Celastrol attenuates human parvovirus B19 NS1-induced NLRP3 inflammasome activation in macrophages

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Abstract. Human parvovirus B19 (B19V) has been strongly associated with a variety of inflammatory disorders, such as rheumatoid arthritis (RA), inflammatory bowel disease and systemic lupus erythematosus. Non-structural protein 1 (NS1) of B19V has been demonstrated to play essential roles in the pathological processes of B19V infection due to its regulatory properties on inflammatory cytokines. Celastrol, a quinone methide isolated from *Tripterygium wilfordii*, has displayed substantial potential in treating inflammatory diseases, such as psoriasis and RA. However, little is known about the effects of celastrol on B19V NS1-induced inflammation. Therefore, cell viability assay, migration assay, phagocytosis analysis, zymography assay, ELISA and immunoblotting were conducted to verify the influences of celastrol on macrophages. The present study reported the attenuating effects of celastrol on B19V NS1-induced inflammatory responses in macrophages derived from human acute monocytic leukemia cell lines, U937 and THP-1. Although the migration was not significantly decreased by celastrol in both U937 and THP-1 macrophages, significantly decreased viability, migration and phagocytosis were detected in both B19V NS1-activated U937 and THP-1 macrophages in the presence of celastrol. Additionally, celastrol significantly decreased MMP-9 activity and the levels of inflammatory cytokines, including IL-6, TNF-α and IL-1β, in B19V NS1-activated U937 and THP-1 cells. Notably, significantly decreased levels of NLR family pyrin domain-containing 3, apoptosis-associated speck-like, caspase-1 and IL-18 proteins were observed in both B19V NS1-activated U937 and THP-1 cells in the presence of celastrol, indicating the involvement of the inflammasome pathway. To the best of our knowledge, the present study is the first to report on the attenuating effects of celastrol on B19V NS1-induced inflammatory responses in macrophages, suggesting a therapeutic role for celastrol in B19V NS1-related inflammatory diseases.

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Introduction

Human parvovirus B19 (B19V), discovered in 1975, is known as a non-enveloped single-stranded DNA virus that spreads through the respiratory tract (1,2). Epidemiological surveys of England and Japan have shown that the prevalence of individuals with antibodies against B19V is 2-15% for children aged <5 years, 15-60% for adolescents aged 6-19 years, 30-60% for adults aged 16-40 years and >85% for the elderly population aged >71 years (3,4). B19V infection has been associated with numerous pathological conditions, such as fifth disease, persistent anemia, myocarditis, hydrops fetalis, arthropathy and autoimmune disorders (2,5-9). The genome of B19V encodes two viral structural proteins [viral envelope protein (VP) 1 and VP2] and three non-structural proteins [non-structural protein 1 (NS1), 7.5-kDa and 11-kDa] that are required for the regulation of viral capsid packaging and DNA replication (10). VP2 protein comprises 95% of the B19V capsid. B19V VP1 is identical to VP2 except for an additional 227 amino acids at the N-terminal, which is known as the VP1 unique region and contributes to B19V infection (11-14). Although the precise mechanism of B19V infection is still unclear, the receptor-binding domain and phospholipase

A2 activity within B19V VP1u play important roles in viral tropism, uptake and subcellular trafficking (11-14).

B19V NS1 is known as a transcriptional activator for DNA replication by binding to the P6 promoter (15). Evidence has also indicated that B19V NS1 can bind TATA box and GC-rich elements, and interact with various DNA-binding proteins, such as the activating transcription factor/cAMP response element binding protein, NF-κB/c-Rel and GC-box binding factors, such as specificity protein 1 (16). In recent decades, B19V NS1 has been demonstrated to induce various cytokines, such as IL-2, IL-6, IL-9, IL-17A, IL-21, IL-22, interferon γ and TNF-α (17,18). Additionally, B19V NS1 has been shown to cause cytotoxic activity by inducing the caspase-3-dependent apoptotic pathway and various inflammatory cytokines, such as IL-1β, IL-6 and IL-18 (19,20), in both parvovirus permissive and nonpermissive cells (21,22), such as nonpermissive THP-1 and nonpermissive U937 monocytic cell lines (19,23). These findings suggested that both B19V NS1 and macrophages play essential roles in inflammatory processes.

Celastrol is a quinone methide from the roots of *Tripterygium wilfordii* and has been demonstrated to exert a protective effect against a variety of disorders, such as psoriasis and RA (24). Substantial evidence has indicated the suppressive effects of celastrol on hepatocellular carcinoma through inhibition of CXCR4-related signaling (25). The beneficial effects of celastrol against neurodegenerative diseases and cardiovascular disorders have also been reported (26,27). Additionally, celastrol is known to inhibit replication of the hepatitis C virus through the JNK MAPK/nuclear factor erythroid 2-related factor 2 pathway (28). Increasing attention has focused on the therapeutic properties of celastrol in inflammatory diseases, such as allergy, rheumatoid arthritis, inflammatory bowel diseases, diabetes and osteoarthritis (24). However, very little is known about the effects and underlying mechanisms of celastrol on B19V NS1-related inflammatory disorders. The present study investigated the ameliorating effects of celastrol on B19V NS1-induced inflammatory responses in U937 and THP-1 human acute monocytic leukemia cell-derived macrophages, as well as its underlying signaling.

Materials and methods

Chemicals and cell culture. All other chemicals used in this study for which no manufacturer was identified were purchased from MilliporeSigma. Celastrol was purchased from ChemFaces. A total of two human acute monocytic leukemia cell lines, U937 [Bioresource Collection and Research Centre (BCRC) 60435] and THP-1 (BCRC 60430), were purchased from the BCRC (Food Industry Research and Development Institute). The cell lines were subjected to short tandem repeat profiling through the National Cheng Kung University Center for Genomic Medicine (Tainan, Taiwan) to confirm their authenticity (report nos. 23070813 and 23070814). The cells were cultured in complete RPMI 1640 medium (Thermo Fisher Scientific, Inc.) supplemented with 10% (v/v) FBS (Gibco; Thermo Fisher Scientific, Inc.) and 100 U/ml penicillin at 37˚C in a humidified atmosphere of 95% air and 5% CO_2. To differentiate monocytes into adherent macrophages, the cells were seeded at a density of $1x10^5$ cells/well in 24-well plates and incubated at 37˚C for 2 days in the presence of 100 nM phorbol 12-myristate 13-acetate (MilliporeSigma). The cells were then washed and incubated in normal growth medium for another 24 h prior to treatment with purified B19V NS1 (1 μg/ml) as described in our previous study (29).

Cell viability. To assess the survival of cells, MTT assay was performed. A total of $2x10^5$ cells were cultured overnight at 37˚C in each well of a 24-well plate. After treatment with different concentrations of celastrol (0, 0.5, 1 and 2 μM) or combinational treatment of 1 μg/ml B19V NS1 and celastrol (0, 0.5, 1 and 2 μM) for another 24 h, the culture medium was removed and MTT reagent (0.5 mg/ml) was added and incubated for another 4 h. To dissolve the formazan crystals, 0.3 ml dimethyl sulfoxide was added to each well of the plate and the absorbance of the medium was detected at 570 nm with a microplate reader (SpectraMax M5®; Molecular Devices, LLC).

Migration assay. Millicell Hanging Cell Culture inserts (pore size, 8 μm; MilliporeSigma) were used to detect the effect of B19V NS1 on cell motility. Briefly, the upper chamber containing cells in serum-free RPMI 1640 medium ($5x10^5$ cells/well) treated with different concentrations of celastrol (0, 0.5, 1 and 2 μM) or combinational treatment of 1 μg/ml B19V NS1 and celastrol (0, 0.5, 1 and 2 μM) was merged with the bottom chamber containing standard medium (RPMI 1640 with 10% FBS) and then incubated at 37˚C for 24 h. The migrating cells were fixed with neutral-buffered formalin (10%) at 25˚C for 2 h and subsequently stained with 0.05% Giemsa stain at 25˚C for 2 h. A total of 10 random fields from each experiment were observed for counting the number of migrated cells under a light microscope (Zeiss Axioskop 2; Zeiss AG) at a magnification of x200 per filter.

Assessment of phagocytosis. Latex beads were used to assess the phagocytosis of macrophages. A total of $2x10^5$ U937 or THP-1 cells were cultured overnight in each well of a 16-well Lab-Tek®II Chamber Slide™ (Thermo Fisher Scientific, Inc.) and then stimulated with 1 μg/ml B19V NS1 recombinant proteins or different concentrations of celastrol (0, 0.5, 1 and 2 μM) for another 16 h at 37˚C before incubation with FITC-labeled latex beads (MilliporeSigma) for 24 h at 37˚C in a cell culture incubator. A total of 100 macrophages in five random fields were counted under a light microscope (Zeiss Axioskop 2; Zeiss AG) at a magnification of x200. The phagocytic index was the number of phagocytosed particles divided by the total number of macrophages and was expressed as a percentage. The phagocytic ratio was the number of cells that swallowed beads divided by the total number of macrophages and was expressed as a percentage.

Zymography assay. U937 and THP-1 cells were stimulated with 1 μg/ml B19V NS1 recombinant proteins or different concentrations of celastrol (0, 0.5, 1 and 2 μM) for 24 h at 37˚C and the activity of MMP-9 in the medium was measured by gelatin-zymography assays. A total of 10 μl culture medium from each treatment was separated by SDS-PAGE on 8% gels containing 0.1% gelatin. After soaking in 2.5% Triton X-100 to remove the SDS at 37˚C for 30 min, the gels were then washed in reaction buffer [40 mM Tris-HCl (pH 8.0),

10 mM $CaCl_2$, 0.02% NaN_3] at 37˚C for another 30 min. The gelatinolytic activity was visualized by staining the gels with 0.5% Coomassie brilliant blue R-250 after being de-stained with a methanol-acetic acid solution. Relative MMP levels were semi-quantified using a gel documentation and analysis system (AlphaImager HP 2200; ProteinSimple, Inc.).

ELISA. The measurement of cytokine levels in cell culture media were performed using ELISA kits for human IL-1β (cat. no. 88-7261-88), IL-6 (cat. no. 88-7066-88) and TNF-α (cat. no. 88-7346-88) according to the manufacturer's instructions (Invitrogen; Thermo Fisher Scientific, Inc.).

Immunoblotting. The cell pellets were collected by centrifugation at 800 x g for 5 min at 4˚C and suspended in 600 μl PRO-PREP™ buffer (iNtRON Biotechnology, Inc.) for lysis. The supernatant was then obtained by centrifugation at 16,600 x g for 5 min at 4˚C. The concentrations of protein were measured using a modified Bradford's assay with a spectrophotometer (Hitachi U 3000; HITACHI) at 595 nm, with BSA (Merck KGaA) as the standard. Protein lysates (25 μg/lane) were separated by SDS-PAGE using 10% gels and were electrophoretically transferred to a polyvinylidene fluoride membrane (Immobilon-E, 0.45 μM; MilliporeSigma). The membrane was blocked with 5% non-fat dry milk in PBS for 2 h at 25˚C with gentle agitation, and then incubated with antibodies against NLR family pyrin domain-containing 3 (NLRP3; 1:2,000; cat. no. A12694; ABclonal), apoptosis-associated speck-like protein (ASC; 1:2,000; cat. no. A1170; ABclonal), caspase-1 (1:2,000; cat. no. A0964; ABclonal Biotech Co., Ltd.), IL-18 (1:1,000; cat. no. 061115; MilliporeSigma) and GAPDH (1:1,000; cat. no. NB300221; Novus Biologicals, LLC) for 3 h at 25˚C. Subsequently, secondary antibodies conjugated with horseradish peroxidase (HRP) (1:5,000; cat. no. sc-2005; Santa Cruz Biotechnology, Inc.) were added and the membranes were incubated for 1 h. Finally, antigen-antibody complexes were visualized using an Immobilon Western HRP Chemiluminescent Substrate kit (MilliporeSigma) and semi-quantified by densitometry (GE ImageQuant TL 8.1; Cytiva).

Statistical analysis. For *in vitro* assays, GraphPad Prism 5 software (GraphPad Software; Dotmatics) was used to calculate the significant differences among groups by one-way analysis of variance followed by Tukey's test. All data are presented as the mean ± standard error of mean of at least three independent experiments. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of celastrol on B19V NS1-induced human macrophage functions. To evaluate the cytotoxicity of celastrol on human acute monocytic leukemia U937 and THP-1 derived macrophages, the viability of U937 and THP-1 macrophages treated with different concentrations of celastrol was determined using an MTT assay. Significantly decreased viability of both U937 and THP-1 macrophages was detected in the presence of celastrol in a dose-dependent manner with an IC_{50} value of 1.77 and 1.39, respectively (Fig. 1A and B). Similar results were observed in both U937 and THP-1 macrophages in the presence of celastrol and 1 μg/ml B19V NS1 with an IC_{50} value of 1.65 and 1.21, respectively, (Fig. 1C and D). In the presence of 1 μg/ml B19V NS1 with no celastrol, no difference in THP-1 macrophage viability was observed, whereas increased viability of U937 macrophages was detected in the presence of 1 μg/ml B19V NS1, thus indicating no significant cytotoxicity of 1 μg/ml B19V NS1 in both cells (Fig. 1C and D).

To further evaluate the effects of celastrol on B19V NS1-induced macrophage functions, cell migration and phagocytosis assays were performed. No significant cell migration was observed in both U937 and THP-1 macrophages in the presence of different concentrations of celastrol alone (Fig. 2A and B). Significant inhibitory effects of celastrol on cell migration were detected in both U937 and THP-1 macrophages treated with 1 μg/ml B19V NS1 (Fig. 2C and D). Additionally, celastrol attenuated the phagocytosis index and ratio in U937 macrophages in a dose-dependent manner but had no significant influence on the phagocytosis index and ratio in THP-1 macrophages (Fig. 3A-D). Notably, celastrol significantly decreased the phagocytosis index and ratio in both U937 and THP-1 macrophages that were activated by 1 μg/ml B19V NS1 (Fig. 3E-H). The representative images of Figs. 2 and 3 are shown in Figs. S1 and S2.

Effects of celastrol on B19V NS1-induced inflammatory responses in human macrophages. To evaluate the effects of celastrol on inflammatory responses in B19V NS1-activated macrophages, MMP-9 activity and inflammatory cytokine levels were measured. Significantly decreased MMP-9 activity was detected only in U937 and THP-1 macrophages treated with 2 μM celastrol, but not in those treated with lower concentrations of celastrol (Fig. 4A and B). Significantly decreased MMP-9 activity was observed in B19V NS1-activated U937 and THP-1 macrophages treated with celastrol in a dose-dependent manner (Fig. 4C and D). High cytotoxicity on both U937 and THP-1 macrophages was exhibited with 2 μM celastrol; therefore, the subsequent experiments in the present study only adopted 0.5 and 1 μM celastrol. No statistical differences in IL-6, TNF-α and IL-1β levels were observed in the medium of U937 macrophages treated with different concentrations of celastrol (Fig. 5A-C). Significantly decreased IL-6 and TNF-α levels were detected in the medium of B19V NS1-activated U937 macrophages in a dose-dependent manner (Fig. 5D and E). Significantly decreased IL-1β level was detected in the medium of B19V NS1-activated U937 macrophages but it was not dose-dependent (Fig. 5F). Significantly increased IL-6 and TNF-α levels, but not IL-1β levels, were observed in the medium of THP-1 macrophages treated with different concentrations of celastrol (Fig. 6A-C). Conversely, significantly decreased IL-6, TNF-α and IL-1β levels were detected in the medium of B19V NS1-activated THP-1 macrophages, but it was not dose-dependent (Fig. 6D-F).

Effects of celastrol on B19V NS1-induced inflammasome signaling in human macrophages. To evaluate the effects of celastrol on B19V NS1-induced inflammasome signaling, the expression levels of NLRP3, ASC, caspase-1 and IL-18

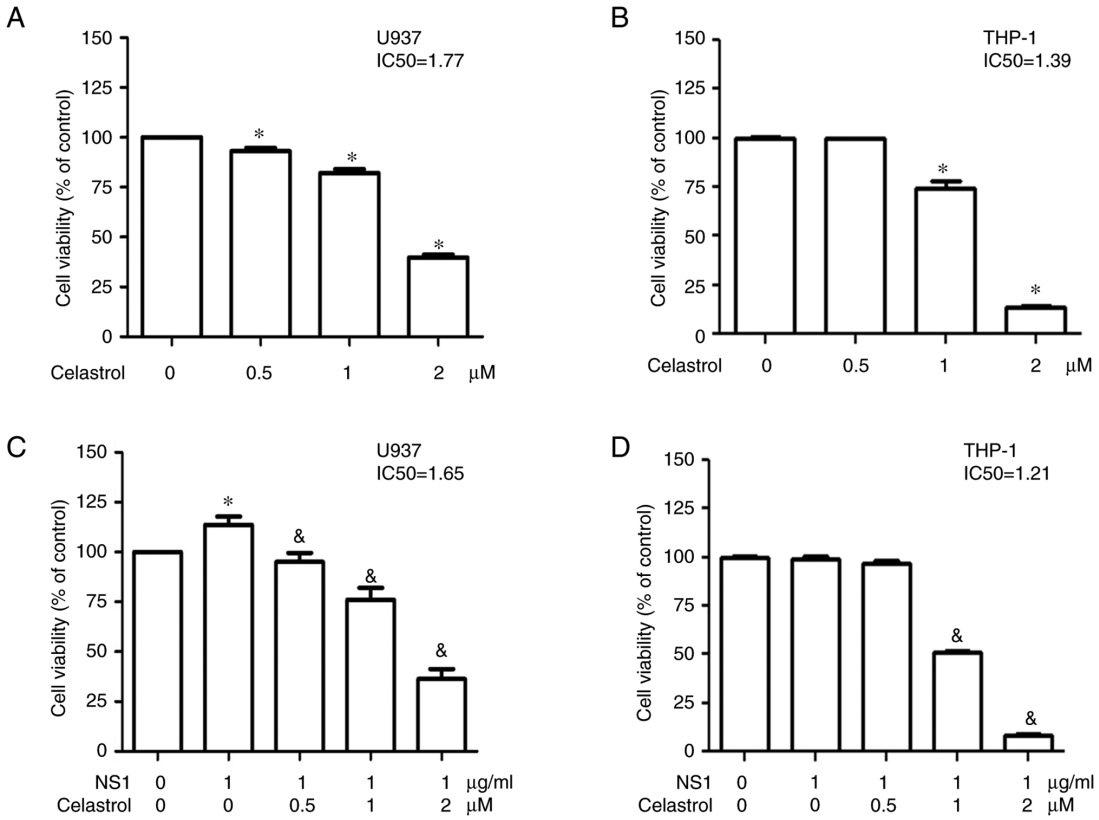

Figure 1. Effects of celastrol and B19V NS1 on survival of macrophages. Cell viability of (A) U937 and (B) THP-1 macrophages in the presence of different concentrations of celastrol. Cell viability of (C) U937 and (D) THP-1 macrophages in the presence of 1 μg/ml B19V NS1 and different concentrations of celastrol. Similar results were obtained in three repeated experiments. *P<0.05 vs. control (0 μM); &P<0. vs. 1 μg/ml B19V NS1. B19V, human parvovirus B19; NS1, non-structural protein 1.

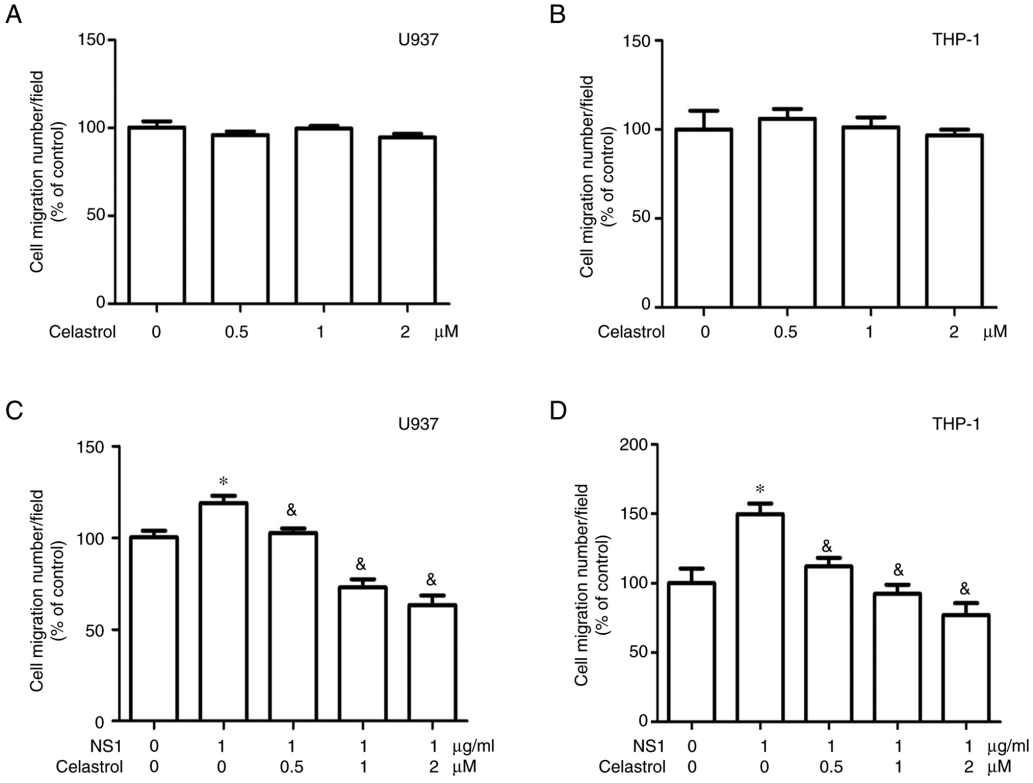

Figure 2. Effects of celastrol and B19V NS1 on the migration of macrophages. Cell migration of (A) U937 and (B) THP-1 macrophages in the presence of celastrol. Cell migration of (C) U937 and (D) THP-1 macrophages in the presence of 1 μg/ml B19V NS1 and different concentrations of celastrol. Similar results were obtained in three repeated experiments. *P<0.05 vs. control (0 μM); &P<0.05 vs. 1 μg/ml B19V NS1. B19V, human parvovirus B19; NS1, non-structural protein 1.

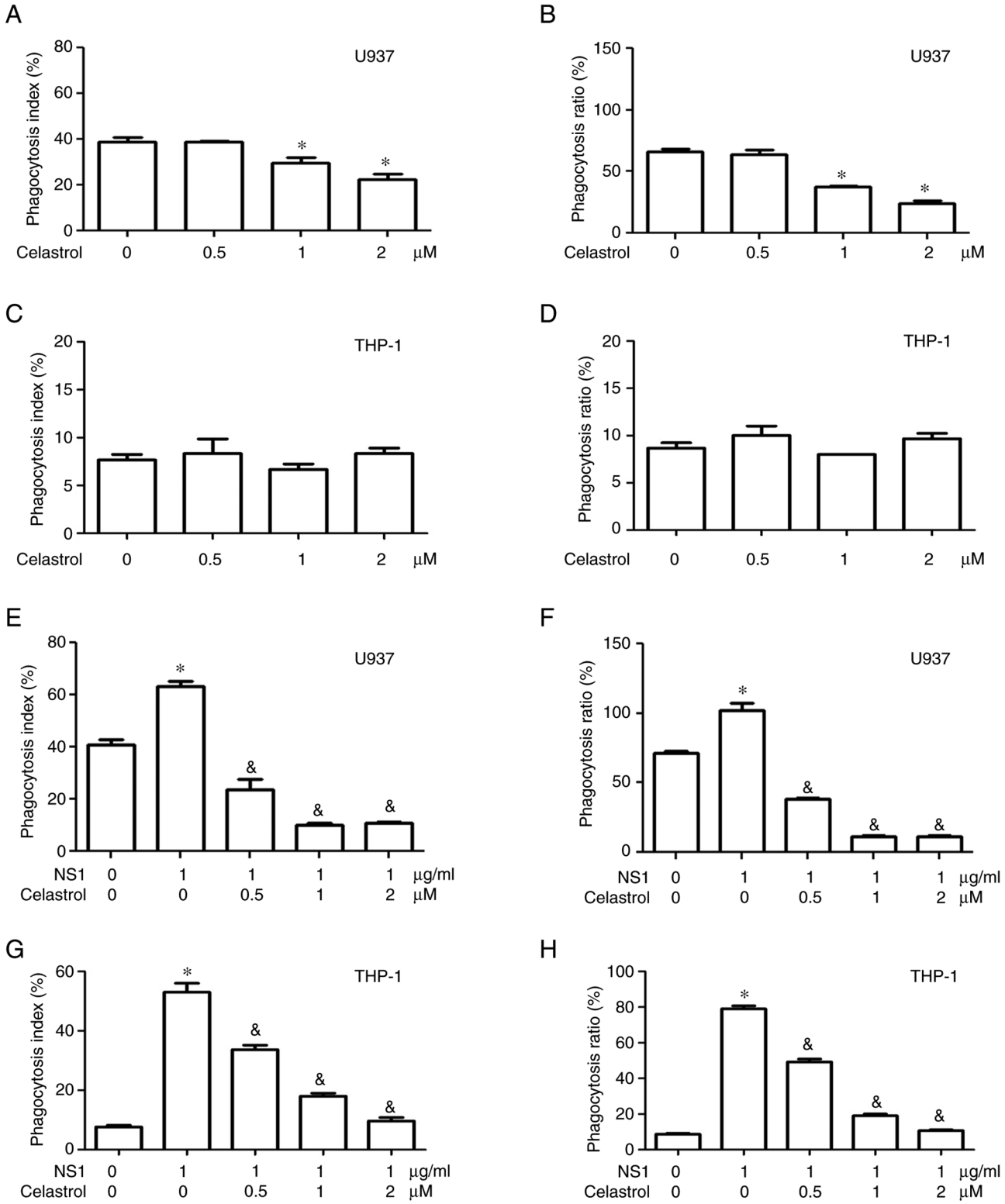

Figure 3. Effects of celastrol and B19V NS1 on phagocytosis of macrophages. (A) Phagocytosis index and (B) phagocytosis ratio of U937 macrophages in the presence of celastrol. (C) Phagocytosis index and (D) phagocytosis ratio of THP-1 macrophages in the presence of celastrol. (E) Phagocytosis index and (F) phagocytosis ratio of U937 macrophages in the presence of 1 μg/ml B19V NS1 and different concentrations of celastrol. (G) Phagocytosis index and (H) phagocytosis ratio of THP-1 macrophages in the presence of 1 μg/ml B19V NS1 and different concentrations of celastrol. Similar results were obtained in three repeated experiments. *P<0.05 vs. control (0 μM); $^{\&}$P<0.05 vs. 1 μg/ml B19V NS1. B19V, human parvovirus B19; NS1, non-structural protein 1.

proteins were detected. Significantly increased amounts of NLRP3, ASC, caspase-1 and IL-18 proteins were observed in both U937 and THP-1 macrophages treated with 1 μg/ml B19V NS1 as compared with those in the untreated control group (Fig. 7). Notably, celastrol significantly decreased the expression levels of NLRP3, ASC, caspase-1 and IL-18 proteins in both B19V NS1-activated U937 and THP-1 macrophages in a dose-dependent manner (Fig. 7).

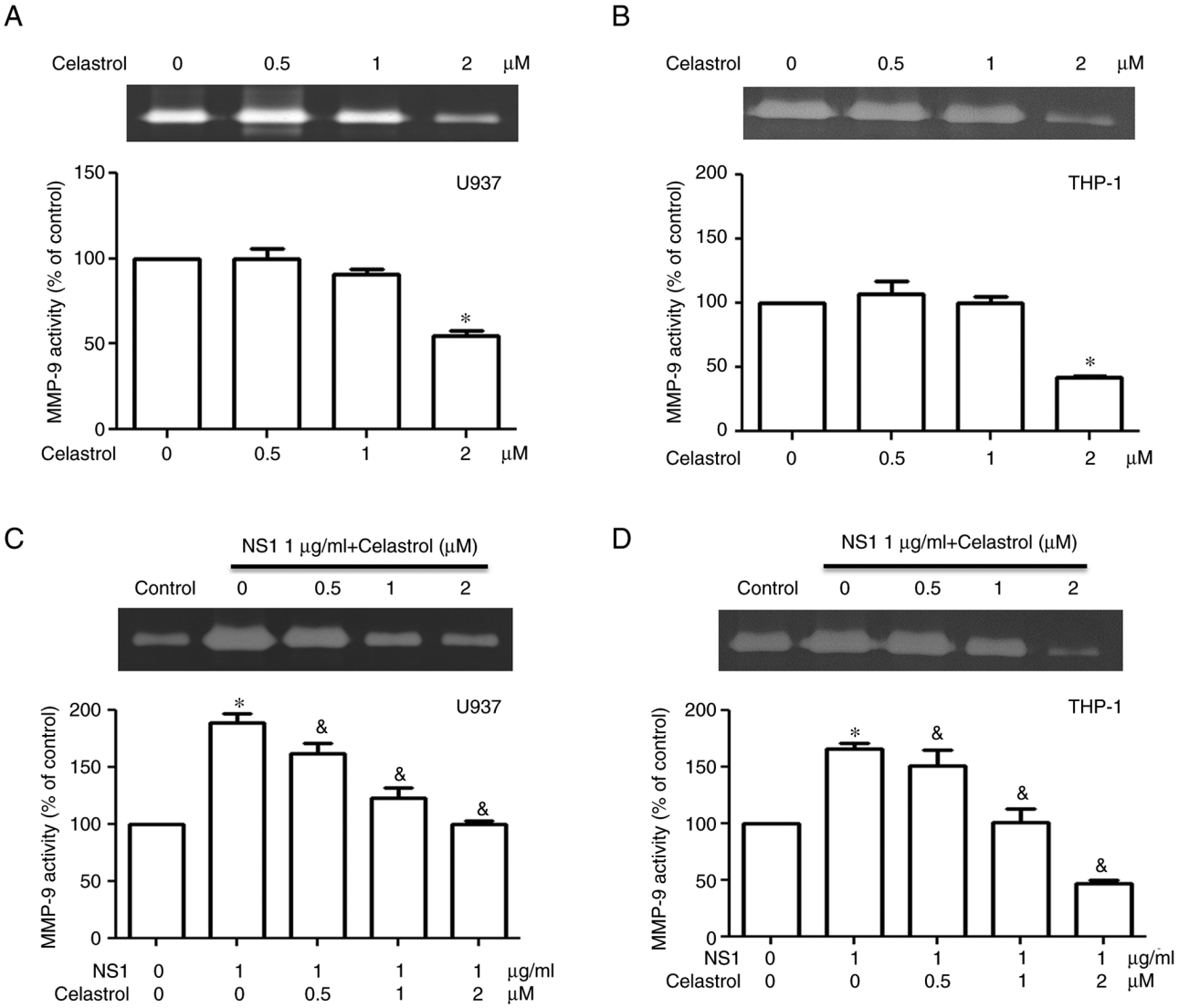

Figure 4. Effects of celastrol and B19V NS1 on MMP-9 activity in macrophages. MMP-9 activity of (A) U937 and (B) THP-1 macrophages in the presence of different concentrations of celastrol. MMP-9 activity of (C) U937 and (D) THP-1 macrophages in the presence of 1 μg/ml B19V NS1 and different concentrations of celastrol. Similar results were obtained in three repeated experiments. *P<0.05 vs. control (0 μM); &P<0.05 vs. 1 μg/ml B19V NS1. B19V, human parvovirus B19; NS1, non-structural protein 1.

Discussion

Although B19V NS1 is known to evade host innate immunity by inhibiting the exogenous type I IFN signaling at interferon-sensitive response element, interferon-stimulated gene, and signal transducer and activator of transcription 1 (STAT1) (30), mounting evidence has demonstrated the pivotal roles of B19V NS1 in various diseases, especially inflammatory and autoimmune disorders (7,18). In *in vivo* studies, B19V NS1 transgenic mice have been demonstrated to exhibit susceptibility to polyarthritis (31) and are considered a model for non-immune hydrops fetalis (32). Additionally, B19V NS1 transgenic mice develop vascular damage in the heart and have been recognized as a mouse model for myocarditis associated with B19V infection (33).

Previous studies have reported that B19V NS1 can induce various inflammatory cytokines in monocytes (17,18,23) with B19V NS1 associated with elevated Th-17-related cytokines, such as IL-17, IL-6, IL-1β and TNF-α in patients with systemic lupus erythematosus presenting with dilated cardiomyopathy (34). Additionally, B19V NS1 has been reported to elevate IL-1β and IL-18 levels in adult-onset Still's disease through activating NLRP3 inflammasome signaling (29). These findings indicated that B19V NS1 can induce inflammatory cytokines and inflammasome signaling in monocytes. Accordingly, the present study reported that B19V NS1 significantly activated human macrophages by increasing migration, phagocytosis, inflammatory cytokines and inflammasome signaling. Notably, celastrol significantly ameliorated the B19V NS1-induced inflammatory responses in human U937 and THP-1 macrophages, including decreased cell migration, MMP-9 activity, phagocytosis, inflammatory cytokines and inflammasome signaling; therefore, this suggested the therapeutic potential of celastrol in B19V NS1-related inflammatory disorders.

Celastrol, a compound from traditional Chinese herbs, has long been used in the treatment of a number of diseases due to its significant anti-inflammatory and antioxidant

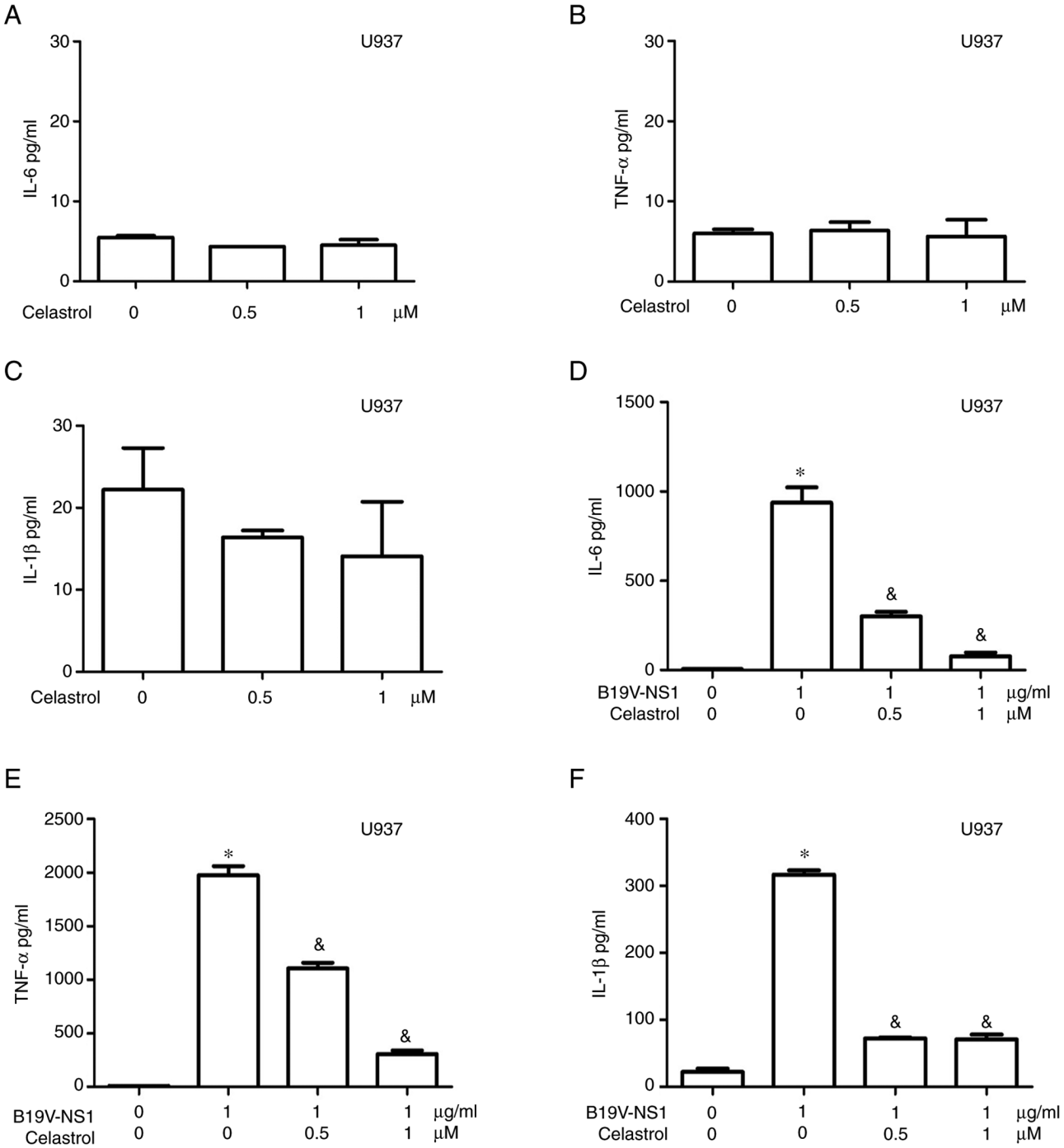

Figure 5. Effects of celastrol on inflammatory cytokine levels in U937 macrophages. The concentrations of (A) IL-6, (B) TNF-α and (C) IL-1β in the medium of U937 macrophages treated with celastrol. The concentrations of (D) IL-6, (E) TNF-α and (F) IL-1β in the medium of U937 macrophages in the presence of 1 μg/ml B19V NS1 and different concentrations of celastrol. Similar results were obtained in three repeated experiments. *P<0.05 vs. controls (0 μM); $^{\&}$P<0.05 vs. 1 μg/ml B19V NS1. B19V, human parvovirus B19; NS1, non-structural protein 1.

properties (24). Celastrol is recognized as a therapeutic agent for numerous pathological diseases, including arthritis, asthma and autoimmune disorders, through inhibition of NF-κB (35). A previous study reported that celastrol inhibits the induction of inducible nitric oxide synthase by reducing the binding activity of NF-κB in lipopolysaccharide-treated macrophages (36,37). Another study also indicated that celastrol can attenuate the migration and invasion of MCF-7 cells by downregulating NF-κB-mediated MMP-9 expression (38). Apart from NF-κB signaling, various signaling pathways, such as MAPK signaling, JAK/STAT signaling and receptor activator of NF-κB/osteoprotegerin signaling were recently reported as specific targets for celastrol (39). Accordingly, a new molecular target for celastrol was reported in a recent study where celastrol ameliorated type 2 diabetes by blocking carbohydrate response element-binding protein and inhibiting its nuclear translocation (40). These findings provide evidence that support the idea that the anti-inflammatory effects of celastrol in B19V NS1-activated macrophages are due to the diverse

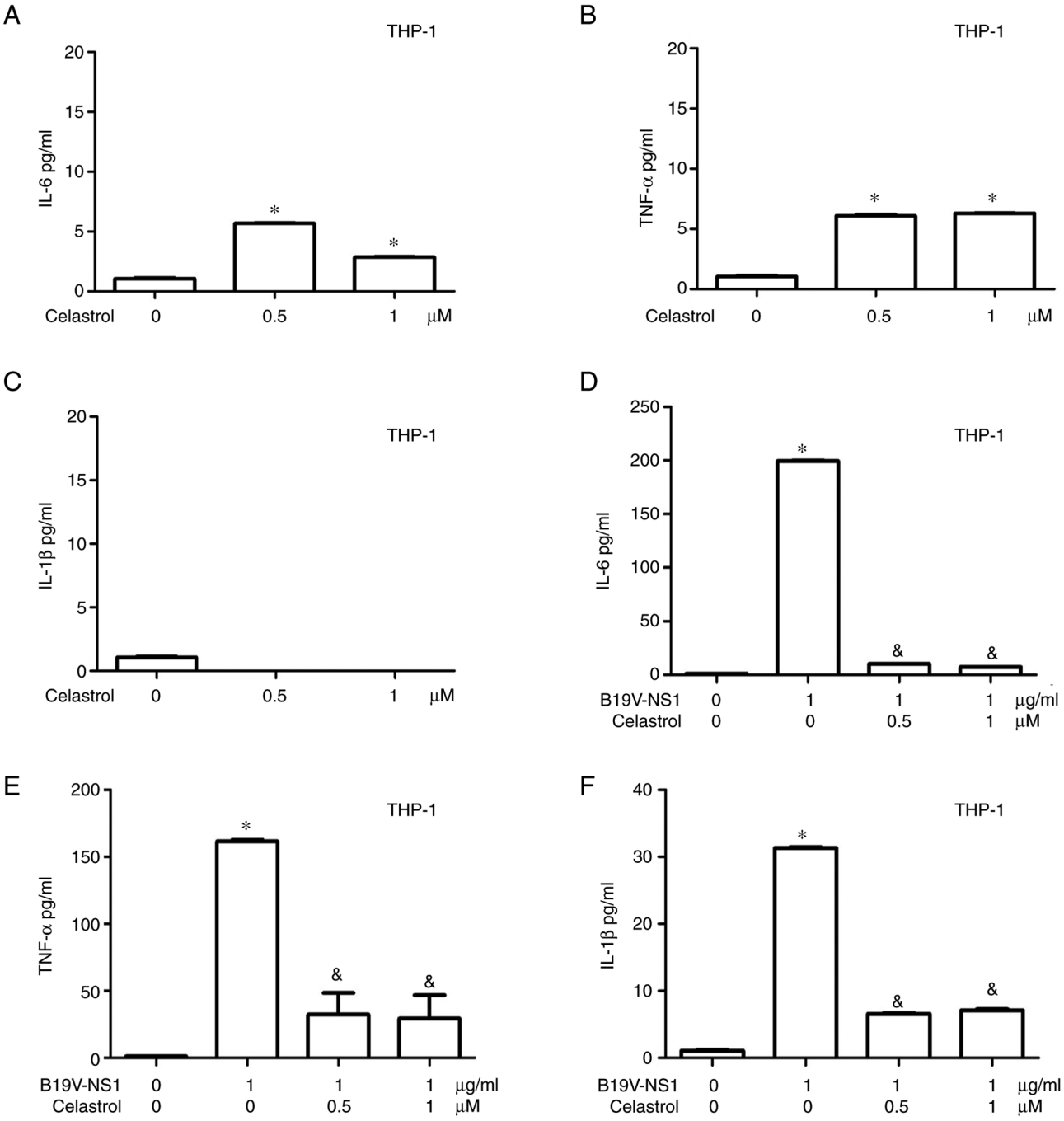

Figure 6. Effects of celastrol on inflammatory cytokine levels in THP-1 macrophages. The concentrations of (A) IL-6, (B) TNF-α and (C) IL-1β in the medium of THP-1 macrophages treated with celastrol and the concentrations of (D) IL-6, (E) TNF-α and (F) IL-1β in the medium of THP-1 macrophages in the presence of 1 μg/ml B19V NS1 and different concentrations of celastrol. Similar results were obtained in three repeated experiments. *P<0.05 vs. controls (0 μM); $^{\&}$P<0.05 vs. 1 μg/ml B19V NS1. B19V, human parvovirus B19; NS1, non-structural protein 1.

influences of celastrol through multiple pathways. However, further investigations are merited to verify the precise mechanisms of celastrol in ameliorating B19V NS1-induced inflammation.

There were some limitations in the present study. Firstly, no significant migration in both U937 and THP-1 macrophages was detected in the presence of celastrol alone. However, significantly decreased migration in B19 NS1-treated U937 and THP-1 macrophages was observed in the presence of celastrol. Although this finding reveals that celastrol significantly attenuates the cell migration of both U937 and THP-1 macrophages activated by 1 μg/ml B19V NS1, further investigations are required to verify the precise mechanisms of action of celastrol in B19 NS1-induced signaling in U937 and THP-1 macrophages. Additionally, *in vivo* studies involving celastrol require further investigation. To further explore the possibility of the clinical applications of celastrol, it is important to understand the toxicity of celastrol, which can be achieved through animal testing, such as use of collagen-induced arthritis mice. A previous study has reported that oral administration of 2.5 μg/g/day celastrol is non-toxic and the lowest effective

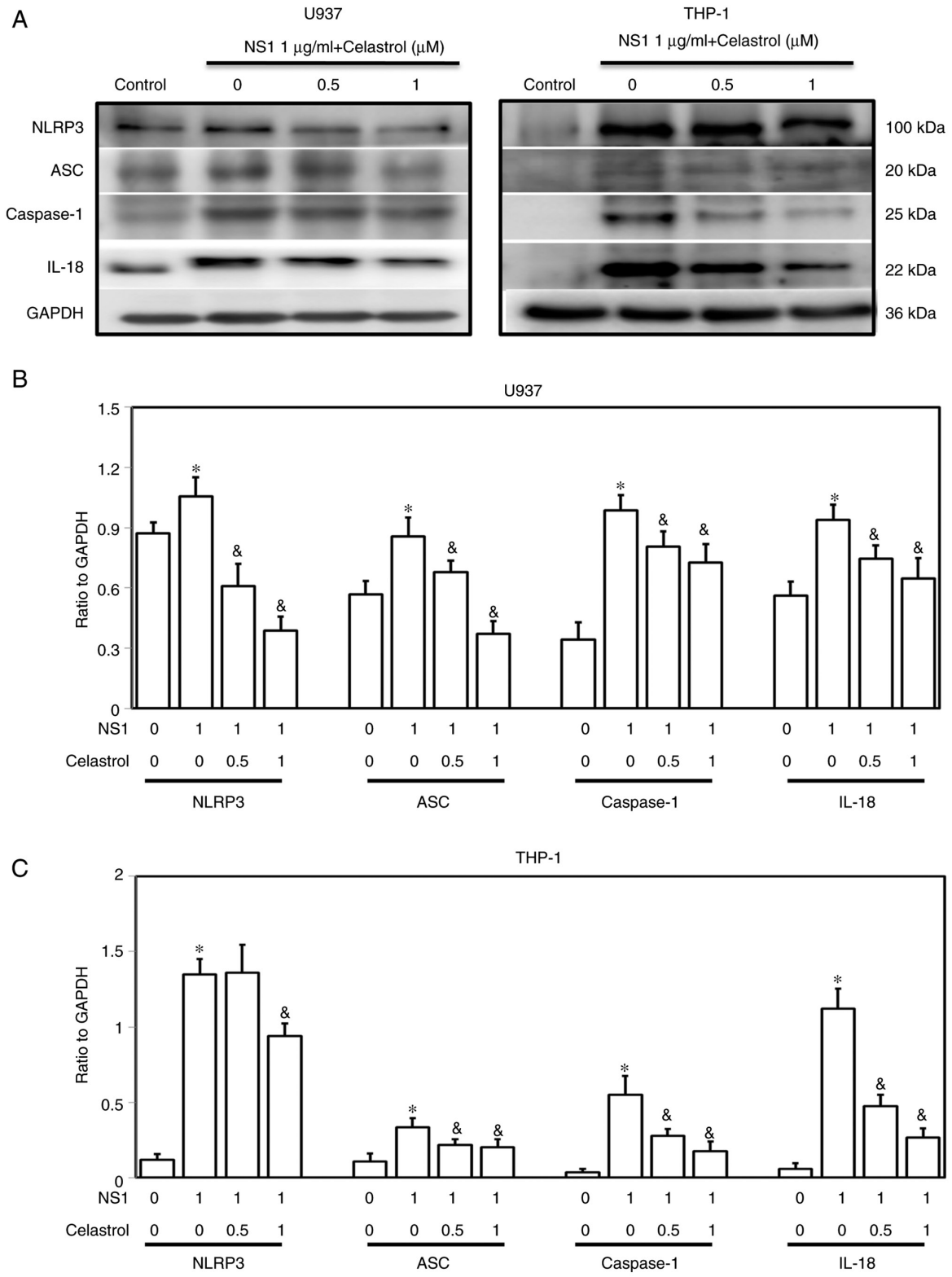

Figure 7. Effects of celastrol on inflammasome signaling in macrophages. (A) Expression levels of NLRP3, ASC, caspase-1 and IL-18 proteins in U937 and THP-1 macrophages. Bar charts represent the ratios relative to the amount of GAPDH in (B) U937 and (C) THP-1 macrophages. Similar results were obtained in three repeated experiments. *P<0.05 vs. controls (0 μM); &P<0.05 vs. 1 μg/ml B19V NS1. B19V NS1. B19V, human parvovirus B19; NS1, non-structural protein 1; NLRP3, NLR family pyrin domain-containing 3; ASC, apoptosis-associated speck-like protein.

dosage of celastrol for rats with adjuvant-induced arthritis. Conversely, a dose of 7.5 μg/g/day can induce severe toxicity, such as thymic and liver lesions (41). Another report also indicated that administration of celastrol by intradermal injection significantly attenuated paw swelling, arthritic scores, pro-inflammatory cytokines and oxidative stresses in rats with collagen-induced arthritis (42). These findings provide insight into the effective and safe dosage of celastrol for future animal experiments on B19 infection while avoiding possible complications or adverse events.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CLH and DYC conceived, reviewed and edited the manuscript. CCT and JWL performed experiments and analysis of data. TCH and BST were involved in the study conception and design, drafting and revising of the manuscript and analysis of data. TCH and BST confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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