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Berberine suppresses IL-33-induced inflammatory responses in mast cells by inactivating NF-κB and p38 signaling

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ABSTRACT

Berberine (BBR) possesses many pharmacological characteristics including anti-inflammation, anti-allergy, anti-angiogenesis and anti-tumor. However, the effects and mechanisms of BBR on IL-33-induced mast cell inflammatory responses are kept unknown. To investigate these, rat peritoneal mast cells (RPMCs) were isolated from the peritoneal cavity and cultured with BBR treatment in combination IL-33 stimulation. Firstly, cytotoxic effect of BBR on RPMCs was detected by MTT assay. Then, IL-33-induced cytokine production and the expression of ST2 receptor, were evaluated by ELISA and real-time PCR, respectively. In addition, NF-κB and MAPK signaling involved in IL-33-mediated mast cell activation were assessed by Western blot, which also was confirmed using the signal transduction inhibitors. Simultaneously, the effect of BBR on IL-33-activated enhancement of IgE-mediated mast cell responses was analyzed. Lastly, SD rats were used to explore the effect of BBR on IL-33-induced inflammation in vivo. BBR treatment significantly reduced IL-33-stimulated cytokine production in RPMCs, such as IL-6, TNF-α, IL-13 and MCP-1, but had little effect in ST2 expression. BBR modulated IL-33 signaling via suppressing IL-33-induced NF-KB transcription and p38 phosphorylation, but not ERK and JNK. Additionally, BBR also hampered the combined effects of IL-33 and IgE-mediated mast cell activation. Decreased cytokine production followed BBR treatment in vitro was consistent with that in vivo, where BBR injection i.p. into rats obviously inhibited IL-33-induced plasma cytokine levels. These findings demonstrated that BBR suppressed IL-33-mediated inflammation in mast cells by inactivating NF-κB and p38 signaling, suggesting its potential application for the treatment of allergic inflammation.

1. Introduction

As a recently described member of interleukin (IL)-1 cytokine family, IL-33 has attracted considerable attention since its discovery [1], which is found and identified in different cell types, such as epithelial cells, endothelial cells, dendritic cells, monocytes, macrophages, and mast cells [2]. In addition to serving as an alarmin to potently enhance activity of the immune system [3], IL-33 also displays a functional property of gene regulation at transcriptional level [4]. Generally, IL-33 exerts its cytokine function though binding to a receptor complex, which consists of IL-1 receptor-related protein-2 (ST2) and IL-1 receptor accessory protein (IL-1RAcP). On the one hand, IL-33 binds to ST2 and forms a favorable conformation to combine with IL-1RAcP. On the other hand, IL-1RAcP recruitment and ST2/IL-1RAcP interaction constitute a heterodimeric complex to initiate subsequent signal cascade reactions, such as the phosphorylation of mitogen-activated protein kinase (MAPK) and activation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) [5]. Increasing evidence indicates that IL-33 plays an important role in a broad range of disorders, including infectious, inflammatory, and autoimmune diseases [6].

Mast cells (MCs) are best known for its major roles in allergic disease, such as asthma, allergic rhinitis, and allergic dermatitis [7]. Accompanying the interactions among allergen, immunoglobulin E (IgE), and the high-affinity IgE receptor (FcεRI), the cross-talking reactions

Abbreviations: BBR, berberine; MCs, mast cells; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; RPMCs, rat peritoneal mast cells; Anti-DNP IgE, anti-dinitrophenyl immunoglobulin E; DNP-HAS, dinitrophenyl-human serum albumin

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facilitate producing different types of chemical mediators in different stage. On the early phase, MCs release tryptases, histamine, leukotrienes, prostaglandins, etc. within minutes of activation. On the late phase, MCs secret majorly synthesized cytokines and chemokines, including tumor necrosis factor-α (TNF-α), IL-4, IL-6, IL-13, macrophage inflammatory protein-1α (MIP-1α), and monocyte chemotactic protein-1 (MCP-1). These biological mediators regulate and contribute to the pathological process of allergic inflammation together with other immune cells [8,9]. Recent study indicated that IL-33 was a potent mast cell activator [10], whereas the relationship between MCs and IL-33 was bidirectional. MCs have been shown to produce IL-33 both in vivo and in vitro, which is also up-regulated after IgE-mediated activation [11]. In turn, IL-33 can activate MCs and IL-33/ST2 axis is critical for the progress of IgE-dependent inflammatory responses [12].

Berberine (BBR), a natural isoquinoline alkaloid, possesses a wide range of pharmacological activities, such as anti-oxidation, anti-angiogenesis, antiatherosclerosis, anti-inflammation, anti-allergy, anti-diabetes and anti-tumor [13]. A previous study reported that anti-inflammatory activity of BBR, which suppressed the release of IL-6, CXCL8, CCL2 and CCL7 in combined IL-31- and IL-33-activated human eosinophils and eosinophils-dermal fibroblasts co-culture [14]. To the best of our knowledge, few studies focus on the effect of BBR on IL-33-induced inflammatory responses in mast cells. Herein, we aimed to investigate the effect and explored the underlying mechanisms of BBR on IL-33-induced mast cell inflammation. Our results showed that BBR treatment reduced IL-33-induced cytokine secretion, inhibited IL-33-mediated p38 phosphorylation and decreased NF-κB transcription. Importantly, BBR injection blunted the increment in the systemic cytokines elicited by IL-33 challenge in vivo. These findings illustrated that BBR was a potent suppressor of IL-33-mediated inflammatory responses in mast cells, suggesting its potential application of BBR in allergic inflammation associated with mast cell activation.

2. Materials and methods

2.1. Animals

The original stock of male 7-week-old Sprague-Dawley rats, weighing 200 to 220 g, were purchased from Hubei Research Center of Laboratory Animals (Wuhan, China) and housed in specific pathogen-free environment at the Animal Care Facility of Hubei University of Chinese Medicine (Wuhan, China). Animal care and use were consistent with the guidelines established by the Animal Care and Use Committee of Hubei University of Chinese Medicine (No.: SYXK2012-0067).

2.2. Isolation and culture of rat peritoneal mast cells (RPMCs)

Rats were anesthetized with 10% Chloral hydrate i.p. (300 mg/kg). Twenty milliliters of DMEM (Hyclone, Logan, UT, USA), containing 10% fetal bovine serum (FBS), penicillin (100 U/mL), streptomycin (100 μg/mL), and heparin (5 U/mL) (Hyclone), were injected into the peritoneal cavity. After gentle massage for 15 min, fluid was collected with the cooled polypropylene tubes. Once the lavage was completed, the rats were euthanized with cervical dislocation after CO_2 application (the flow rate > 30% of the chamber volume/minute). The cells were centrifuged and collected at 400 ×*g* for 15 min at room temperature. Then, cell pellets were resuspended in 1 mL serum-free DMEM, and macrophages were separated by centrifugation using a Percoll solution as previous description [15]. The isolated RPMCs were cultured in DMEM with 10% FBS and 100 U/mL penicillin/streptomycin at 37 °C in a 5% CO_2 humidified incubator.

2.3. Preparation of berberine (BBR)

BBR was purchased from Sigma-Aldrich (St. Louis, MO, USA) and prepared by dissolving with dimethyl sulfoxide (Sigma-Aldrich). Dilutions were made in phosphate buffered saline (PBS) and filtered through 0.22 μm syringe filter.

2.4. MTT assay

Cell viability was determined by MTT assay. Briefly, RPMCs (1×10^5) were cultured with various concentrations of BBR (0, 5, 10, 25, 50, 75 and 100 μM) for 24 h or indicated time. Then, MTT (Sigma-Aldrich) working solution (0.5 mg/mL) was added. After incubation for an additional 4 h at 37 °C, the medium was replaced with dimethyl sulfoxide. The absorbance was measured at 540 nm with a microplate reader (Bio-Rad Laboratories, Inc., Richmond, CA, USA). The inhibitory rate of cells viability (%) = (OD value of control group − OD value of test group) × 100% / OD value of control group.

2.5. Cytokine assay by enzyme-linked immunosorbent assay (ELISA)

The cytokine production induced by IL-33 (50 ng/mL) (NBP2-35248, Novus Biological, Littleton, Colorado, USA), including IL-6, TNF-α, IL-13, and MCP-1, were determined by ELISA (R&D systems, Minneapolis, MN, USA) according to the manufacturer's instruction and measured using a micro-plate reader (Bio-Rad Laboratories). All experiments were done in triplicate.

2.6. Quantitative real-time PCR (qRT-PCR)

Total RNA for RPMCs was extracted by TRizol reagent (Invitrogen, Carlsbad, CA, USA). RNA (50 ng) was used for primer-specific reverse transcription (RT) using the RT reagent Kit (Applied Biosystems Life Technologies, Foster City, CA, USA) following the manufacturer's instructions. Quantitative real-time PCR was performed using the SYBR-Green Master PCR Mix (Applied Biosystems). Amplification conditions: 95 °C for 5 min followed by 40 cycles of 95 °C for 15 s, 58 °C for 30 s, and 72 °C for 1 min. The PCR primers were listed as follows: ST2-forward CGCCTGTTCAGTGGTTTA, ST2-reverse TGGTTCCGTTCTCCG TGT; GAPDH-forward GGCCCCTCTGGAAAGCTGTG, GAPDH-reverse CCGCCTGCTTCACCACCTTCT. Expression of GAPDH was used as the internal control. Data were analyzed by the $2^{-\Delta\Delta Ct}$ method.

2.7. Cytosolic protein and nuclear protein extraction

RPMCs were collected for protein extraction on ice using a Nuclear Protein and Cytosolic Protein Extraction Kit (#P0028) (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's instructions. Briefly, cells were washed with cold PBS, resuspended in the protein extraction buffer A containing 1 mM PMSF (#ST506, Beyotime Institute of Biotechnology), and vortexed for 30 s at the highest speed. After incubated on ice for 15 min, the protein extraction buffer B was added, vortexed for 30 s, incubated on ice for 1 min, and then centrifugated at 12,000*g* for 5 min at 4 °C. The supernatants containing extracted cytoplasmic protein were completely drawn into a pre-cooled plastic tube. Next, remained precipitation was resuspended with the extraction buffer for nuclear protein, incubated on ice for 30 min (vortexed for 30 s every 2 min), and centrifugated at 12,000*g* for 10 min at 4 °C. Then, the supernatants containing extracted nuclear protein were completely drawn into a pre-cooled plastic tube.

2.8. Western blot analysis

Extracted supernatant of cytosolic protein nuclear protein were separately quantitated for protein concentration with a BCA Protein Assay kit (Pierce, Rockford, IL, USA). Samples (30 μg) were loaded and separated by 10% serum dodecyl sulfate-polyacrylamide gels (SDS-PAGE). Then, the gels were electro-transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% non-fat dry milk for 2 h at room temperature, the

membranes were incubated with the primary antibodies against IκBα (ab32518), NF-κB (p65) (ab28856), p-ERK (ab4819), ERK (ab17942), p-JNK (ab76572), JNK (ab208035), p-p38 (ab4822), p38 (ab27986), β-actin (ab8227) and α-tubulin (ab4074) (all from Abcam, Cambridge, MA, USA) overnight at 4 °C. Subsequently, the horseradish peroxidase-conjugated secondary antibody (ab6721) (Abcam) were used to incubate the membranes for 2 h at room temperature. Peroxidase-labeled protein bands were detected by enhanced chemiluminescence reagents (Millipore) and the protein intensity was analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA). Sample loading was normalized by quantities of β-actin or α-tubulin detected parallel.

2.9. Signal transduction inhibitors assay

NF-κB inhibitor (BAY 11-7085, 5 μM), ERK inhibitor (PD98059, 10 μM), JNK inhibitor (SP600125, 10 μM) and p38 inhibitor (SB203580, 10 μM) were obtained from Cell Signaling Technology Inc. (Beverly, Massachusetts, USA). Inhibitors were added to culture 2 h prior to stimulation with IL-33 (50 ng/mL), respectively. Supernatants were collected 24 h later for ELISA analysis.

2.10. IgE-mediated mast cell activation

RPMCs were sensitized overnight with DNP-specific IgE (500 ng/mL). Then, cells were washed, resuspended and stimulated with IL-33 (50 ng/mL) or antigen (DNP-HAS, 100 ng/mL) in the presence or absence of BBR. After 24 h, the supernatants were collected for assessment of cytokine production.

2.11. Berberine injection in vivo

Rats (n = 5 per group) were injected i.p. daily with BBR (1 mg/kg) or the same volume PBS for continuous 1 week, separately. On the eighth day, IL-33 (5 μg) was injected intraperitoneally. Six hours after IL-33 injection, rats were decapitated and trunk blood was collected. Plasma isolated from blood samples was analyzed for cytokine expression.

2.12. Statistical analysis

Data were presented as means ± standard error of means (SEM) and analyzed using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA). Comparisons between two groups were done using an unpaired Student *t*-test, and comparisons between multiple groups were done using one-way ANOVA with a Tukey post hoc test. $P < 0.05$ indicated statistical significance.

3. Results

3.1. Effect of BBR on the cytotoxicity of rat peritoneal mast cells

Firstly, we detected the cytotoxic effect of BBR on the RPMCs using MTT assay. As shown in Fig. 1A, the cytotoxicity was markedly increased following the up-regulation of BBR concentration (≥25 μM). However, both 5 μM and 10 μM BBR showed little effect on the cell viability. Next, we compared the indicated concentration of BBR (5, 10, 25 μM) on the cytotoxicity of RPMCs for different time up to 72 h. The result indicated that 25 μM BBR showed obvious cytotoxicity from 60 h (Fig. 1B). According to these findings, 10 μM BBR was chosen for follow-up experiments.

3.2. BBR suppresses IL-33-induced cytokine production

To determine the role of BBR on IL-33-induced cytokine production, RPMCs were treated with IL-33 in the presence of BBR for 24 h. As shown in Fig. 2, BBR significantly suppressed IL-33-induced cytokine release, such as IL-6, TNF-α, IL-13, and MCP-1, compared with those in the single IL-33-stimulated group. Due to the role of ST2 on the biological effects of IL-33 signaling as its specific receptor, we investigated the expression of ST2 among different groups. The result showed that there was no distinct difference in ST2 receptor expression among the forth groups (Fig. 3).

3.3. BBR inhibits IL-33-mediated NF-κB and p38 activation

Because of little effect of BBR treatment on ST2 expression, it suggested that the inhibition of IL-33 signaling might be involved in the suppressive effect of BBR. Following IL-33 stimulation, the cytosol IκBα was clearly decreased and nuclear NF-κB (p65) was distinctly increased. BBR treatment effectively curbed the degradation of IκBα and NF-κB (p65) translocation (Fig. 4A). Simultaneously, p38 phosphorylation was evidently curbed in the presence of BBR, but not ERK or JNK (Fig. 4B). Furthermore, NF-κB or p38 inhibitor completely abolished IL-33-mediated cytokine production as the same with BBR, but ERK and JNK inhibitors showed no effect (Fig. 5). Thus, BBR inhibited multiple IL-33 signaling cascades, yielding excessive suppression of the inflammatory responses.

3.4. BBR hampers IL-33-induced enhancement of IgE-mediated responses in mast cells

Considering the enhancement of IL-33 on FcεRI-mediated cytokine and chemokine production in mast cells [16]. We assessed the effect of BBR on the synergistic effects of IL-33 and IgE/antigen stimulation. As shown in Fig. 6, combined stimulation with additional antigen and IL-33 amplified the cytokine production. Importantly, BBR treatment visibly hampered IL-33-induced enhancement of IgE-mediated responses in mast cells.

3.5. BBR curbs IL-33-induced cytokine production in vivo

Lastly, we investigated the effect of BBR on IL-33-induced cytokine production in vivo. As expected, IL-33 injection increased plasma inflammatory cytokine levels, such as IL-6, TNF-α, IL-13, and MCP-1. BBR treatment thoroughly restrained the cytokine production induced by IL-33 (Fig. 7). These data were consistent with our findings in vitro and suggested that BBR effectively antagonized IL-33 function in vivo.

4. Discussion

As a traditional Chinese medicine, BBR has attracted more and more attention and is increasingly used as immunomodulator in recent years. Our previous study showed that BBR obviously ameliorated collagen-induced arthritis in rats, suggesting its anti-inflammatory property of BBR [17]. Additionally, BBR significantly attenuated delayed-type hypersensitivity in mice, attributing to the inhibition of Th1-mediated cytokines including IFN-γ and TNF-α [18]. Recent publication reported that BBR reduced mast cell infiltration in rats with allergic contact dermatitis and inhibited inflammatory cytokine production, such as IFN-γ and IL-4 [19]. These findings emphasized the anti-inflammatory potent of BBR in inflammation and allergic diseases.

Increasing evidence shows that IL-33 can initiate and exacerbate inflammatory responses as well as enhance IgE production and histamine release in naïve wild-type mice and human mast cells [20,21]. IL-33 stimulates mast cells to secrete IL-6, IL-13, TNF-α, MCP-1 and prostaglandin D [22]. TNF-α is released in allergic responses from both mast cells and macrophages via IgE-dependent mechanisms [23], which also participated in pathological process of chronic inflammation and illnesses [24–26]. TNF-α-treated MCs induced a shift in cytokine expression from a Th1 to a Th2 profile (decreased IFN-γ production, and increased secretion of IL-4 and IL-10) and showed the inhibition of MC

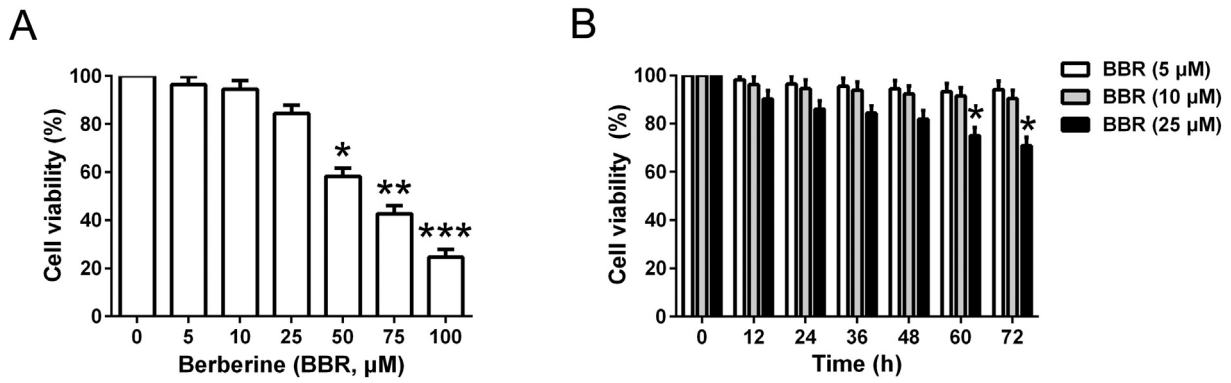

Fig. 1. Cytotoxic effects of BBR on RPMCs. (a) RPMCs (1×10^5) were isolated from the peritoneal cavity and cultured with various concentrations of BBR (0–100 μM) for 24 h. Then, cell viability was determined by MTT assay. (b) RPMCs (1×10^5) were cultured and treated with different concentrations of BBR (5, 10, 25 μM) for various time (0–72 h), respectively. After incubation, MTT assay was performed for evaluation of cell viability. Results shown were representative of three experiments done in triplicate. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, comparison with the group without BBR treatment.

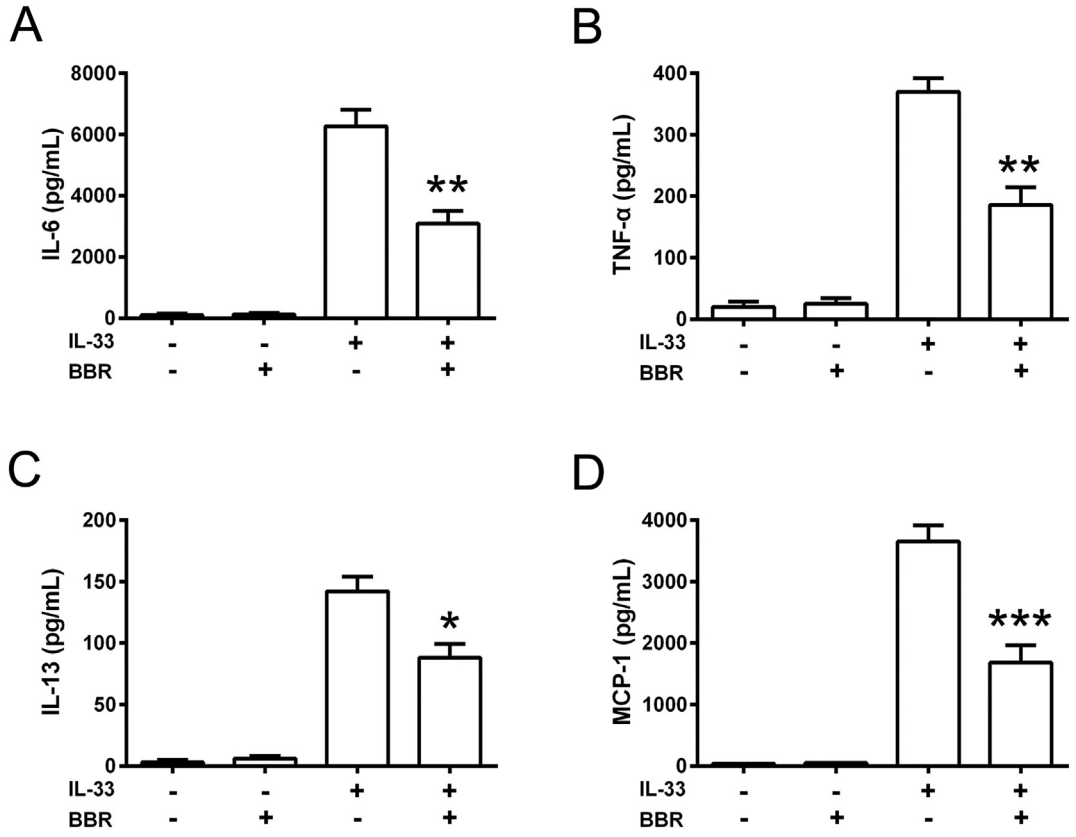

Fig. 2. BBR suppresses IL-33-induced cytokine production in the RPMCs. RPMCs (1×10^5) were isolated and cultured with IL-33 (50 ng/mL) in the presence or absence of BBR (10 μM). After 24 h, supernatants were collected and cytokine productions were analyzed by ELISA, including (a) IL-6, (b) TNF-α, (c) IL-13 and (d) MCP-1. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, comparison with the IL-33-stimulated group without BBR treatment.

degranulation [27]. As the pleiotropic inflammatory cytokine, IL-6 could enhance MC proliferation, maturation, and reactivity after FcεRI aggregation [28]. Simultaneously, MC activation in response to FcεRI aggregation also increased IL-6 production [29]. IL-1β has the ability to upregulate genes expression involved in the inflammatory process, including IL-6 and IL-8 [30]. The maturation of pro-IL-1β is linked to inflammasome, a large caspase-1 containing protein complex. Release of IL-1β may lead to the activation of NF-κB associated with inflammation [31]. Due to its potent chemoattractant, MCP-1 plays a role in the activator of monocytes and macrophages. IL-33 induced the secretion of MCP-1, which enhanced mast cell dependent recruitment of monocytes to lesions during allergic inflammation [32]. Here we further confirmed that the involvement of anti-inflammatory effect of BBR on IL-33-induced cytokine production, such as IL-6, TNF-α, IL-13, and MCP-1.

MCs play a critical role in the regulation of allergic responses through the release of biological mediators [33]. The ability of MCs to induce IL-33 release has been well established, where unstimulated-MCs possessed very low basal of IL-33 [34]. Increased-IL-33 production was induced by antigen-crosslinked IgE with FcεRI via a calcium-dependent pathway [35]. Meanwhile, combination of IL-33 and ST2 also promoted MC activation and maturation [36]. Binding to ST2, IL-33 triggered the downstream signaling events, such as MAPK (including ERK, JNK and p38), NF-κB, and Janus kinase 2 (JAK2) [37,38]. A recent

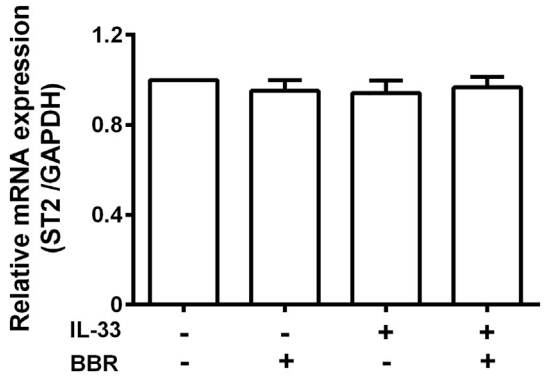

Fig. 3. ST2 receptor expression in the RPMCs following BBR application. Isolated RPMCs were stimulated with IL-33 (50 ng/mL) in the presence or absence of BBR (10 μM) for 24 h, and then the cells were collected. ST2 receptor expression at mRNA level was measured by Real-time PCR. Results were presented from three experiments done in triplicate.

study showed that lactic acid suppressed IL-33-mediated mast cell inflammation via inhibiting phosphorylation of ERK, JNK, and NF-κB, but not p38 activation [39]. Using bone marrow-derived mast cells, TGF-β1 inhibited IL-33-activated Akt and ERK phosphorylation as well as NF-κB- and AP-1-regulated transcription, leading to the repression of IL-33-mediated mast cell function [40]. In the present study, BBR had little effect on ST2 expression, which reinforced our rationale to investigate the molecular mechanisms involved in IL-33 signaling pathway. Similar with the above studies, BBR curbed NF-κB translocation and p38 phosphorylation, suggesting that BBR was a potent inhibitor of IL-33-mediated inflammation in mast cells.

A previous study demonstrated that IL-33 signaling could enhance antigen-driven IgE-dependent MC degranulation and cytokine production in vitro. Importantly, the potentiating effect of IL-33 on MC degranulation was specific because it was blocked by neutralizing anti-ST2 mAb [41]. However, IL-33 by itself caused no MC degranulation [42]. Consistent with these reporters, the present study indicated that BBR evidently hampered the synergistic effects of IL-33 and IgE/antigen stimulation on cytokine production, suggesting BBR had the ability to suppress the synergistic responses. It was also worth noting that BBR injection i.p. into rats obviously inhibited IL-33-induced plasma cytokine levels in vivo, which further supported the anti-inflammatory effect of BBR in vitro. How to affect and regulate MC degranulation accompanying BBR treatment in IL-33 stimulation in vitro and in vivo, more studied were needed to explain and clarify the underlying details.

As we know, IL-33/ST2 cross-linking leads to the activation and recruitment of MyD88 adapter protein in cytoplasm, followed by the connection and activation of IL-1R-associated kinase1 (IRAK1), IRAK4, and TNFR-associated factor 6 (TRAF6) [43]. Subsequently, the signaling cascades further incur the transcriptional activation of NF-κB and MAPK (ERK, JNK and p38) phosphorylation, initiating and promoting the production of inflammatory mediators in mast cells [44,45]. Based on the background and our results in this study, we speculated that BBR treatment suppressed IL-33-induced NF-κB transcription and p38 MAPK activation in mast cells, coupled with subsequent inhibition

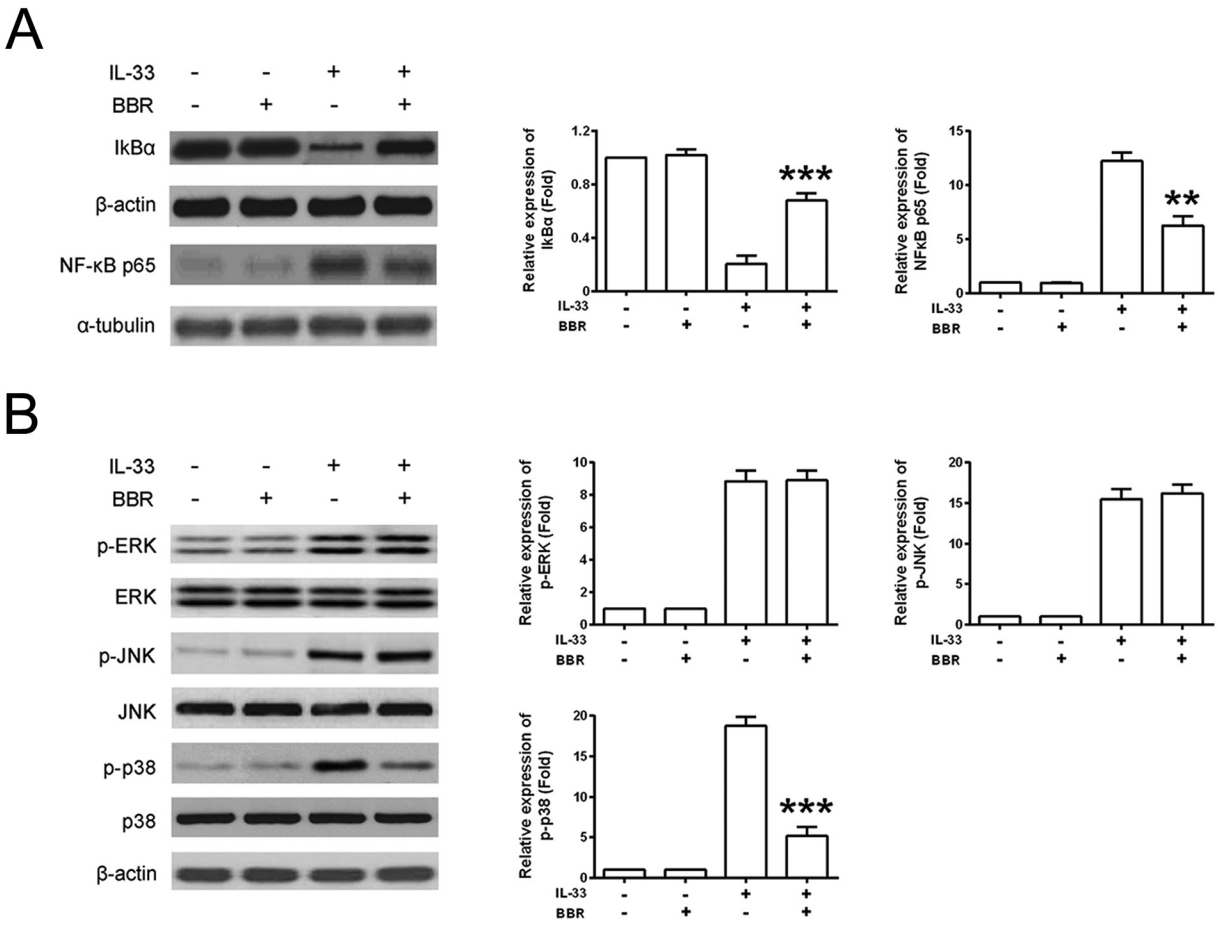

Fig. 4. BBR treatment inhibits IL-33 signaling in the RPMCs. RPMCs (1×10^5) were isolated and cultured with IL-33 (50 ng/mL) accompanying BBR (10 μM). After 24 h, the cells were collected and lysates were analyzed by Western blotting, including (a) the expression of cytosolic IκBα and nuclear NF-κB (p65) as well as (b) the expression of cytosolic ERK, p-ERK, JNK, p-JNK, p38 and p-p38. β-Actin and α-tubulin were used to confirm equal sample loading, respectively. Representative results were shown. $^{**}P < 0.01$ and $^{***}P < 0.001$, comparison with the IL-33-stimulated group without BBR treatment.

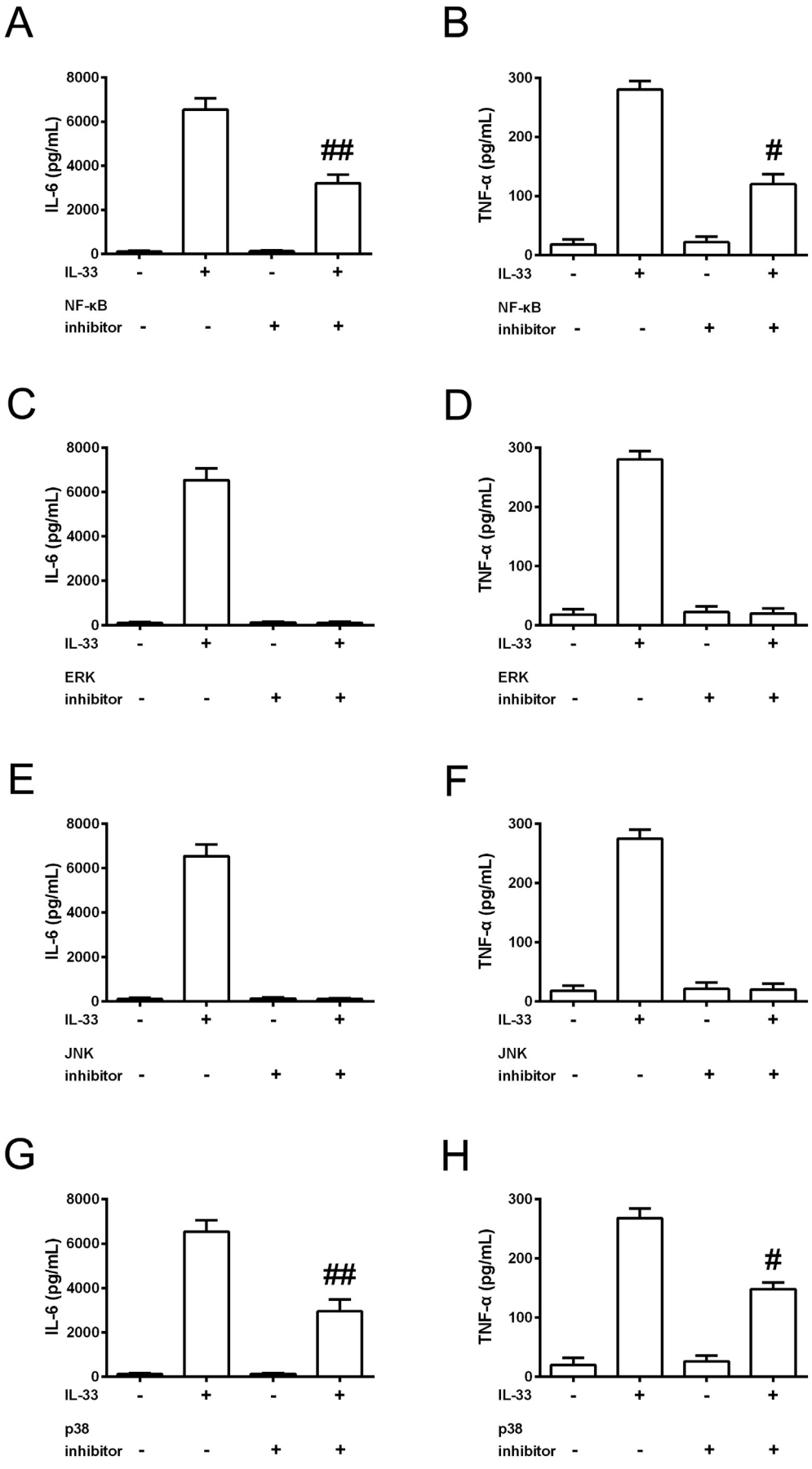

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Fig. 5. Signal transduction inhibitors reproduce berberine-regulated suppression on IL-33-induced mast cell inflammatory responses. RPMCs were pretreated with different signal transduction suppressors including (a) NF-κB inhibitor (BAY 11-7085, 5 μM), (b) ERK inhibitor (PD98059, 10 μM), (b) JNK inhibitor (SP600125, 10 μM) and (d) p38 inhibitor (SB203580, 10 μM) for 2 h. Then, the cells were activated with IL-33 (50 ng/mL) for 24 h and the supernatants were collected for ELISA analysis. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, comparison with the alone IL-33-stimulated group.

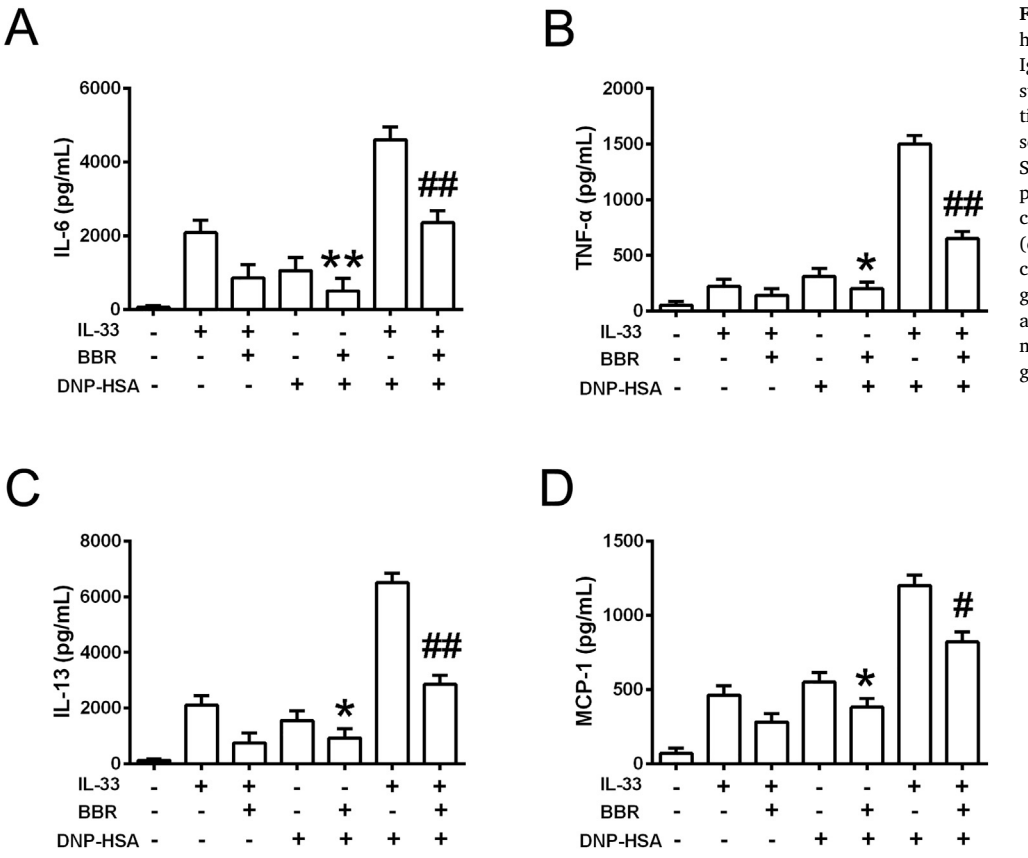

Fig. 6. BBR restrains IL-33-induced enhancement of IgE-mediated responses. After IgE sensitization overnight, RPMCs were stimulated with IL-33 (50 ng/mL) or antigen (DNP-HAS, 100 ng/mL) in the presence or absence of BBR (10 μM) for 24 h. Supernatants were collected and cytokine productions were analyzed by ELISA, including (a) IL-6, (b) TNF-α, (c) IL-13, and (d) MCP-1. $^{*}P < 0.05$ and $^{**}P < 0.01$, comparison with the antigen-stimulated group without BBR treatment. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, comparison with the simultaneous IL-33- and antigen-stimulated group without BBR treatment.

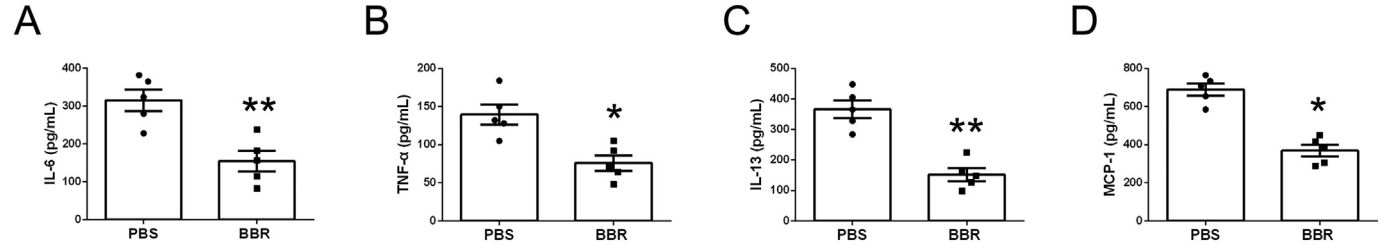

Fig. 7. BBR curbs IL-33-induced cytokine production in vivo. BBR (1 mg/kg) or the same volume PBS was injected i.p. daily into rats for continuous 1 week, separately. On the eighth day, IL-33 (5 μg) was injected i.p. to induce inflammatory cytokine production. After 6 h, rats were euthanized and blood was collected for cytokine analysis by ELISA, including (a) IL-6, (b) TNF-α, (c) IL-13, and (d) MCP-1. $^{*}P < 0.05$ and $^{**}P < 0.01$, comparison with PBS-treated group (n = 5).

of NF-κB p65 transcription and p38 phosphorylation into nucleus, resulting in the reduction of cytokine production, including IL-6, TNF-α, IL-13, and MCP-1 (Fig. 8).

5. Conclusions

In summary, our results demonstrated that the anti-inflammatory effect of BBR on IL-33-induced mast cell inflammation was regulated by the inhibition of IL-33/ST2 signaling and the subsequent downstream NF-κB and p38 pathway. Our findings suggest its potential of BBR as an alternative and promising drug for the treatment of allergic and inflammatory diseases.

Acknowledgments

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Conflict of interest statement

The authors declare no conflicts of interest.

Author contribution

Weihua Li, Nina Yin, and Zhigang Wang designed the study and wrote the manuscript, Weihua Li, Nina Yin, and Wenting Tao performed majorly the experiment and analyzed the data, Qian Wang and Hong Fan contributed to the work in vivo, Zhigang Wang helped revise

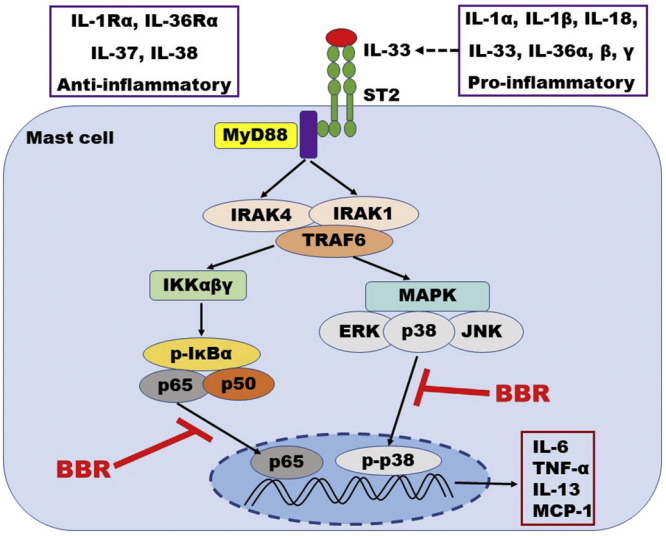

Fig. 8. Schematic diagram of proposed mechanisms for BBR on IL-33-induced mast cell inflammatory responses. BBR treatment suppressed IL-33-induced NF-κB activation and p38 MAPK phosphorylation. As a result, BBR markedly inhibited IL-33-stimulated inflammatory cytokine production including IL-6, TNF-α, IL-13, and MCP-1, suggesting its potent of berberine as a specific drug for the treatment of inflammatory diseases associated with IL-33-induced mast cell activation.

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