



Effects of lentivirus-mediated CCR3 RNA interference on the function of mast cells of allergic rhinitis in mice

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ABSTRACT

The CC chemokine receptor 3 (CCR3) expressed by eosinophils, mast cells and Th2 cells is closely related to allergic diseases. The objective of this study was to explore whether silencing of CCR3 with short hairpin RNAs (shRNAs) delivered by a lentiviral vector could impact the function of mast cells in a murine model of allergic rhinitis (AR) in vivo. The murine model of allergic rhinitis (AR) inducing by ovalbumin (OVA) was constructed, and the BALB/c mice were divided into normal control group, AR group, controlshRNA treated group and lentiviral CCR3-shRNA treated group. The recombinant lentivirus vectors which express a short hairpin RNA (shRNA) targeting the CCR3 were dropped into the nasal cavity of OVA-sensitized mice before the challenges. Real-time fluorescence quantitative PCR and western blotting were performed to observe inhibitory effect of CCR3 gene. Nasal symptoms of mice and OVA-specific IgE in each group were assessed. Concentrations of histamine, tryptase and Prostaglandin D2 (PGD2) in bone marrow, peripheral blood and nasal mucosa were analyzed. Furthermore, histological analysis and electron microscopy analysis were applied to detect the histology changes of nasal mucosa and the infiltration of mast cells in nasal mucosa. The results showed that administration of CCR3shRNA could effectively inhibit the expression of the CCR3 gene in bone marrow, peripheral blood and nasal mucosa, which reduced the nasal symptoms, the level of OVA-specific IgE, the inflammatory cells and mast cells infiltration into nasal cavity, and relieved the histopathological changes of nasal mucosa. In addition, intervention of CCR3shRNA could reduce the levels of the histamine, tryptase and PGD2 in bone marrow, peripheral blood and nasal mucosa. These results suggest that inhibition of CCR3 gene expression by shRNAs lentiviral vectors can effectively attenuate migration, infiltration and degranulation of mast cells in local tissues and alleviate the inflammation of allergic rhinitis mice.

1. Introduction

Allergic rhinitis (AR) is chronic upper airway disease characterized by inflammation of the nasal mucosa due to infiltration and activation of several inflammatory cells such as mast cells, eosinophils, lymphocytes and so on. Among these cells, mast cells are one of the most important effector cells in the pathogenesis of allergic reaction in AR. Accumulating evidences indicate that mast cells play an important role in the pathogenesis of allergic and non-allergic inflammatory diseases such as allergic rhinitis, asthma and allergic conjunctivitis [1–4]. Mast cells express a vast array of activating receptors, including the IgE high-affinity receptor FcεRI, the SCF receptor, the C-C chemokine receptor type 3 and other activating receptors [5,6]. Activation of the above related receptors will result in rapid degranulation of mast cells and release of preformed mediators, denovo lipid mediators, and newly

synthesized cytokines, such as leukotrienes, histamine, tryptase, PGD2 and so on [7,8]. Those mediators have an influence on different processes occurring during inflammation, including promoting mucus secretion, dilating blood vessels, increasing vascular permeability and inducing the recruitment of other inflammatory cells into affected site. However, the release of mast cell mediators and their effects form highly complex are not fully understood mechanism, further research is needed on the role of mast cells in AR.

The main symptoms of AR include nasal obstruction, nasal itching, runny nose and sneezing. Some of them are often accompanied with itchy eyes and conjunctival congestion [9–11]. Currently, the effective ways to alleviate these symptoms of AR mainly include avoiding specific allergens, drug treatment and allergen immunotherapy [12]. However, avoiding known allergens has no significant effect on symptoms. Antihistamines and corticosteroids have a transient efficacy and

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increasing side effects. Allergen immunotherapy takes a long time, which reduces most patients' compliance [13–16]. Therefore, development of new better treatment for this disease is urgently needed.

RNA interference (RNAi) is a phenomenon of post-transcriptional gene silencing which uses double-stranded RNA to induce special degradation of homologous target gene's mRNA and inhibit gene expression in most eukaryotic cells [17,18]. The ability of RNAi on specifically down-regulate target genes makes it an effective way to treat a variety of diseases. In recent years, RNAi technology has been widely used to study various diseases in vivo and in vitro, including allergic diseases [19,20] and other non-allergic diseases, such as cancer [21], and satisfactory results have been obtained. Naked siRNA or siRNA-liposome complexes have the disadvantages of low delivery efficiency in vivo and transient gene silencing, which can be improved by viral vectors. Human immunodeficiency virus-derived lentiviral vectors can infect non-dividing cells and can be safely delivered to tissues for sustained gene expression [22]. Hence, a lentivirus-based viral vector system is well suited to mediate RNAi, which can significantly increase the efficiency and duration of RNAi.

It is well established that AR is characterized by the migration and infiltration of mast cells, eosinophils, lymphocytes and basophils into inflammatory tissues, and what is important is that chemokines and their receptors play a key role in the transport of these leukocytes to inflammatory tissues. As a G protein-coupled receptor, CCR3 is a high affinity receptor for CC chemokines eotaxin-1/CCL11, eotaxin-2/CCL24 and eotaxin-3/CCL26. There is ample evidence that blocking CCR3 can prevent eosinophil mobilization and reduce its infiltration and degranulation in inflammatory tissues, revealing the central role of CCR3 in eosinophil migration [23,24]. However, CCR3 is not only expressed on eosinophils, but also on Th2 cells, basophils and mast cells. There are still many controversies about its role in mast cells of allergic diseases. For example, Collington et al. [25] found that CCR3 did not affect chemotaxis of bone marrow-derived mast cells in mice. Miyazaki D et al. [26] found that blocking CCR3 could significantly inhibit allergen-mediated hypersensitivity and IgE-mediated degranulation of mast cells. A CCR3 gene knockout mouse study showed that an increase in the number of mast cells in the tracheal epithelium of mice after allergen challenge [27]. In contrast, there was no significant change in the number of skin mast cells in the allergic skin model. It can be seen that CCR3 expressed by mast cell progenitor cells and mature tissue mast cells may be involved in the process of guiding progenitor cells from the bone marrow to the inflammatory tissue.

So far, although CCR3 has made some progress in determining mast cell tissue localization and regulating mast cell function, most of these research results come from allergic diseases other than allergic rhinitis and is little known about the role of CCR3 in mast cells of allergic rhinitis. In our previous study, we successfully constructed an RNAi lentiviral expression vector targeting CCR3 gene and found that it can down-regulated the expression levels of CCR3 mRNA and protein in mouse mast cells. Based on our previous findings, the present study was designed to explore whether inhibition of CCR3 expression through RNAi technology could impact the functions of mast cells in a murine model of AR in vivo.

2. Materials and methods

2.1. Experimental animals

Six to eight-week-old BALB/c mice in a SPF grade were purchased from Hunan SJA Laboratory Animal Co., Ltd. All animals were housed in the Transgenic Animal Center of the Institute of Translational Medicine, Nanchang University in accordance with standards of SPF animal feeding. The present study protocol and use of animals was approved by the Institutional Animal Care and Use Committee of Nanchang University School of Medicine. The mice were randomly divided into four groups (n = 8 per group): normal group, AR group,

controlshRNA group and CCR3shRNA treatment group.

2.2. Construction and preparation of lentiviral CCR3-shRNA vector

The shRNA targeting sequence for CCR3 (CCR3-shRNA) was 5'-GCAGCATTGCCTGAATT TATC-3' and the RNA sequence of mismatch (control-shRNA) used as negative control was 5'-TTCTCCGAAC GTGTCACGT-3'. These shRNAs were firstly synthesized and inserted into the lentiviral vector. Then, combined with lentivirus packaging plasmids were co-transfected into 293T cells to obtain lentivirus particles. Next, the supernatants containing lentivirus particles were collected and concentrated by ultracentrifugation. Finally, it was stored at -80°C for further use.

2.3. Murine model of experimental allergic rhinitis and lentiviral intervention

The mice were sensitized with ovalbumin (Grade V; Sigma Chemical, St. Louis, MO) and aluminium hydroxide (Alum Imject; Pierce Chemical, Rockford, IL) and challenged with ovalbumin to construct a mouse model of AR. The mice in the AR, controlshRNA and CCR3shRNA group were sensitized intraperitoneally by injection of 100 μg OVA emulsified in 4 mg of aluminum hydroxide in a total volume of 200 μl on days 1, 8 and 15. In addition, local challenge was performed twice daily with 20 μl OVA solution by intranasal administration on days 21–27.

On days 1, 7, and 14, mice in AR, controlshRNA and CCR3shRNA group were intranasally administered with 20 μl PBS, controlshRNA and CCR3shRNA respectively. Similarly, the same drugs were provided to continue intervention three hours before each challenge on days 21–27, and mice in normal control group were given with normal saline.

2.4. Evaluation of nasal symptoms and specimen preparation

After the last local challenge with OVA, nasal symptom score including sneezing and nose scratching were recorded for 30 min to evaluate early allergic responses. The above symptom scores standard are as mentioned previous [28]. Then, the mice were sacrificed and the bone marrow, peripheral blood, nasal mucosa were collected and stored in a refrigerator at -80°C for later use, and the bone marrow, peripheral blood and nasal mucosa were centrifuged to obtain supernatant for ELISA analysis. Besides, the level of CCR3mRNA and CCR3 protein in bone marrow, peripheral blood and nasal mucosa in each mice were measured by real-time fluorescence quantitative PCR (q-PCR) and western blotting (WB), and the cells were extracted from the peripheral blood to detect the expression of CCR3 gene. In addition, the nasal mucosa stored at room temperature were assayed by H&E staining, toluidine blue staining and electron microscopy.

2.5. Quantitative real-time PCR

Total RNA of tissues or cells was isolated by using Trizol reagent (Thermo Fisher Scientific, Yokohama, Japan) and converted to cDNA by using a reverse transcription kit according to the manufacturer's instructions. Then, they were performed on the machine for qPCR analysis. Expression of target mRNA was normalized to expression of the GAPDH, and the data was analysed using the $2^{-\Delta\Delta\text{CT}}$ method. The CCR3 (148 bp) primers were: upstream primer, 5'-ATTGGAAGAGTTA CTTGAGA-3' and downstream primer, 5'-CCTACTTACAGAGCATTA TTG-3'. The GAPDH (133 bp) primers were: upstream primer, 5'-CCT GTCCTCGTAGACAAAATG-3' and downstream primer, 5'-TGAGGTCA ATGAAGGGGTGCT-3'.

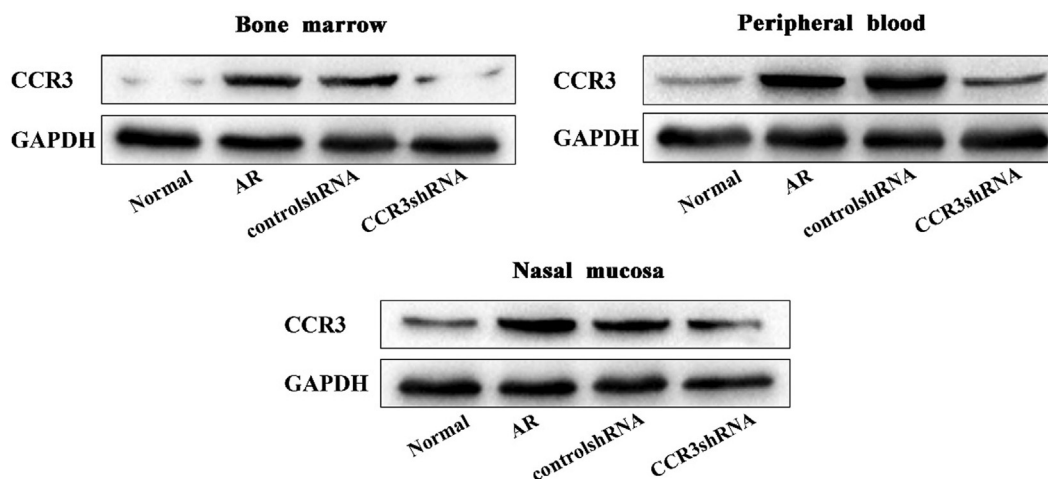


Fig. 1. Expression of CCR3 protein in bone marrow, peripheral blood and nasal mucosa.

2.6. Western blot

First of all, collected the bone marrow, peripheral blood cells and nasal mucosa of each group for total protein extraction with RIPA buffer supplemented with protease inhibitors, and then determined the concentration of lysate. The protein samples were loaded on 10% SDS-PAGE for protein separation and subsequently transferred onto PVDF membranes. Next, blocked with 5% skimmed milk for 2 h at room temperature. After that, the PVDF membranes were incubated with the primary antibody (rabbit anti-mouse CCR3, Abcam; rabbit anti-mouse GAPDH, Abcam) at 4°C overnight and with the secondary antibody for 1 h at room temperature. Finally, specific protein bands were displayed using the ECL chemiluminescence reaction kit.

2.7. H&E staining and toluidine blue staining

After dissection of nasal cavity of the mice, the nasal mucosa was separated and washed with normal saline, and fixed in 10% paraformaldehyde solution. Then, the nasal mucosa tissue were embedded into a block with paraffin, sliced into 4 μm and stained sections with hematoxylin and eosin to observe the histology changes of nasal mucosa under light microscope. In order to detect whether down regulation of CCR3 can reduce infiltration of mast cells into nasal mucosa or not, we performed toluidine blue staining on the nasal mucosa tissue.

2.8. Analysis of electron microscopy

After dissection of nasal cavity of the mice, the nasal mucosa was separated and the nasal mucosa tissues were quickly cut into several pieces of tissue at about 1 mm × 1 mm × 1 mm with a new sharp blade. Immediately, the tissues were put into 2.5% glutaraldehyde at 4 °C for 4 h and then fixed into 1% cesium tetrachloride for 2 h at room temperature. After dehydration, tissue embedding and ultrathin section, 2% uranium acetate, the sections were stained with uranyl acetate followed by lead citrate, and then observation of transmission electron microscopy was performed.

2.9. ELISA assay

Nasal mucosa and cell lysates were homogenized in RIPA lysis buffer supplemented with protease inhibitor. Then the supernatant were centrifuged and collected. At the same time, the serum was harvested by centrifugation at 3000 rpm for 15 min at 4 °C after collecting the blood sample in a centrifuge tube. The levels of histamine, tryptase and PGD2 in mucosa, bone marrow, and serum were measured via an enzyme-linked immunosorbent assay (ELISA) according to the

manufacturer's instructions. Besides, the OVA-specific immunoglobulin E (IgE) in serum was assayed by using ELISA kit.

2.10. Statistical analysis

The data are expressed as mean ± SEM of at least three separate tests. The statistical significance of between the groups was determined by One-way ANOVA using GraphPad version 7 (GraphPad Software, Inc.). Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Inhibition the expression of CCR3 protein by intranasal instillation of CCR3 shRNA lentivirus

To study the effect of intranasal instillation of CCR3 shRNA lentivirus on CCR3 protein expression in a murine model of AR, the mice were sensitized with OVA by intraperitoneal injection and challenged with OVA nasal drip. Then, the mice were treated with CCR3 shRNA lentivirus or lentiviral vector expressing the control shRNA before sensitization and challenge. We obtained bone marrow, peripheral blood and nasal mucosa samples from each group, and detected protein levels of CCR3 in four groups of mice. Besides, it should be noted that the expression of CCR3 gene was detected by extracting the cells from the peripheral blood in our experiment. As shown in Fig. 1, the levels of CCR3 in bone marrow, peripheral blood and nasal mucosa of AR group and controlshRNA group were significantly higher than those in normal group after model establishment. However, compared with the AR group and the controlshRNA group, the CCR3shRNA treatment group had a relatively low level of CCR3 protein expression, which indicated that silencing the CCR3 gene by lentiviral-mediated RNA interference could effectively reduce the expression of CCR3 protein.

3.2. Effect of CCR3 shRNA lentivirus on CCR3mRNA levels in AR mice

To further investigate whether CCR3 shRNA lentivirus could silence the expression of the CCR3 gene, we measured the levels of CCR3mRNA in bone marrow, peripheral blood and nasal mucosa by quantitative real-time PCR. Among them, for peripheral blood, we first extract the cells and then detect the CCR3 gene. It was found significant upregulation of CCR3mRNA levels in AR mice compared with normal control mice and the difference was statistically significant (Fig. 2). Moreover, after intervention with lentiviral-mediated CCR3 shRNA, level of CCR3 gene in the CCR3shRNA group was significantly down regulated compared with the AR group.

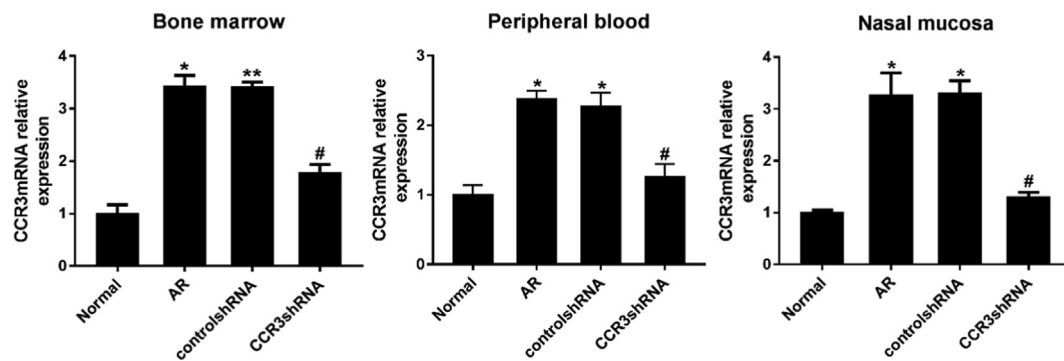


Fig. 2. Effect of lentiviral-mediated CCR3 shRNA treatment on CCR3mRNA levels in animal specimens. ** $P < 0.01$, * $P < 0.05$ versus the Normal group, # $P < 0.05$ versus the AR group.

3.3. Effect of CCR3 shRNA lentivirus on the nasal symptoms and OVA-specific IgE in OVA-induced murine model of allergic rhinitis

To explore the effect of CCR3 shRNA lentivirus on nasal symptoms, we assessed the numbers of sneezing and nasal rubbing movements for 30 min in the final challenge in mice and performed symptom scores according to the scoring standard. The results (Table 1 and Fig. 3A) show a significant increase in frequency of sneezing, nasal rubbing in the AR group and the controlshRNA group. However, the symptom score of the CCR3shRNA treatment group was significantly lower than that of the AR group, and the difference was statistically significant. These results indicate that silencing the CCR3 gene by lentiviral-mediated RNA interference can effectively suppress the allergic rhinitis response. Furthermore, in order to investigate the effects of CCR3 on allergic response in AR mice, we measured the OVA-specific IgE in serum (Fig. 3B). The result showed that the concentration of OVA-specific IgE was significantly increased in AR mice compared to the normal mice. However, intranasal administration of CCR3 shRNA lentivirus inhibited the OVA-specific IgE concentration.

3.4. Effect of CCR3 shRNA lentivirus treatment on histopathological changes of nasal mucosa in mice

In order to explore the effect of CCR3 shRNA treatment on the morphology of mouse nasal mucosa, the nasal mucosa specimens were collected, and the pathological changes of nasal mucosa in each group were observed by H&E staining. As shown in Fig. 4, there was no obvious abnormality of mucosa in normal control group. In contrast, the AR group and the controlshRNA group showed the following changes such as mucosal epithelial shedding, infiltration of eosinophils, mucosal layer thickening and edema of nasal mucosa. However, these changes were improved after treatment with CCR3shRNA. The results revealed that local administration of CCR3shRNA in nasal cavity could reduce the degree of nasal mucosal damage and the infiltration of inflammatory cells.

Table 1

Symptom score after the last challenge in mice ($\bar{x} \pm s$).

Group	Symptom score
Normal	0.875 $\pm$ 0.354
AR	5.500 $\pm$ 0.756**
Controls hRNA	5.500 $\pm$ 0.535**
CCR3 shRNA	1.625 $\pm$ 0.744#

Note: ** $P < 0.01$ versus the Normal group, # $P < 0.05$ versus the AR group.

3.5. Effect of CCR3 shRNA lentivirus treatment on mast cell infiltration of nasal mucosa in mice.

To further determine whether CCR3 shRNA lentivirus can reduce the migration and infiltration of mast cells into nasal mucosa or not, we performed toluidine blue staining and counted the number of mast cells that had infiltrated in the nasal mucosa of each group. The method for assessing mast cell infiltration in the nasal mucosa was performed as described previously [29]. Compared with the normal control group, the number of mast cells was shown to be significantly increased in the AR group and the controlshRNA group. Contrarily, Lentivirus-mediated CCR3 shRNA interference decreased such infiltration of mast cells into nasal cavity, which indicated that CCR3 shRNA treatment could reduce the migration and infiltration of mast cells into the nasal mucosa of allergic rhinitis mice (Fig. 5). In addition, numbers of mast cell in each group were shown in Fig. 6.

3.6. Effect of CCR3 shRNA lentivirus treatment on structure of nasal mucosa cilia in mice

In order to further examine the pathological differences of nasal mucosa between different groups of experimental mice, electron microscopy was used to observe the cilia structure of nasal mucosa in this study. As depicted by electron microscopy analysis, a large number of neatly arranged, uniform thickness of cilia can be observed in normal control group. In the AR group and the control shRNA group, electron microscopy showed increased secretion of the epithelial surface of the nasal mucosa, and the large area of the cilia was reduced, sparse, and disordered arrangement in AR group and control shRNA group, nasal mucosal epithelial secretion increased, large area of cilia shedding decreased, sparse and disordered arrangement. Compared with AR group, the cilia of nasal mucosa in CCR3shRNA group were arranged neatly and uniformly (Fig. 7).

3.7. Effect of CCR3 shRNA lentivirus treatment on histamine, tryptase and PGD2 levels in mucosa, bone marrow and serum

To further investigate whether interference with CCR3 gene can affect the process of mast cells degranulation or not, ELISA was used to detect the concentration of histamine, tryptase and PGD2 in bone marrow, peripheral blood and nasal mucosa of mice. As shown in Fig. 8, compared to mice in the AR group and controlshRNA group, the administration of CCR3 shRNA lentivirus significantly decreased histamine levels in the bone marrow, peripheral blood and nasal mucosa of mice in CCR3 shRNA group. In addition, tryptase level was also significantly reduced by Lentivirus-mediated CCR3shRNA administration. Moreover, the concentration of PGD2 in all samples of mice was significantly decreased in the CCR3 shRNA group.

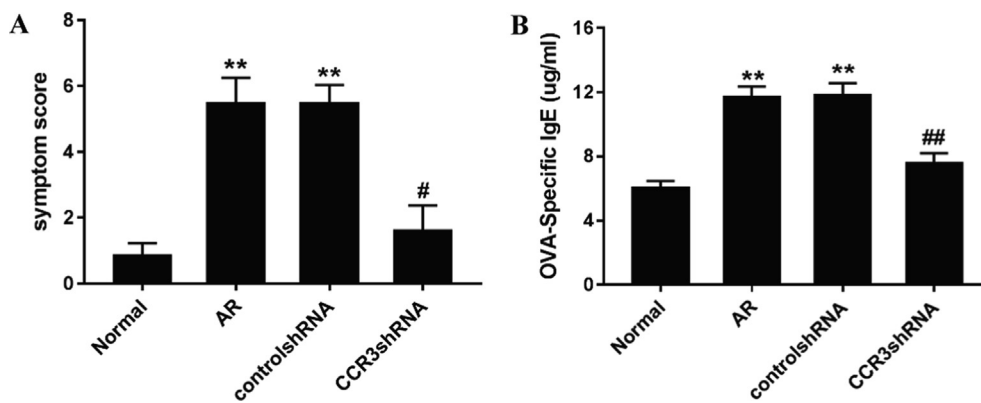


Fig. 3. Effect of CCR3 shRNA lentivirus on the nasal symptoms and OVA-specific IgE. (A) The frequencies of rubbing and sneezing were assessed for 30 min in the final challenge in mice, and symptom scores were calculated. (B) The concentration of OVA-specific IgE in serum were measured using ELISA. The results were expressed as mean $\pm$ SEM. ** $P < 0.01$ versus the normal group, # $P < 0.05$ or ## $P < 0.01$ versus the AR group.

4. Discussion

In this study, we explored whether silencing of CCR3 with short hairpin RNAs (shRNAs) delivered by a lentiviral vector could impact the function of mast cells in a murine model of AR in vivo. The results demonstrated that administration of CCR3shRNA lentivirus could effectively inhibit the expression of the CCR3 gene in bone marrow, peripheral blood and nasal mucosa. Further investigation suggested that inhibiting the expression of CCR3 gene could reduce the nasal symptoms, inflammatory cells and mast cells infiltration into nasal cavity, and relieve the histopathological changes of nasal mucosa. In addition, we also found that intervention of CCR3shRNA could decrease the levels of the histamine, tryptase and PGD2 in bone marrow, peripheral blood and nasal mucosa.

RNAi is a new technology for studying the regulation of gene expression. It is a phenomenon of post-transcriptional gene silencing initiated by double-stranded RNA homologous to the target gene

sequence in eukaryotes. Due to its high specificity and low toxicity, RNAi has been used as an effective tool in gene function or gene therapy research, and each gene causing human disease may be a potential target for RNAi therapy [30], which provides a new way for us to target gene therapy for diseases. siRNA and shRNA are the two major types that mediate silencing gene expression.

Due to concentration of siRNA in cells decreases rapidly during continuous cell division, it can only have a short gene silencing effect. By contrast, shRNA expression vector can synthesize a large number of shRNAs in cells, and the shRNA expression cassette can be stably integrated into the genome of target cells, which is more conducive to inducing long-lasting gene silencing effect [31–34]. Besides, a lentivirus-based viral vector system can significantly increase the efficiency and duration of RNAi. Consequently, in this paper, we selected a lentiviral vector expressing shRNA to generate recombinant lentiviral CCR3 shRNA against CCR3. In our previous study, we successfully constructed an RNAi lentiviral expression vector targeting CCR3 gene,

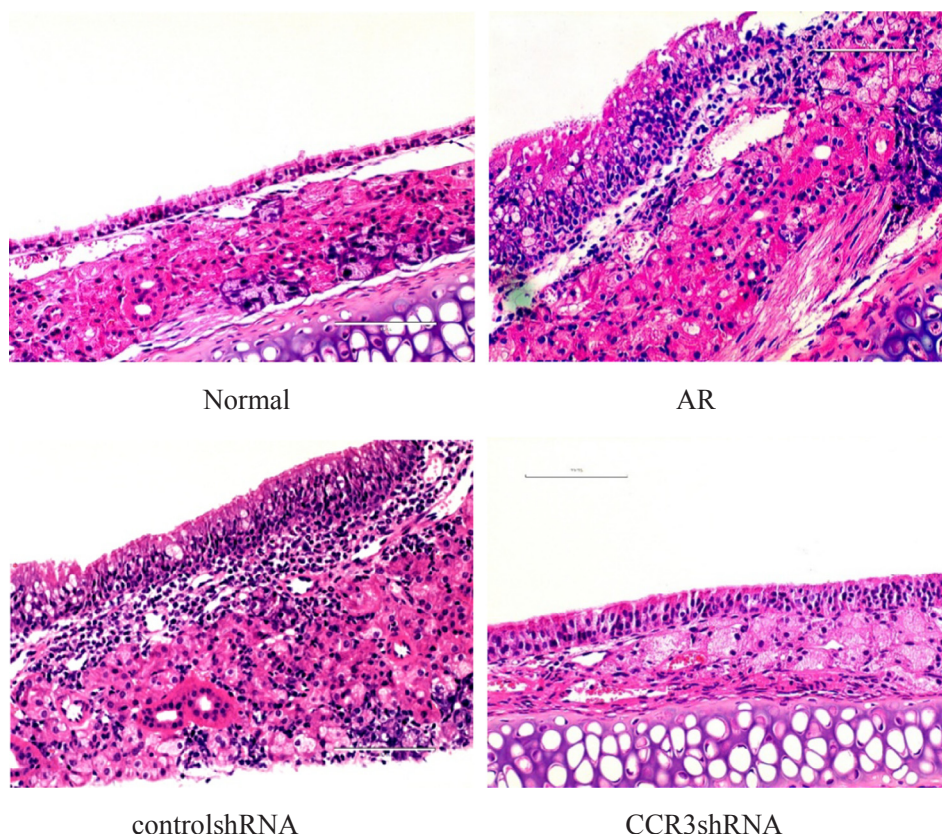


Fig. 4. The pathological changes of nasal mucosa in each group were observed by H&E staining. Representative images were shown ($\times 400$ magnifications).

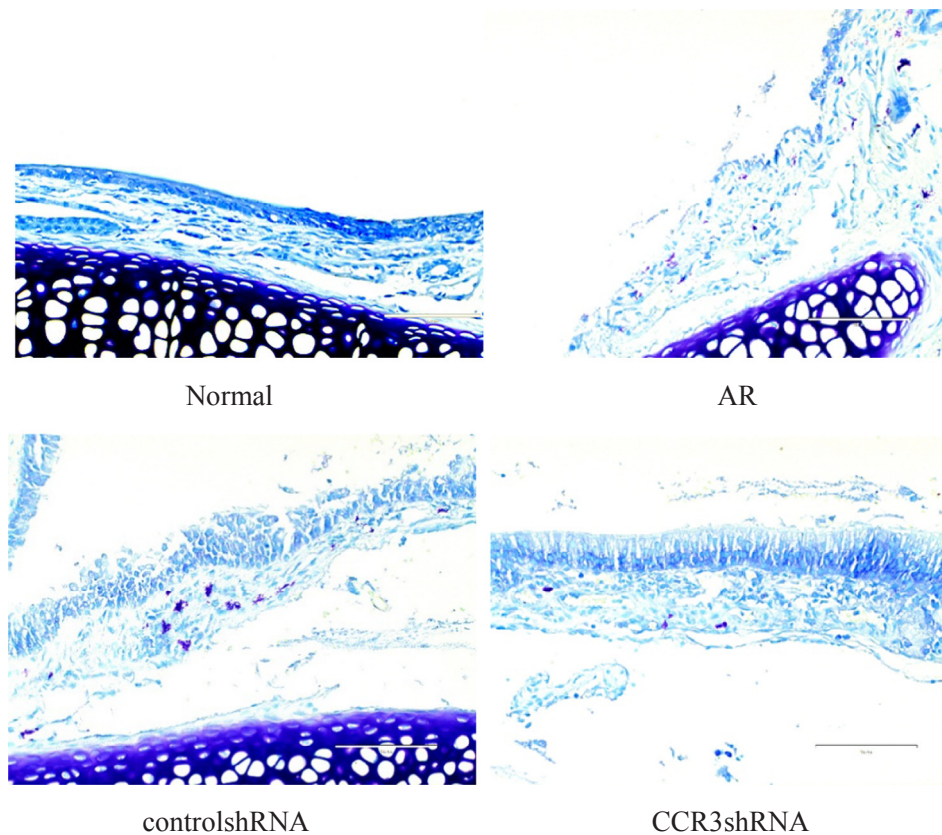


Fig. 5. The infiltration of mast cells of nasal mucosa in each group were observed by toluidine blue staining. Representative images were shown ($\times 400$ magnifications).

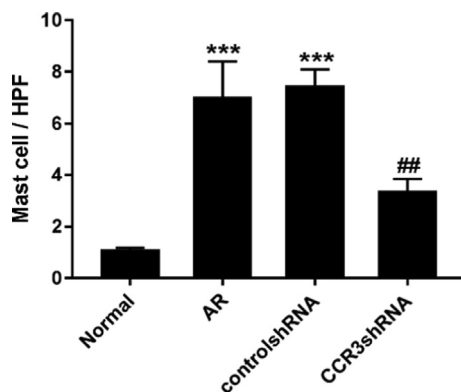


Fig. 6. Analysis of mast cells number in nasal mucosa of mice. The nasal mucosa sections were observed by microscope ($\times 400$ magnifications). Data are presented as mean $\pm$ SEM. *** $P < 0.001$ versus the Normal group, ## $P < 0.01$ versus the AR group.

and found that it can down-regulated the expression levels of CCR3 mRNA and protein in mouse mast cells.

CCR3 is expressed on a variety of inflammatory cells. Accumulating data has shown that CCR3 plays an important role in a variety of inflammatory diseases. One of the studies inhibited eosinophilic inflammation and mucosal damage in a mouse model of eosinophilic gastroenteritis by using CCR3 monoclonal antibodies [35]. Another CCR3-deficient mouse study showed that CCR3 receptors regulated both early and late responses of IgE-mediated allergic conjunctivitis [24]. Moreover, our previous studies [23] on eosinophils also found that down-regulation of CCR3 gene could promote eosinophil apoptosis, inhibit eosinophil proliferation and degranulation, thereby inhibiting the allergic reaction in allergic rhinitis mice. However,

although CCR3 is also expressed in mast cells, there are still many controversies about the effect of CCR3 on mast cells. For example, Collington et al. [25] found that CCR3 did not affect chemotaxis of bone marrow-derived mast cells in mice. Miyazaki D et al. [26] found that blocking CCR3 could significantly inhibit allergen-mediated hypersensitivity and IgE-mediated degranulation of mast cells. In this paper, we found that recombinant lentiviral CCR3 shRNA specifically reduced the expression of CCR3 mRNA and protein of mast cells in bone marrow, peripheral blood and nasal mucosa, which showed that local administration of CCR3 shRNA might have some inhibitory effect on the expression of CCR3 in vivo, including whole body to local tissue.

As is known to all, AR is characterized by nasal obstruction, nasal itching, runny nose and sneezing. The mast cells in the body are mainly distributed in the skin, respiratory tract, and gastrointestinal tract. As an important effector cell in early allergic reactions, it is essential for the development of allergic rhinitis and considerable researches have already explained it [36,37]. Mast cells are initially in the bone marrow in the form of pluripotent hematopoietic progenitors. After leaving the bone marrow, they enter the circulation and migrate to the tissues, and differentiate into mature cells under the influence of local growth factors. However, in this process, the chemokine CCR3 receptor is continuously expressed, suggesting that CCR3 may play a role in mast cell localization and regulation of mast cell function. The bone marrow and systemic hematopoietic mechanisms play an important role in the occurrence and maintenance of allergic inflammation of local tissues. After being stimulated by allergen, the body initiates the bone marrow to produce specific inflammatory cells including mast cell progenitor cells and eosinophil progenitor cells, which were transported to local tissues through peripheral blood circulation and activated to cause local inflammation. CCR3 and its ligands may play an important role in this process. This experiment confirmed that down-regulating the expression of CCR3 in mast cell improved the pathological morphology of the

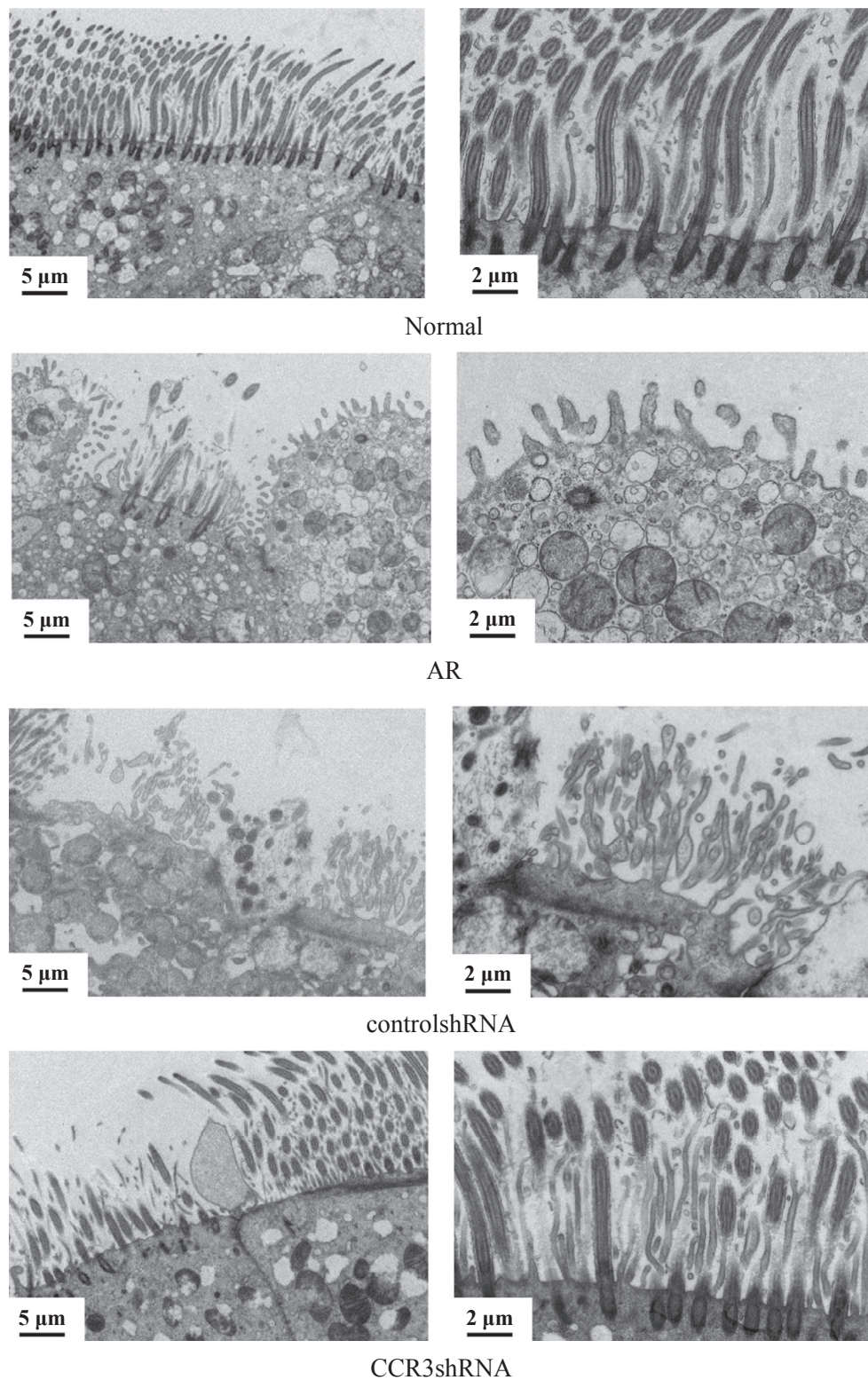


Fig. 7. Analysis of structure of nasal mucosa cilia of mice. The nasal mucosa sections were observed by electron microscopy.

nasal mucosa and the ultrastructural changes of nasal mucosa and inhibited the infiltration of inflammatory cells and mast cells into the nasal cavity, which suggested that CCR3 is involved in the migration and infiltration process of mast cells. Activation of the receptor results in transient calcium flux formation, activation of p38 and EPK2 mitogen-activated protein kinases, actin polymerization, and consequent degranulation release. The activated mast cells will immediately

degranulate and release mast cell products such as histamine, mast cell-specific protease, PDG2 and leukotriene C4. Therefore, in this experiment we tested the histamine, tryptase and PDG2 to explore whether inhibition of CCR3 receptors can affect activated degranulation of mast cells or not. Although the use of OVA caused an increase of OVA-specific IgE in serum, the level of IgE in the lentiviral vector intervention group was decreased. The application of CCR3 shRNA reduced the

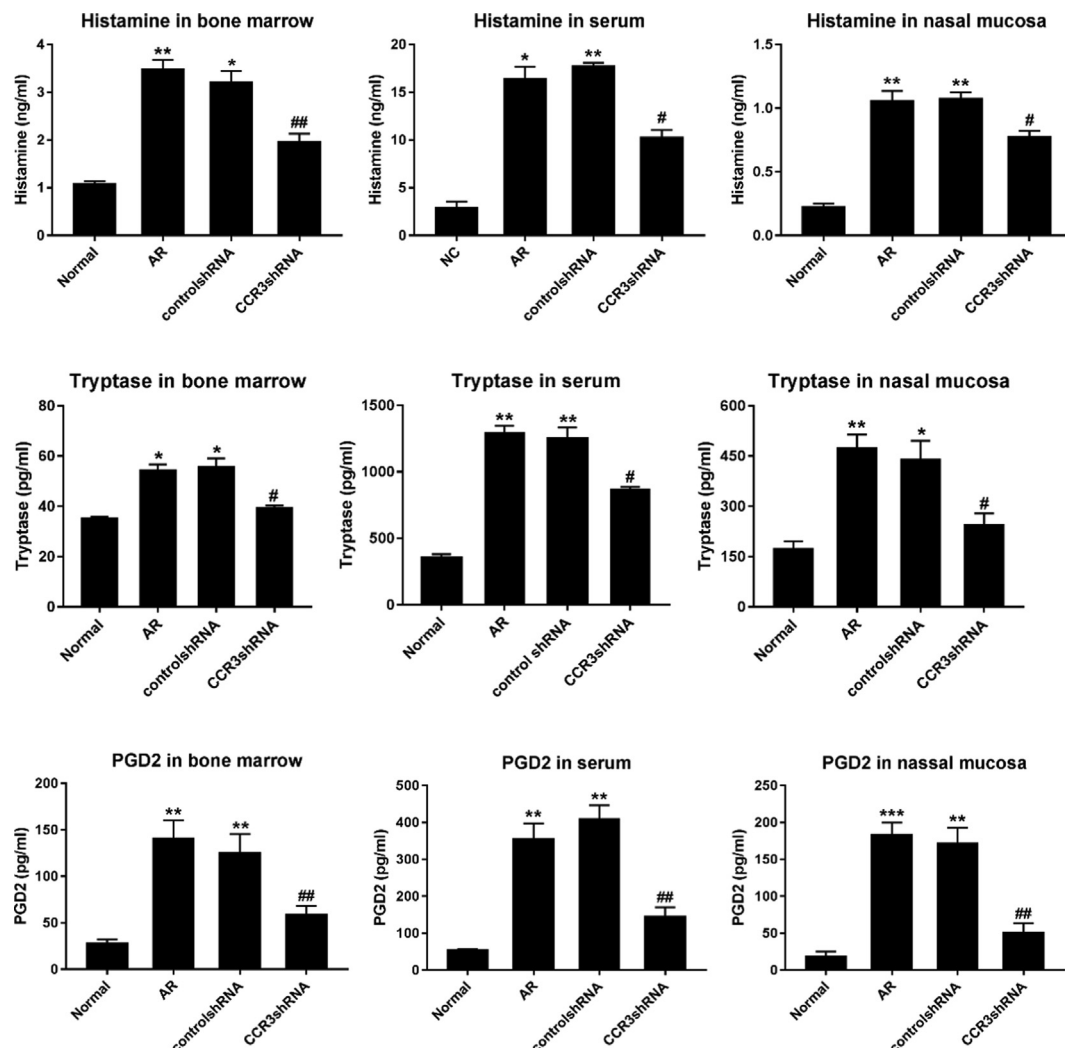


Fig. 8. The ELISA result of histamine, tryptase and prostaglandin D2 (PGD2) in bone marrow, peripheral blood and nasal mucosa. * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$ versus the Normal group, # $P < 0.05$ or ## $P < 0.01$ versus the AR group.

symptom score of the CCR3shRNA group, indicating that the down-regulation of CCR3 can effectively alleviate the symptoms of rhinitis in mice, which may be related to the improvement of inflammatory cell infiltration, the degranulation and the IgE reduction. Histamine can not only stimulate the sensory nerves to cause nasal itching and sneezing reflex behavior by acting on the H1 receptor, but also cause dilation of blood vessels, mucosal edema and nasal obstruction. Related experiments have demonstrated that histamine can weaken the barrier function of epithelial cells and enhance the pathogenic effect in the early stage of allergic rhinitis reaction [38]. In recent years, mechanisms for alleviate allergic reactions by blocking histamine release or preventing mast cell degranulation as therapeutic targets have been confirmed [39]. Tryptase is stored in secretory granules of mast cells and is also a marker of mast cell activation. Mast cell products including tryptase can induce further degranulation of mast cells, promote further release of mediators and enhance further recruitment of granulocytes through positive feedback mechanism [40,41]. Prostaglandins such as PGD2 can also cause nasal edema due to vasodilation and increase vascular permeability. From the results of this experiment, we found that after the inhibition of CCR3 gene by RNAi technology, the release of histamine, tryptase and PGD2 in the CCR3shRNA treatment group was significantly reduced compared with the AR group. Therefore, we believe that CCR3 have an effect on the degranulation function of mast cells. In conclusion, we found that CCR3 lentivirus vectors have inhibitory effects on migration, infiltration and degranulation of mast cells in local

tissues, thereby alleviated the inflammation of allergic rhinitis.

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Declaration of Competing Interest

The authors declare no conflict of interest

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