Allergy, Asthma & Immunology Research

AAIR

Original Article

Check for updates

Activation of Dopamine D2 Receptor Alleviates Neuroinflammation in a Mouse Model of Allergic Rhinitis With Olfactory Dysfunction

Peiqiang Liu [ID],[1,2] Danxue Qin [ID],[1,2] Hao Lv [ID],[1,2] Wenjun Fan [ID],[1,2] Fangwei Zhou [ID],[1,2] Ziang Gao [ID],[1,2] Zezhang Tao [ID],[1,2] Yu Xu [ID] [1,2*]

[1]Department of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan, China
[2]Research Institute of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan, China

OPEN ACCESS

Correspondence to
Yu Xu, MD
Department of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuhan 430060, China.
Tel: +86-027-88041911
Fax: +86-027-88041911
E-mail: xuy@whu.edu.cn

ORCID iDs
Peiqiang Liu [ID]
https://orcid.org/0000-0002-4479-1583
Danxue Qin [ID]
https://orcid.org/0000-0001-5776-8596
Hao Lv [ID]
https://orcid.org/0000-0003-0890-6862
Wenjun Fan [ID]
https://orcid.org/0000-0003-2222-1413
Fangwei Zhou [ID]
https://orcid.org/0000-0002-2984-1263

ABSTRACT

Purpose: Allergic rhinitis (AR) is a common otolaryngology disease and one of the clinical causes of olfactory dysfunction (OD). The olfactory bulb serves as a transfer station for olfactory information transmission, and alleviating its neuroinflammation may be expected to improve AR-induced OD. Recent studies have suggested that the dopamine D2 receptor acts as a key target in regulating immune functions and neuroinflammatory reaction. However, the effect of dopamine D2 receptor on AR-induced neuroinflammation is still unknown.
Methods: An AR mouse model with OD induced by ovalbumin were constructed. The buried food pellet test was to evaluate the olfactory function of the mice. Immunofluorescence staining, hematoxylin and eosin staining, enzyme-linked immunosorbent assay and western blotting were also used to investigate the molecular mechanisms underlying the anti-inflammatory effects of the dopamine D2 receptor in AR-induced OD.
Results: We found that AR-induced OD has a relationship with inflammatory responses in the olfactory bulb. Nasal administration of quinpirole (Quin, a dopamine D2 receptor agonist, 3 mg/kg) improved olfactory function in mice, inhibited the expression of toll-like receptor 4 (TLR4)/nuclear factor-κB (NF-κB) signalings and the levels of tumor necrosis factor-α, interleukin (IL)-1β and IL-6 in the olfactory bulb. *In vitro*, Quin (20 μmol/L) inhibited the release of TLR4/NF-κB signalings-dependent inflammatory cytokines in cultured microglia.
Conclusions: Activation of the dopamine D2 receptor inhibits the release of inflammatory cytokines through TLR4/NF-κB signaling in the olfactory bulb microglia, and protects olfactory function.

Keywords: Allergic rhinitis; dopamine D2 receptor; microglia; inflammation

INTRODUCTION

Allergic rhinitis (AR) is a common clinical otorhinolaryngology disease, which has a huge impact on public health. Olfactory dysfunction (OD) is one of the symptoms of AR, and its mechanism is not fully understood.[1,2] Recent studies have shown that airway allergen exposure may damage the brain's related functions.[3-5] Th2-type cytokines are produced in the prefrontal cortex and

Allergy, Asthma & Immunology Research
AAIR

Ziang Gao
https://orcid.org/0000-0002-7952-5058
Zezhang Tao
https://orcid.org/0000-0002-5404-4186
Yu Xu
https://orcid.org/0000-0001-7751-6345

olfactory bulb of AR mouse model,[5] and tumor necrosis factor (TNF)-α and interleukin (IL)-6 are up-regulated in the hippocampus.[6] In addition, nasal corticosteroids inhibit the activation of microglia and the release of inflammatory mediators in the hippocampus and the prefrontal cortex caused by allergic airway inflammation,[7] reduced the expression of neuronal nitric oxide synthase in the olfactory bulb,[8] and helps reduce the loss of neurons. Therefore, the olfactory bulb serves as a transfer station for olfactory information transmission, and alleviating its neuroinflammation may be expected to improve AR-induced OD.

Dopamine D2 receptor, a member of the rhodopsin-like heptahelical receptor family, can be used as an important bridge molecule to connect the nervous and immune system.[9] Increasing evidence indicates a protective role for dopamine D2 receptor in regulating immune functions and inflammatory reaction.[10] For example, dopamine D2 receptor agonist LY171555 inhibited the activation of NLR family pyrin domain containing 3 (NLRP3) inflammasomes in the substantia nigra of Parkinson's disease (PD) mice, thereby reducing MPTP-induced neurons loss.[11,12] In animal models of ischemic stroke, activation of dopamine D2 receptor reduced the neuroinflammatory response through αβ-crystallin.[13-15] In allergic encephalomyelitis, dopamine D2 receptor agonist inhibited dendritic cell-mediated Th17 cell differentiation, and alleviates the occurrence of encephalomyelitis and quadriplegia.[16] In addition, injecting dopaminergic neurotoxins into the olfactory bulb produced olfactory deficits, and induced a decrease in the number of dopaminergic neurons in the olfactory bulb, which were reversed by dopamine receptor agonists.[17,18] Furthermore, dopamine D2 receptor is the main subtype of dopamine receptor in the olfactory bulb.[19] Therefore, we speculate that dopamine D2 receptor may be a potential therapeutic target for AR-induced OD.

Numerous studies have found that the toll-like receptor 4 (TLR4)/nuclear factor-κB (NF-κB) pathway in microglia is a key link in inducing neuroinflammatory damage.[20-22] In the brain, TLR4 is mainly expressed in microglia and can be specifically recognized by lipopolysaccharide (LPS). When TLR4 is triggered by LPS, the downstream protein NF-κB of the TLR4 signaling pathway is activated to release inflammatory cytokines. Previous studies have shown that inhibition of TLR4 signaling pathway can reduce neuroinflammation.[23] Based on the above studies, we speculate that dopamine D2 receptor may inhibit the TLR4/NF-κB pathway in microglia, thereby protecting olfactory function.

MATERIALS AND METHODS

Establishment of the AR model with OD

The 6- to 8-week-old C57BL/6 mice were provided by the Animal Experiment Center of Renmin Hospital of Wuhan University. All experimental procedures were approved by the Animal Ethics Committee of Renmin Hospital of Wuhan University (License No. WDRM 20190419). The AR model with OD was established as described previously.[24] On days 0, 7, and 14, 300 μL phosphate buffered saline (PBS) containing 100 μg ovalbumin (OVA, grade V; Sigma-Aldrich, Taufkirchen, Germany) and 5 mg of aluminum hydroxide was administered by intraperitoneal injection for sensitization. On the 21st day, 20 μL PBS containing 200 μg OVA was administered intranasally in the model group (n = 22) once a day for 2 consecutive weeks for the challenge. The mice in the control (Ctrl) group (n = 8) were given the same amount of PBS in the nasal cavity. Finally, the AR mice with OD were selected by the buried food pellet test (BFPT; **Fig. 1A**). To verify this model, histopathology, OVA-specific immunoglobulin E (IgE) and allergic symptoms were evaluated.
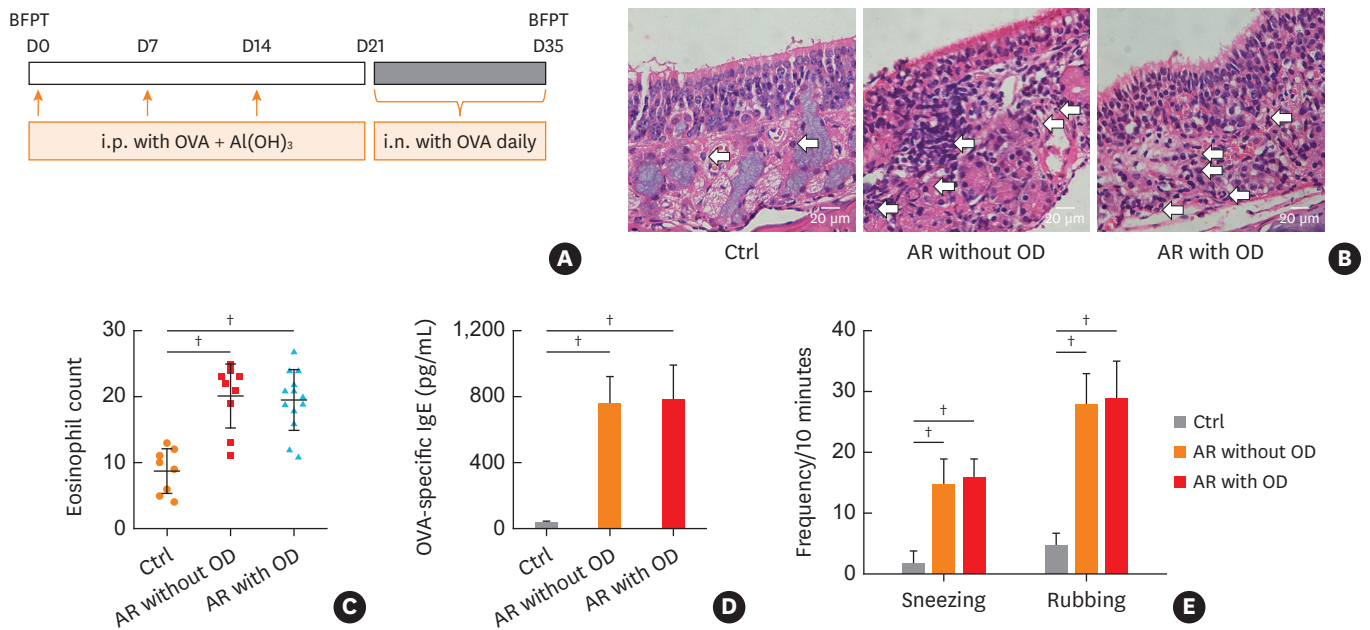
Fig. 1. Establishment of the AR mouse model with OD. After the last challenge, histopathology, OVA-specific IgE and allergic symptoms were evaluated. (A) Protocol for the mice model of AR with OD. (B, C) Eosinophils in the nasal mucosa were counted in random high-power fields (×400) by H&E staining, and eosinophils were labeled by arrows. (D) OVA-specific IgE in serum was measured by enzyme-linked immunosorbent assay. (E) Symptoms of sneezing and rubbing were counted 10 minutes after final challenge. Ctrl (n = 8), AR without OD (n = 9), AR with OD (n = 13).
BFPT, buried food pellet test; i.p., intraperitoneal; OVA, ovalbumin; i.n., intranasal; Ctrl, the control group challenged with phosphate buffered saline; AR without OD, the group of allergic rhinitis without olfactory dysfunction; AR with OD, the group of allergic rhinitis with olfactory dysfunction; IgE, immunoglobulin E.
[†]$P<0.01$.

Buried food pellet test (BFPT)

The BFPT was used to evaluate the olfactory function of mice as previously described.[25] Briefly, the mice were fasted 18–24 hours before the test. In the test cage (45 cm length × 24 cm width × 20 cm height), a food pellet of approximately 4g was buried at a depth of 0.5 cm under the bedding. After 1 hour of habituation, the time it took the mice to grasp the food pellets with their front paws or teeth was recorded. If the food pellets were not found within 300 seconds (an average of 3 tests), it was identified as OD. In addition, we measured the latency time of each mouse after the drug treatments.

Drug treatment and experimental grouping

The AR mice with OD were identified and randomly divided into the following groups: quinpirole (Quin, 3 mg/kg; Sigma-Aldrich) was administered nasally every day for 3, 6, 9, and 12 days (n = 8 for each group), and the Ctrl group (n = 8) was given PBS in equal doses. The drug concentration and route of administration used were based on the previously published article,[14] and the nasal-brain pathway allows the drugs to bypass the blood-brain barrier and enter the central nervous system (CNS). In our preliminary experiments, we have found that both nasal administration and intraperitoneal injection improved olfactory function, but Quin administered nasally at the same drug dose achieved drug effects earlier and more effectively. During this period, the mice were challenged with OVA every other day. The olfactory function was evaluated 24 hours after the administration, and the mice were used for the following experiments after being sacrificed.

Culture of microglia

Magnetic bead sorting maintains the *in vivo* state of microglia to a greater extent, and recapitulates the relationship between microglia and neuroinflammation. As previously reported,[26,27] the microglia were isolated from the olfactory bulbs after successful modeling. The olfactory bulbs were enzymatically digested and filtered through 70-μm cell strainers to prepare a single cell suspension. The microglia were sorted with CD11b magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). After 3 rounds of resuspending, loading, and washing, the positive cells on the sorting column were collected. The cells were resuspended with DMEM/F12 medium containing 10% fetal bovine serum (Gibco, Carlsbad, CA, USA), inoculated into a polylysine-coated culture plate, and cultured a humidified CO_2 (5%) incubator at 37°C. Simultaneously, microglia were cultured with Quin (20 μmol/L) for 24 hours.

Hematoxylin and eosin (H&E) staining

After the mice were sacrificed, their noses and olfactory bulbs were fixed in 4% paraformaldehyde for 48 hours. Additionally, the noses were decalcified in decalcified solution for 2 weeks and then made into paraffin sections. After the paraffin sections were deparaffinized, hematoxylin was used to stain the nucleus and eosin was used to stain the cytoplasm. The morphology of the nasal mucosa and olfactory bulbs were then observed under a light microscope (×400). Five fields on each slice were randomly selected, in which the number of eosinophils was counted under the microscope and then averaged.

Immunofluorescence staining

The olfactory bulbs were fixed with 4% paraformaldehyde, embedded in slices, and baked. After the paraffin sections were completely dewaxed with xylene, 10% calf serum was added, and the section were placed at room temperature for 10 minutes. The sections were incubated with rabbit polyclonal anti-CD11b antibody (1:500, ab184308; Abcam, Cambridge, UK) at 4°C overnight, followed by a FITC-labeled goat anti-rabbit IgG (ab150080; Abcam) at room temperature for 30 minutes. The sections were washed with water, blown dry, sealed with glycerin, and followed by observation under a fluorescence microscope (Olympus, Tokyo, Japan).

Enzyme-linked immunosorbent assay (ELISA)

The olfactory bulbs, blood and culture media were collected after drug treatments. The olfactory bulbs on both sides of a mouse were taken, and 200 μL of lysate was added. After homogenization, the lysate was split on ice for 30 minutes and centrifuged at 12,000 rpm for 15 minutes. The protein concentration of the supernatant was measured by a BCA kit (Absin, Shanghai, China), and ensured that the total protein of each sample was 50 μg. Similarly, supernatants of blood and culture media were retained. The levels of inflammatory mediators were measured using ELISA kits of TNF-α, IL-6, IL-1β (BD Biosciences, San Jose, CA, USA) and OVA-specific IgE (Bioswamp, Wuhan, China) according to the manufacturer's instructions, respectively. Briefly, 50 μL ELISA diluent and 50 μL sample were added to each well, and incubated at room temperature for 2 hours. After aspirating and washing 5 times, 100 μL of the working detector was added and incubated at room temperature for 1 hour. Then, 100 μL of TMB one-step substrate reagent was added and incubated at room temperature for 30 minutes. After adding 50 μL stop solution, read at 450 nm within 30 minutes. The experiments were repeated for 3 times.

Western blot

After the drug treatment, the total protein was extracted. Briefly, RIPA lysis buffer containing a protease inhibitor and a phosphatase inhibitor was added to the tissues or cells, which were

then placed on ice for 30 minutes. After centrifugation at 12,000 rpm for 15 minutes at 4°C, the supernatant was extracted. The protein concentration was measured by a BCA kit (Absin), and samples of 40 μg were loaded onto the gel for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis. After SDS-PAGE electrophoresis, the proteins were transferred to a membrane, which was blocked in 5% skim milk for 1.5 hours. The following primary antibodies, which were incubated at 4°C in a shaker overnight, were used: TLR4 (1:500, sc-293072; Santa Cruz, Dallas, TX, USA), MyD88 (1:500, sc-74532; Santa Cruz), NF-κB p65 (1:1,000, sc-8008; Santa Cruz), NF-κB phospho-p65 (1:1,000, sc-166748; Santa Cruz) and β3-tubulin (1:1,000, ab78078; Abcam). The secondary antibody was labeled with HRP (Servicebio, Wuhan, China) and incubated with the membrane at room temperature for 1 hour. The blots were washed 3 times for 10 minutes. The bands were visualized with enhanced chemiluminescence (Millipore, Burlington, MA, USA) using a gel imaging system (Bio\xe2\x80\x90Rad, Hercules, CA, USA). ImageJ was used to determine the gray value. Using β3-tubulin as the reference protein, the relative intensities of each target protein band were calculated.

Statistical analysis

All results are represented as mean ± standard error of mean. The data and graphs were analyzed by GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). The results were analyzed by 1-way analysis of variance followed by *post hoc* Tukey's tests for multiple comparisons. A *P* value of < 0.05 was considered significant.

RESULTS

Establishment of the mouse model of AR with OD

To verify this model, histopathology, OVA-specific IgE and allergic symptoms were evaluated, and the AR mice were divided into groups with or without OD by the BFPT. In this study, we used 8 and 22 mice in the Ctrl and AR groups, respectively. Out of 22 mice, 13 mice had OD and 9 mice did not have OD. The incidence of OD observed in AR mice was 59.09% (13/22), which is consistent with that in the AR population being from 21% to 88%.[2] The reason why some AR mice did not develop OD may be that OD is a common symptom of AR, but not all AR is accompanied by OD. H&E staining showed that the infiltration of eosinophils was significantly increased in the submucosa of the AR mice with or without OD, compared with the Ctrl group (**Fig. 1B and C**). The levels of OVA-specific IgE and the frequency of sneezing and rubbing in the AR mice with or without OD were significantly increased, compared with the Ctrl group (**Fig. 1D and E**). This finding suggests that the AR mice with or without OD exhibit the characteristics of AR, and the AR mice with OD selected met the needs of the subsequent experiments.

Neuroinflammation in the olfactory bulb of AR mice with OD

Microglia, being the resident immune cells of the CNS, play an important role in maintaining tissue homeostasis and contribute towards brain development under normal conditions.[28] However, overactivation of microglia will release a wide spectrum of proinflammatory cytokines, neurotoxic factors and chemokines, including TNF-α, IL-1β, IL-6, monocyte chemotactic protein-1, nitric oxide and prostaglandin E2 that induce neuroinflammation.[29] To verify the relationship between AR-induced OD and neuroinflammation in the olfactory bulb, we detected the expression of microglial marker CD11b and the related cytokines TNF-α, IL-1β and IL-6 in the olfactory bulb. Our research found that the cells in the olfactory bulb of AR mice with OD were arranged irregularly, and substantial neuron loss (**Fig. 2A**). Compared

with the group without OD, the expression of CD11b, TNF-α, IL-1β and IL-6 in the group with OD were all significantly increased (**Fig. 2A-C**). To verify the role of TLR4 in AR-induced OD, the expression of the TLR4/NF-κB pathway in microglia sorted from the olfactory bulb was detected. The expression of TLR4, MyD88 and NF-κB phospho-p65/p65 in microglia of the group with OD were all significantly increased, compared with the group without OD, respectively (**Fig. 2D and E**). However, the above proteins in the group without OD was not significantly different from that of the Ctrl group, which further reveals that AR-induced OD may be caused by neuroinflammation of the olfactory bulb, and be related to the TLR4/NF-κB pathway in microglia.
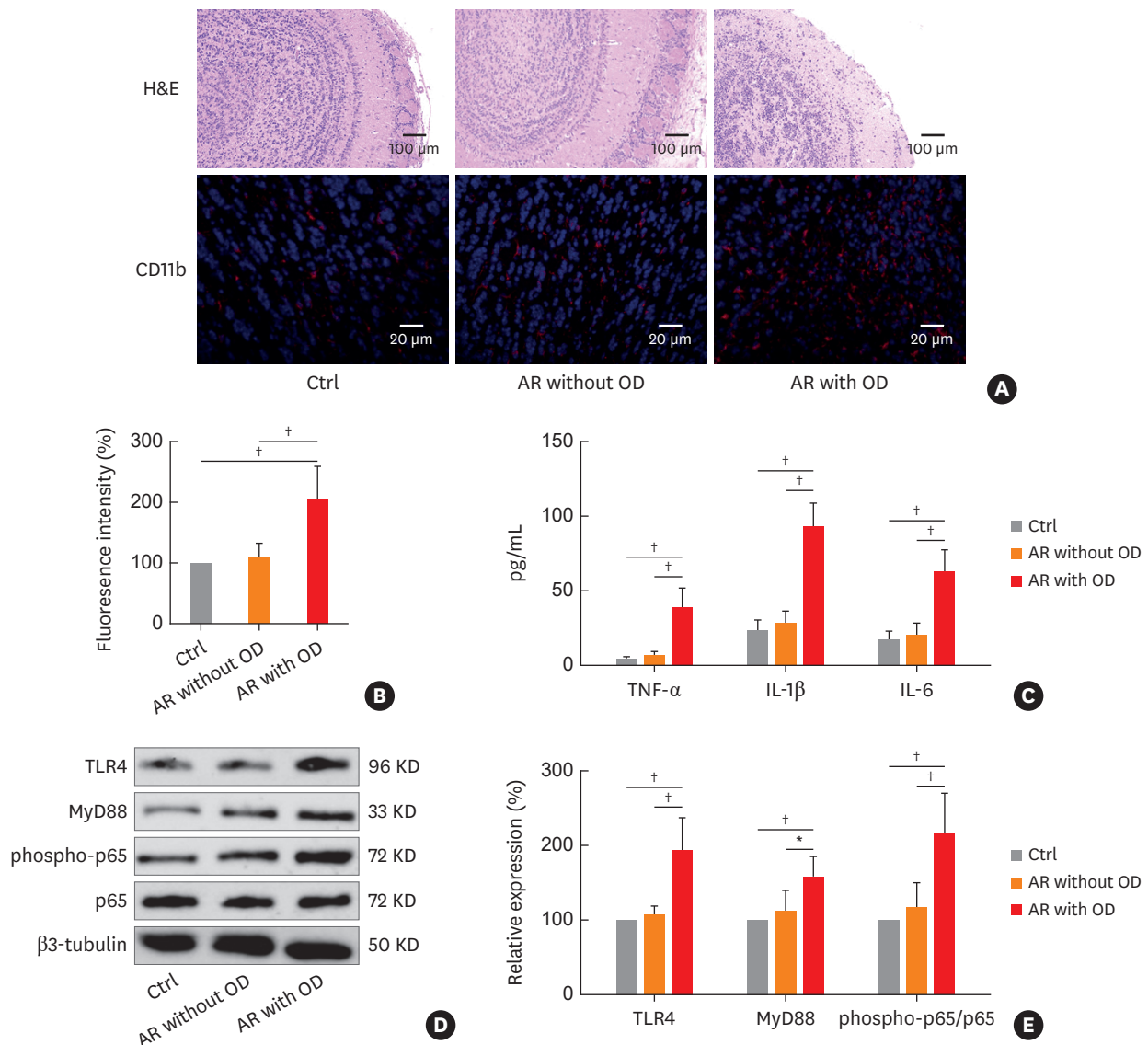
Fig. 2. Neuroinflammation in the olfactory bulb of AR mice with OD. (A, B) Morphology of the olfactory bulb was detected by H&E staining. The expression of CD11b in the olfactory bulb was detected by immunofluorescence, and fluorescence intensity in each group of mice was analyzed. (C) The expression of TNF-α, IL-1β and IL-6 in the olfactory bulb were detected by ELISA. (D, E) The expression of TLR4, MyD88, NF-κB p65 and NF-κB phospho-p65 in microglia sorted from the olfactory bulbs for each group were detected by Western blot. Ctrl (n = 8), AR without OD (n = 9), AR with OD (n = 13).
H&E, hematoxylin and eosin; Ctrl, the control group challenged with phosphate buffered saline; AR without OD, the group of allergic rhinitis without olfactory dysfunction; AR with OD, the group of allergic rhinitis with olfactory dysfunction; TNF-α, tumor necrosis factor-α; IL, interleukin; TLR4, toll-like receptor 4; NF-κB, nuclear factor-κB.
*$P < 0.05$, †$P < 0.01$.

Quin alleviates neuroinflammation in AR mice with OD

To verify the effect of dopamine D2 receptor on the olfactory mucosa and olfactory bulb, Quin, a dopamine D2 receptor agonist, was administered intranasally to assess the olfactory function, the morphology of olfactory mucosa and the expression of CD11b, TNF-α, IL-1β and IL-6 in the olfactory bulb. The BFPT found that Quin could shorten the time to find the food pellets, and the most significant improvement in olfactory function occurred on day 12 (**Fig. 3B**). Therefore, olfactory mucosa and olfactory bulb on the 12th day of treatment were selected for further analysis. Compared with AR mice with OD (no treatment), the olfactory mucosa eosinophils were not significantly increased fter 12 days of OVA challenge, but the expression of CD11b in the olfactory bulb was increased. Quin had no significant effect on eosinophil infiltration in olfactory epithelium, but reduced the activation of microglia in the olfactory bulb (**Fig. 3C**), compared with OVA challenge only. Furthermore, after treatment with Quin, the expression of TNF-α in the olfactory bulb was decreased on the 6th, 9th and 12th days, the expression of IL-1β was decreased on the 9th and 12th days, and the expression of IL-6 was also decreased on the 9th and 12th days (**Fig. 3D-F**), compared with OVA treatment alone. In order to further study the anti-inflammatory effects of Quin, we sorted the microglia from the olfactory bulbs after successful modeling. After 24 hours of treatment with Quin, TNF-α, IL-1β and IL-6 in the culture medium were also significantly decreased (**Fig. 3G**). The results suggest that Quin reverses neuroinflammation in the olfactory bulb and improve olfactory function.

Quin inhibits the TLR4/NF-κB pathway *in vivo*

Previous studies have shown that TLR4/NF-κB is a vital pathway by which microglia release inflammatory factors.[20-22] Therefore, we explored Quin's effect on the expression of TLR4, MyD88, NF-κB p65 and NF-κB phospho-p65 in the microglia sorted from the olfactory bulbs for each group. Compared with OVA treatment alone, after nasal administration of Quin, the expression of TLR4 was decreased on the 3rd, 6th, 9th, and 12th days; the expression of MyD88 was decreased on the 6th, 9th, and 12th days; the expression of phospho-p65/p65 was also decreased on the 6th, 9th, and 12th days, respectively (**Fig. 4**). The results suggest that the reversal of neuroinflammation by Quin may be related to the TLR4/NF-κB pathway.

Quin exerts anti-inflammatory effects through the TLR4/NF-κB pathway

To further study the direct correlation between Quin and the TLR4/NF-κB pathway, we explored Quin's effect *in vitro* on the expression of TLR4, MyD88, NF-κB p65 and NF-κB phospho-p65 in the microglia sorted from the olfactory bulbs for each group. After 24 hours of treatment with Quin in vitro, TLR4, MyD88, and phospho-p65/p65 in the microglia of AR mice with OD were reduced significantly (**Fig. 5A and B**). Then, we sorted the microglia of normal mice and observed the effect of Quin on microglia treated with TLR4 agonist LPS. After 24 hours of treatment with Quin, TLR4, MyD88 and phospho-p65/p65 were also significantly decreased compared with LPS treatment alone, respectively (**Fig. 5C and D**). Concurrently, TNF-α, IL-1β and IL-6 were decreased compared with LPS treatment alone, respectively (**Fig. 5E**). This suggests that Quin plays an anti-inflammatory role through the TLR4/NF-κB pathway directly.

DISCUSSION

The mechanism by which AR causes OD is not fully understood. The previous view believed that swollen nasal mucosa caused by inflammation was the main cause of OD.[30] However,
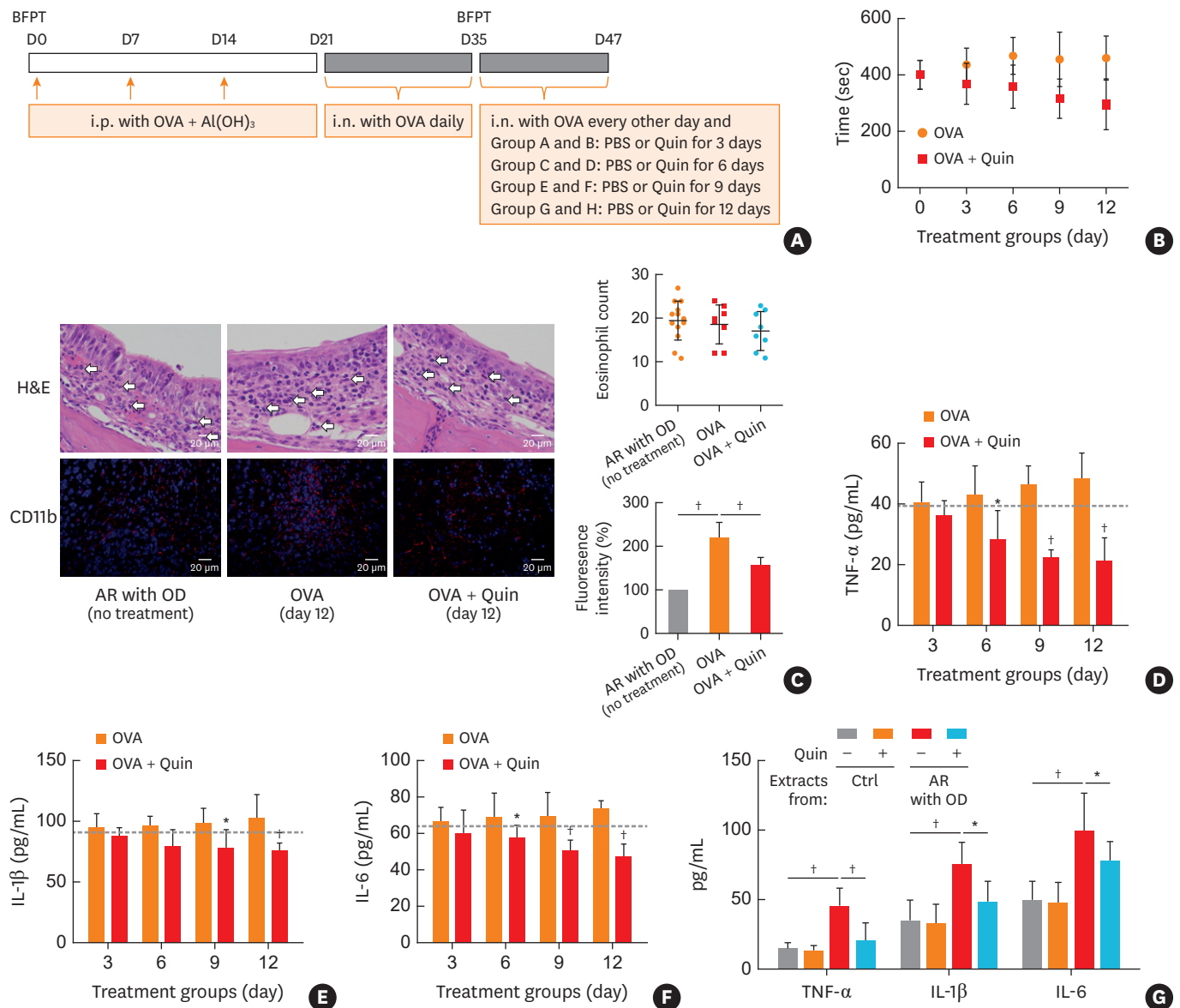
Fig. 3. Quin alleviates neuroinflammation in AR mice with OD. After the AR mice with OD were picked out, Quin (3 mg/kg) was administered nasally every day for 3, 6, 9 and 12 days, and the OVA group was given PBS and OVA in equal doses. (A) Protocol for the mouse model of AR with OD and drug treatment grouping. (B) The effect of Quin for mice to find buried pellets. The time for mice to find buried pellets after treatment with Quin on days 3, 6, 9 and 12 (n = 8 per group). (C) After 12 days of treatment with OVA or OVA + Quin, eosinophils in the nasal mucosa were counted in random high-power fields (×400) by H&E staining, and the expression of CD11b in the olfactory bulb was detected by immunofluorescence. (D-F) The expression of TNF-α, IL-1β and IL-6 in the olfactory bulb after treatment with Quin on days 3, 6, 9 and 12 (n = 8 per group). (G) The expression of TNF-α, IL-1β and IL-6 in medium supernatant of microglia sorted from the olfactory bulbs after treatment with Quin (20 μmol/L) for 24 hours (n = 6 per group). "---" represented the average on day 0.
BFPT, buried food pellet test; i.p., intraperitoneal; i.n., intranasal; H&E, hematoxylin and eosin; PBS, phosphate buffered saline; Ctrl, the control group, normal mice; AR with OD, the group of allergic rhinitis with olfactory dysfunction; OVA, the group of mice treated with ovalbumin and PBS; OVA + Quin, the group of mice treated with ovalbumin and quinpirole; TNF-α, tumor necrosis factor-α; IL, interleukin.
*$P < 0.05$, †$P < 0.01$.

recent studies reported that the degree of nasal obstruction is not directly related to AR-induced OD. For instance, nasal mucosal decongestant does not restore normal olfactory function in AR patients. Studies by Cowart et al.[31] also showed that although nasal resistance was significantly higher among AR patients than among normal Ctrls, it was not related to olfactory threshold in either group. Wang et al.[24] found that the inflammatory response of AR led to pathological changes in the olfactory mucosa, the expression of olfactory marker
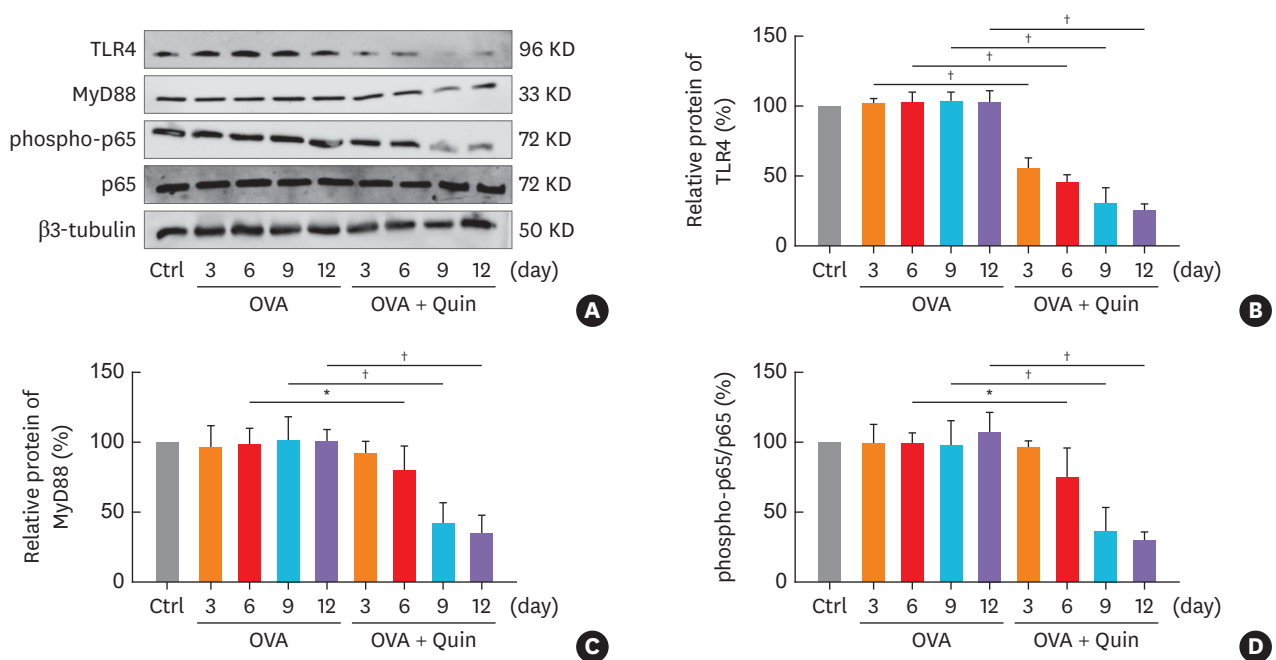
Fig. 4. Quin inhibits the TLR4/NF-κB pathway *in vivo*. (A-D) The expression of TLR4, MyD88, NF-κB p65 and NF-κB phospho-p65 in microglia sorted from the olfactory bulbs were detected by western blot after treatment with Quin for 3, 6, 9 and 12 days (n = 8 per group).
Ctrl, the control group, the olfactory dysfunction mice without treatment; OVA, the group of mice treated with ovalbumin and phosphate buffered saline; OVA + Quin, the group of mice treated with ovalbumin and quinpirole; TLR4, toll-like receptor 4; NF-κB, nuclear factor-κB.
*$P < 0.05$, †$P < 0.01$.

protein decreased, and the number of olfactory receptor neurons decreased. In addition, our research found that the cells in the olfactory bulb of AR mice with OD are irregularly arranged, and substantial neuron loss. Besides, the microglia of the olfactory bulb were abnormally activated, which may be one of the reasons for AR-induced OD.

Several studies on humans and rodents have shown that intranasal or nebulized inhalation of allergens in sensitized animals induced avoidance behavior and activated limbic brain areas.[5-8] This study also showed that AR mice with OD showed abnormal activation of microglia and release of inflammatory cytokines, but not in AR mice without OD (**Fig. 2A-C**), which further proved that AR-induced OD is closely related to neuroinflammation of the olfactory bulb. Similar to the findings, Tonelli *et al*.[5] reported that OVA or pollen-induced AR rats produced TH2 cytokines in the olfactory bulb and prefrontal cortex, but not in the temporal cortex and hypothalamus, and increased brain activity was observed by functional MRI. Asthma induces activation of the microglia in the hippocampus and the prefrontal cortex, elevated levels of TNF-α and IL-1β, and a significant loss of neurons in the brain.[7] In some other allergic diseases, the migration of microglia and the production of ROS increase, which ultimately enhance the neurotoxicity induced by glutamate.[32-34] However, the mechanism that causes neuroinflammation of the olfactory bulb is unclear.

Microglia are important immune cells of CNS.[28] In homeostasis, microglia monitor the microenvironment and protect astrocytes and neurons. Under pathological conditions, microglia are activated and morphologically altered to protect nerve cells by phagocytic fragments and altered secretions. However, overactivation of microglia can lead to neuronal damage and therefore plays an important role in neuroinflammation.[29] Numerous studies have shown that TLR4 is a key molecule that regulates the immune response during

https://doi.org/10.4168/aair.2021.13.6.882
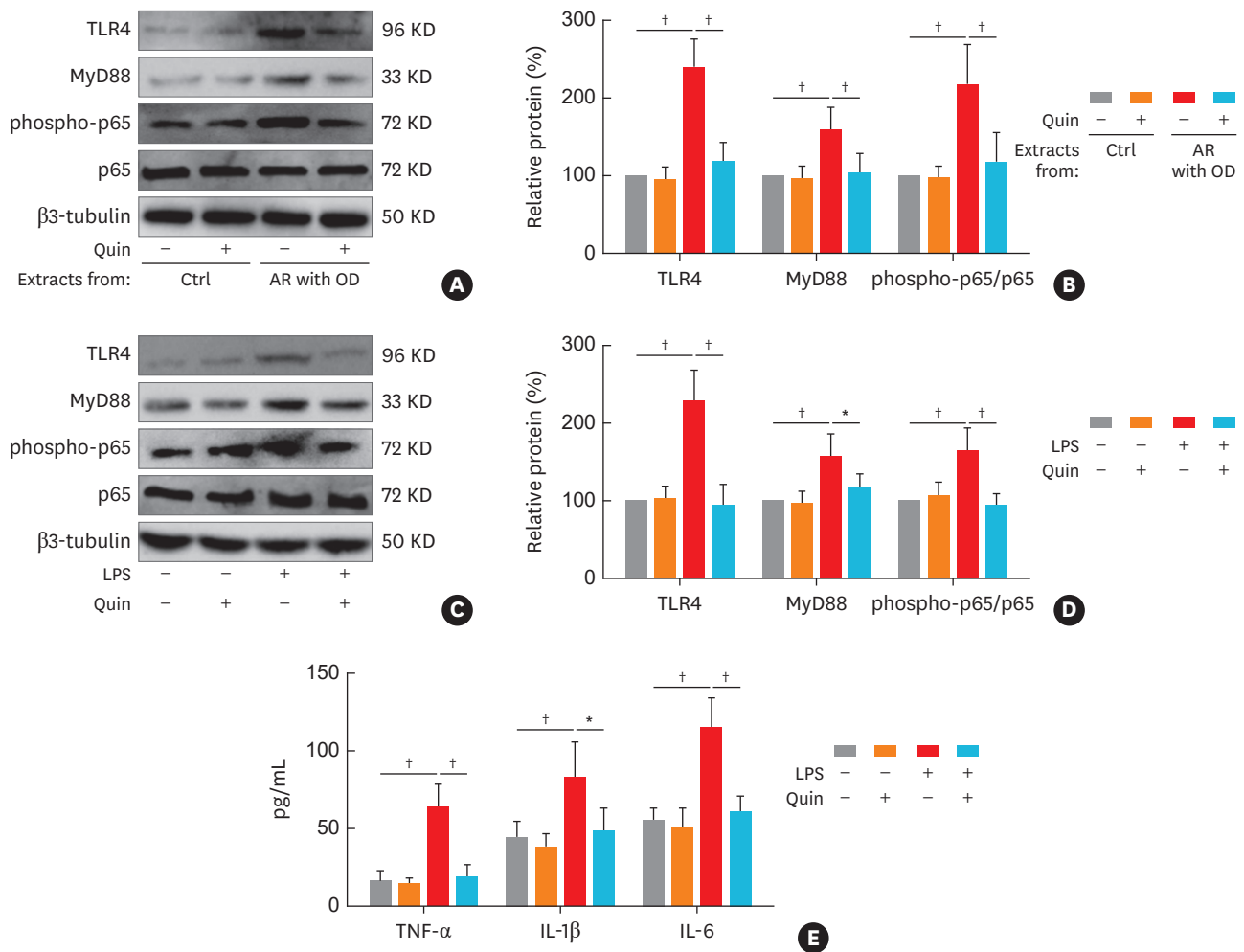
Fig. 5. Quin exerts anti-inflammatory effects through the TLR4/NF-κB pathway. (A, B) In the cultured microglia extracted from the olfactory bulbs after successful modeling, the expression of TLR4, MyD88, NF-κB p65 and NF-κB phospho-p65 of microglia after treatment with Quin (20 μmol/L) for 24 hours. (C, D) The effect of Quin (20 μmol/L) on microglia sorted from the normal mice after 24 hours of LPS (TLR4 agonist, 1 μg/mL) treatment. (E) The effect of Quin (20 μmol/L) on TNF-α, IL-1β and IL-6 in medium supernatant of microglia sorted from the normal mice after 24 hours of LPS treatment (n = 6 per group).
TLR4, toll-like receptor 4; Quin, quinpirole; Ctrl, the control group, normal mice; AR with OD, the group of allergic rhinitis with olfactory dysfunction; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; IL, interleukin; NF-κB, nuclear factor-κB.
$*P < 0.05$, $†P < 0.01$.

CNS infection and injury,[20] which is mainly expressed in microglia and can be specifically recognized by LPS. After activation in the brain, TLR4 binds to MyD88 to relieve the inhibitory effect of IκB on NF-κB, promote NF-κB nuclear translocation, stimulate inflammation-related gene expression, and promote the synthesis and release of TNF-α, IL-1β and IL-6.[20-22] In Alzheimer's disease, PD, amyotrophic lateral sclerosis and other neurodegenerative diseases, TLR4 activates the expression of key pro-inflammatory cytokine genes.[35-37] In our research, TLR4/NF-κB pathway, TNF-α, IL-1β and IL-6 was significantly increased in the olfactory bulb microglia in AR mice with OD, but not in AR mice without OD (**Fig. 2D and E**). In addition, TLR4 knockout significantly reduced neuroinflammation and dysfunction in animal studies.[36,38] Therefore, TLR4 may be a potential therapeutic target for AR-induced OD.

Recently, the anti-neuroinflammatory effects of the dopamine D2 receptor have been highlighted, related to NLRP3 inflammasome, renin-angiotensin system and αB-crystallin.[12,13,39] Nasal administration of Quin not only reduced systemic side effects,[40,41] but

also entered the olfactory bulb more efficiently than systemic delivery. For example, studies have found that after 30 minutes of intranasal administration of dopamine, the uptake of [³H] dopamine in the brain was significantly higher than that of intravenous administration.[42] After [³H]dopamine was administered to the unilateral nasal cavity (right), the content of [³H] dopamine in the right olfactory bulb was 27 times higher than that in the left olfactory bulb at 4 hours.[43] In our study, the dopamine D2 receptor agonist Quin inhibited the expression of TLR4/NF-κB pathway *in vivo* and *in vitro* (**Figs. 4**, **5A and B**), and the release of TNF-α, IL-1β and IL-6 (**Fig. 3D-G**), finally improved olfactory function in mice (**Fig. 3B**). In addition, Quin inhibited TLR4/NF-κB pathway and the release of inflammatory cytokines induced by TLR4 agonist LPS (**Fig. 5C-E**). Consistent with these results, Quin suppressed the expression of TLR4/NF-κB pathway in PD mice by increasing the expression of βArr2.[23] This suggests that dopamine D2 receptor activation can reduce the TLR4/NF-κB-dependent inflammatory response of the olfactory bulb and protect olfactory function.

There are some limitations to our study. The relationship between dopamine receptors and olfactory loss caused by AR would have been further investigated. This study mainly focuses on the pharmacological mechanism of dopamine D2 receptor. It is believed that the study of the relationship between dopamine receptors and olfactory loss can provide a more comprehensive understanding of the pathogenesis of AR with OD.

In conclusion, the microglia of the olfactory bulb were abnormally activated in AR with OD, with the up-regulation of TLR4/NF-κB signaling, TNF-α, IL-1β and IL-6. Quin, a dopamine D2 receptor agonist, can improve TLR4/NF-κB signaling-dependent neuroinflammation and help restore olfactory function. Our findings suggest an association among dopamine D2 receptor, neuroinflammation and olfactory function, providing a novel target for the treatment of OD induced by AR.

ACKNOWLEDGMENTS

REFERENCES

1. Guss J, Doghramji L, Reger C, Chiu AG. Olfactory dysfunction in allergic rhinitis. ORL J Otorhinolaryngol Relat Spec 2009;71:268-72.
   PUBMED | CROSSREF

2. Stuck BA, Hummel T. Olfaction in allergic rhinitis: a systematic review. J Allergy Clin Immunol 2015;136:1460-70.
   PUBMED | CROSSREF

3. Rosenkranz MA, Busse WW, Johnstone T, Swenson CA, Crisafi GM, Jackson MM, et al. Neural circuitry underlying the interaction between emotion and asthma symptom exacerbation. Proc Natl Acad Sci U S A 2005;102:13319-24.
   PUBMED | CROSSREF

4. Costa-Pinto FA, Basso AS, Britto LR, Malucelli BE, Russo M. Avoidance behavior and neural correlates of allergen exposure in a murine model of asthma. Brain Behav Immun 2005;19:52-60.
   PUBMED | CROSSREF

5. Tonelli LH, Katz M, Kovacsics CE, Gould TD, Joppy B, Hoshino A, et al. Allergic rhinitis induces anxiety-like behavior and altered social interaction in rodents. Brain Behav Immun 2009;23:784-93.
    PUBMED | **CROSSREF**

6. Yang S, Wu J, Zhang Q, Li X, Liu D, Zeng B, et al. Allergic rhinitis in rats is associated with an inflammatory response of the hippocampus. Behav Neurol 2018;2018:8750464.
    PUBMED | **CROSSREF**

7. Xia MX, Ding X, Qi J, Gu J, Hu G, Sun XL. Inhaled budesonide protects against chronic asthma-induced neuroinflammation in mouse brain. J Neuroimmunol 2014;273:53-7.
    PUBMED | **CROSSREF**

8. Chen SD, Wen ZH, Chang WK, Chan KH, Tsou MT, Sung CS, et al. Acute effect of methylprednisolone on the brain in a rat model of allergic asthma. Neurosci Lett 2008;440:87-91.
    PUBMED | **CROSSREF**

9. Sarkar C, Basu B, Chakroborty D, Dasgupta PS, Basu S. The immunoregulatory role of dopamine: an update. Brain Behav Immun 2010;24:525-8.
    PUBMED | **CROSSREF**

10. Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 2011;63:182-217.
    PUBMED | **CROSSREF**

11. Yan Y, Jiang W, Liu L, Wang X, Ding C, Tian Z, et al. Dopamine controls systemic inflammation through inhibition of NLRP3 inflammasome. Cell 2015;160:62-73.
    PUBMED | **CROSSREF**

12. Zhu J, Hu Z, Han X, Wang D, Jiang Q, Ding J, et al. Dopamine D2 receptor restricts astrocytic NLRP3 inflammasome activation via enhancing the interaction of β-arrestin2 and NLRP3. Cell Death Differ 2018;25:2037-49.
    PUBMED | **CROSSREF**

13. Shao W, Zhang SZ, Tang M, Zhang XH, Zhou Z, Yin YQ, et al. Suppression of neuroinflammation by astrocytic dopamine D2 receptors via αB-crystallin. Nature 2013;494:90-4.
    PUBMED | **CROSSREF**

14. Zhang Y, Chen Y, Wu J, Manaenko A, Yang P, Tang J, et al. Activation of dopamine D2 receptor suppresses neuroinflammation through αB-crystalline by inhibition of NF-κB nuclear translocation in experimental ICH mice model. Stroke 2015;46:2637-46.
    PUBMED | **CROSSREF**

15. Qiu J, Yan Z, Tao K, Li Y, Li Y, Li J, et al. Sinomenine activates astrocytic dopamine D2 receptors and alleviates neuroinflammatory injury via the CRYAB/STAT3 pathway after ischemic stroke in mice. J Neuroinflammation 2016;13:263.
    PUBMED | **CROSSREF**

16. Nakano K, Higashi T, Hashimoto K, Takagi R, Tanaka Y, Matsushita S. Antagonizing dopamine D1-like receptor inhibits Th17 cell differentiation: preventive and therapeutic effects on experimental autoimmune encephalomyelitis. Biochem Biophys Res Commun 2008;373:286-91.
    PUBMED | **CROSSREF**

17. Lazarini F, Gabellec MM, Moigneu C, de Chaumont F, Olivo-Marin JC, Lledo PM. Adult neurogenesis restores dopaminergic neuronal loss in the olfactory bulb. J Neurosci 2014;34:14430-42.
    PUBMED | **CROSSREF**

18. Höglinger GU, Alvarez-Fischer D, Arias-Carrión O, Djufri M, Windolph A, Keber U, et al. A new dopaminergic nigro-olfactory projection. Acta Neuropathol 2015;130:333-48.
    PUBMED | **CROSSREF**

19. Gutièrrez-Mecinas M, Crespo C, Blasco-Ibáñez JM, Gracia-Llanes FJ, Marqués-Marí AI, Nácher J, et al. Distribution of D2 dopamine receptor in the olfactory glomeruli of the rat olfactory bulb. Eur J Neurosci 2005;22:1357-67.
    PUBMED | **CROSSREF**

20. Lin ST, Wang Y, Xue Y, Feng DC, Xu Y, Xu LY. Tetrandrine suppresses LPS-induced astrocyte activation via modulating IKKs-IkappaBalpha-NF-kappaB signaling pathway. Mol Cell Biochem 2008;315:41-9.
    PUBMED | **CROSSREF**

21. Zhang Z, Qin P, Deng Y, Ma Z, Guo H, Guo H, et al. The novel estrogenic receptor GPR30 alleviates ischemic injury by inhibiting TLR4-mediated microglial inflammation. J Neuroinflammation 2018;15:206.
    PUBMED | **CROSSREF**

22. Guo LT, Wang SQ, Su J, Xu LX, Ji ZY, Zhang RY, et al. Baicalin ameliorates neuroinflammation-induced depressive-like behavior through inhibition of toll-like receptor 4 expression via the PI3K/AKT/FoxO1 pathway. J Neuroinflammation 2019;16:95.
    PUBMED | **CROSSREF**

23. Du RH, Zhou Y, Xia ML, Lu M, Ding JH, Hu G. α-Synuclein disrupts the anti-inflammatory role of Drd2 via interfering β-arrestin2-TAB1 interaction in astrocytes. J Neuroinflammation 2018;15:258.
    PUBMED | CROSSREF

24. Wang X, Zhu Y, Ni D, Lv W, Gao Z, Qi F. Intranasal application of glucocorticoid alleviates olfactory dysfunction in mice with allergic rhinitis. Exp Ther Med 2017;14:3971-8.
    PUBMED | CROSSREF

25. Talaga AK, Dong FN, Reisert J, Zhao H. Cilia- and flagella-associated protein 69 regulates olfactory transduction kinetics in mice. J Neurosci 2017;37:5699-710.
    PUBMED | CROSSREF

26. Holt LM, Stoyanof ST, Olsen ML. Magnetic cell sorting for *in vivo* and *in vitro* astrocyte, neuron, and microglia analysis. Curr Protoc Neurosci 2019;88:e71.
    PUBMED | CROSSREF

27. Nikodemova M, Watters JJ. Efficient isolation of live microglia with preserved phenotypes from adult mouse brain. J Neuroinflammation 2012;9:147.
    PUBMED | CROSSREF

28. Subhramanyam CS, Wang C, Hu Q, Dheen ST. Microglia-mediated neuroinflammation in neurodegenerative diseases. Semin Cell Dev Biol 2019;94:112-20.
    PUBMED | CROSSREF

29. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 2007;8:57-69.
    PUBMED | CROSSREF

30. Seiden AM, Duncan HJ. The diagnosis of a conductive olfactory loss. Laryngoscope 2001;111:9-14.
    PUBMED | CROSSREF

31. Cowart BJ, Flynn-Rodden K, McGeady SJ, Lowry LD. Hyposmia in allergic rhinitis. J Allergy Clin Immunol 1993;91:747-51.
    PUBMED | CROSSREF

32. Teixeira MM, Wells TN, Lukacs NW, Proudfoot AE, Kunkel SL, Williams TJ, et al. Chemokine-induced eosinophil recruitment. Evidence of a role for endogenous eotaxin in an *in vivo* allergy model in mouse skin. J Clin Invest 1997;100:1657-66.
    PUBMED | CROSSREF

33. Parajuli B, Horiuchi H, Mizuno T, Takeuchi H, Suzumura A. CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. Glia 2015;63:2274-84.
    PUBMED | CROSSREF

34. Isobe N, Kanamori Y, Yonekawa T, Matsushita T, Shigeto H, Kawamura N, et al. First diagnostic criteria for atopic myelitis with special reference to discrimination from myelitis-onset multiple sclerosis. J Neurol Sci 2012;316:30-5.
    PUBMED | CROSSREF

35. Baldwin AG, Brough D, Freeman S. Inhibiting the inflammasome: a chemical perspective. J Med Chem 2016;59:1691-710.
    PUBMED | CROSSREF

36. Song M, Jin J, Lim JE, Kou J, Pattanayak A, Rehman JA, et al. TLR4 mutation reduces microglial activation, increases Aβ deposits and exacerbates cognitive deficits in a mouse model of Alzheimer's disease. J Neuroinflammation 2011;8:92.
    PUBMED | CROSSREF

37. Lee JY, Lee JD, Phipps S, Noakes PG, Woodruff TM. Absence of toll-like receptor 4 (TLR4) extends survival in the hSOD1 G93A mouse model of amyotrophic lateral sclerosis. J Neuroinflammation 2015;12:90.
    PUBMED | CROSSREF

38. De Virgilio A, Greco A, Fabbrini G, Inghilleri M, Rizzo MI, Gallo A, et al. Parkinson's disease: autoimmunity and neuroinflammation. Autoimmun Rev 2016;15:1005-11.
    PUBMED | CROSSREF

39. Dominguez-Meijide A, Rodriguez-Perez AI, Diaz-Ruiz C, Guerra MJ, Labandeira-Garcia JL. Dopamine modulates astroglial and microglial activity via glial renin-angiotensin system in cultures. Brain Behav Immun 2017;62:277-90.
    PUBMED | CROSSREF

40. Borovac JA. Side effects of a dopamine agonist therapy for Parkinson's disease: a mini-review of clinical pharmacology. Yale J Biol Med 2016;89:37-47.
    PUBMED

41. Yu XX, Fernandez HH. Dopamine agonist withdrawal syndrome: a comprehensive review. J Neurol Sci 2017;374:53-5.
    PUBMED | **CROSSREF**

42. Dahlin M, Jansson B, Björk E. Levels of dopamine in blood and brain following nasal administration to rats. Eur J Pharm Sci 2001;14:75-80.
    PUBMED | **CROSSREF**

43. Dahlin M, Bergman U, Jansson B, Björk E, Brittebo E. Transfer of dopamine in the olfactory pathway following nasal administration in mice. Pharm Res 2000;17:737-42.
    PUBMED | **CROSSREF**