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Inhibition of NLRP3 inflammasome-mediated pyroptosis in macrophage by cycloastragenol contributes to amelioration of imiquimod-induced psoriasis-like skin inflammation in mice

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ABSTRACT

Psoriasis is a common chronic inflammatory skin disease, and the infiltrated macrophages in psoriatic skin lesions play a key role in the progression of this uncontrolled cutaneous inflammation. However, the current therapeutic strategies for patients with psoriasis are not satisfactory. Here, we report that cycloastragenol (CAG), a natural active small compound isolated from *Astragalus membranaceus,* significantly ameliorated imiquimod (IMQ)-induced psoriasiform dermatitis in mice by targeting proinflammatory macrophages. CAG significantly reduced the clinical scores, decreased the epidermal thickness, and ameliorated the deteriorating histopathology observed in IMQ-induced mice. CAG treatment specifically reduced the dermal infiltration of macrophages, rather than of dendritic cells, neutrophils, or T lymphocytes, into psoriatic skin. CAG dose-dependently decreased the level of proinflammatory cytokines, including IL-1β, TNF-α and IL-6, in murine psoriatic skin and serum, as well as in IMQ-stimulated, bone-marrow-derived macrophages. When compared to the control group, CAG significantly decreased IMQ-triggered NLRP3 inflammasome activation and gasdermin D-mediated cell pyroptosis in these proinflammatory macrophages. CAG also suppressed the assembly of the NLRP3 inflammasome complex. Taken together, the results show that CAG selectively modulates macrophage function by inhibiting NLRP3 inflammasome-mediated pyroptosis to ameliorate IMQ-induced psoriasis-like skin inflammation in mice. Our findings also identify an effective drug candidate for the treatment of psoriasis.

1. Introduction

Psoriasis is a common hereditary chronic inflammatory skin disease mediated by the innate and adaptive immune systems. It affects approximately 2% of the worldwide population and is mainly manifested in the skin and as arthritis, with a great impact on the physical and mental health of patients [1–3]. The histological characteristics of psoriasis mainly include abnormal keratinocyte differentiation, massive infiltration of immune cells, and excessive angiogenesis [4–6]. However, the exact mechanisms underlying the initiation of psoriasis remain unclear, and none of the endlessly emerging drugs has fully satisfied patients with psoriasis [1,7,8]. Therefore, finding potential therapeutic targets or effective compounds for the treatment of psoriasis remains a significant challenge.

Accumulating evidence now shows that the recruitment and activation of macrophages in psoriatic skin is a key pathogenic event in the development and maintenance of psoriatic disease [9–11]. The skin lesions in psoriasis are infiltrated by a large number of macrophages, which secrete many pro-inflammatory factors, including IL-1β, TNF-α, and IL-6, during the progression of psoriasis [12,13]. The IL-1β released by macrophages can, in turn, promote the secretion of IL-17 by Th17 cells. IL-6 helps effector T cells to evade Treg that inhibits the

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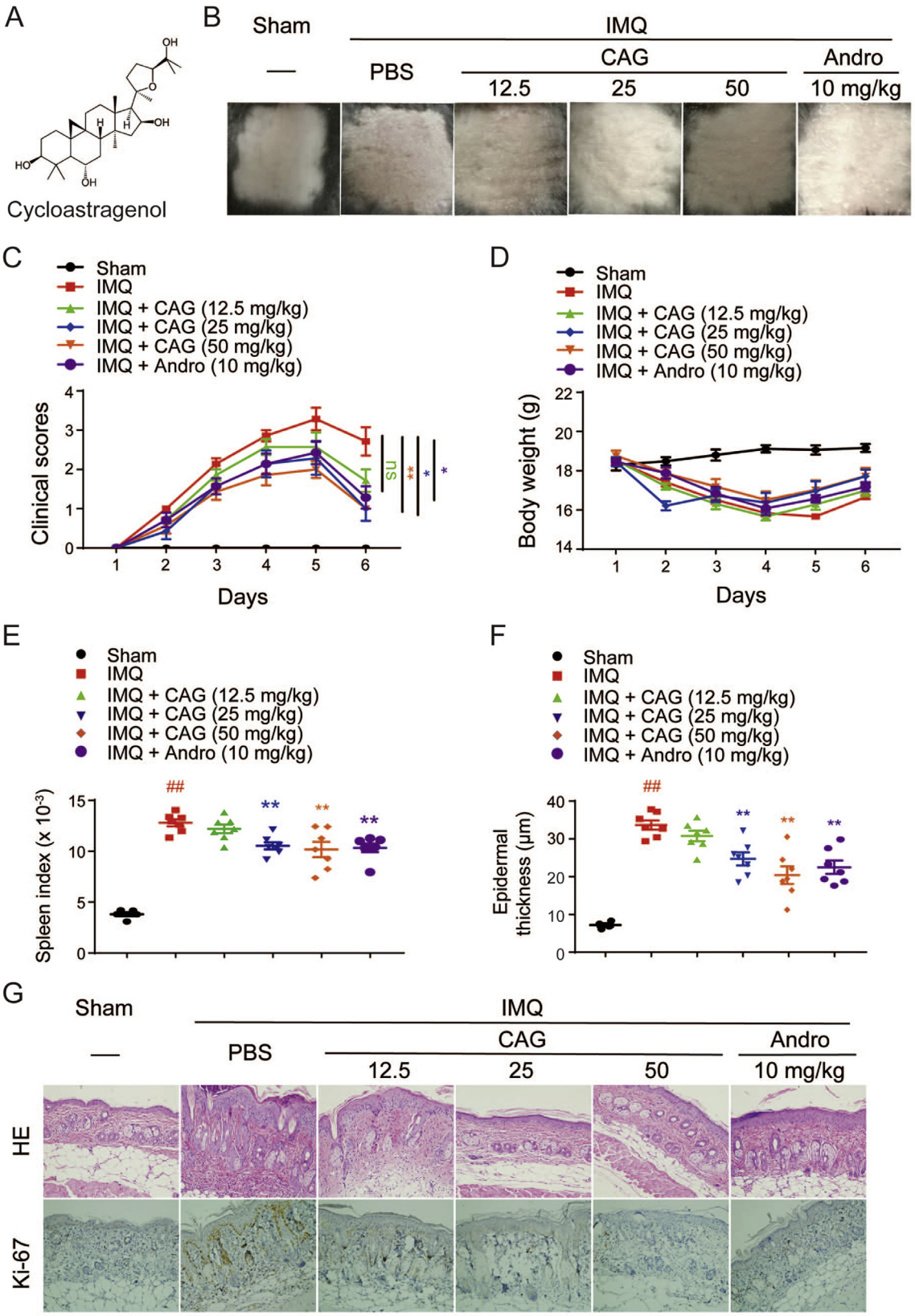

Fig. 1. Cycloastragenol ameliorated IMQ-induced psoriasis-like symptom in C57BL/6 mice. (A) Molecular structure of cycloastragenol. (B) Photograph of mice back skin was taken on day 6. (C) Clinical score of IMQ-induced psoriasis-like dermatitis in mice was monitored. (D) Body weight of mice was monitored every day. (E) Spleen index of mice was examined on day 6. (F) Epidermal thickness of mice was measured on day 6. (G) H&E and Ki-67 staining of back skin sections were examined on day 6, respectively. Data represent the mean ± S.E.M of 7 mice per group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs IMQ group. IMQ: miquimod; CAG: cycloastragenol; Andro: andrographolide.

involvement of Th17 in inflammatory response. TNF-α is identified as an activator of IL-23 synthesis in dendritic cells, which together aggravate the progression of psoriasis [14–18]. Therefore, the specific inhibition of macrophage functions involved in pathogenesis of this chronic inflammatory skin disease is highly desirable.

One important component of innate immunity is the NLRP3 inflammasome, which plays a vital role in the immune response and disease occurrence [19,20]. When the body perceives a danger signal stimulus, it activates the innate immune system to recruit and activate ASC and caspase-1 following recognition of the pathogen-associated molecular patterns or host-derived risk signal damage-associated molecular patterns [21–23]. Activated caspase-1 splices pro-IL-1β and pro-IL-18 to generate IL-1β and IL-18 [24,25]. The NLRP3 inflammasome also regulates the splicing of gasdermin D by activated caspase-1 into cleaved-gasdermin D, and then accumulates and forms pores on plasma membrane. This is accompanied by a large release of LDH (lactate dehydrogenase) and proinflammatory cytokines, resulting in programmed cell pyroptosis under inflammatory and stress pathological conditions [26–29]. However, no agent capable of inhibiting inflamed macrophage pyroptosis has been reported for the treatment of psoriasis.

Recently, many effective components extracted from traditional Chinese medicine have shown promising effects in the treatment of psoriasis [30–32]. Cycloastragenol (CAG), an active triterpenoid saponin component isolated from the traditional Chinese medicine *Astragalus membranaceus*, has various pharmacological effects, such as anti-viral, anti-bacterial, anti-aging, anti-inflammation, and anti-tumor activities [33–37]. CAG is reported to prevent cardiac fibrosis in mice by inhibiting the NLRP3 inflammasome [36]. However, what is not yet clear is whether CAG can ameliorate psoriasis, a classic cutaneous inflammatory disease. In this study, our aim was to assess the action and mechanism of CAG on IMQ-induced psoriasis-like skin inflammation in mice.

2. Materials and methods

2.1. Reagents

Cycloastragenol (CAG, purity > 99%) was purchased from Jiangsu Yongjian Pharmaceutical Technology Co., Ltd. Lipopolysaccharides (LPS) and Andrographolide (Andro) were purchased from Sigma-Aldrich (Shanghai, China). Imiquimod cream (IMQ) was purchased from Inova Pharmaceuticals (Singapore) pet. limited. TNF-α (1217202), IL-1β (1210122) and IL-6 (1210602) ELISA kits were purchased from Dakewei (Shenzhen, China). LDH enzyme activity detection kit was purchased from Nanjing Jiancheng Institute of Biological Engineering. Anti-mouse antibodies of CD3-FITC, CD11c-FITC, F4/80-FITC, F4/80-APC, CD45-APC-Cy7, CD4-Percp-Cy5.5, CD11b-Percp-Cy5.5 and Ly6G-PE were purchased from Biolegend. Caspase-1 was purchased from Proteintech. Gasdermin D and Actin were purchased from Abcam. NLRP3, and ASC were purchased from Santa Cruz Biotechnology.

2.2. Mice maintenance, induction of psoriasis and clinical scoring

Female C57BL/6 mice (6–8 weeks old) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). Mice in keeping the 12 h light and dark cycle, the temperature of 22 °C environment adaptability to feed for a week. The area about 2 cm × 2 cm was scraped off the back of the mice, and IMQ cream (3 mg per mouse) was applied evenly every day. Two hours later, mice in each group were intragastric (i.g.) administration, CAG group were given with doses of 12.5, 25, 50 mg/kg, and the Sham group and IMQ group were given the same amount of PBS every day. The scoring criteria of the mouse psoriasis model were based on the clinical psoriasis area and severity index (PASI) to conduct an objective scoring system. For erythema and scale from 0 to 4, 0, none; 1, slight; 2, moderate; 3, marker; 4, very obvious [38]. The weight and scores were recorded daily.

2.3. BMDMs isolation and culture

Bone marrow cells were isolated from the femurs and tibias of C57BL/6 mice. After centrifugation at 300*g* for 5 min, DMEM was added with 10% fetal bovine serum and 20 ng/mL GM-CSF. The culture medium was changed every 3 days with 20 ng/mL GM-CSF was added. Under these conditions, adherent macrophages were obtained within 6–7 days. Cells were digested with trypsin, centrifuged at 300*g* for 5 min, and then laid in a 12-well plate with every 2×10^5 cells [39].

2.4. Flow cytometry

The axillary lymph nodes of mice were isolated and the single-cell suspension was prepared. Surface staining was performed with surface antigen antibodies in the FACS buffer (PBS containing 1% FBS) and stained on ice with appropriate antibodies for 30 min. Reactive dyes (eBioscience) are used to eliminate dead cells. Intracellular cytokine staining was performed with BD cell fixative solution/extracellular membrane solution, and the cells were fixed and permeated, and then stained with antibodies against cytokines in Perm/Wash buffer (BD Biosciences) [40].

2.5. Immunohistochemistry (IHC) and immunofluorescence (IF)

The skin tissue was fixed at 10% formalin and embedded in paraffin. After the tissue sections were dewaxed with xylene and dehydrated with ethanol, the antigen epitopes were repaired with sodium citrate antigen repair solution. Background was removed with 3% hydrogen peroxide and then sealed with 5% goat serum. Overnight incubation with rabbit polyclonal antibodies (1:200 dilution) against caspase-1 and Ki-67 or anti-mouse flow antibodies against CD4-FITC, F4/80-FITC and CD11c-FITC was performed. After incubation with secondary antibody or stained with DAPI, the samples were photographed under the microscope.

2.6. Co-immunoprecipitation assay and Western blotting

Cells or mouse skin tissues protein was lysed with lysis buffer and proteasome inhibitor, and then quantified by BCA methods. For Co-IP, proteins from cells were incubated with 5 μg of appropriate antibody and precipitated with protein A/G-agarose beads. These proteins were then isolated in 12.5% SDS PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore Corp., Bedford, MA, USA). The appropriate strip, blocked by 5% BSA for 1.5 h at room temperature, was incubated with a primary antibody (1:1000) under 4 °C overnight. Then HRP-conjugated secondary antibody was incubated for 2 h at room temperature. Detection was performed using the medical X-ray film developing machine (Hu.q, Jiangsu, China; HQ-320XT).

2.7. mRNA isolation and real-time PCR

Total RNA was isolated from cells or mouse tissues using Trizol (TaKaRa, Dalian, China) and 2 μg of total RNA was transcribed into cDNA. Real-time PCR was performed on a CFX 100 (Bio-Rad, Hercules, CA) cycler using the primers as GAPDH, Forward: 5′-TGACCTCAACT ACATGGTCTACA-3′, Reverse: 5′-CTTCCCATTCTCGGCCTTG-3′; TNF-α, Forward: 5′-CCTGTAGCCCACGTCGTAG-3′, Reverse: 5′-GGGAGTAGAC AAGGTACAACCC-3′; IL-23A, Forward, 5′-CAGCAGCTCTCTCGGAAT CTC-3′, Reverse, 5′-TGGATACGGGGCACATTATTTTT-3′; IL-17A, Forward, 5′-TTTAACTCCCTTGGCGCAAAA-3′, Reverse, 5′-CTTTCCCTC CGCATTGACAC-3′; IL-1β, Forward, 5′-GAAATACCACCTTTTGACA GTG-3′, Reverse, 5′-TGGATGCTCTCATCAGGACAG-3′; IL-6, Forward, 5′-TCTATACCACTTCACAAGTCGGA-3′, Reverse, 5′-GAATTGCCATTGC ACAACTCTTT-3′; IL-22, Forward, 5′-ATGAGTTTTTCCCTTATGGG

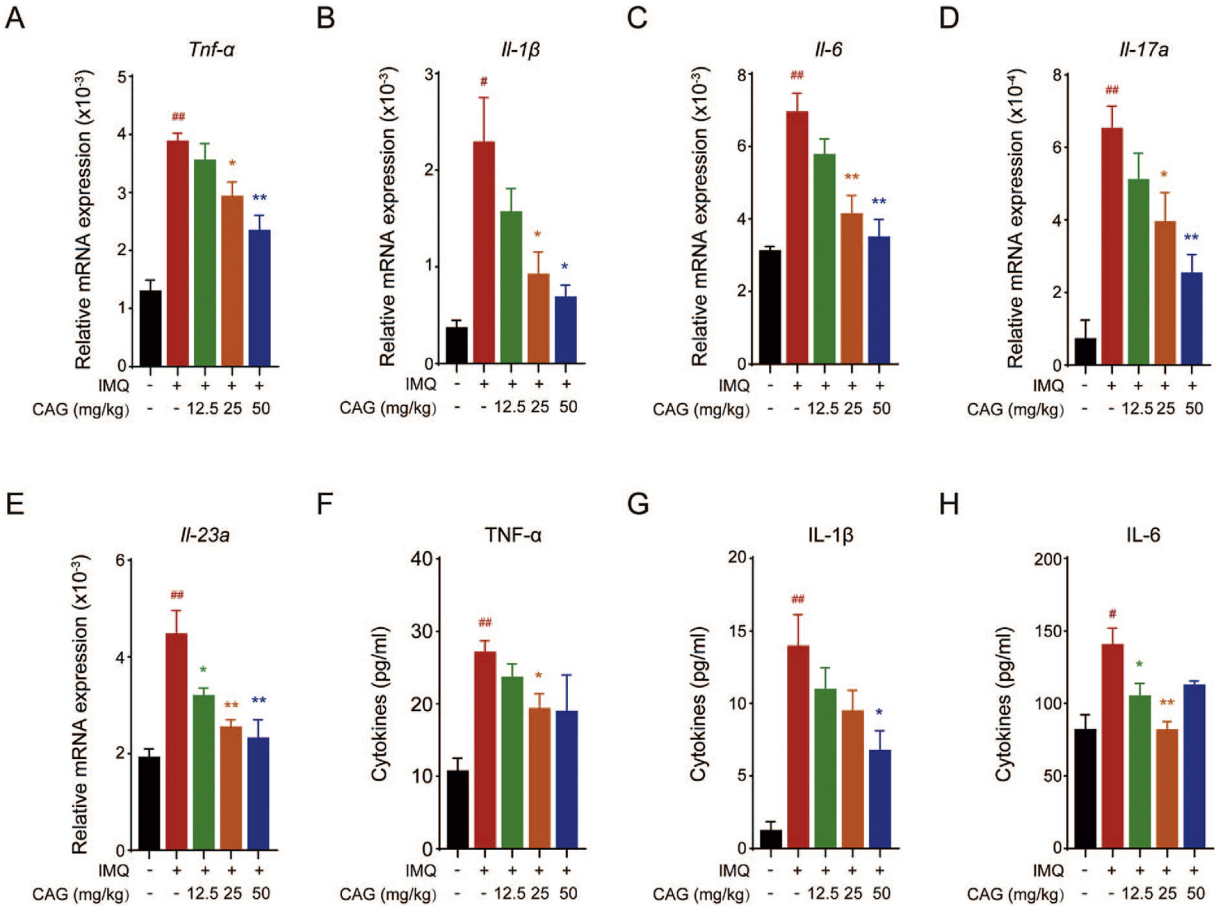

Fig. 2. Cycloastragenol decreased the proinflammatory cytokine expression in IMQ-induced murine psoriasis model. (A–E) Relative mRNA expression of *Tnf-α*, *Il-1β*, *Il-6*, *Il-17a*, *Il-23a* in skin lesions was measured using real-time PCR. (F–H) Cytokines of TNF-α, IL-1β and IL-6 in serum were detected by ELISA. Data represent the mean ± S.E.M of 7 mice per group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs IMQ group. IMQ: imiquimod; CAG: cycloastragenol.

GAC-3′, Reverse, 5′-GCTGGAAGTTGGACACCTCAA-3′; CCL2, Forward, 5′-TTAAAAACCTGGATCGGAACCAA-3′, Reverse, 5′-GCATTAGCTTCAG ATTTACGGGT-3′; CCR2, Forward, 5′-ATCCACGGCATCTATCAAC ATC-3′, Reverse, 5′-CAAGGCTCACCATCATCGTAG-3′; CXCL10, Forward, 5′-CCAAGTGCTGCTGCCGTCATTTC-3′, Reverse, 5′-GGCTCG CAGGGATGATTTCAA-3′; CXCR3, Forward, 5′-TACCTTGAGGTTAGTG AACGTCA-3′, Reverse, 5′-CGCTCTCGTTTTCCCCATAATC-3′; CX3CL1, Forward, 5′-ACGAAATGCGAAATCATGTGC-3′, Reverse, 5′-CTGTGTCG TCTCCAGGACAA-3′; CX3CR1, Forward, 5′-GAGTATGACGATTCTGCT GAGG-3′, Reverse, 5′-CAGACCGAACGTGAAGACGAC-3′; Here augmentation procedure was as follows: 95 °C for 2.5 min, and 44 cycles for 15 s at 95 °C, 60 °C for 30 s. Dissociation curves are analyzed at the end of magnification. The GAPDH mRNA expression level was used for normalization [41].

2.8. LDH release assay and enzyme-linked immuno sorbent assay (ELISA)

LDH activity determination using cytotoxic® 96 non-radioactive cytotoxicity test kit (Promega G1780). Cytokines of TNF-α, IL-1β and IL-6 in mouse serum and BMDMs culture supernatant were detected by ELISA.

2.9. Statistical analysis

All statistical analyses were performed using SPSS software (version 19.0). All data in this study are expressed as the mean ± SEM. For data that showed a normal distribution and homogeneity of variance, one-way ANOVA was performed for comparisons among more than two groups using a Bonferroni analysis. Two-tailed Student's *t*-tests were performed to evaluate significant differences between two groups. *P* value < 0.05 was considered significant.

3. Results

CAG effectively alleviated IMQ-induced psoriasis-like skin inflammation in C57BL/6 mice.

The potential for CAG to ameliorate psoriasis-like symptoms was examined by inducing a mouse psoriasis model with imiquimod and evaluating the model using human clinical PASI scoring. CAG significantly reduced the scaling and redness of the epidermis (Fig. 1B) and decreased the clinical scores (Fig. 1C) in IMQ-induced psoriasis-like skin inflammation in C57BL/6 mice, but it had little effect on body weight (Fig. 1D). CAG also markedly down-regulated the spleen weight and the epidermal thickness (Fig. 1E and F). In addition, CAG at doses of 25 or 50 mg/kg significantly reduced cortical thickening and inflammatory infiltration, as well as the expression of Ki-67, in skin tissue (Fig. 1G).

The expression of inflammatory cytokines was significantly increased in the IMQ-induced murine psoriasis model; therefore, we determined the mRNA expression of inflammatory factors in mouse back skin tissues. We found that the mRNA expression of *Tnf-α*, *Il-1β*, *Il-6*, *Il-17a,* and *Il-23a* was significantly inhibited by CAG treatment in a dose-dependent manner (Fig. 2A–E). In addition, the protein levels of TNF-α, IL-6, and IL-1β were also decreased by CAG treatment (Fig. 2F–H). These results suggest that CAG ameliorates IMQ-induced psoriasis-like cutaneous inflammation.

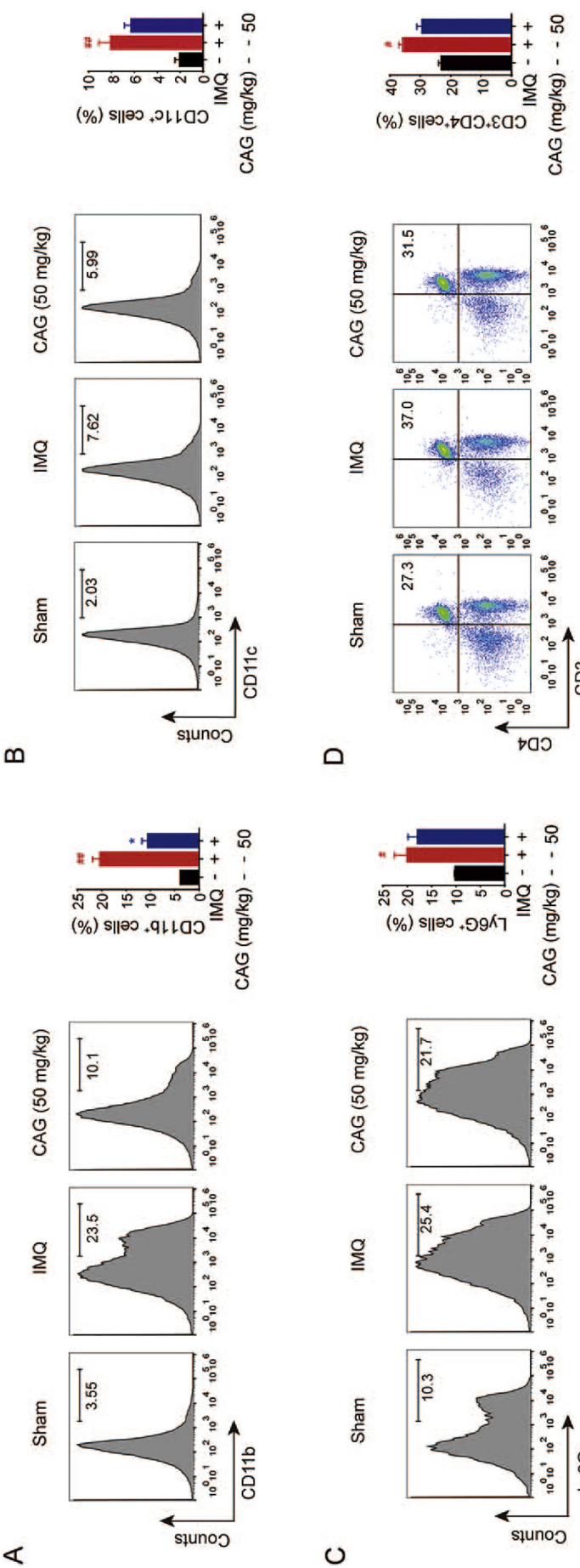

Fig. 3. Infiltration of macrophages rather than dendritic cells, neutrophils or $CD4^+$ T cells was significantly reduced by cycloastragenol in IMQ-induced murine psoriasis model. Cells in inflamed draining lymph nodes were stained with CD11b-Percp-Cy5.5 (A), CD11c-FITC (B), Ly6G-PE (C) or CD4-Percp-Cy5.5 (D) respectively and then analyzed by flow cytometry. Data represent the mean ± S.E.M of three independent experiments, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* Sham group;$^{*}P < 0.05$ vs IMQ group. IMQ: imiquimod; CAG: cycloastragenol.

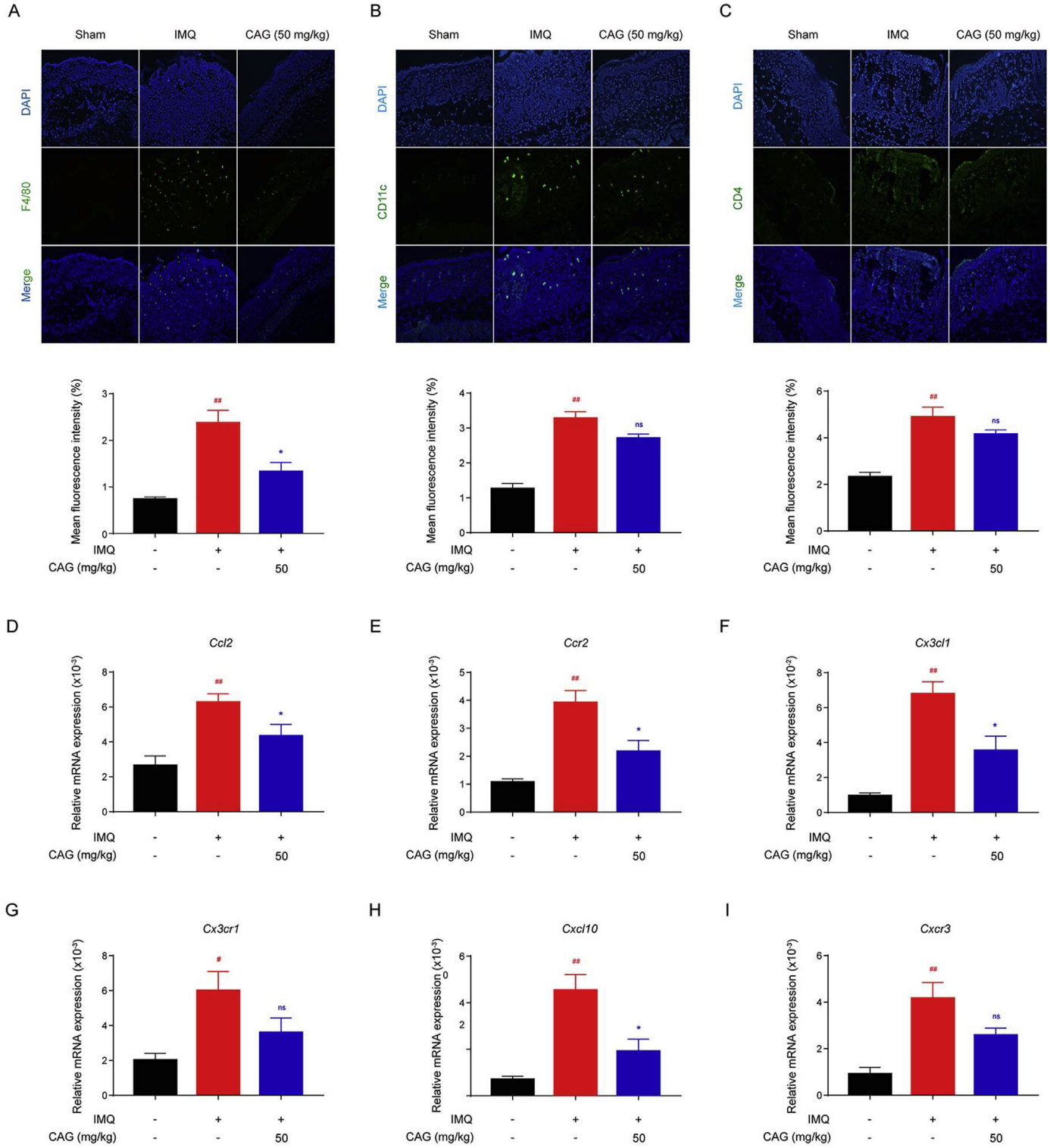

Fig. 4. Macrophages infiltration in inflamed skin lesions was remarkably decreased by cycloastragenol. Skin sections were stained with F4/80-FITC (green) (A), CD11c-FITC (green) (B), or CD4-FITC (green) (C) to indicate macrophage, dendritic cell and T cell infiltration, respectively. Cell nuclei were stained with DAPI (blue). (D-I) Relative mRNA expression of *Ccl2, Ccr2, Cx3cl1, Cx3cr1, Cxcl10* and *Cxcr3* in skin lesions was measured using real-time PCR. Data represent the mean ± S.E.M of three independent experiments, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Sham group; $^{*}P < 0.05$ vs IMQ group. IMQ: imiquimod; CAG: cycloastragenol; ns: no significance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CAG mainly inhibited infiltration of macrophages rather than dendritic cells, neutrophils or T cells to alleviate IMQ-induced psoriasis.

We investigated how CAG ameliorates IMQ-induced psoriasis using flow cytometry to characterize the infiltration of immune cells in this murine model. We found that CAG at 50 mg/kg dramatically reduced the infiltration of $CD11b^{+}$ macrophages into inflamed lymph nodes, while the infiltration of $CD11c^{+}$ dendritic cells, $Ly6G^{+}$ neutrophils, and $CD4^{+}$ T cells was only mildly affected by CAG (Fig. 3A–E). Immunofluorescence staining also showed that the infiltration of macrophages rather than dendritic cells and $CD4^{+}$ T cells into inflamed skin

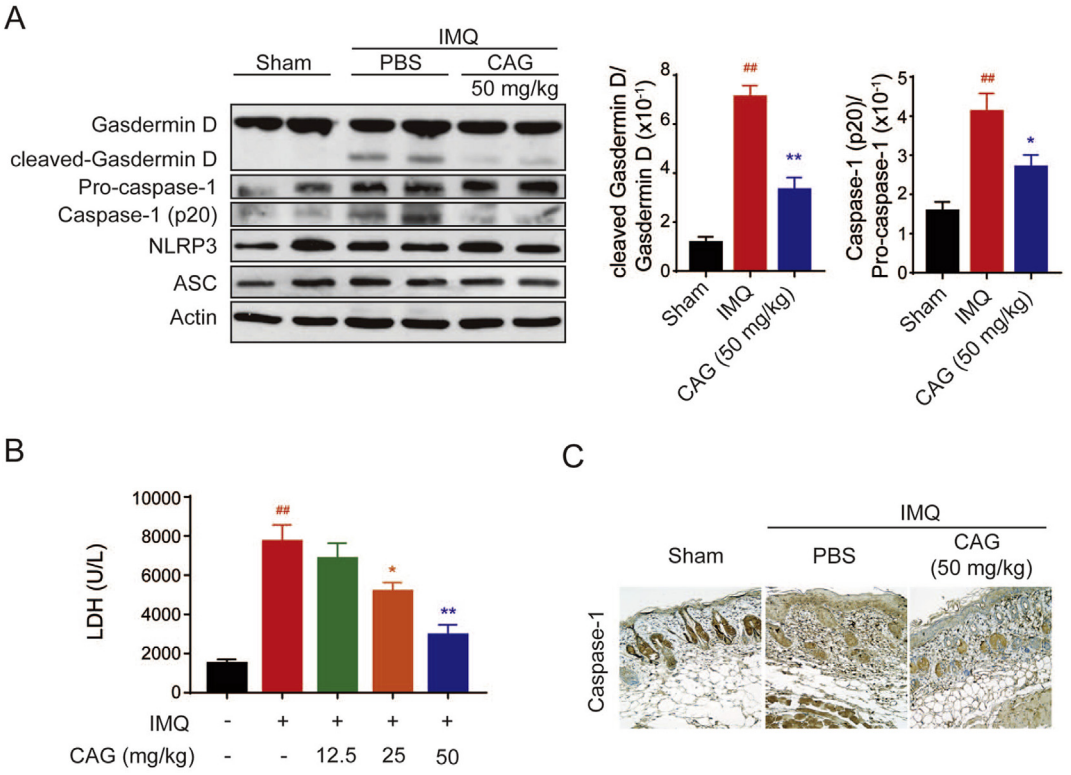

Fig. 5. Cycloastragenol inhibited IMQ-induced NLRP3 inflammasome activation and pyroptosis. (A) The activations of Caspase-1 and Gasdermin D from mice skin tissues were examined by Western blot. (B) Enzyme activity of LDH in serum was detected by LDH assay kit. (C) Caspase-1 expression in skin was analyzed by immunohistochemistry. Data represent the mean ± S.E.M of three independent experiments. $^{\#\#}P < 0.01$ vs Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs IMQ group. IMQ: imiquimod; CAG: cycloastragenol.

lesions was significantly decreased by CAG (Fig. 4A–C). We also detected the mRNA expression levels of chemokines and their receptors related to macrophages in skin tissues, and found that CAG significantly reduced the mRNA expression levels of Ccl2, Ccr2, Cx3cl1 and Cxcl10 (Fig. 4D–I). Therefore, CAG appeared mainly to target inflammatory macrophages to alleviate IMQ-induced psoriasis in mice.

CAG inhibited NLRP3 inflammasome-mediated pyroptosis in macrophage.

We speculated that CAG is likely to improve IMQ-induced psoriasis by inhibiting the activation of NLRP3 in macrophage. Western blot analysis showed that CAG at 50 mg/kg inhibited the expression of caspase-1 (p20) and cleaved-gasdermin D (Fig. 5A) in inflamed skin lesions. The enzyme activity of LDH (Fig. 5B) was also decreased in serum of mice treated with 50 mg/kg CAG. Immunohistochemical staining of skin tissues showed that caspase-1 expression (Fig. 5C) was significantly reduced by CAG treatment. CAG therefore appeared to ameliorate IMQ-induced psoriasis by inhibiting NLRP3 inflammasome-mediated pyroptosis in macrophage.

CAG reduced mRNA levels of inflammatory cytokines induced by IMQ in BMDMs but not in T cells in vitro.

We further explored the specific mechanism underlying the improvement of IMQ-induced psoriasis by CAG by isolating the primary BMDMs of mice and stimulating them with IMQ. CAG dose-dependently reduced the mRNA expression levels of *Tnf-α*, *Il-6*, and *Il-1β* (Fig. 6A–C). CAG also inhibited the mRNA expression level of *Tnf-α*, *Il-6*, and *Il-1β* (Fig. 6D–F) in a dose-dependent manner in BMDMs stimulated with LPS. Fig. 2 shows that CAG also inhibited the mRNA expression of *Il-17a* and *Il-23a*. However, CAG did not affect the mRNA expression of *Tnf-α, Il-1β, Il-6, Il-17a, Il-22,* and *Il-23a* in IMQ-stimulated splenic T cells, indicating that CAG has no direct effect on T cells (Fig. 6G–L). These results suggest that the beneficial effects of CAG on IMQ-induced psoriasis are obtained mainly through effects on macrophage function.

CAG inhibited NLRP3 inflammasome-mediated pyroptosis in BMDMs in vitro.

We found that caspase-1^{+} PI^{+} cells were decreased after CAG treatment of cultured mouse BMDMs (Fig. 7A), the levels of TNF-α, IL-1β, and IL-6 cytokines in serum were significantly reduced (Fig. 7B–D), and the enzyme activity of LDH was also decreased in a dose-dependent manner (Fig. 7E). The protein expression of caspase-1 (p20) and cleaved-Gasdermin D was also decreased by CAG (Fig. 7F). In addition, CAG also inhibited the assembly of the NLRP3 inflammasome (Fig. 7G). Taken together, CAG inhibits the activation of the NLRP3 inflammasome and further suppresses IMQ-induced pyroptosis in macrophage.

4. Discussion

Psoriasis is an immune-mediated genetic disease with complex etiology [3]. The mechanism of psoriasis has been studied extensively, but the none of the large number of drugs used presently to treat psoriasis are deemed satisfactory by patients with psoriasis [7,8]. In this study, we found that CAG, a small molecule derived from *Astragalus membranaceus,* could ameliorate IMQ-induced psoriasis in mice. Both in vivo and in vitro experiments confirmed that CAG could inhibit NLRP3 inflammasome-mediated pyroptosis and suppress the release of inflammatory cytokines and the infiltration of macrophages (Fig. 8).

The IL-17/IL-23 axis is believed to play an important role in the pathogenesis of psoriasis [42]. Current data from clinical trials of monoclonal antibodies (ixekizumab, secukinumab, and brodalumab) targeting IL-17 signaling and newer IL-23p19 antagonists (risankizumab, guselkumab, and tildrakizumab) also highlight the important role of these cytokines in psoriasis [43]. CAG inhibited the mRNA expression of *IL-17a* and *IL-23a* in skin lesions (Fig. 2D and E), indicating that CAG primarily alleviates the inflammatory progression of psoriasis. However, these cytokines are secreted by T cells or dendritic cells. In

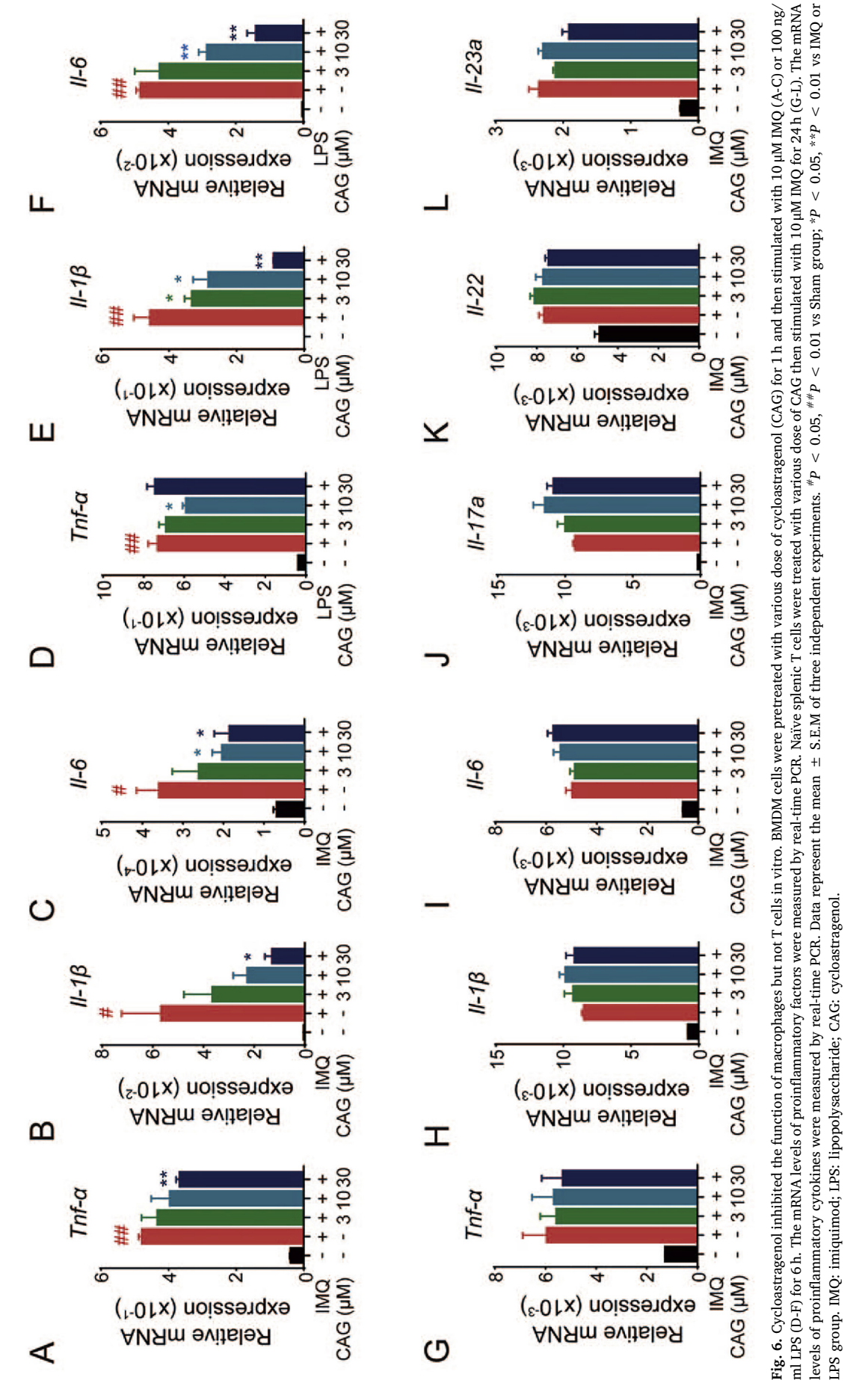

Fig. 6. Cycloastragenol inhibited the function of macrophages but not T cells in vitro. BMDM cells were pretreated with various dose of cycloastragenol (CAG) for 1 h and then stimulated with 10 μM IMQ (A-C) or 100 ng/ml LPS (D-F) for 6 h. The mRNA levels of proinflammatory factors were measured by real-time PCR. Naïve splenic T cells were treated with various dose of CAG then stimulated with 10 μM IMQ for 24 h (G-L). The mRNA levels of proinflammatory cytokines were measured by real-time PCR. Data represent the mean ± S.E.M of three independent experiments. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs IMQ or LPS group. IMQ: imiquimod; LPS: lipopolysaccharide; CAG: cycloastragenol.

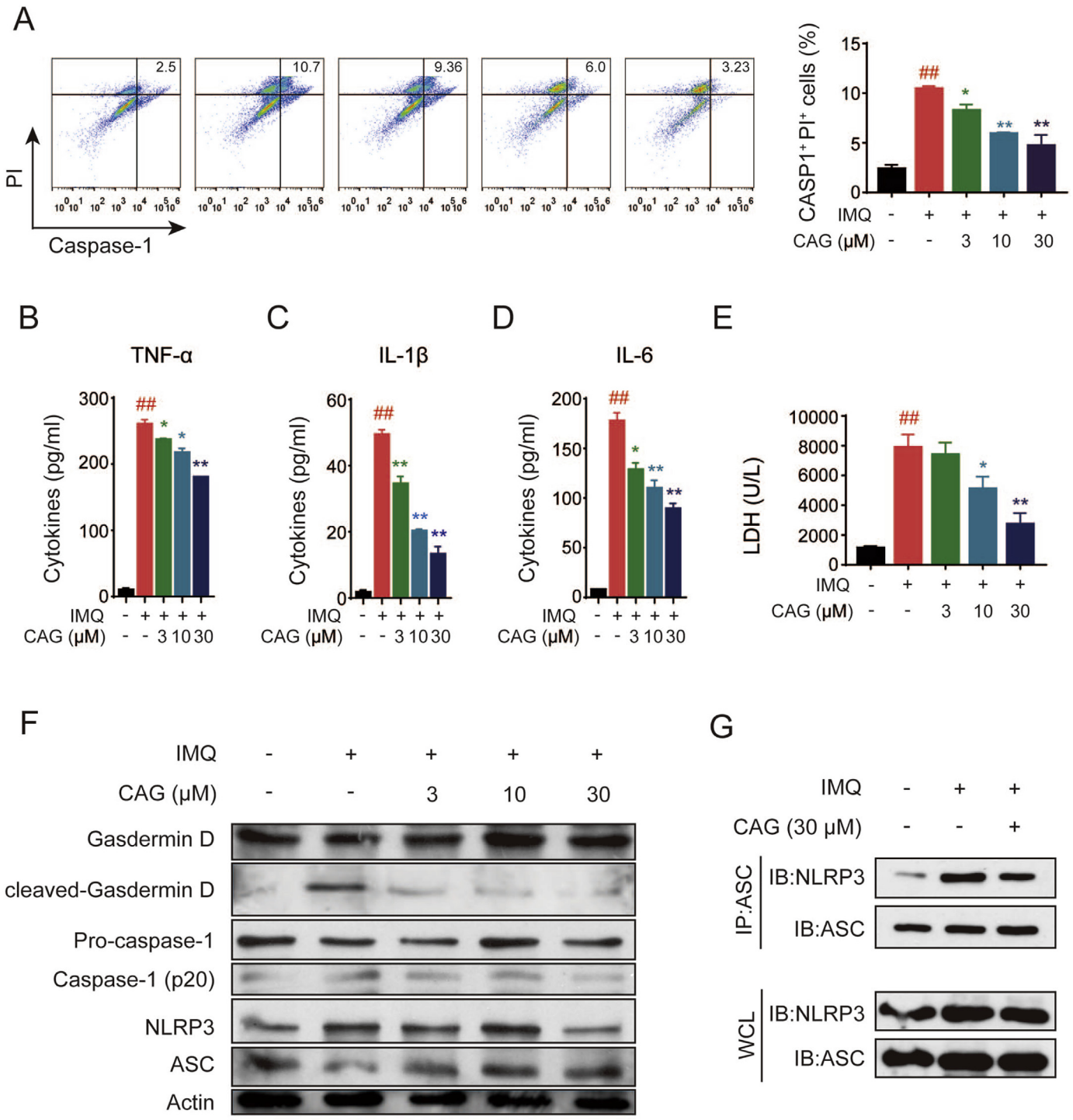

Fig. 7. Cycloastragenol suppressed IMQ-induced NLRP3 inflammasome activation and pyroptosis in BMDM cells. BMDM cells were stimulated with 10 μM IMQ for 6 h after incubation with various dose of cycloastragenol for 1 h. (A) Double positivity of Caspase-1 and PI cells were detected by flow cytometry. (B-D) The levels of TNF-α, IL-1β and IL-6 in the supernatant were detected by ELISA. (E) LDH activity in the supernatant was measured by LDH assay kit. (F) The activations of Caspase-1 and Gasdermin D from BMDMs stimulated with IMQ for 6 h were analyzed by Western blot. (G) Co-immunoprecipitation of ASC with NLRP3 was performed. The immunoprecipitated proteins or whole cell lysates were analyzed by Western blot. Data represent the mean ± S.E.M of three independent experiments. $^{\#\#}P < 0.01$ vs Sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs IMQ group. IMQ: imiquimod; CAG: cycloastragenol.

the present study, we saw slightly changed the infiltration of dendritic cells, neutrophils and T cells in the inflamed draining lymph nodes (Fig. 3B–D) and inflamed skin lesions (Fig. 4B and C), indicating that CAG may affect other cells.

The role of TNF-α, IL-1β, and IL-6 signaling in psoriasis is now recognized [44–47]. CAG treatment clearly inhibited the mRNA expression of *Tnf-α*, *IL-1β*, or *IL-6* (Fig. 2A–C) in skin lesions and decreased the protein levels of TNF-α, IL-1β and IL-6 in the serum (Fig. 2F–H), where with the most pronounced effects observed on IL-1β. These findings indicate that CAG mainly decreases macrophage infiltration (Fig. 3A, Fig. 4A), a critical process for the progression of psoriasis [48,49]. In addition, CAG significantly reduced the mRNA expression levels of *Ccl2, Ccr2, Cx3cl1* and *Cxcl10* (Fig. 4D–I) in skin tissues, suggesting inhibition of macrophage chemotaxis is responsible for the ameliorating

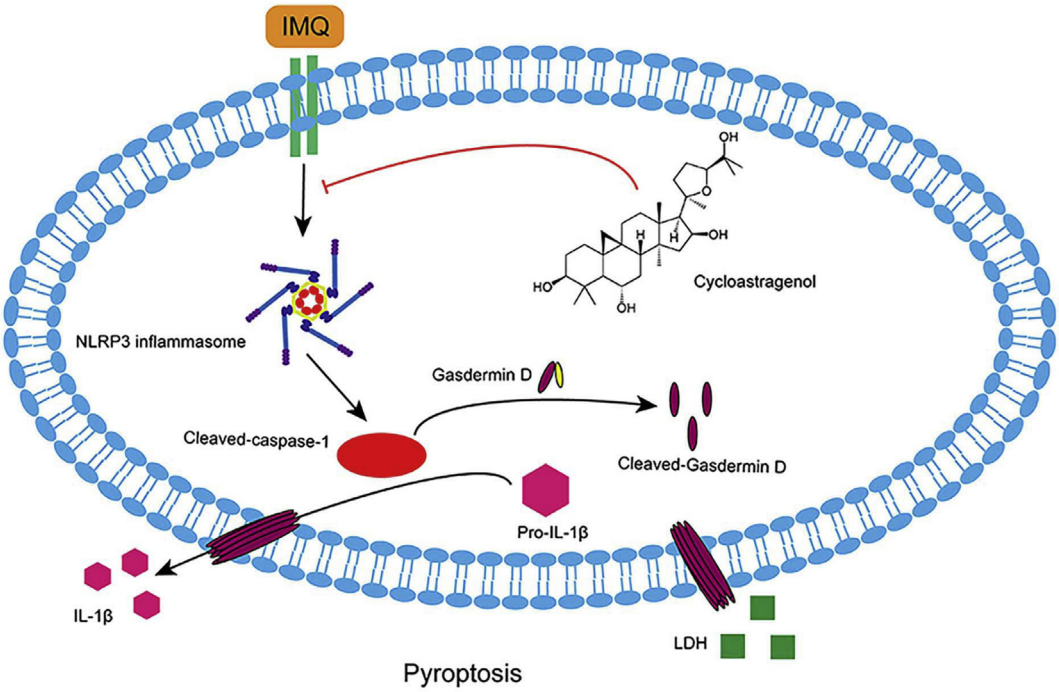

Fig. 8. Mechanism of cycloastragenol for ameliorating IMQ-induced psoriasis-like skin inflammation. Cycloastragenol inhibits IL-1β production and Gasdermin D-mediated pyroptosis through inhibiting NLRP3 inflammasome-dependent caspase-1 activation in macrophage, which contributing to amelioration of imiquimod (IMQ)-induced psoriasis-like skin inflammation in mice.

effect of CAG on murine psoriasis model.

Many previous reports have demonstrated that NLRP3 inflammasome-mediated IL-1β production accounts for psoriasis development [50,51]. The NLRP3 inflammasome orchestrates infection and immune response to cellular stress by recruiting ASC and activating caspase-1 to shear pro-IL-1β and release mature IL-1β [39,52]. Nevertheless, the contribution of pyroptosis in macrophages to psoriasis remains unclear. Our results showed that IMQ-induced cell death and caspase-1 activation in skin tissue could be suppressed by CAG treatment (Fig. 5), indicating that CAG ameliorates IMQ-induced psoriasis by inhibiting caspase-1-mediated cell death. CAG markedly decreased caspase-1 and PI double-positive BMDMs (Fig. 7A), suggesting inhibition of pyroptosis by CAG. The further inhibition of cleaved gasdermin D and release of LDH to the supernatant confirmed this suppression of pyroptosis (Fig. 7B–E). Mechanistically, CAG inhibited the assembly of NLRP3 inflammasome, thereby suppressing IMQ-induced pyroptosis in BMDMs (Fig. 7F and G).

In summary, CAG significantly ameliorates IMQ-induced psoriasis-like skin inflammation in mice by preventing NLRP3 inflammasome-mediated pyroptosis in macrophage (Fig. 8). To our knowledge, this is the first report that a small-molecule compound inhibits macrophage pyroptosis to ameliorate psoriasis-like symptom in mice. This study confirms the beneficial effects of CAG in IMQ-induced psoriasis and provides an in-depth understanding of pathogenesis in this uncontrolled chronic skin inflammation.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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