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ORIGINAL ARTICLE

NLRP3 inhibition attenuates the allergic rhinitis symptoms in a mouse model

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Abstract

Background: Recent human and animal studies have demonstrated that Nod-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome is closely involved in the development of allergic diseases.

Objective: To identify the mechanism underlying the activation of NLRP3 inflammasome signaling pathway in an ovalbumin (OVA)-induced allergic rhinitis (AR) mice model and to validate the effect of a specific inhibitor of the NLRP3, MCC950.

Methods: Mice were divided into three groups and each group consisted of ten mice (saline group, the negative control group; OVA group, the OVA-induced AR model group; and OVA+MCC group, treated with 10 mg/kg MCC950). MCC950 was administered intraperitoneally every second day. Multiple parameters of AR, including NLRP3, caspase-1, interleukin (IL)-1β, and IL-18 were evaluated by using ELISA, RT-qPCR, histopathology, and immunohistochemistry.

Results: The mRNA and protein levels of NLRP3, caspase-1, IL-1β and IL-18 were upregulated in the OVA group compared with those of the saline group. MCC950 significantly inhibited the mRNA and protein levels of NLRP3, caspase-1, IL-1β and IL-18 in nasal tissue. Further, AR symptoms and eosinophil count were normalized after MCC950 treatment. However, OVA-specific IgE was not restored in the OVA+MCC group.

Conclusions: NLRP3 inflammasome signaling pathway may be an alternative pathway to induce AR symptoms in OVA-induced AR model. MCC950 is a specific inhibitor of NLRP3 cascade, which attenuates AR symptoms regardless of IgE.

Key words: Allergic rhinitis, Animal models, Inflammasomes, MCC950, NLRP3 protein

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Introduction

Allergies, also known as allergic diseases, have become an important worldwide public health problem in recent decades, as their incidence has steadily increased and now they affect millions of people. Between 20% and 30% of residents in developed countries suffer from at least one type of allergy-related disease.[1] Additionally, 15% to 18% of the western population suffers from allergic rhinitis (AR).[2] Despite the advances in the understanding of the immune system in the last century, the physiological mechanisms underlying allergy development still remain unknown.[3]

Recent human and animal studies have demonstrated that Nod-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome is closely involved in the development of allergic diseases. NLRP3 inflammasome is a multiprotein complex known to be associated with numerous inflammatory diseases.[4] In response to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), NLRP3 acts as a pathogen recognition receptor and undergoes a conformational change to enable the assembly of NLRP3 inflammasome complex, which activates caspase-1. Then, active caspase-1 cleaves the precursors of the inflammatory cytokines interleukin (IL)-1β and IL-18,[5] thereby producing the corresponding mature products. IL-1β, a cytokine mainly secreted by macrophages, promotes the differentiation of T helper (Th)17 cells and the production of Th17-related cytokines, thereby accelerating allergic reactions.[6] On the other hand, IL-18 can induce T cells, mast cells, and basophils to secrete Th2 cytokines like IL-4 and IL-13, as well as enhance Th1 and Th2 cell-mediated immune reactions.[7] A recent study of the expression profile of AR patients revealed upregulation of type 2 inflammation involving IL-1β.[8] Moreover, elevated levels of IL-18 have been reported in nasal secretions of seasonal and persistent AR patients, further suggesting an association of IL-18 with AR.[9] Similarly, NLRP3 has also been found to be significantly upregulated in the nasal mucosal epithelium of AR patients, but downregulated once symptoms were controlled.[10] Finally, Oh et al. reported that IL-1β and caspase-1 were increased in the serum or nasal mucosa of ovalbumin (OVA)-induced AR mice compared to those in control mice. The administration of anti-allergic drugs to these mice reduced IL-1β and caspase-1 expression levels, as well as attenuated the allergic symptoms.[11] Altogether, these results highlight the involvement of NLRP3 in AR and the potential benefits of administering specific NLRP3 inflammasome pathway inhibitors, which have been already demonstrated by animal studies.[12]

IL-1β blockade using anti-IL-1β immunoglobulin (Ig) yolk has been suggested to be a promising strategy for preventing and treating AR.[13] The targeted inhibition of NLRP3 by microRNA-133b reduced the expression levels of caspase-1, apoptosis-associated speck-like protein containing a CARD (ASC), IL-1, and IL-18; the eosinophil and mast cell infiltration in the nasal mucosa, and pathological alterations in AR mice.[14] In line with this, treatment with the specific caspase-1 inhibitor Belnacasan ameliorated the development and progression of AR in an OVA-induced AR mice model.[15] Therefore, these compounds may represent potential therapeutic methods for the management of clinical allergic disorders.[16]

MCC950 is a potent, selective, small-molecule inhibitor of NLRP3 that blocks canonical and noncanonical NLRP3 activation.[17] MCC950 directly interacts with the Walker B motif within the NLRP3 NACHT domain, blocking ATP hydrolysis and inhibiting NLRP3 activation and inflammasome formation.[18] In a mice model of severe, steroid-resistant asthma, inhibiting NLRP3 with MCC950 had more potent effects than inhibiting caspase-1, suggesting that inhibiting NLRP3 directly, instead of targeting inflammasome-mediated caspase-1 or IL-1β activation, may have additional therapeutic benefits.[19]

On the basis of the above results, we hypothesized that directly inhibiting NLRP3 in AR might present a better therapeutic outcome than inhibiting caspase-1, IL-1β, or IL-18. In this study, we investigated the effects of MCC950 in an OVA-induced AR mice model.

Materials and Methods

Animal model

Thirty female BALB/c mice (4 weeks of age) that weighed 19-21 g were purchased from Koatech (Pyeongtaek, Korea). All animals were maintained under specific pathogen-free conditions. All animal experiments in the present study followed the guidelines and ethics of the Institutional Animal Care and Use Committee of the Biomedical Research Institute of Seoul National University Hospital (IACUC No.: 18-0026-S1A0(3)) and animals were maintained in the facility accredited AAALAC International (#001169) in accordance with Guide for the Care and Use of Laboratory Animals 8th edition, NRC (2010). This study was reported in accordance with ARRIVE guidelines. The AR mice model was induced by OVA as previously described.[20] Mice were divided into three groups as follows: the saline group (n = 10) as the negative control group, the OVA group (n = 10) as the positive control group, and the OVA+MCC group (n = 10) as the treatment group. The mice were sensitized by an intraperitoneal injection of a mixture containing 25 µg of OVA (Sigma-Aldrich, St. Louis, MO, USA) and 1 mg of aluminum hydroxide (Alum; ThermoFisher Scientific, Waltham, MA, USA) on days 0, 7, and 14. The mice were then subjected to intranasal challenges with 100 µg of OVA on 7 consecutive days, from days 21 to 27. The negative control mice were intraperitoneally injected and intranasally challenged with phosphate-buffered saline (PBS) instead of OVA following the same schedule until day 27. Mice in the OVA+MCC group were intraperitoneally injected with 10 mg/kg of MCC950 (AdipoGen, San Diego, CA, USA) every second day from days 21 to 27 immediately after intranasal OVA challenge, with reference to previous study.[16] The saline group and OVA group mice were intraperitoneally injected with PBS in the same manner (**Supplementary Figure 1**).

Symptom score

On day 27, after intranasal allergen provocation with 100 µg of OVA, the numbers of sneezing and nose rubbing bouts were counted for 15 minutes to evaluate early allergic responses by blinded observers as previously described.[20]

Tissue preparation

Serum samples from each mouse were obtained under isoflurane anesthesia 24 h after the last OVA challenge. Then the mice were euthanized (Cervical dislocation after carbon dioxide inhalation). Spleens were harvested for cell culture. The heads of five mice from each group were removed en bloc and then fixed in formaldehyde solution. Nasal mucosa was obtained from the other five mice, and then was immediately immersed in liquid nitrogen and stored at −70°C until use for RNA extraction.

Quantitative reverse transcription PCR (RT-qPCR) analysis of cytokines in the nasal mucosa

Total RNA was isolated from the nasal mucosa using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized using a cDNA synthesis kit (Gendepot, Katy, TX, USA). For the analysis of the expression levels of *NLRP3* (Mm00840904_m1), *caspase-1* (Mm00438023_m1), *IL-1β* (Mm00434228_m1), *IL-18* (Mm00434226_m1), *IL-4* (Mm00445259_m1), *IL-5* (Mm00439646_m1), *IL-10* (Mm00439616_m1), *IL-13* (Mm00434204_m1), *interferon (IFN)-γ* (Mm99999071_m1) and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) (Mm99999915_g1), the corresponding probes were purchased from ThermoFisher Scientific. The cDNA for these transcripts was amplified in MicroAmp optical 96-well reaction plates (Applied Biosystems, Foster City, CA, USA) using a QuantStudio™ 3 RT-PCR System (Applied Biosystems). The average transcript levels were normalized to those of *GAPDH* and expressed as ΔΔCT.

Nasal histology and immunohistochemistry

For the histological evaluation of nasal tissue, the heads of mice in each group were fixed with 10% formaldehyde solution. The nasal tissues were decalcified with ethylenediaminetetraacetic acid solution, embedded in paraffin, sectioned coronally into 4-µm slices, and stained with hematoxylin and eosin. The number of eosinophils was counted in five fields of the nasal septal mucosa by a single-blind observer using alight microscope (×400 magnification).

Immunohistochemistry was performed using anti-NLRP3 (#ab214185; Abcam, Cambridge, UK), anti-caspase-1 (#ab138483; Abcam), anti-IL-1β (#12242; Cell signaling, Danvers, MA, USA), and anti-IL-18 (#ab71495; Abcam) antibodies. The total area and the stained area were measured in four fields of the nasal septal mucosa. The percentage of the stained area was calculated using ImageJ software.

Serum levels of total and OVA-specific immunoglobulins

Serum samples from each mouse were obtained at the time of sacrifice. Serum levels of total IgE and OVA-specific IgE, IgG_1 and IgG_{2a} were measured using enzyme-linked immunosorbent assay (ELISA). For the analysis of total IgE, 96-well flat-bottom plates were coated with purified rat anti-mouse IgE (#553413; BD Bioscience, San Jose, CA, USA). A purified mouse IgE isotype (#557079; BD Bioscience) was used to construct the standard curve. Nonspecific antigen-antibody reactions were blocked with 3% bovine serum albumin. To detect total IgE, horseradish peroxidase (HRP)-conjugated anti-mouse Ig (#1130-05; SouthernBiotech, Birmingham, AL, USA) was added to the wells. For the analysis of OVA-specific IgE, serum samples were added to OVA-coated 96-well flat-bottom plates. After washing, 100 µL of biotin-conjugated rat anti-mouse IgE mAb (#553414; BD Bioscience) were added, followed by the addition of streptavidin-HRP (#554066; BD Bioscience). For the analysis of OVA-specific IgG_1 and IgG_{2a}, serum samples were added to OVA-coated 96-well flat-bottom plates. After washing with 0.05% tween20-PBS, biotinylated rat anti-mouse IgG_1 and IgG_{2a} (#553341 and #553388, respectively; BD Bioscience) were added to each well and incubated at room temperature for 1 h, washed, and streptavidin-HRP was added to each well. The reactions were developed using 3,3′,5,5′-tetramethylbenzidine SeraCare, Milford, MA, USA) and terminated by adding 1 M HCl. The absorbance was measured using a microplate reader at 450 nm.

Measurement of cytokine levels in the spleen cell culture

Spleen single-cell suspensions from each mouse were plated in 24-well cell culture plates at a final concentration of 5×10^6 cells/well using Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum and supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco, Grand Island, NY, USA). The cells were incubated in a CO_2 incubator at 37°C for 72 h and stimulated with OVA for 72 h. The culture supernatant was collected and stored at −70°C until cytokines were measured. Cytokine levels in the culture supernatant were assayed using a DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. After measuring the absorbance at 450 nm, the concentrations of IL-4, IL-5, IL-10 and IFN-γ were determined by interpolation from the respective standard curves. All data were expressed in pg/mL.

Statistics

The data are presented as mean ± standard error mean. Mann-Whitney U test was used to compare results between the negative and positive control groups and between the treatment and positive control groups. A P value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS 22.0 software (IBM Co., Armonk, NY, USA) and GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA).

Results

Symptom score

The mean numbers of nose rubbing, sneezing, and sum of symptoms in the OVA group (positive control) were significantly higher than those in the saline group (negative control) ($p = 0.0003$, $p < 0.0001$, and $p = 0.0001$, respectively). Mice in the OVA+MCC group (MCC950 treatment group) showed significantly smaller numbers of nose rubbing, sneezing, and sum of symptoms than those in the OVA group ($p = 0.0047$, $p < 0.0001$, and $p = 0.0184$, respectively) (**Figure 1**).

Levels of total and OVA-specific immunoglobulins in the serum and levels of IL-4, 5, 10, and IFN-γ in the spleen supernatant

According to the serum ELISA results, the OVA group presented significantly higher levels of total IgE, OVA-specific IgE, IgG_{2a}, and IgG_1 levels than those of the saline group, confirming the successful establishment of the AR mice model ($p < 0.0001$, $p < 0.0001$, $p = 0.0015$, and $p = 0.0011$, respectively). These increases were not attenuated by intraperitoneal MCC950 treatment ($p = 0.9766$, $p = 0.8534$, $p = 0.1333$, and $p = 0.7394$, respectively) (**Figure 2**). Analysis of spleen supernatant using ELISA

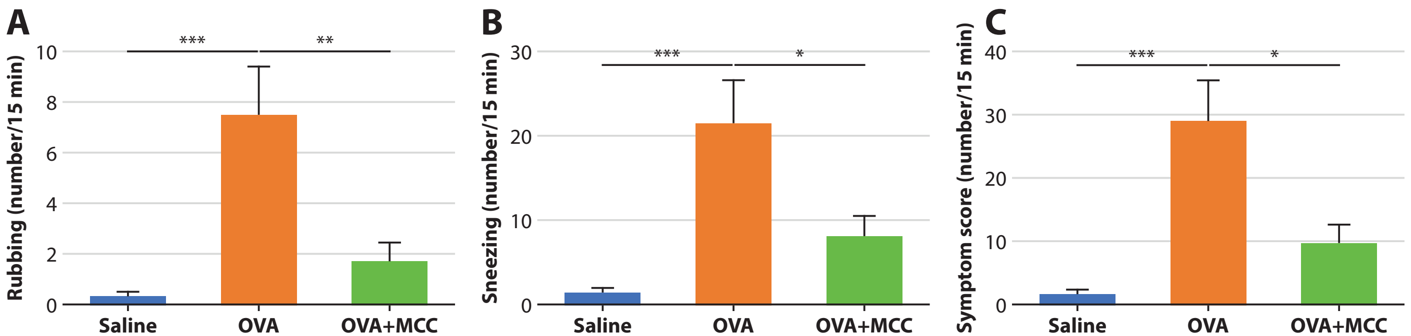

Figure 1. Symptom score calculated from the frequencies of nasal rubbing and sneezing. (A, B) The number of nasal rubbing and sneezing among three groups for 15 minutes. (C) The symptom score is the sum of the number of nasal rubbing and the number of sneezing. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

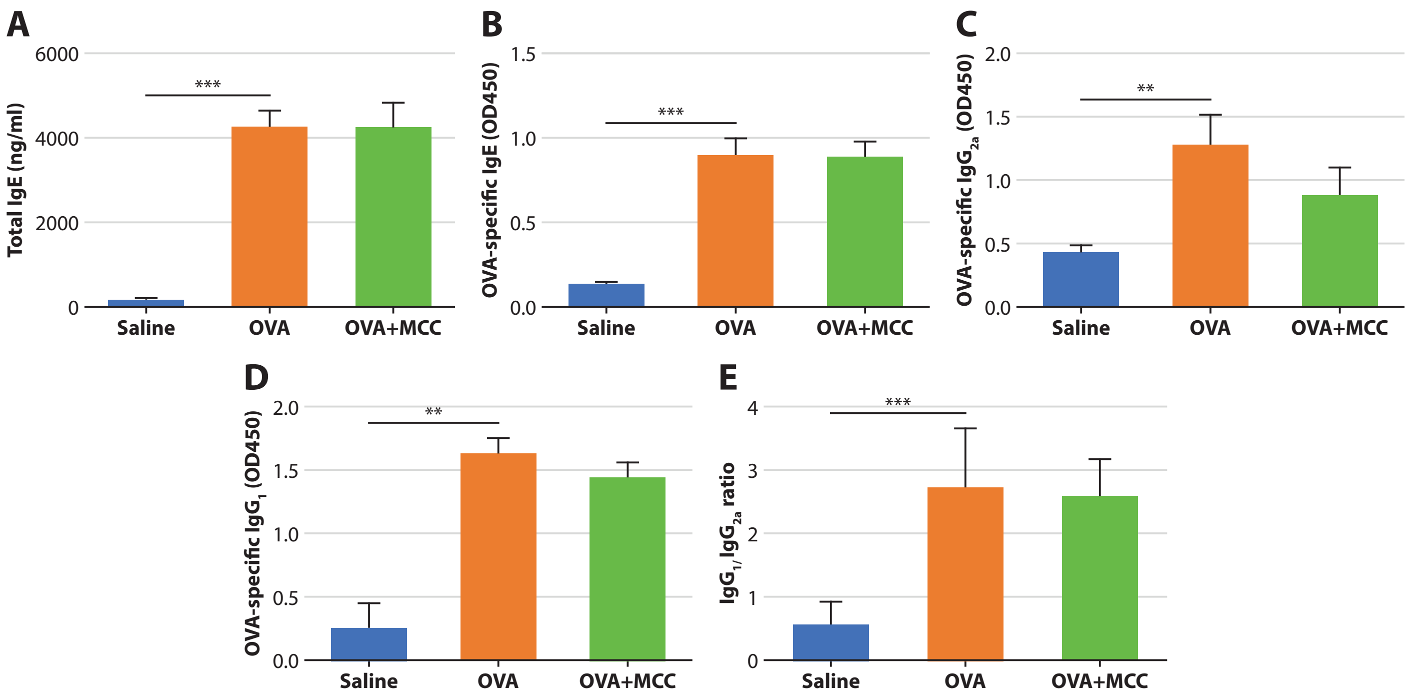

Figure 2. (A-D) Serum levels of total IgE, ovalbumin (OVA)-specific IgE, OVA-specific IgG_{2a}, and OVA-specific IgG_1 measured using an enzyme-linked immunosorbent assay. (E) OVA-specific IgG_1/IgG_{2a} ratio was calculated to identify the balance between T helper 1 and 2 cells. The increases of total IgE, OVA-specific IgE, IgG_{2a}, IgG_1 levels and IgG_1/IgG_{2a} ratio in the OVA group were not attenuated by MCC950 treatment. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

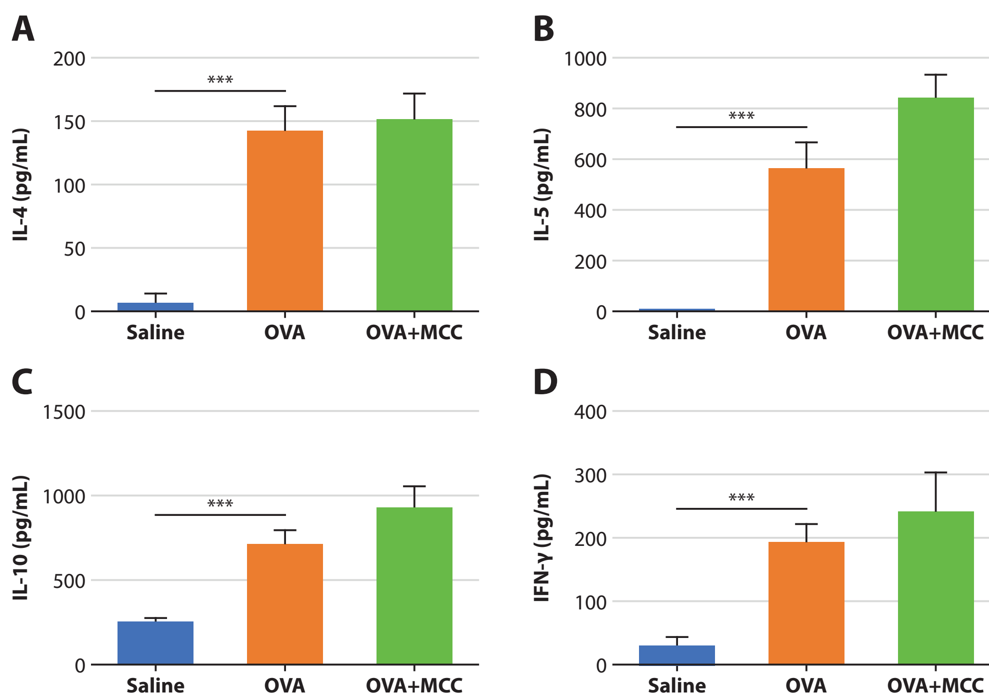

Figure 3. Systemic cytokine levels of interleukin (IL)-4, IL-5, IL-10, and interferon (IFN)-γ in the spleen cell culture measured using an enzyme-linked immunosorbent assay. The increases of IL-4, 5, 10, and IFN-γ levels in the OVA group were not attenuated by MCC950 treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

showed that the OVA group presented higher levels of IL-4, 5, 10, and IFN-γ than those of the saline group (p = 0.0005, p = 0.0008, p = 0.0002, and p = 0.0006, respectively), which were not restored with intraperitoneal MCC950 treatment (p = 0.8665, p = 0.0721, p = 0.2319, and p = 1.0000, respectively) (**Figure 3**).

Expression levels of NLRP3, caspase-1, IL-1β, IL-18, IL-4, IL-5, IL-10, IL-13, and IFN-γ transcripts in the nasal tissue

NLRP3, caspase-1, IL-1β, and IL-18 mRNA levels were significantly elevated in nasal tissues from the OVA group (p = 0.0095, p = 0.0303, p = 0.0079, and p = 0.0079, respectively) and significantly reduced in the OVA+MCC group (p = 0.0286, p = 0.0357, p = 0.0079, and p = 0.0159, respectively) compared with those in the saline group (**Figure 4A-D**). Additionally, IL-4 and IL-5 mRNA levels were upregulated in the OVA group compared with those in the saline group (p = 0.0025, and p = 0.0159, respectively), and tended to be downregulated after MCC950 treatment, although this reduction was not significant (p = 0.1508, and p = 0.4206, respectively) (**Figure 4E, F**). Additionally, IL-10 and IL-13 mRNA levels also tended to increase in the OVA group and decrease to normal levels in the OVA+MCC without statistical significance (**Figure 4G-I**). To further investigate the effect of OVA and MCC950 treatment in maintaining Th2/Th1 balance, the IL-4/IFN-γ and IL-5/IFN-γ ratios were quantified (**Figure 4J, K**). Both ratios were significantly increased in the OVA group (p = 0.0159, and p = 0.0159, respectively), and decreased in the OVA+MCC group (p = 0,0286, and p = 0.0286, respectively) compared with those of the saline group.

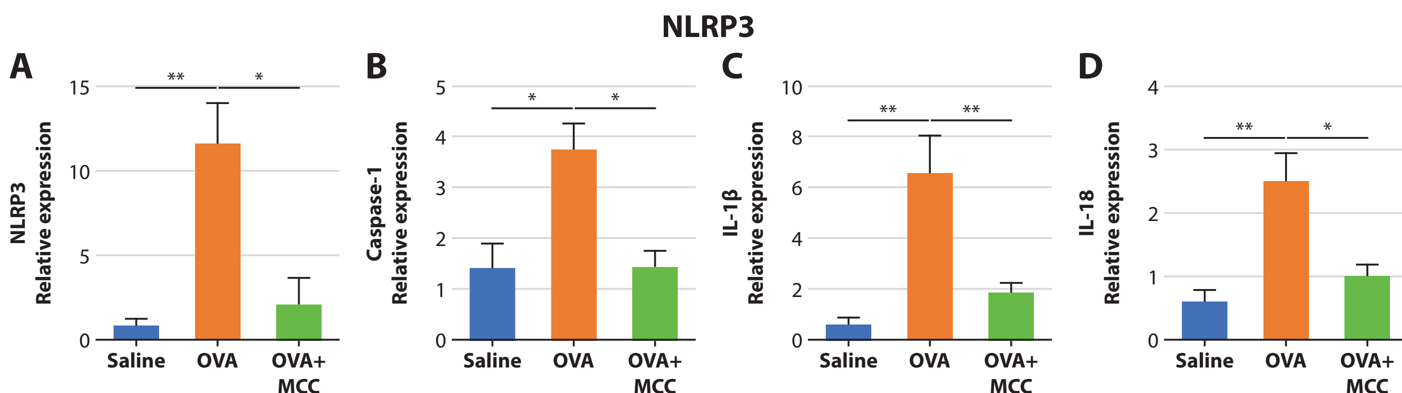

Figure 4. (A-I) mRNA level of nasal cytokines measured using quantitative reverse transcription PCR (RT-qPCR). (J-K) Interleukin (IL)-4 or 5/IFN-γ ratios were calculated to identify the balance between T helper 1 and 2 cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Th2

E
IL-4 Relative expression
0 10 20 30 40 50
**
Saline OVA OVA+ MCC

F
IL-5 Relative expression
0 10 20 30 40 50
*
Saline OVA OVA+ MCC

G
IL-13 Relative expression
0 20 40 60 80
Saline OVA OVA+ MCC

Th1

H
IFN-γ Relative expression
0.0 0.5 1.0 1.5 2.0
Saline OVA OVA+ MCC

Treg

I
IL-10 Relative expression
0 1 2 3 4
Saline OVA OVA+ MCC

Th2/1 balance

J
IL-4 / IFN-γ ratio
0 20 40 60 80
* *
Saline OVA OVA+ MCC

K
IL-5 / IFN-γ ratio
0 1 2 3 4
* *
Saline OVA OVA+ MCC

Figure 4. (Continued)

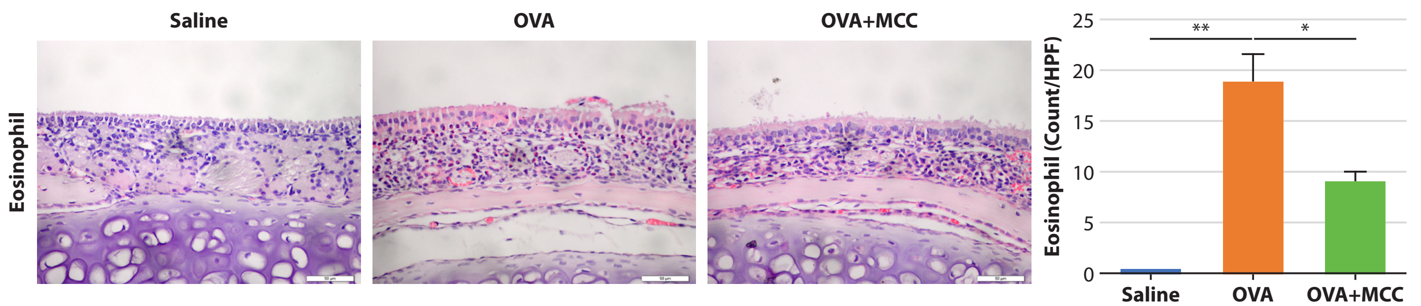

Figure 5. Nasal histology stained with hematoxylin and eosin (200× magnification). Eosinophil infiltration in the nasal septal mucosa was quantified in the ovalbumin (OVA) group and the OVA+MCC group compared with that of the saline group. Eosinophil count was reduced after treatment with MCC950. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

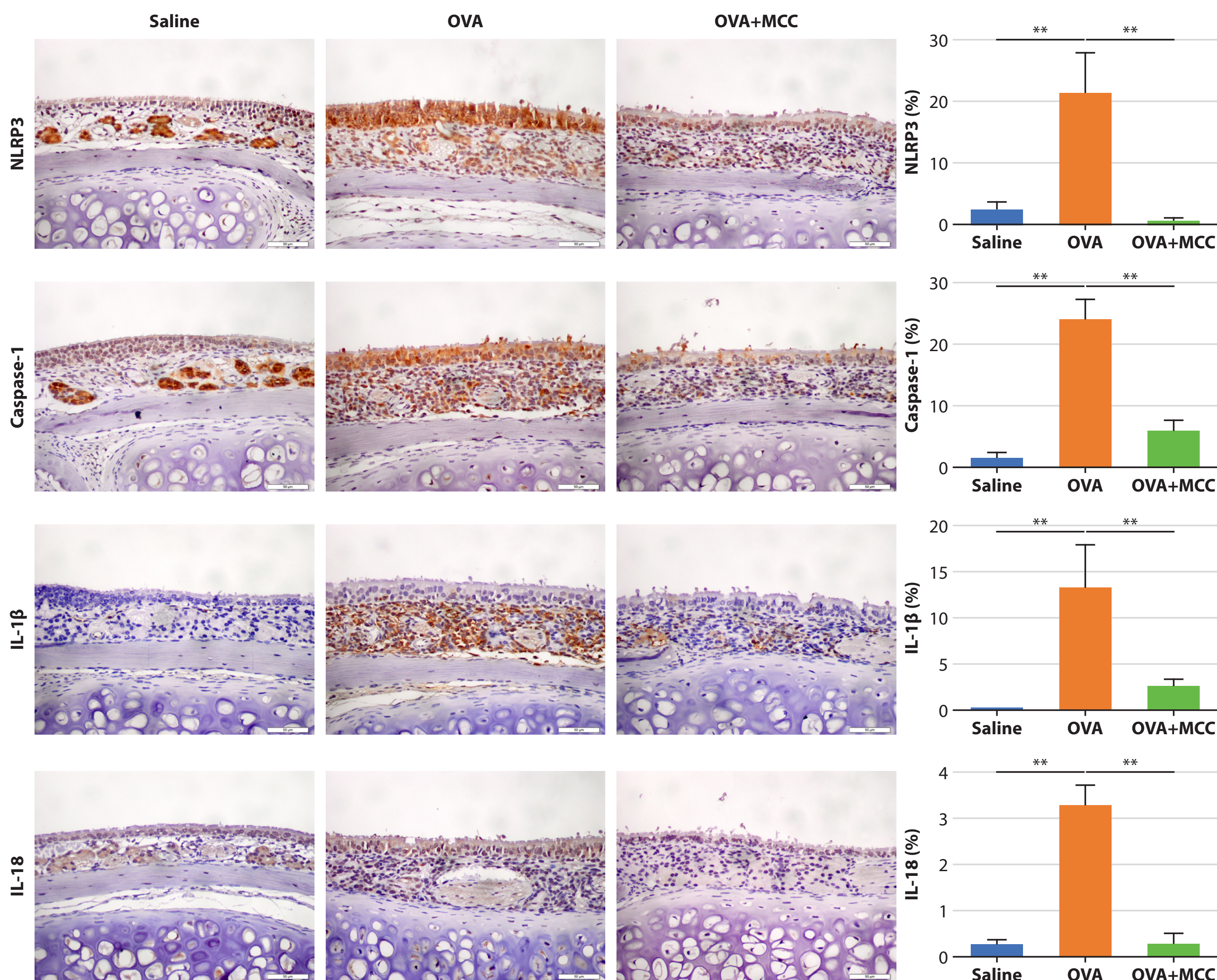

Figure 6. Immunohistochemical staining of nasal septal tissues using anti-NLRP3, anti-caspase-1, anti-IL-1β, and anti-IL-18 antibodies (200× magnification). The proportion of the stained area was calculated using ImageJ software. The stained areas of NLRP3, caspase-1, IL-1β, and IL-18 increased in the ovalbumin (OVA) group, and were significantly reduced after MCC950 treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Immunohistochemistry and histological evaluation of the nasal tissues

Histological analysis revealed that the OVA group presented an increased infiltration of eosinophils compared with that of the saline group. However, eosinophil infiltration was significantly reduced in the OVA+MCC group (p = 0.0049, and p = 0.0160, respectively) (**Figure 5**). Additionally, immunohistochemical staining of nasal tissues revealed that NLRP3, caspase-1, IL-1β and IL-18 protein levels were upregulated in the OVA group compared with those in the saline group (p = 0.0025, p = 0.0043, p = 0.0025, and p = 0.0022, respectively). Nonetheless, the expression level of these proteins was significantly diminished after MCC950 treatment, indicating that MCC950 efficiently inhibited the NLRP3 inflammasome pathway (p = 0.0079, p = 0.0043, p = 0.0087, and p = 0.0022, respectively) (**Figure 6**).

Discussion

AR is an allergic disorder characterized by nasal obstruction, nasal pruritus, rhinorrhea, and sneezing. It is caused by the inhalation of allergens, which trigger an IgE-mediated inflammatory reaction mediated by allergen-specific Th2 cells. Th2 cells activate B cells and induce IgE class switching, which in turn induce the activation and degranulation of mast cells and basophils, which cause the acute AR symptoms. Further, activated Th2 cells produce IL-4, IL-5, and IL-13, which favors eosinophils infiltration. However, much is still unknown about the mechanisms of AR development and further investigations are required.[19,21] NLRP3 inflammasome activation has been reported in diverse infectious and nonmicrobial diseases, including gout, autoimmune diseases, Alzheimer's disease,

and diabetes.[22] Additionally, it has also been reported in allergic diseases such as asthma, dermatitis, rhinitis, and conjunctivitis.[7] Currently, it is known that NLRP3 forms part of the innate immune response. Inflammatory stimuli such as PAMPs and DAMPs activate monocytes and macrophages, which lead to NLRP3 inflammasome assembly, which in turn activates pro-IL-1β and pro-IL-18. The molecular mechanism of NLRP3 inflammasome assembly is still uncertain. To date, three potential mechanisms have been reported, including changes of K^+ influx/efflux, Ca^{2+} induced lysosomal damage, and production of reactive oxygen species.[5]

A recent study reported results similar to those of the present study. Zhang et al. reported that intranasal administration of MCC950 had a beneficial effect in an OVA-induced AR mouse model, reducing the allergic symptoms and the mRNA and protein levels of cytokines to the levels achieved by dexamethasone treatment.[23] In this study, however, the MCC treatment failed to reduce the total IgE, OVA-specific IgE, OVA-specific IgG_1, and OVA-specific IgG_{2a} levels in serum, and the systemic levels of cytokines including IL-4, IL-5, IL-10, and IFN-γ as assessed in spleen supernatant. However, the treatment tended to reduce the mRNA levels of IL-4, IL-5, IL-10, and IFN-γ in nasal tissue. Local, rather than systemic, downregulation of Th1, Th2, and regulatory T cell reactions was obvious after intraperitoneal administration of MCC950 in this study. Additionally, in line with our results, Yang et al. also reported that the activation of NLRP3 inflammasome signaling pathway promoted the development of AR.[15] In their study, nasal lavage fluid and nasal mucosal tissue from AR patients were evaluated, and caspase-1, IL-1β and IL-18 were found to be upregulated. Additionally, they constructed an OVA-induced AR mouse model, in which upregulated IL-1β was found in nasal lavage fluid and nasal tissue. Moreover, NLRP3 knockout (KO) mice were induced by OVA. NLRP3-KO OVA-induced AR mice exhibited decreased AR symptoms, levels of caspase-1, and proinflammatory cytokines and chemokines such as IL-1β compared with those of the WT OVA-induced AR mice group. However, no difference was detected in the serum IgE level between the two groups. Treatment with the caspase-1 inhibitor Belnacasan lead to the attenuation of AR symptoms, and reduced the protein levels of caspase-1 and IL-1β and the mRNA levels of cytokines and chemokines in nasal tissue. Nonetheless, serum IgE level was not restored after Belnacasan treatment. In another study, Xiao et al. reported that upregulation of microRNA-133b suppressed NLRP3 inflammasome activation. MicroRNA-133b directly targeted NLRP3, reduced AR symptoms, serum OVA-specific IgE, the levels of cytokines such as IL-4, 5, and IFN-γ from cervical lymph nodes cell suspensions and serum, and the mRNA level of AR cytokines in nasal mucosa tissue. Immunohistochemistry analysis revealed that microRNA-133b overexpression reduced the protein expression levels of NLRP3, caspase-1, ASC, IL-1β, and IL-18.[14] In conclusion, it is still unclear whether the NLRP3 pathway is associated with the mounting of an IgE-mediated reaction or not in OVA-induced AR mice models.[14,15,23] Classical type I hypersensitivity in AR is induced by mast cells and basophils cross-linked with antigen-specific IgE. An IgE-independent response in AR can result from the induction of mast cell/basophil-independent histamine release due to endotoxin-containing allergen exposure.[24] The application of endotoxin-containing OVA triggered type 1 hypersensitivity-like symptoms in mice, which were dependent on CD4 T cell, histamine, and monocytes/macrophages. Monocytes and macrophages are considered the major effector cells in IgE-mediated responses and key elements in mast cell/basophil-independent nasal hypersensitivity. In addition, a severe, steroid-resistant asthma mice model induced by *Chlamydia* and *Haemophilus* infection presented upregulation of NLRP3 inflammasome, caspase-1, and IL-1β, as well as infiltration of neutrophils and macrophages into the airways without eosinophilia.[18] This suggests that the mechanism of NLRP3-related allergic reaction may be associated with IgE-independent monocyte/macrophage-dominant hypersensitivity, rather than with the establishment of a classical allergic reaction.

In this study, local Th2 cells tended to be suppressed after inhibition of NLRP3, but local Th1 cells did not. This was unexpected, as we hypothesized that NLRP3 might be closely linked with Th1-related inflammation. In line with the effect observed, previous IgE-independent AR models, such as lipopolysaccharides- or double stranded DNA or RNA -induced rhinitis mice models, have showed IL-17 dominancy, which can explain the possible correlation between NLRP3 and Th17 rather than that with Th1.[25,26] The role of IL-17 in allergic rhinitis had recently been elucidated by recruiting neutrophils,[27] and IL-17 is known to be associated with the severity of allergic rhinitis.[28] In the future, additional studies are required to assess the correlation between NLRP3 and IgE-independent, Th17-related allergic reaction, with optimized methods.

Finally, the findings of this study should be considered under the light of its limitations. The main limitation was that the method of administration and dose of MCC950 were not very effective. MCC950 was administered intraperitoneally at the dose reported in previous studies.[16,17] For this reason, MCC950 was applied through an intraperitoneal route in this study, when was before referring to the later published study by Zhang et al.[23] Further study is necessary to elucidate which route is more effective to activate NLRP3 pathway. Additionally, the small sample size of the study may have influenced the results. And this AR model showed some different mechanisms compared to conventional AR model. Because it may be a different allergic mechanism in real world, further study is necessary to elucidate it. In conclusion, the conventional OVA-induced AR mice model was associated with the activation of NLRP3 inflammasome signaling pathway. Specific inhibition of NLRP3 by MCC950 treatment attenuated NLRP3, caspase-1, IL-1β and IL-18, but did not restore OVA-specific IgE elevation.

Conflict of interest

none

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Author contributions

- Conceptualization: ML, Y-LZ, S-WC, DWK, HJK, CSR.
- Data curation: ML, Y-KK, CHG, KME.
- Formal analysis: ML, Y-KK, CHG, KME, SWC, DWK.
- Funding acquisition: ML, S-WC, C-SR.
- Methodology: ML, Y-LZ, S-WC, DWK. HJK. CSR.
- Project administration: ML.
- Visualization: ML, Y-KK, CHG, KME.
- Writing – original draft: ML, Y-KK, S-WC.
- Writing – review & editing: ML, Y-KK, Y-LZ, S-WC, DWK, HJK, C-SR.

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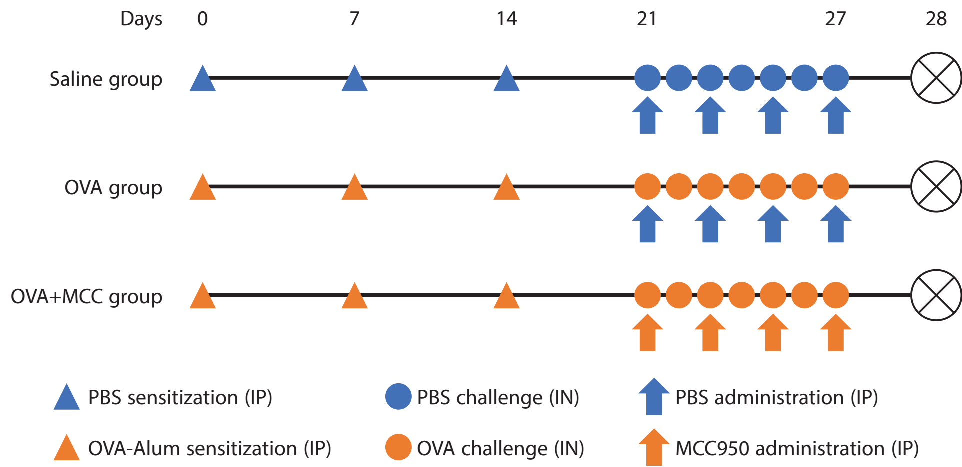
Supplementary Material



Supplementary figure 1. Experimental protocol for the ovalbumin (OVA)-induced rhinitis model and MCC950 treatment.