

Original Article

Effects of Dectin-1 on the mast cells in allergic conjunctivitis and its underlying mechanism

Lijuan Wei¹, Jinsong Zhang², Yuan Wang³, Lixiao Zhou⁴

¹Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, China; ²Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; ³Department of Ophthalmology, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou, China; ⁴Department of Ophthalmology, The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Received November 6, 2015; Accepted January 3, 2016; Epub February 1, 2016; Published February 15, 2016

Abstract: Our previous investigations have shown that Dectin-1 plays a crucial role in allergic conjunctivitis (AC) and the mast cells mediate this allergic reaction. Here, we tried to investigate the possible effects and underlying signal pathway of Dectin-1 on the mast cells in allergic conjunctivitis mice. 30 Balb/c mice were randomly divided into control and AC groups. The mice were immunized with phosphate buffer solution (PBS) or ovalbumin (OVA) to establish control or allergic conjunctivitis model. Prostaglandin D₂ (PGD₂), histamine (HIS), interleukin 4 (IL-4), and tumor necrosis factor-alpha (TNF-α) in the serum of the mice were detected using enzyme-linked immunosorbent assay (ELISA). Additionally, conjunctival tissues in mice were separated and RNA was extracted, the expression of Dectin-1 was detected using real-time PCR. Moreover, the peritoneal mast cells were separated and then stimulated using allergen OVA or control allergen BSA and mRNA levels of PGD₂, histamine, IL-4 and TNF-α were detected. Furthermore, western blot was used to examine the expressions of Dectin-1, and two downstream signal molecules Syk and Bcl-10 to elucidate the possible effects and underlying mechanism of Dectin-1 on allergic conjunctivitis. The expression of Dectin-1 was significantly up-regulated in the conjunctiva tissues in AC group. Importantly, the expression of Dectin-1 in the OVA treated mast cells was dramatically elevated compared to BSA groups. Moreover, Syk and Bcl-10 were up-regulated after OVA stimulation. These data indicates that Dectin-1 via Syk-Bcl signal pathway triggers immune responses, which promotes the development of allergic conjunctivitis.

Keywords: Allergic conjunctivitis, allergic response, Bcl-10, Dectin-1, Syk

Introduction

Allergic conjunctivitis (AC) is a class of conjunctival reactive inflammation and very common in department of ophthalmology. AC is a type I hypersensitivity disease mediated by the mast cells and IgE [1, 2]. To note, the pathogenesis of AC is complicated. The allergens are extensively broad in different individuals. The most common allergens are pollens, dusts, microbial proteins and polysaccharide components, etc. Innate immune responses depend on a series of pattern recognition receptors (PRRs) on the surface of the cell membrane. PRRs can identify the conserved structure-pathogen-associated molecular pattern (PAMPs), of the microorganisms. PAMPs are highly conserved microbial structures that serve as ligands for pattern rec-

ognition receptors (PRRs). When the PRR_s recognizes PAMPs, the body will produce the corresponding innate immune responses, and ultimately form the specific adaptive immune response [3]. The cell walls of fungi are mainly composed of carbohydrates, including β-dextran and chitosan, etc. These polysaccharides can act as PAMPs, which can be identified by the corresponding PRRs and then activate the immune signaling pathway. β-dextran receptors dendritic cell associated C-type Lectin-1 (Dectin-1) is an important PRR_s [4, 5]. Previous investigations have shown that Dectin-1 is required to absorb, killing and early inflammatory reaction in fungi. The expression of Dectin-1 are found in epithelial cells and mast cells, which can trigger the immune signaling pathways and tyrosine kinase Syk plays an impor-

Mechanism of Dectin-1 in allergic conjunctivitis

tant role to mediate the signal transduction [6, 7]. Briefly, investigations about Dectin-1 will provide important theoretical bases to elucidate the possible mechanisms of allergic conjunctivitis [8, 9].

In the current work, we investigate the expressions of Dectin-1 in allergic conjunctivitis effects in preclinical animal model. Furthermore, we explore the underlying mechanisms of Dectin-1 at mRNA and protein level using separated mast cells in AC mice.

Materials and methods

6-8 weeks old female Balb/c mice were maintained in a climate-controlled room on a 12 hr light/dark cycle, and food and water were available ad libitum. The room temperature was maintained at 18-25°C. 30 mice were used in the current study and 5 mice were in each cage. Mice were purchased from Shanghai SLRC Laboratory Animal Co., Ltd. All experiments were performed in accordance with the policies and recommendations of the International Association for the Study of Pain and the National Institutes of Health guidelines for the handling and use of laboratory animals and received approval from the Institutional Animal Care and Use Committee of the Zhengzhou University. All efforts were made to minimize animal suffering and reduce the number of animals used.

Main materials and reagents

Ovalbumin (OVA, A5503) and aluminum hydroxide (V900163) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). PGD₂, Histamine (HIS), IL-4 and TNF- α ELISA kits were obtained from USCN Business Co., Ltd. (Wuhan, China). The catalog numbers were as follows: SEB640Mu, CEA927Ge, SEA077Mu and SEA133Mu, respectively. Total RNA extraction kit was from Tiangen Biotech (DP430, Beijing, China) Co., Ltd. Reverse transcription Kit was purchased from Applied Biosystems (No. 4366597, New York, USA). Real-time PCR fluorescence quantitative reagent kit was from Bio-Rad (No. 172-5264, California, USA). Percoll cell liquid separation was from Pharmacia Company (New York, USA) and ReadyPrep protein extraction kit was purchased from Bio-Rad (California, USA). BCA protein quantitative kit was obtained from Vazyme Biotech (Nanjing,

China). Dectin-1, Syk, Bcl-10 monoclonal antibodies were from Santa Cruz Biotech (Texas, USA) and the catalog numbers were sc-73897, 573 and 5611, respectively. Horseradish peroxidase (HRP) labeled second antibody was from ZSGB-Bio (Beijing, China). ECL chemiluminescent substrate reagent kit was purchased from Life Technologies (WP20005, New York, USA).

CO₂ cell culture box was purchased from Thermo Scientific (New York, USA). Nucleic acid quantitative analyzer was from Qubit Fluorometer (Massachusetts, USA). Fluorescence quantitative PCR detection system was from Bio-rad-CFX96 Touch (California, USA).

Establishment of allergic conjunctivitis model, isolation and culture of mast cells

30 Balb/c mice were randomly divided into 2 groups: Ctrl group and AC group. 15 mice were in each group. After fed for 7 days, the mice were carried out to establish the AC model. AC model was established by two steps: sensitization and inspiration. Sensitization was carried out by intraperitoneal injection of 100 mg ovalbumin (OVA) and the adjuvant 5 mg Al (OH)₃ dissolved in 200 ml every week and continued for three weeks. The mice were treated with 400 mg OVA dissolved in 100 ml physiological saline into the right eyes once every day for 7 days. After that, 400 mg OVA dissolved in 100 ml PBS (pH 7.7) was used to allergen attack the eyes and induce AC symptoms.

After establishing the AC model, the blood was taken from the tail vein. The mice were killed by cervical dislocation. The abdomen was disinfected by 75% alcohol and 5 ml sterile Tyrode's solution without calcium was injected intraperitoneally. Next, the abdomen was gently massaged and opened on the super clean bench. The peritoneal fluid was drawn and centrifuged for 10 min (1500 rpm) at 4°C. Then, the supernatant was discarded. 1 ml Tyrode's solution without calcium was added and the cells were suspended. After that, 80% Percoll separation medium was added and centrifuged about 15 min (2500 rpm) at 4°C. The cells at the division were collected and put in RPMI 1640 culture liquid containing 10% calf serum (5% CO₂) at 37°C.

Mechanism of Dectin-1 in allergic conjunctivitis

Table 1. The primers used in Real-time PCR

Gene	GenBank Accession	Primer (5'-3')	Length	Tm
Dectin-1	NM_020008.3	For: GACTTCAGCACTCAAGACATCC	22	60.6
		Rev: TTGTGTCGCCAAAATGCTAGG	21	61.2
Interleukin-4	NM_021283.2	For: GGTCTCAACCCCAAGCTAGT	20	62.8
		Rev: GCCGATGATCTCTCTCAAGTGAT	23	61.5
TNF-a	NM_013693	For: CAGGCGGTGCCTATGTCTC	19	62.2
		Rev: CGATCACCCCGAAGTTCAGTAG	22	62.1
GAPDH	NM_008084.3	For: AGGTCGGTGTGAACGGATTTG	21	60.8
		Rev: GGGGTCGTTGATGGCAACA	19	61.4

For, forward; Rev, reverse.

Table 2. The synthetic system of inverse transcription

Components	Volume per Reaction
5×iScript reaction mix	4 µl
iScript reverse transcriptase	1 µl
RNA template(1 µg RNA)	1 µg
Nuclease-free water	Up to 20 µl

iScript reaction mix was from Bio-Rad (California, USA);
Nuclease-free water was from Invitrogen (New York, USA).

Table 3. The synthetic system of PCR

Components	Volume per Reaction
SsoAdvanced SYBR Green	
Super mix	5 µl
Forward primer (10 µM)	0.3-0.4 µl (300-400 nM)
Reverse primer (10 µM)	0.3-0.4 µl (300-400 nM)
cDNA template	100 ng
Nuclease-free water	Up to 10 µl

SsoAdvanced SYBR Green Super mix was from Bio-Rad (California, USA).

Detection of related inflammatory factors using ELISA

The blood was collected from the tail vein and put it in the room temperature for 2 hours, and the serum was centrifuged. The quantitative analysis of TNF- α , IL-4 and PGD₂ in serums was detected using ELISA assay kits. Briefly, the standard wells, the sample wells, and the blank wells were set up. 7 standard wells were established and the different concentrations of the standards were added. Then, the tested samples were added and incubated for 2 h at 37°C. Next, the biotinylated primary antibodies were added about 100 µl and incubated for

1 h at 37°C. The solution in the wells was discarded and washed with 350 µl washing buffer for 1-2 min three times. 100 µl HRP labeled secondary antibodies were added followed by incubation for 30 min at 37°C. Next, 90 µl TMB substrate was added to visualize the results for 15-25 min at 37°C and the light should be avoided. When the first 3

standard wells appeared an obvious blue gradient, the enzymatic reaction was terminated and 50 µl 2M H₂SO₄ was added. Immediately, the optical density (OD) values were measured using micro-plate reader at the absorbance 450 nm wavelengths.

According to the standard curve, the target protein concentration was calculated, and the data were expressed as mean \pm SD. T-test was used to compare the differences between two groups. *P<0.05, **P<0.01.

Real-time PCR

The collected cells were washed using RNase free PBS and the total RNA was extracted with a RNA Extraction Kit. The concentration and purity of RNA were tested using Qubit Fluorometer (Massachusetts, USA). In brief, total RNA was reversed according to the reverse transcription kit to cDNA and the expression of the relevant genes was detected using real-time PCR. The mRNA sequences of Dectin-1, IL-4 and TNF- α gene were searched in the National Center for Biotechnology Information (NCBI) database and the real time PCR primers were designed. All the primers were synthesized by Shanghai Generay Biotech Co., Ltd. The specific sequences were shown in **Table 1**. The relative expression levels of target genes were calculated using double Δ Ct value. The Ct values were obtained from the average values in three parallel experiments. Δ Ct = CT (Target Gene) - CT (internal reference), $\Delta\Delta$ Ct = Δ Ct (sample) - Δ Ct (control). Therefore, the relative expression levels of target genes was $2^{-\Delta\Delta C_t}$ and $2^0 = 1$ [10] in the control group. The synthetic system of inverse transcription and the synthetic system of PCR were shown in **Tables 2** and **3**.

Mechanism of Dectin-1 in allergic conjunctivitis

Table 4. The levels of PGD₂, HIS, IL-4 and TNF-α in the serum using ELISA

	PGD ₂	HIS	IL-4	TNF-α
Ctrl	276.34±41.07	12.79±1.06	22.78±0.98	107.01±12.03
AC ^a	403.66±52.2 ^{a,b}	19.06±1.88 [*]	46.16±0.93 [*]	235.48±16.87 ^{**}

a. Vs. Ctrl ^{*}P<0.05; ^{**}P<0.01; b. Mean ± SD, ng/l.

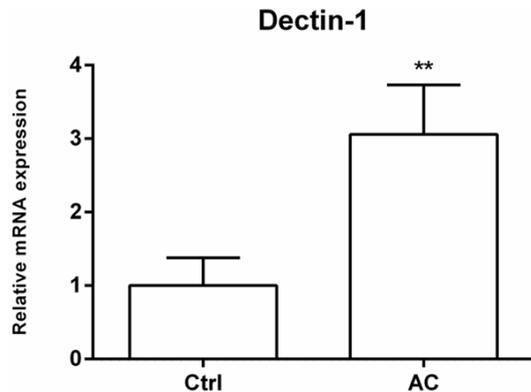


Figure 1. Expressions of Dectin-1 in the allergic conjunctivitis tissues. The relative mRNA expressions of Dectin-1 was dramatically higher in AC that in the control group (^{**}P<0.01).

Western blot

The collected cells were washed twice using PBS. Total protein was extracted using ReadyPrep protein extraction kit. Briefly, the cells were placed on ice for 30 min and the ultrasound was performed to lyse cells. The lysis was centrifuged for 20 min at 4°C and the speed was 13,000 r/min. Protein concentration was determined with BCA method.

20 g total protein samples was electrophoresed using SDS-PAGE. Then, the gel was transferred into transfer buffer for 10 min. After that, proteins were transferred onto PVDF for 10-15 min at 100 mA. The membrane was blocked in 5% (w/v) non-fat milk TBS/T for 1 h at the room temperature and then treated with the primary antibody (diluted with 1% (w/v) non-fat milk in TBS-T), for 2 h at the room temperature. Next, the membrane was washed using TBS-T for 5-10 min each time and three washes. Then, the membrane was treated with HRP labeled secondary antibody (1:10000, diluted with 0.05% (w/v) non-fat milk in TBS-T) for 1 h at room temperature. Next, the membrane was washed using TBS-T for 5-10 min each time and three washes. After exposure, photographs

were preserved. The experiments were repeated for three times. The bands were observed and analyzed with a gel analysis system Quantity one v4.62. The relative protein expression of target protein was calculated as follows: relative expression of target protein = OD target protein/OD GAPDH, and GAPDH served as an internal reference.

Statistical analysis

All the data was expressed as mean±SD. Paired t-test was used to compare the differences between treatment group and control group. P<0.05 was considered to be statistically significant. The data were analyzed using SPSS 11.5 statistical software.

Results

Establishment of allergic conjunctivitis mice model

30 Balb/c mice were randomly divided into two groups, namely Ctrl group and AC group, 15 in each group. In the process of modeling, two mice in the AC group were died because of infection, and finally 13 mice in AC group. After the second allergen attack into the eyes using 400 µg OVA solved in 100 µl pH 7.7 PBS, blood samples were collected from tail vein and the serum was separated. The concentrations of PGD₂, HIS, IL-4 and TNF-α in the serum of AC group and Ctrl group were detected by ELISA. The results showed that **Table 4**. The concentrations of PGD₂, HIS, IL-4 and TNF-α in AC group were significantly higher than that in the control group (P<0.05). Real-time PCR suggested that Dectin-1 in the conjunctivitis tissues was higher than that in the control groups, as shown in **Figure 1**.

Expression of IL-4, TNF-α and Dectin-1 in the peritoneal mast cells of allergic conjunctivitis

The peritoneal mast cells in mice were isolated in AC group and followed by OVA or BSA stimulation. 5 mice in AC group were sacrificed. The peritoneal mast cells was extracted and then cultured. The livability of the cultured peritoneal mast cells was 87.21%, and the purity was 72.33%. 4 hours after OVA or BSA stimulation, the cells were collected and total RNA was extracted. Real-time PCR indicated that the

Mechanism of Dectin-1 in allergic conjunctivitis

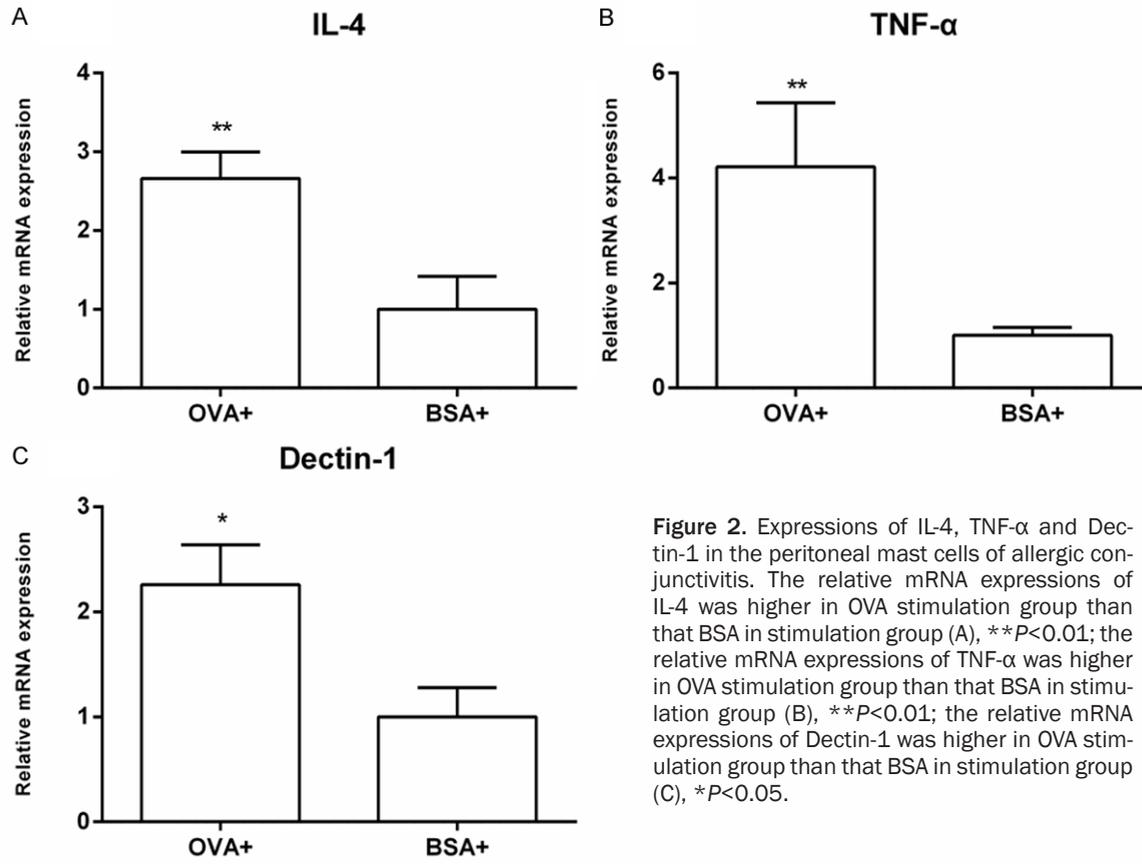


Figure 2. Expressions of IL-4, TNF- α and Dectin-1 in the peritoneal mast cells of allergic conjunctivitis. The relative mRNA expressions of IL-4 was higher in OVA stimulation group than that BSA in stimulation group (A), ** $P<0.01$; the relative mRNA expressions of TNF- α was higher in OVA stimulation group than that BSA in stimulation group (B), ** $P<0.01$; the relative mRNA expressions of Dectin-1 was higher in OVA stimulation group than that BSA in stimulation group (C), * $P<0.05$.

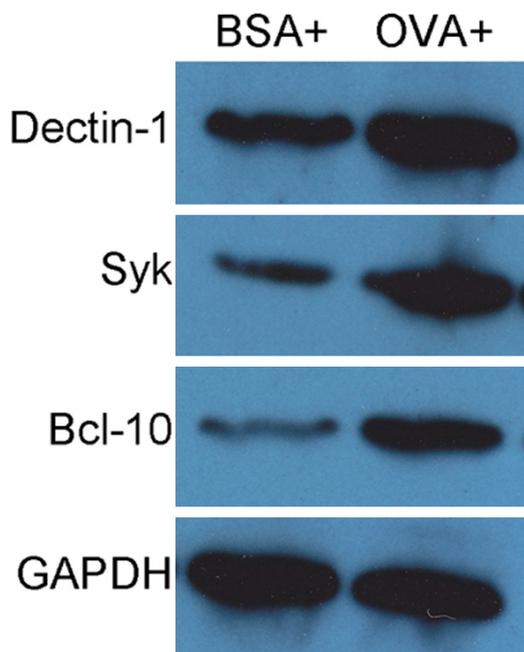


Figure 3. Expressions of Dectin-1 and its downstream molecules Syk and Bcl-10 in allergic conjunctivitis mice. The expressions of Syk, Bcl-10 and Dectin-1 were significantly up-regulated after OVA stimulation compared with that after BSA stimulation.

expressions of IL-4, TNF- α and Dectin-1 in OVA group ($n = 7$) were 2.66 ± 0.34 , 4.21 ± 1.22 , and 2.26 ± 0.38 , respectively, and higher than that in BSA group (the relative expression is 1; $n = 6$), as shown in **Figure 2**.

Expression of Dectin-1 and its downstream molecules Syk and Bcl-10 in allergic conjunctivitis mice

Western blot was used to detect the expressions of Dectin-1 and its downstream molecules Syk and Bcl-10 in AC mice. We found that the expression of Dectin-1 in OVA group was higher than that in BSA group ($P<0.05$). The expression of Syk and Bcl-10 in Dectin-1 signaling pathway was significantly up-regulated after OVA stimulation ($P<0.01$), as shown in **Figure 3**. The data from Western blot was consistent with that from real-time PCR.

Discussion

Allergic conjunctivitis (AC) is well-known as allergic reaction conjunctivitis, which is a hypersensitivity reaction of the conjunctiva induced by the external allergen. AC is a type I hypersen-

Mechanism of Dectin-1 in allergic conjunctivitis

sitivity disease mediated by the mast cells and Ig E [1, 2]. The pathogenesis of AC is not clear yet. The allergens are extensively broad in different individuals. The most common allergens are pollens, dusts, microbial proteins and polysaccharide components, etc. They are pathogen-associated molecular patterns (PAMPs) and can trigger allergic responses in the body.

Except for toll-like receptor (TLR) family, Dectin-1 is discovered in recent years that can induce the own intracellular signal pattern recognition receptors. Dectin-1 can trigger phosphorylation of tyrosine kinase under Src family kinases (SFKs) and activate the downstream signal pathway after the receptors bind to the ligands [8]. Dectin-1 was expressed in epithelial cells and mast cells. The main component of fungi cell walls is β -dextran, which is PAMPs of Dectin-1. Previous investigations have shown that except for β -dextran, Dectin-1 can recognize other polysaccharides and trigger the immune responses [11-13]. In the current work, we investigate the expressions of Dectin-1 in allergic conjunctivitis effects in preclinical animal model. Furthermore, we explore the underlying mechanisms of Dectin-1 using AC mast cells at mRNA and protein levels.

To note, allergic conjunctivitis animal model is established via the secondary sensitization of PAMPs, such as *Cunninghamia lanceolata* pollen with aluminium hydroxide adjuvant immunized Hartley guinea pigs, ovalbumin (OVA) with aluminium hydroxide adjuvant immunized Balb/c mice. In the current investigation, we used ovalbumin (OVA) with aluminium hydroxide adjuvant immunized Balb/c mice to establish AC model and elucidate our hypothesis. Degranulation of mast cells is considered as crucial responses in type I hypersensitivity reactions. Currently, treatments on allergic conjunctivitis are mast cell stabilizer and can inhibit its degranulation, which can effectively relieve the symptoms of AC patients. Degranulation of mast cells can induce the secretion of chemokine, pro-inflammatory factor and some substances which can induce spasm. The common mediators in this process are prostaglandin D_2 (PGD_2), histamine (HIS), interleukin 4 (IL-4) and tumor necrosis factor (tumor necrosis factor-alpha, $TNF-\alpha$) [15, 16]. In the current investigation, we tested the above-mentioned factors and found that concentrations of PGD_2 , HIS, IL-4 and $TNF-\alpha$ in the serum

in the OVA stimulated AC model were much higher than that in the control group, which is consistent with the previous reports and indicates that AC model is stable and the data is creditable in the current investigation. The immune signal pathways were activated and further secreted a variety of inflammatory cytokines in AC model. Up-regulation of PGD_2 suggests that substances secreted by mast cell degranulation produce pathological manifestations such as smooth muscle spasm and other pathological manifestations. Moreover, we separated the conjunctiva and extracted RNA and found that Dectin-1 level in the conjunctiva of AC mice was dramatically increased compared with that of control group. The data indicates that Dectin-1 plays a crucial role in the early stage of AC.

To further explore the effects of Dectin-1 on a mast cell in the AC model, we separated the mast cells according to literatures [17, 18]. We administrated the allergen OVA or the control BSA to stimulate the separated mast cells and analyzed the mRNA expression of IL-4 and $TNF-\alpha$. Importantly, average livability of mast cells was 87.21% and the purity was 72.33%, which validates the method and experimental procedures in the current investigation. After 4 hours stimulation with OVA or BSA, we found that the expressions of IL-4, $TNF-\alpha$ and Dectin-1 were significantly increased in AC group, compared with that in the control group. The data indicates that Dectin-1 plays an important role on the mast cells mediated allergic conjunctivitis. Moreover, western blot data demonstrates that the expressions of Dectin-1 and its downstream signal molecules, such as Syk, Bcl-10, dramatically up-regulated after OVA stimulation and didn't have a significant change after in BSA stimulation, which is consistent with the data at the mRNA level and further confirm our hypothesis. Syk and Bcl-10 are the middle molecules in the signal pathway, which receive the signals from upstream PRR_s and then trigger the downstream NF-kappa-B signal pathway, and further express and secret a variety of inflammatory molecules and induce the development of allergic conjunctivitis [19, 20].

In summary, we have demonstrated that Dectin-1 via Syk-Bcl signal pathway triggers immune responses, which promotes the development of allergic conjunctivitis. The current investigation provides further evidences to elu-

Mechanism of Dectin-1 in allergic conjunctivitis

elucidate the underlying mechanisms of allergic conjunctivitis. The underlying action sites and mechanisms involved into allergic conjunctivitis and possible mechanisms will increase understanding of allergic conjunctivitis mechanism and may offer translational opportunities for the discovery of novel therapeutic strategies for allergic conjunctivitis. Dectin-1 would be a valuable target to affectively treat allergic conjunctivitis in patients.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jinsong Zhang, Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450000, China. Tel: 86-371-66916420; Fax: 86-371-66916420; E-mail: ophzjs@yahoo.com.cn

References

- [1] Chigbu DI. The pathophysiology of ocular allergy: a review. *Cont Lens Anterior Eye* 2009; 32: 3-15.
- [2] Shen ZH, Song JT, Yang W, Gao JS. Effects of Chuanjiao Decoction on substance pin allergic conjunctivitis with mice. *J Traditional Chin Ophthalmol* 2011; 25: 87-90.
- [3] Diacovich L, Gorvel JP. Bacterial manipulation of innate immunity to promote infection. *Nat Rev Microbiol* 2010; 8: 117-28.
- [4] Pyz E, Marshall AS, Gordon S, Brown GD. C-type lectin-like receptors on myeloid cells. *Ann Med* 2006; 38: 242-51.
- [5] Brown GD. Dectin-1: a signaling non-TLR pattern-recognition receptor. *Nat Rev Immunol* 2006; 6: 33-43.
- [6] Osorio F, LeibundGut-Landmann S, Lochner M, Lahl K, Sparwasser T, Eberl G, Reis e Sousa C. DC activated via dectin-1 convert Treg into IL-17 producers. *Eur J Immunol* 2008; 38: 3274-81.
- [7] Whibley N, Gaffen SL. Brothers in arms: Th17 and Treg responses in *Candida albicans* immunity. *PLoS Pathog* 2014; 10: e1004456.
- [8] Xiuli Yi, Bin Li, Xianlong Qi. Recent insights from Dectin-1-pattern recognition receptor. *Chin J Mycol* 2012; 7: 121-124.
- [9] Hernández-Santos N, Huppler AR, Peterson AC, Khader SA, McKenna KC, Gaffen SL. Th17 cells confer long-term adaptive immunity to oral mucosal *Candida albicans* infections. *Mucosal Immunol* 2013; 6: 900-10.
- [10] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-(\Delta\Delta C(T))}$ Method. *Methods* 2001; 25: 402-8.
- [11] Pandey S, Singh S, Anang V, Bhatt AN, Natarajan K, Dwarakanath BS. Pattern recognition receptors in cancer progression and metastasis. *Cancer Growth Metastas* 2015; 8: 25-34.
- [12] Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defense. *Nat Rev Immunol* 2007; 7: 179-90.
- [13] Suaini NH, Zhang Y, Vuillermin PJ, Allen KJ, Harrison LC. Immune modulation by vitamin D and its relevance to food allergy. *Nutrients* 2015; 7: 6088-108.
- [14] Ondas O, Keles S. Central corneal thickness in patients with atopic keratoconjunctivitis. *Med Sci Monit* 2014; 20: 1687-90.
- [15] Yang H, Li T, Wei J, Zhang H, He S. Induction of tumor necrosis factor (TNF) release from subtypes of T cells by agonists of proteinase activated receptors. *Mediators Inflamm* 2013; 2013: 165453.
- [16] Gehlsen U, Cursiefen C, Steven P. Basic immunology and current therapeutic concepts in ocular allergy. *Klin Monbl Augenheilkd* 2014; 231: 490-5.
- [17] Gu YJ, He ML, Yin LH, Dong GQ. The three ways to separate the peritoneal mast cells in mice. *China Animal Husbandry & Veterinary Medicine* 2014; 41: 115-118.
- [18] Gong J, Yang NS, Croft M, Weng IC, Sun L, Liu FT, Chen SS. The antigen presentation function of bone marrow-derived mast cells is spatiotemporally restricted to a subset expressing high levels of cell surface FcεR1 and MHC II. *BMC Immunol* 2010; 30: 11:34.
- [19] Roth S, Ruland J. Caspase recruitment domain-containing protein 9 signaling in innate immunity and inflammation. *Trends Immunol* 2013; 34: 243-50.
- [20] Mócsai A, Ruland J, Tybulewicz VL. The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat Rev Immunol* 2010; 10: 387-402.