL-1β and IL-18 in the cell supernatants after *M. intracellulare* infection by ELISA. The results showed that after NLRP3 and AIM2 gene functions were knocked down, the secretion of IL-1β and IL-18 induced by *M. intracellulare* infected mouse macrophages was significantly reduced (Fig. 2a, b).

In view of the above results, we found that the secretion of IL-1β and IL-18 induced by Mycobacteria-infected mouse macrophages is associated with the function of both NLRP3 and AIM2. Therefore, we speculate that *M. intracellulare* may trigger the formation of NLRP3 and AIM2 inflammasomes in mouse macrophages. We detected the expression of NLRP3 and AIM2 inflammasomes components using fluorescence quantitative PCR and Western blot. The results showed that *M. intracellulare* infection induced the expression of NLRP3, AIM2, and ASC gene proteins in mouse macrophages. (Fig. 2c, d). These results suggest that infection of mouse macrophages with *M. intracellulare* induces the assembly of NLRP3 and AIM2 inflammasomes.

Next, we treated mouse macrophages with 0.1 μM, 1 μM, or 10 μM of Glibenclamide and Ac-yVAD-CHO for 1 h. The results showed a negative correlation between the expression levels of NLRP3 and AIM2 inflammasomes components and the concentrations of Glibenclamide and Ac-yVAD-CHO (Fig. 2e, f). This suggests that the assembly of NLRP3 and AIM2 inflammasomes is regulated by K + and caspase-1.

M. intracellulare induces caspase-1 activation mediates pyroptosis

The cytotoxicity kit was used to detect LDH release in macrophages treated with Ac-yVAD-CHO. The results showed that after Ac-yVAD-CHO treatment of macrophages, the proportion of cell membrane damage and death cells in the *M. intracellulare* group decreased (Fig. 3a). The gene and protein expression of GSDMD in macrophages after infection were measured by qRT-PCR and WB. Compared with the PBS group, the GSDMD gene was significantly expressed, and GSDMD-NT with activity was produced in the *M. intracellulare* group (Fig. 3b, c).

Discussion

Inflammation is an early symptom of many diseases and belongs to one of the body's normal immune defense reactions, but excessive inflammation will lead to dysregulation of the immune system, ultimately causing the deterioration of the disease, which is a serious threat to human life and health (Qian et al. 2021). According to research reports, NTMs that can activate inflammatory vesicles are *Mycobacterium ulcerans* (Foulon et al. 2020), *Mycobacterium abscess* (Kim et al. 2020), *Mycobacterium Kansas*, Mycobacterium fortuitum, Mycobacterium smegmatis (Shah et al. 2013). However, whether there is an association between inflammatory vesicles and intracellular mycobacterial infection has not yet been reported. Therefore, in the present study, by analyzing the association between the release of inflammatory factors and inflammatory vesicles after *M. intracellulare* infection, which in turn provides a new perspective on the immune pathogenesis of *M. intracellulare* infections and provides a certain scientific basis for the prevention and control of tuberculosis and non-tuberculosis diseases.

Pyroptosis is an emerging mechanism of intrinsic cell death, a pro-inflammatory cell death mode that relies on the cysteine aspartic protease (caspase) family. When a foreign signal stimulates an infected cell, the pattern recognition receptor (PRR) is automatically activated and participates in the formation of the inflammasomes, which specifically recognize and cleave Pro-caspase-1/4/5/11 to form mature caspase (Kayagaki et al. 2015; Shi et al. 2015). Then, the GSDMD-N terminal is depolymerized into the cell membrane to form holes, resulting in cell permeation, swelling, lysis and necrosis, and cell pyroptosis (Aglietti et al. 2016; Ding et al. 2016; Liu et al. 2016; Malik and Kanneganti 2017). This process releases activated inflammatory cytokines interleukin-1β and IL-18 outside the cell and recruits more inflammatory cells to amplify the inflammatory response. Three key findings are clarified in this paper. First, infecting J774A.1 cells with *M. intracellulare* induced the secretion of IL-1β and IL-18 and the activation of Caspase-1. Secondly, infecting J774A.1 cells with *M. intracellulare* induces the assembly of NLRP3 and AIM2 inflammasomes, which are regulated by K + channel and caspase-1. Finally, *M. intracellulare* can cause J774A.1 cells to pyroptosis.

Caspase-1 exists as an inactive precursor in the cytoplasm in vivo. Activated caspase-1 can cleave pro-IL-1β

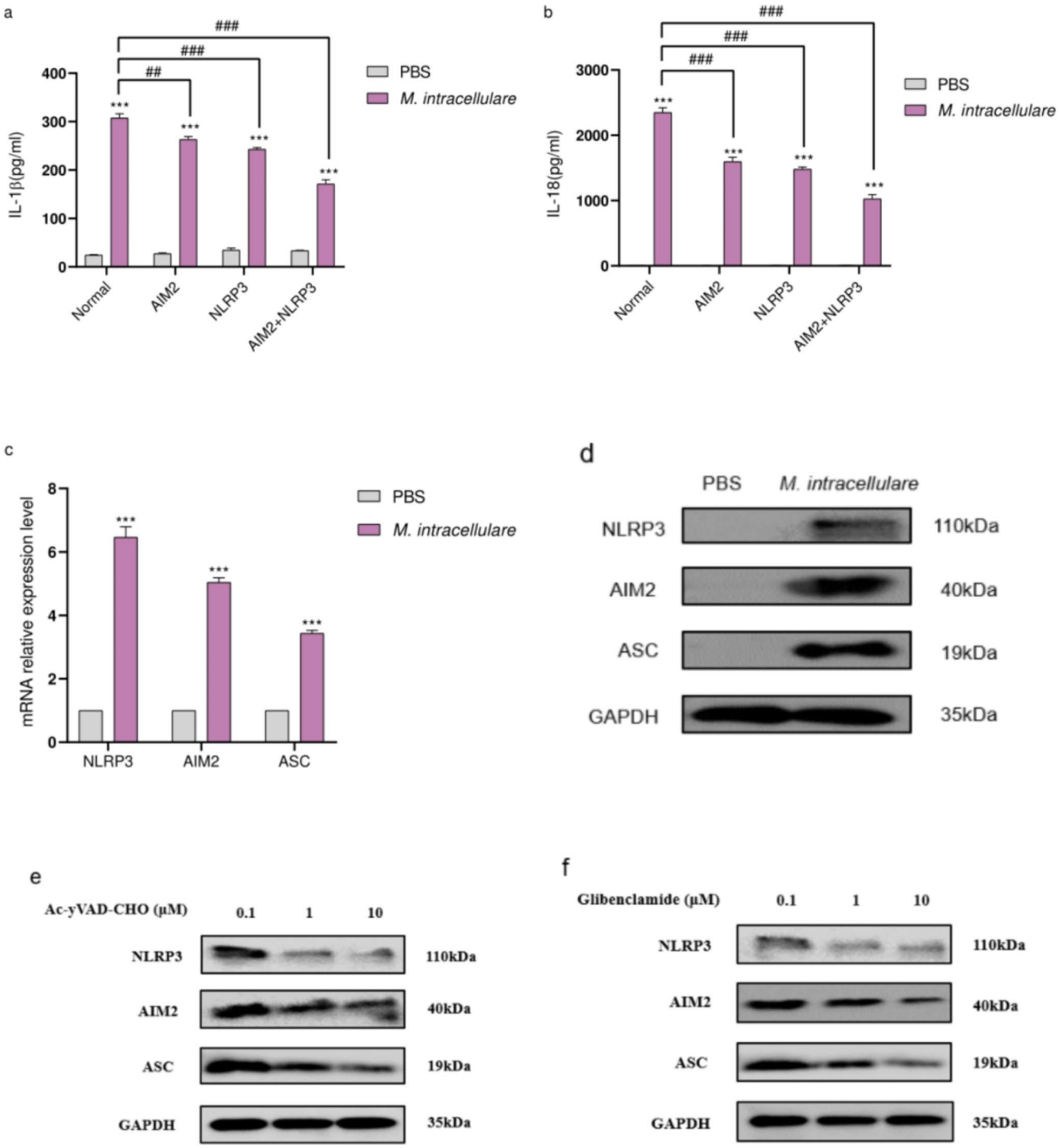

Fig. 2 *M. intracellulare* infection of macrophages induces NLRP3 and AIM2 inflammasome assembly, which is regulated by caspase-1 and K+, and IL-1β and IL-18 are dependent on the activation of NLRP3 and AIM2 inflammasomes. **a**, **b** Small interfering RNA transfection silenced the expression of NLRP3 and AIM2 mRNA genes, IL-1β and IL-18 secretion were detected by ELISA. **c** mRNA expression of NLRP3, AIM2 and ASC was detected by qRT-PCR. **d** Western blot analysis of NLRP3, AIM2 and ASC protein expression. **e** The cells were treated with Ac-yVAD-CHO (caspase-1 specific inhibitor) at different concentrations, and the protein expression of NLRP3, AIM2 and ASC was detected by Western blot. **f** Cells treated with different concentrations of glibenclamide (ATP-dependent selective K + channel inhibitor) were examined by Western blot for NLRP3, AIM2 and ASC protein expression. Data are expressed as the mean ± SEM ($n = 3$), $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$

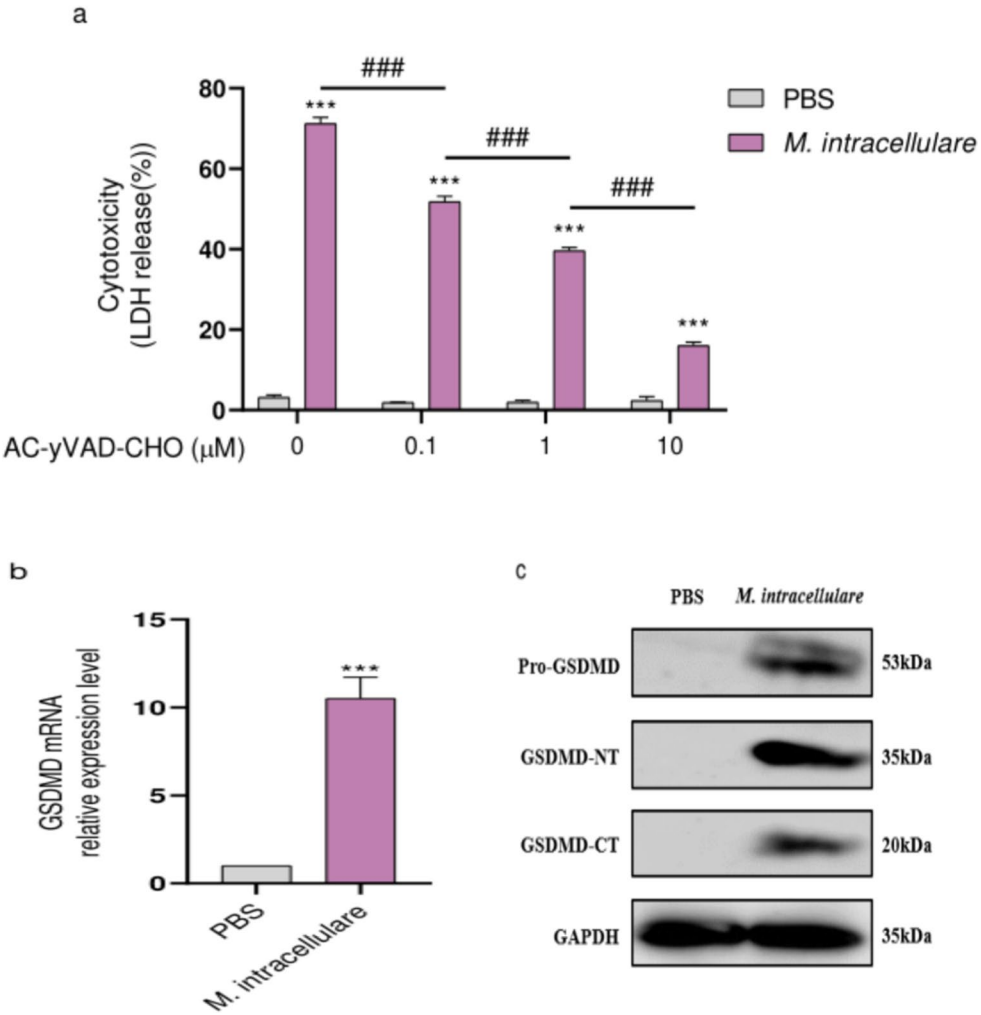

Fig. 3 *M. intracellulare* can induce mouse macrophages to activate Caspase-1-mediated pyroptosis. **a** Cell membrane damage was detected by cytotoxicity kit after Ac-yVAD-CHO (caspase-1 specific inhibitor) treatment of mouse macrophages. **b** mRNA expression of GSDMD was detected by qRT-PCR. **c** Western blot analysis of GSDMD protein expression. Data are expressed as the mean ± SEM ($n = 3$), $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$

and pro-IL-18 to produce active IL-1β and IL-18 (Malik and Kanneganti 2017). Numerous studies have demonstrated that the secretion and signaling of IL-1β and IL-18 play a crucial role in the progression of mycobacterial infection (Miao et al. 2010; Liu et al. 2016; Sborgi et al. 2016; Malik and Kanneganti 2017). In this study, we initially investigated the secretion of IL-1β and IL-18 in mouse macrophages induced by *M. intracellulare*. The results showed a significant increase in the secretion of IL-1β and IL-18, reaching their peak levels two hours after infection. We also identified the expression of IL-1β, IL-18, and caspase-1 using fluorescence quantitative PCR and Western blot analysis. The expression and protein levels of IL-1β, IL-18, and caspase-1 were significantly increased, indicating that *M. intracellulare* infection induced the activation of caspase-1 and the maturation and secretion of IL-1β and IL-18. Next, mouse macrophages were treated with various concentrations of a caspase-1 inhibitor (Ac-yVAD-CHO) and an ATP-dependent selective K + channel inhibitor (Glibenclamide), and then infected with *M. intracellulare*. The results indicated that the secretion of IL-1β and IL-18 in mouse macrophages decreased in a dose-dependent manner. These results suggest that the secretion of IL-1β and IL-18 in mouse macrophages induced by *M. intracellulare* depends on the activation of caspase-1 and K^+ efflux channels.

With the discovery of inflammatory vesicles, mechanisms of caspase-1 activation and IL-1β and IL-18 maturation were revealed. The main role of inflammatory vesicles is the maturation of IL-1 superfamily cytokines (IL-1β, IL-18, and IL-33), which also cleave gasdermin D and promote the release of IL-1β, generating an osmotic imbalance that leads to cell lysis and death (Miao et al. 2011; Sborgi et al. 2016). The main role of inflammatory vesicles is the maturation of IL-1 superfamily cytokines (IL-1β, IL-18, and IL-33), which also cleave gasdermin D and promote the release of IL-1β, generating an osmotic imbalance that leads to cell lysis and death (Krishnan et al. 2013; Ma et al. 2021; Briken 2013). Numerous studies have shown that NLRP3 and AIM2 inflammasomes play a key role in Mycobacterium infections (Carlsson et al. 2010; Chen et al. 2012; Marim et al. 2016; Malik and Kanneganti 2017; Foulon et al. 2020; Zhang et al. 2021; Ma et al. 2021). In addition,

the above study showed that *M. intracellulare* infection of mouse macrophages was strongly associated with activation of caspase-1 and secretion of IL-1β and IL-18. We hypothesize that *M. intracellulare* infection induces the activation of macrophage inflammatory vesicles. To further determine the secretion of IL-1β and IL-18 in mouse macrophages regulated by that inflammatory vesicles, we synthesized siRNA sequences of NLRP3 and AIM2, and targeted knockdown of the expression of NLRP3 and AIM2 genes in mouse macrophages by cellular transfection. The results showed that IL-1β and IL-18 were significantly reduced in macrophages of mice in the treated group, suggesting that the NLRP3 and AIM2 inflammasome mediated the secretion of IL-1β and IL-18. Next, to further verify that Mycobacterium infects mouse macrophages induces NLRP3 and AIM2 inflammasomes assembly. We examined gene and protein expression of NLRP3 and AIM2 inflammatory-related components. The results showed that the expression of NLRP3, AIM2 and ASC was induced by *M. intracellulare* infection, indicating that the assembly of NLRP3 and AIM2 inflammasomes were induced by *M. intracellulare* infection.

Studies have shown that when Streptococcus pneumoniae infects macrophages, K + regulates IL-1β secretion, caspase-1 activation, and ASC oligomerization, suggesting that the AIM2 inflammasome is involved in the regulation of IL-1β maturation and secretion during the process of Streptococcus pneumoniae infection of macrophages (Saiga et al. 2012; Yang et al. 2013). In this study, Glibenclamide-was used to inhibit K + channel activation in target cells infected by *M. intracellulare*, and WB results showed that the expression levels of NLRP3 and AIM2 inflammasome components were negatively correlated with Glibenclamide concentration. Studies have shown that most of the inflammasome activators associated with pyroptosis in Mycobacterium tuberculosis are mediated by caspase-1. We treated the cells with Ac-yVAD-CHO, and WB results showed that the expressions of NLRP3 and AIM2 inflammasomes were negatively correlated with the concentration of Ac-yVAD-CHO. From this, we can conclude that *M. intracellulare* infection may induce macrophages to activate the NLRP3 and AIM2 inflammasome, and this process is regulated by K + channel and caspase-1.

Pyroptosis is a newly discovered programmed cell death mode in recent years, which mainly depends on the activation of caspase-1/-11 (Thornberry et al. 1992). A recent study showed that Salmonella invasion into macrophage cytoplasm can be recognized by the NLRC4 and NLRP3 inflammasome, thus activating mediated Pyroptosis (Yu et al. 2021; Li 2022). Both Mycobacterium extracellular and Salmonella typhimurium-infected mouse macrophages can cause GSDMD cleavage and pyroptosis (Subbarao and Sanchez-Garrido 2020). To investigate the pyrodeath of mouse macrophages induced by *M. intracellulare* infection. We first used cytotoxicity kits to detect the release of LDH in the cytoplasm and found that *M. intracellulare* infection induced cell membrane damage in macrophages. It is suggested that *M. intracellulare* infection may cause pyroptosis of cells. Therefore, we also treated macrophages with Ac-yVAD-CHO. It was found that Ac-yVAD-CHO could significantly inhibit membrane damage and the cell death rate of mouse macrophages induced by *M. intracellulare* infection. Next, we further investigated whether *M. intracellulare* infection could induce GSDMD cleavage. The results showed that *M. intracellulare* infection induced macrophage GSDMD cleavage and produced GSDMD-NT. Therefore, it is almost certain that *M. intracellulare* infection can cause pyrodeath of macrophages.

There are some limitations to this paper. As the article derives changes in relevant cytokines and even gene expression from in vitro experiments, this is not a complete reflection of the in vivo situation, for which further research is needed.

Conclusion

M. intracellulare infection of macrophages induces the activation of caspase-1 and the secretion of IL-1β and IL-18, and activates AIM2 and NLRP3 inflammasomes, suggesting the process of pyroptosis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11259-024-10505-x .

Author contributions All authors contributed to the study conception and design. Material preparation was performed by R. J., data collection was performed and analysis was performed by S. Y.The first draft of the manuscript was written by S. Y. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Financial interests The authors have no relevant financial or non-financial interests to disclose.

Competing interests The authors declare no competing interests.

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