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Chlamydia trachomatis ct143 stimulates secretion of proinflammatory cytokines via activating the p38/MAPK signal pathway in THP-1 cells

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ABSTRACT

Chlamydia trachomatis (Ct) infections can cause bacterial sexually-transmitted and preventable blindness. The Ct infections induced excessive cytokines generation which attributed to pathologic changes in host cells. However, the precise mechanisms of Ct-induced cytokines production are still unclear.CT143 protein was identified as a novel Ct specific protein with high immunogenicity. In the present study. The CT143 fusion protein was recombined and purified. The mice immune serum was prepared by immunizing BALB/c mice with the purified fusion protein. The specificity of the antibody was confirmed using Immunoblotting. Indirect immunoflurescence assay (IFA) and Immunoblotting assays were performed to detect the temporal and spatial characteristics of CT143 in Ct infected cells. ELISA was performed to analyze the secretion of proinflammatory cytokines IL-1β, IL-8 and TNF-α by human macrophages under the stimulation of CT143 protein. Finally, the involvement of p38 signaling in CT143-induced cytokine secretion was validated. CT143 protein was located in the inclusion body and represented an Elementary body (EB)-related protein, which may be encoded by the mid- and late-stage expressing genes. CT143 protein could stimulate the secretion of inflammatory cytokines in macrophages which differentiated from THP-1 This induction may be mediated by the activation of p38 signaling. In summary, CT143 protein is involved in inflammatory processes during Ct infection.

1. Introduction

Chlamydia trachomatis (Ct) are Gram-negative obligate intracellular bacteria which can cause infectious blinding and neonatal pneumonia in developing countries (Stary et al., 2015; Taylor et al., 1987). Ct are also the most common global pathogens in sexually transmitted disease (STD) and could increase the risk of HIV and HPV infection (Cheng et al., 2008a; Lucas et al., 2015; Wang et al., 2010a). Although antibiotics can effectively cure Ct infections, delayed diagnosis and treatment can cause chronic persistent infection, leading to severe complications such as endometritis, salpingitis, pelvic inflammatory disease, and infertility (Stamm, 1999).

Infection of Ct can affect the metabolism of host cells by synthesizing proteins, interfering with the normal physiological behavior of the host cells, and thus forming a microenvironment conducive to the maintenance of Ct persistence. For example, Ct infection of Hela cells could inhibit the activation of Bax and Bak, the release of cytochrome C from mitochondria, and induce the degradation of the pro-apoptotic factors (Bik, Bim, Puma, Bad, etc.) in the host cells, thus inhibiting cell apoptosis and facilitating Ct proliferation in cells (Dong et al., 2005; Zhong et al., 2006). In our previous study, we identified a total of 27 dominant antigens, which could be recognized by > 50% patients' immune sera, in the whole-genome of Ct (Wang et al., 2010a). Of them, 12 were previously reported to play a vital role in Ct infection by participating in the interaction between Ct and host cells and potentially inducing immunoprotection against Ct infection (Chen et al., 2006; Heinz et al., 2010; Murthy et al., 2011; Olsen et al., 2010; Wang et al., 2009; Xu et al., 2011). Of the rest 15 newly described antigens, CT143 has been considered as a Ct specific protein with the highest immunogenicity. High concentrations of anti-CT143 antibodies were detected in serum in 84% of patients (Wang et al., 2010a). Further investigating the role and mechanism of CT143 will provide new directions for chlamydial vaccines or the discovery of new virulence factors.

Infection with Ct induces a wide array of inflammatory cytokines and chemokines (Cheng et al., 2008b) (Buchholz and Stephens, 2006; Gervassi et al., 2004; Maxion and Kelly, 2002), which would contribute to chlamydia-induced inflammatory pathologies (Stephens, 2003). The

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Ct infection-induced pathological changes in genital and ocular trachoma are results from aggressive inflammatory responses. Previous studies have demonstrated that MAPKs contribute to the production of different cytokines during chlamydial infection (Srivastava et al., 2011; Vignola et al., 2010). Zhou et al. (Zhou et al., 2013) reported that MAPKs were involved in the production of TNF-α, IL-1β, and IL-8 induced by pORF5, which was also a critical Ct protein contributing to chlamydia-induced pathologies.

In the present study, a recombinant CT143 protein was produced and purified. Further, whether CT143 can activate monocytes to produce pro-inflammatory cytokines (TNF-α, IL-1β, and IL-8) and whether MAPKs are required for CT143-induced cytokine expression by monocyte cells are investigated.

2. Materials and methods

2.1. Expression and purification of the GST-CT143 fusion protein

A pGEX-6p-2/CT143 recombinant plasmid was transformed into *Escherichia coli* BL21 host cells for expression of the GST-CT143 fusion protein. Cells were cultured in 3 ml of Luria-Bertani (LB) liquid medium containing 100 μg/ml of ampicillin (Sangon Biotech, Shanghai, China) at 37 °C overnight. The culture was expanded at a ratio of 1:100 the next day. After 2.5 h, when the OD_{600} reached 0.6, Isopropyl β-D-Thiogalactoside (IPTG) (Sangon Biotech, Shanghai, China) with a final concentration of 0.1 mmol/L was added to induce the expression of the target protein at 16 °C. The bacteria were collected by centrifugation (12,000 × *g*), washed twice in PBS, and lysed with Phenylmethanesulfonyl fluoride (PMSF) (Sigma, CA, USA). After ultrasonication and high-speed centrifugation (12,000 × *g*), the supernatant was incubated with Glutathione Sepharose4B beads (ThermoFisher, MA, USA) at 4 °C for 8 h. The beads were washed three times with PBST and PBS to remove non-specifically bound hetero proteins. PreScission protease (Sangon Biotech, Shanghai, China) was added. The supernatant was collected by centrifugation (2500 × g) and finally concentrated by an ultrafiltration centrifuge tube (Millipore, MA, USA) to obtain purified, untagged CT143 protein. Proteins were analyzed by 10% SDS-PAGE and validated by Coomassie blue staining (Beyotime, Shanghai, China).

2.2. Fusion protein immunized animals

Four-week-old female BALB/c mice (obtained from SLAC experimental animal company, Changsha, China) were used for antibody production. A total of 100 μg of recombinant protein and 250 μl of Freund's Adjuvant Complete (FCA) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) were mixed in equal volumes. After phacoemulsification, the mice were vaccinated by intraperitoneal injection. A second vaccination was given on the 21 st day after the initial immunization with a half initial dose mixed with 250 μl of Freund's Adjuvant Inomplete (FICA) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) after phacoemulsification. The third and fourth vaccination was given 15 days after the last vaccination with a half initial dose mixed with 250 μl of FICA after phacoemulsification. Ten days after the last vaccination, the orbital blood was collected, and the mice were sacrificed. Peripheral blood was collected and hold for 1 h at room temperature, 4 h at 4 °C and then centrifuged at 1000 × g for 10 min. Serum was separated, an equal volume of glycerol (ThermoFisher, MA, USA) was added and mixed. The mixture was stored at −20 °C. The antibody titer was detected by indirect ELISA.

2.3. Specificity of CT143 antibody in antiserum

The specificity of the CT143 antibody in the prepared mice antiserum was analyzed by Immunoblotting. Purified CT143, *Chlamydia trachomatis*-like activity factor (CPAF), IncA, and HSP60 fusion proteins were electrophoresed on 10% SDS-PAGE, and the proteins were transferred to polyvinylidene fluoride (PVDF) membrane. Mouse anti-GST-CT143 immune serum was used as the primary antibody (1:200 dilutions), and Horseradish Peroxidase (HRP)-labeled horse anti-mouse IgG (1:5000 dilutions, Beyotime, Shanghai, China) was used as the secondary antibody.

2.4. Cell lines and cell culture

THP-1 cells (TIB-202; ATCC, VA, USA) were cultured in RPMI 1640 (GIBCO, MD, USA) supplemented with 10% fetal bovine serum (FBS; GIBCO) at 37 °C/5% CO_2. Hela229 cells were obtained from ATCC and cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS (GIBCO).

2.5. Localization of endogenous CT143 protein

Indirect immunofluorescence analysis (IFA) was performed to analyze the endogenous CT143 protein localization. HeLa229 cells were plated in a 24-well plate with cell slides at a density of 2×10^4 cells/ml at 37 °C, 5% CO_2 for about 16 h. When the cell density reached 60%, the cells were infected with *Chlamydia trachomatis* (Ct) at 1:800. The chlamydia infection fluid was discarded 2 h later, 1 ml of prewarmed complete medium containing 2 μg/ml cycloheximide (Sangon Biotech, Shanghai, China) was added, and the cells were cultured for 40 h. 42 h after chlamydial infection, the slides were collected, washed three times with cold PBS and fixed in 4% paraformaldehyde (Beyotime, Shanghai, China) for 30 min at 4 °C. Next, 0.2% Triton X-100 (Beyotime, Shanghai, China) was added to allow permeabilization of cells. Cells were then blocked with 1% BSA for 1 h at room temperature and incubated with primary antibody rabbit anti-*Chlamydia* antibody (Abcam, Cambridge, UK) and mouse anti-GST-CT143 polyclonal antibody at 1:100 dilutions for 2 h at room temperature. Then, cells were further incubated with secondary antibody (Cy2-labeled goat anti-rabbit IgG, Cy3-labeled goat anti-mouse IgG) (Beyotime, Shanghai, China) at 1:500 dilutions. Hoechst 33,342 was used to stain DNA (Beyotime, Shanghai, China). The cell slides were then mounted with an anti-fluorescence quencher (Beyotime, Shanghai, China). Cells observed under a fluorescence microscope and photographed.

2.6. The time phase of CT143 protein expression

Hela229 cells were infected with Ct at 1:500 as above described. The time phase of CT143 protein expression was analyzed using Immunoblotting and IFA assays. At 0 h, 6 h, 12 h, 24 h, 36 h, 42 h, and 48 h after infection, cellular proteins were collected, and protein concentration was measured using a BCA protein concentration assay kit. The extracted protein was mixed with 5× loading buffer and boiled for denaturing. After 10% SDS-PAGE, Immunoblotting and IFA assays were performed as above described using mouse anti-GST-CT143 polyclonal antibody (1:200 dilutions), mouse anti-β-actin antibody (1:1000 dilutions) (Abcam, Cambridge, UK) as primary antibodies and HRP-labeled horse anti-mouse IgG (1:5000 dilution, Beyotime, Shanghai, China) as the secondary antibody.

2.7. RNA interference

Specific small interfering RNA (Si-RNA) of p38 (si-p38, sense: antisence:) and negative control (si-NC, sense: 5′-UUC UCC GAA CGU GUC ACG UTT -3′; antisense: 5′-ACG UGA CAC GUU CGG AGA ATT -3′) were obtained from GenePharma, Shanghai, China. THP-1 cells (2×10^5 cells/ml) grown in 6-well plates were transfected with 100 pmol si-p38 or si-NC using RFect siRNA transfection reagent (BIOGEN, MA,USA). Six hours later, PMA (50 ng/ml) (Sigama, MA, USA) was added to induce the differentiation of THP-1 cells towards macrophages (cell identification was shown in Fig.S1). The cells were harvested after 48 h.

ELISA or other experiments were performed.

2.8. ELISA for cytokine detection

THP-1 cells were cultured in 6-well plates at a density of 1×10^6 cells/ml. PMA (50 ng/ml) (Sigama, MA, USA) was added to induce the differentiation of THP-1 cells towards macrophages (cell identification was shown in Fig.S1). Twenty-four hours after induction, purified, unlabeled CT143 protein (6, 12, 24 and 48 μg/ml) was added to stimulate THP-1 cells for 24 h. PBS and LPS (100 μg/ml) was used as a negative and positive control, respectively. The cellular supernatant was collected and stored at −80 °C. The concentrations of TNF-α, IL-1β, and IL-8 were measured using ELISA kits (R&D Systems, Inc., MN, USA), according to the manufacturer's instructions. The absorbance was measured at 405 nm using a microplate reader (Molecular Devices Corporation, CA, USA) and cytokine concentrations calculated.

2.9. Immunoblotting

The protein levels of total p38 and p-p38 were examined using immunoblotting. Cells were lysed cultured or transfected in 1% PMSF supplemented RIPA buffer (Beyotime, Shanghai, China). Protein was loaded onto SDS-PAGE minigel, and then transferred onto PVDF membrane ((Beyotime, Shanghai, China)). The blots were probed with the following antibodies (Abcam, Cambridge, MA, USA): anti-p38, anti-p-p38 and anti-GAPDH at 4 °C overnight and incubated with HRP-conjugated secondary antibody (1:5000). Signals were visualized using ECL Substrates (Millipore, MA, USA). The protein expression was normalized to endogenous GAPDH.

3. Statistical analysis

Data from three independent experiments are presented as the mean ± standard deviation (SD). GraphPad was used to analyze results with Student's *t*-test followed by one-way analysis of variance (ANOVA). A *P*-value less than 0.05 was considered statistically significant.

4. Results

4.1. Obtaining of recombinant CT143 protein and polyclonal antibody against CT143

Recombinant plasmid pGEX-6p-2/CT143 was transformed into *E. coli* BL21. After induction with IPTG at 16 °C for 8 h, the bacteria were collected, and the bacterial proteins were extracted. The proteins were purified by SDS-PAGE and stained with Coomassie brilliant blue. As shown in Fig.1A, the induced recombinant plasmid had a significantly brighter band at 57 kDa compared with the uninduced group, and its molecular weight was consistent with that of the GST-CT143 fusion protein. In addition, there was another significantly brigher band at the molecular weight of 26 kDa, which was consistent with the GST protein (Fig.1A). Next, the expressed product was purified by Glutathione Sepharose4B Beads, eluted with reduced glutathione, and concentrated. A bright band was observed at a relative molecular weight of approximately 57 kDa (Fig.1B), consistent with the predicted GST-CT143 fusion protein.

The antibody titer of mice antiserum was then measured using an indirect ELISA assay. The results showed that the serum of non-immune mice did not recognize CT143 protein and the reaction was negative (Fig.1C). On the contrary, mice antiserum could recognize CT143 protein and showed a positive response. When the dilution of antiserum reached 1:8000, the response was still positive (Fig.1C). It is suggested that the collected antiserum can recognize and bind to the CT143 protein with a titer higher than 1:8000.

Moreover, SDS-PAGE was performed to validate the specificity of mice antiserum using purified CT143, CPAF, IncA and HSP60 fusion proteins using mice antiserum as the primary antibody. As shown in Fig.1D, there was a distinct band in the CT143 lane with a relative molecular weight of about 31 kDa, while other Chlamydia proteins do not show a specific band at the corresponding molecular weight (Fig.1D), suggesting that the mice antiserum prepared and used in this experiment can specifically recognize the CT143 protein but no other Chlamydia proteins.

4.2. CT143 was produced during the Ct developmental cycle

After preparation of CT143 protein and antibody, the localization and time phase of CT143 protein in HeLa229 cells was examined using IFA and Immunoblotting assays. The results showed that the spatial distribution of CT143 was consistent with that of Ct, mainly in the inclusion body of Ct (Fig.2A). The fluorescence intensity of CT143 was the same as that of Ct, and the position was consistent with that of Ct (Fig.2A), suggesting that CT143 protein was located in the inclusion body of Ct.

Next, the time phase of CT143 expression was analyzed by Immunoblotting. The level of CT143 protein could be detected in Hela229 cells from 24 h after Ct infection (Fig.2B), and the protein level was significantly higher at 30 h and 36 h after infection than that at 24 h ($P < 0.01$, Fig.2B). Interestingly, the protein levels of CT143 were strongly increased 42 h and 48 h after infection than that at 30 and 36 h ($P < 0.005$, Fig.2B). The protein level of CT143 at 48 h was almost three times of that at 30 and 36 h.

Further IFA results showed that the expression of CT143 could be detected in Hela229 cells within 6 h after Ct infection (Fig.2C), but disappeared at 12 h, and a small amount of CT143 was detected again at 18 h, and the expression of CT143 protein gradually increased along with the time. These data suggested that the CT143 protein may be a mid-late protein encoded by the mid-late gene of Ct.

4.3. CT143 protein induces proinflammatory cytokines secretion in THP-1 cells

Next, THP-1 cells were stimulated with a series of concentrations of CT143 protein (6, 12, 24 and 48 μg/ml) and the levels of the secreted cytokines were examined using ELISA. Compared with the negative control group, the levels of TNF-α, IL-1β and IL-8 were significantly increased by CT143 protein from the concentration of 6 μg/ml. As the protein concentration increased, the IL-1β secretion level gradually increased and reached a peak value upon 24 μg/ml CT143 protein stimulation (Fig.3A). IL-8 secretion peaked upon 12 μg/ml CT143 protein stimulation and decreased slightly upon 24 and 48 μg/ml CT143 protein stimulation (Fig.3B). The levels of TNF-α secretion was not significantly different upon 6, 12, 24 and 48 μg/ml CT143 protein stimulation (Fig.3C).

THP-1 cells were then stimulated by 24 μg/ml CT143 protein for 0, 6, 12, 24, 36 and 48 h and examined for the secretion of TNF-α, IL-1β, and IL-8. The levels of all cytokines increased in a time-dependent manner and reached peak values at 24 h of stimulation, then decreased at 36 and 48 h.

4.4. The p38/MAPK pathways are involved in pro-inflammatory cytokine secretion induced by CT143 in THP-1 cells

After confirming that CT143 protein could stimulate the secretion of cytokines, the signaling pathway involved was investigated. THP-1 cells were stimulated with 12 and 24 μg/ml CT143 protein and examined for p38 and p-p38 protein levels. As shown in Fig. 4A, the ratio of p-p38/p38 was significantly increased by CT143 protein in a concentration-dependent manner. Next, THP-1 cells were co-treated with 24 μg/ml CT143 and 20 u M/l SB203580, an inhibitor of the p38/MAPK signaling, and then examined for p-p38/p38 ratio and cytokine secretion.

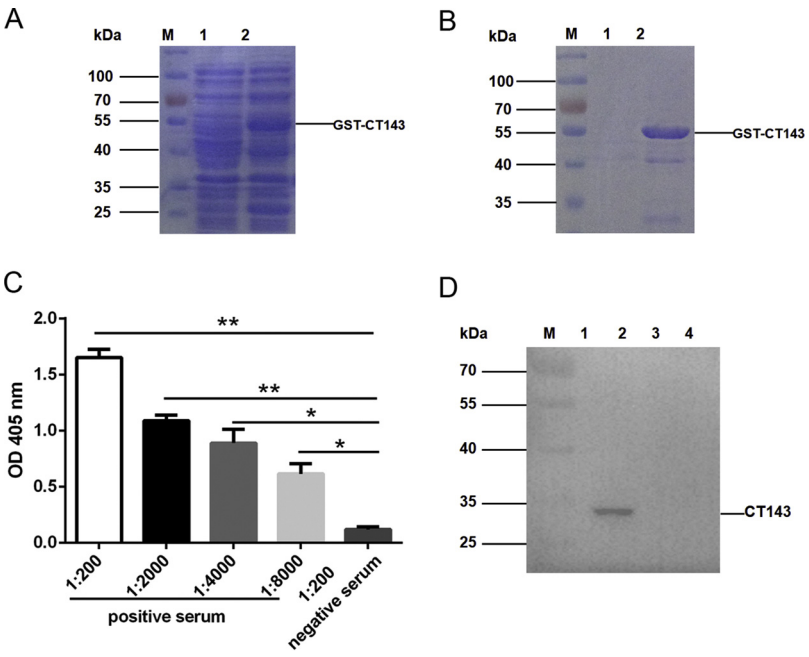

Fig. 1. Preparation of recombinant CT143 protein and polyclonal antibody against CT143 (A) Induction and confirmation of GST-CT143 fusion protein. M: protein marker; 1: the protein expressed by pGEX-6p-2/CT143 transformed strain without IPTG induction at 16 °C for 8 h; 2: the protein expressed pGEX-6p-2/CT143 transformed strain induced by 0.1 mM IPTG at 16 °C for 8 h. (B) Purify of the GST-CT143 fusion protein. M: protein marker; 1: the supernatant after the expressed product was purified by Glutathione Sepharose4B Beads and centrifuged (2500 × g); 2. the precipitate after the expressed product was purified by Glutathione Sepharose4B Beads and then pelleted by centrifugation (2500 × g). (C) The titration of antiserum by Indirect ELISA. (D) The specificity analysis of immune serum by Immunoblotting. M: protein marker; 1: CT143 (31 kDa); 2: CPAF (66 kDa); 3: IncA (30 kDa); 4: HSP60 (56 kDa).

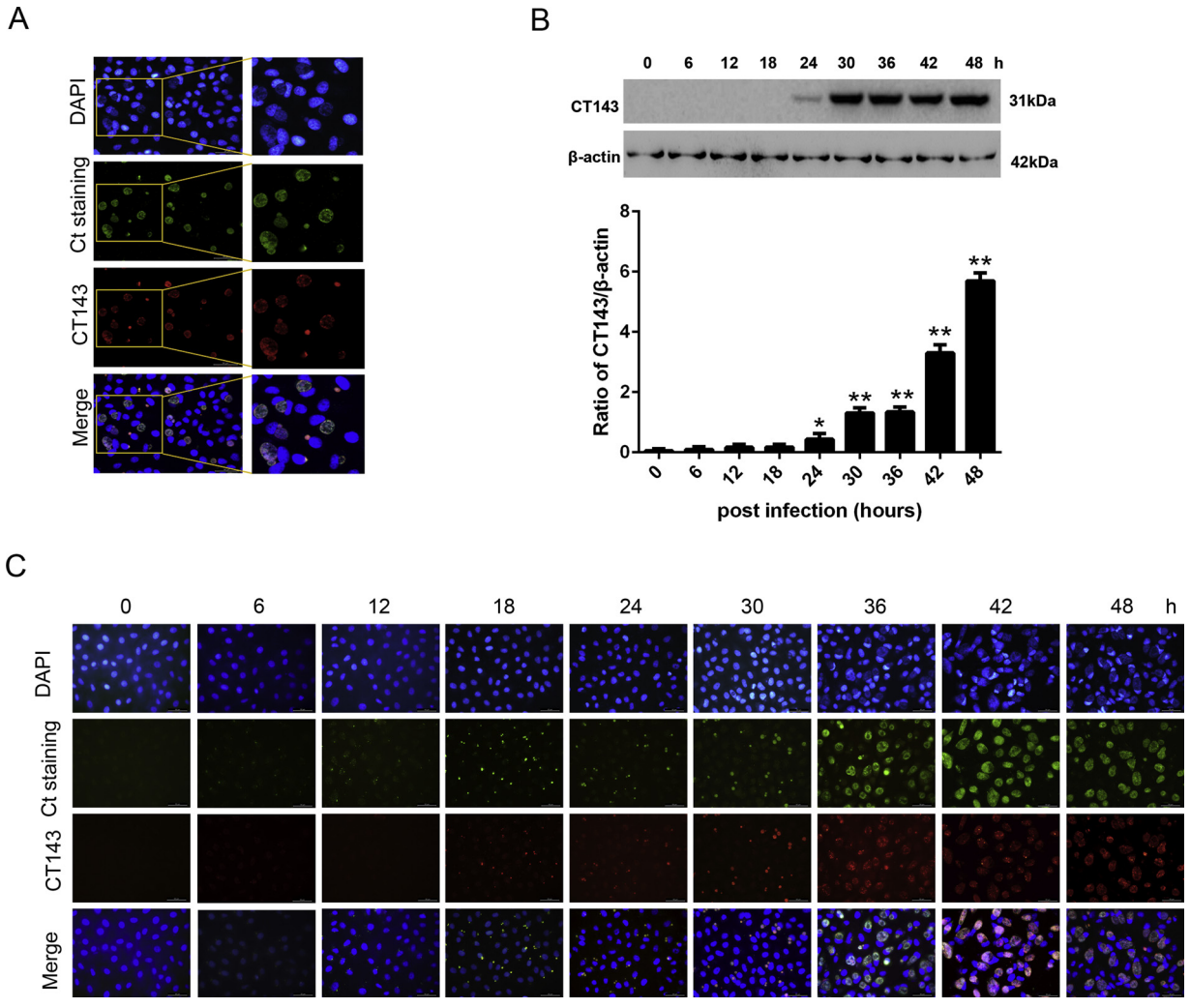

Fig. 2. CT143 was produced during the Ct developmental cycle (A) The location of the endogenous CT143 protein in Hela229 cells detected by IFA. (B) The temporal expression of the endogenous CT143 protein in Hela229 cells detected by Immunoblotting. (C) The temporal expression of the endogenous CT143 protein in Hela229 cells detected by IFA (10 × 40). $^{*}P < 0.05$, $^{**}P < 0.01$.

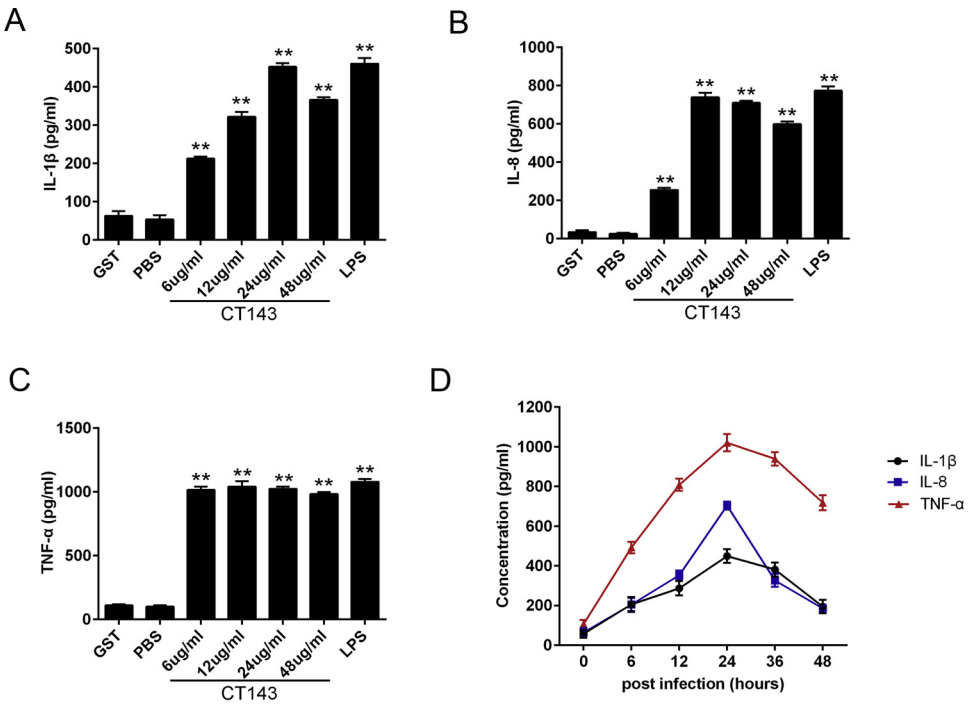

Fig. 3. CT143 protein induces proinflammatory cytokines secretion in THP-1 cells (A–C) The secretion of IL-1β, IL-8, and TNF-α by THP-1 cells stimulated by GST, PBS, LPS or CT143 protein (6, 12, 24 and 48 μg/ml). (D) The secretion of IL-1β, IL-8, and TNF-α by THP-1 cells stimulated by 24 μg/ml CT143 protein for 0, 6, 12, 24, 36 and 48 h. $^{*}P < 0.05$, $^{**}P < 0.01$.

As shown by Immunoblotting and ELISA assays, SB203580 treatment alone only caused slight alterations on p-p38/p38 ratio and cytokine secretion while CT143 protein stimulation significantly induced p-p38 protein levels and the secretion of IL-1β, IL-8 and TNF-α(Fig. 4B-E); however, SB203580 treatment alone only caused limited effect, and partially reversed the effect of CT143 protein (Fig. 4B-E). In addition, we also used si-RNA of p38 (si-p38) to further confirm the effect of the p38/MAPK pathways on CT143-induced cytokines secretion in THP-1 cells. As expected, si-p38 dramatically inhibited CT143-induced upregulation of p-p38 levels and cytokine secretion (Fig. 4F-I).

5. Discussion

Chlamydia trachomatis are obligate intracellular bacterias that replicate in the inclusion within host cells (Witkin et al., 2017). Ct immunodominant proteins play a key role in invading host cells and inducing inflammation in host cells (Wang et al., 2009; Zhou et al., 2013). Our previous study found that CT143 protein is a new immunogenic chlamydial-specific protein (Wang et al., 2010a). Therefore, we further investigated the localization and expression phase of CT143 protein and its function on the induction of proinflammatory cytokines.

In this study, the pGEX-6p-2/CT143 recombinant plasmid was transformed into *E. coli* BL21 competent cells, and the soluble CT143 fusion protein was successfully induced and expressed. After purification, four-week-old BALB/c female mice were immunized with the fusion protein, and specific antiserum against mouse CT143 protein was obtained. The titer of antibodies detected by indirect ELISA was over 1:8000, indicating that CT143 can stimulate an antigen-specific humoral immune response in mice. The antiserum can specifically recognize the CT143 protein without recognizing other proteins of the chlamydia. The localization and the time phase of CT143 protein in Hela229 cells were confirmed. Moreover, CT143 could stimulate the secretion of cytokines, including IL-1β, IL-8, and TNF-α, through the activation of the p38 signaling pathway in THP-1 cells.

A large number of animal experiments have proved that although *Chlamydia* infection can induce the production of a large number of antibodies, which can only provide weak protective immunity in the primary infection of *Chlamydia* (Brunham and Rey-Ladino, 2005; Hafner et al., 2008; Moore-Connors et al., 2015). However, passive immunization of animals with an antiserum containing anti-*Chlamydia* antibodies can enhance the immune response of Th1 cells and produce protective immunity against secondary infections of *Chlamydia* (Moore-Connors et al., 2015). It remains to be further studied whether anti-CT143 antibodies have protective effects on the body.

Ct has a unique biphasic reproductive cycle, consist of an infectious but non-reproductive elementary body (EB) period and a non-infectious but reproductive reticulate body (RB) period (Wang et al., 2010b). The invasion of host cells by EB is a critical step in *Chlamydial* infection and pathogenesis. After adhering to the surface of susceptible cells, EB enters the cells through phagocytosis (Byrne and Moulder, 1978; Murray and Ward, 1984), pinocytosis (Wyrick et al., 1989), or receptor-mediated cyto-granulation (Prain and Pearce, 1989) to form inclusion bodies. Then, EB differentiates into RB and rapidly proliferates in the host cells. According to the expressing time, the chlamydial genes can be divided into three types: early expression genes that begin to express at 1.5 to 8 h after infection; mid-phase expression genes that begin to express at 12 to 18 h after infection; late-expression genes, encoded some proteins involved RB to EB transformation, that begin to express at 24 h after infection (Beeckman et al., 2008). In the present study, CT143 began to express at 30 h after infection and reached a peak at 36 h and continued to be expressed for 48 h. It was also found that CT143 expression disappeared from 6 to 18 h after chlamydial infection. In that period the Chlamydia was in the RB period (Wang et al., 2009). At 18 h after infection, CT143 resumed expression and gradually increased as the infection time prolonged. Moreover, the CT143 protein was located in the inclusion body. Cunha et al. study also found that CT143 protein could be detected from 20 to 26 h post-infection and located with the inclusion (Cunha et al., 2017). Those findings suggesting that CT143 is an EB associated proein and secreted into the inclusion. Therefore, the CT143 protein may play an essential role in chlamydia invasion into host cells and cause inflammatory reactions.

C. trachomatis is an obligate intracellular bacterial pathogen. During intravacuolar growth, *C. trachomatis* must secrete factors into the inclusion membrane (Li et al., 2008a, 2011) or the host cell cytosol (Lei et al., 2011; Li et al., 2008b; G. Zhong, 2011) for interacting with and/or manipulating host cell signaling pathways (Greene et al., 2004; Su et al., 2004; Xiao et al., 2004). As a result of Chlamydia-host interactions, host cell genes encoding inflammatory cytokines and chemokines

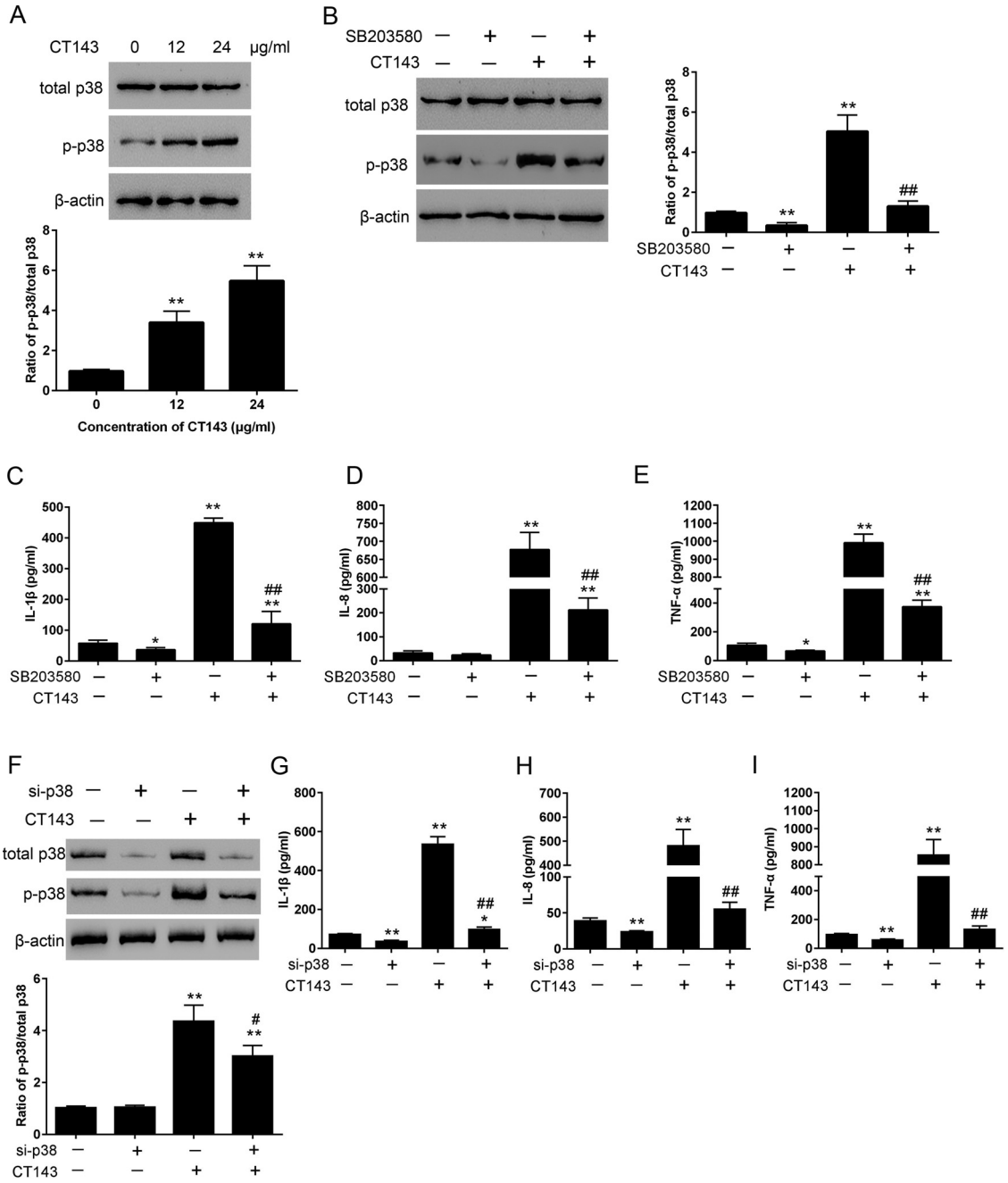

Fig. 4. The p38/MAPK pathways are involved in pro-inflammatory cytokine secretion induced by CT143 in THP-1 cells (A) THP-1 cells were stimulated with 0, 12 and 24 μg/ml CT143 protein and examined for protein levels of total p38 and p-p38. (B) THP-1 cells were co-treated with 24 μg/ml CT143 protein and 20 u M/l SB203580, a p38 signaling inhibitor, and examined for protein levels of total p38 and p-p38 and (C–E) the secretion of IL-1β, IL-8 and TNF-α by THP-1 cells. (F) After transfected with si-p38 for 24 h, THP-1 cells then treated with 24 μg/ml CT143 protein for 24 h. The expression of total p38 and p-p38 and (G–I) the secretion of IL-1β, IL-8 and TNF-α by THP-1 cells were determined. $*P < 0.05$, $**P < 0.01$, compared to control group; $\#\#P < 0.01$, compared to CT143 group.

are often activated. Previous studies have shown that a wide array of inflammatory cytokines and chemokines, including IL-1α, IL-6, IL-8, IL-18, TNF-α (Buchholz and Stephens, 2006; Gervassi et al., 2004; Maxion and Kelly, 2002), are produced in vitro and/or in vivo. These are believed to contribute to chlamydia-induced pathologies (Stephens, 2003). In the present study, CT143 protein stimulation significantly induced the secretion of cytokines, including IL-1β, IL-8, and TNF-α. In the meantime, the p38 signaling pathway was activated by CT143 protein, which could be partially blocked by pathway inhibitor, and si-p38. These findings further indicated that CT143 might induce the secretion of cytokines, and this process is mediated by the p38 signaling pathway.

In summary, CT143 protein is a new highly immunogenic Chlamydia-specific protein which can stimulate the secretion of cytokines through p38 signaling. Further study of the mechanism by which CT143 causes host cells to secrete these pro-inflammatory factors is needed. Hope to help reveal the pathological damage mechanism of *C. trachomatis* infection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.molimm.2018.12.007.

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